STATE OF THE ART REPORT ON QUINOA AROUND THE WORLD IN 2013

Quinua 2013 International Year

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FOREWORD

In his address given at the launch of the **International Year of Quinoa**, FAO Director-General José Graziano da Silva described quinoa as an important ally in the fight against hunger. In this respect, we must capitalize on technical and scientific breakthroughs and use all the available knowledge on this noble crop and superior source of nutrition, not only to maximize the benefits of this golden grain but also to understand the challenges and related risks.

In 2002, in a first great endeavour, FAO published the document entitled "**Quinua (*Chenopodium quinoa*); ancestral cultivo andino, alimento del presente y futuro**". Only a decade after this publication, in this era of new technologies, further research, innovations and knowledge were emerging.

In this context, and within the framework of the International Year of Quinoa, it was considered indispensable to renew efforts to compile all the progress made in recent years towards better understanding quinoa, through this document entitled "***State of the Art Report on Quinoa around the World***". This report aims to become a hallmark document for making better and more informed decisions on quinoa.

FAO therefore called upon the International Cooperation Centre of Agricultural Research for Development (CIRAD), which agreed to rise to the great challenge of conducting research and coordinating with the authors of the chapters in this report.

Through this initiative, FAO and the Regional Office for Latin America and the Caribbean are pleased to be fulfilling one of the Organization's main goals, which is to distribute and share specialized information throughout the global community.

Raúl Benítez

FAO Regional Representative for Latin America and the Caribbean

Introduction to the State of the Art Report on Quinoa around the World

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Why write a report on the state of the art of quinoa in the world in 2013?

In 2013, the United Nations declared the International Year of Quinoa. It gave global priority to quinoa, fostering expectations and highlighting challenges. The scientific studies and articles compiled herein describe with precision the potential contribution of quinoa and its limitations with regard to its cultivation, and promote its consumption in different parts of the world.

The state of the world's quinoa tracks the "footsteps" of quinoa to determine current sectorial trends in 2013 for this exceptional crop which, due to its nutritional qualities, its diversity and its resistance to drought and cold, has been identified as an important alternative to contribute to global food security, especially in areas where the population has no access to adequate sources of protein, or where there are environmental constraints to food crop production.

In this context, the main aim of the **State of the Art Report on Quinoa around the World** is to bring together, within a single document, up-to-date technical and scientific data on growing quinoa so as to encourage the dissemination of this knowledge, promote dialogue and debate amongst partners in the development of quinoa worldwide, and generate new expectations for the crop around the world, in view of its contributions to food security and the family farming economy and also considering the inherent risks of uncontrolled expansion. Special emphasis is given to the need to regulate the use of plant genetic resources, sustainability of agricultural systems and the fair and equitable distribution of benefits from using quinoa outside the Andean region.

This book is divided into six sections comprising currently available data on the various topics of interest related to growing quinoa around the world.

In Section 1, aspects of "Botanics, Domestication and Exchanges of Genetic Resources" are presented. Quinoa (*Chenopodium quinoa* Willd.) is an annual plant with a wide diversity of cultivars and varieties. It is among the species domesticated around Lake Titicaca, between Peru and Bolivia, a location considered to be the birthplace of quinoa and where the greatest diversity of species is conserved *in situ*, together with its wild relatives. An analysis of the current state of conservation of the genetic resources of quinoa allows us to then understand the importance of having instruments to regulate the circulation of these plant genetic resources according to their usage.

In Section 2, the "Agronomic and Ecological Aspects" are addressed to understand the requirements for the development of quinoa crops, with particular attention to quinoa's tolerance to salinity or drought. The chapter on "Plant Breeding" provides a historical overview of the development of modern varieties of quinoa.

In Section 3, we examine the “Nutritional and Technical Aspects”. After harvesting, saponin must be removed before human consumption. Several chapters in this section address the high nutritional value of quinoa in human and animal diets, considering the grain’s gluten-free benefits for coeliac persons and the emerging outlook for nutraceuticals.

In Section 4, “Social and Economic Aspects”, we address the importance of quinoa worldwide, both from an economic perspective and in terms of the relationships between countries. Nevertheless, since quinoa is a staple food before being an export product for Andean communities, the chapter on “Marketing Diversity” presents the different ways in which groups of producers in Andean countries approach the market. This allows us to understand the logic and strength of small-scale farmer associations regarding quinoa.

In Section 5, the various chapters present updated data on “Quinoa Crops in Andean Countries”: Bolivia, Peru, Ecuador, Chile and Argentina.

Section 6 addresses “Experimentation and Current Distribution” of quinoa in new producer countries. We examine the adaptation of quinoa in Mediterranean countries in Europe, its introduction in Asia based on the analysis of the case of India and Pakistan, its experimentation in Africa, the United States of America and Brazil.

The conclusion presents global outlooks in view of the geography and geopolitics of quinoa in an international setting, global challenges, and the role of quinoa in achieving the goal of zero hunger.

The Long Journey of quinoa: Who wrote its history?

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Quinoa (*Chenopodium quinoa* Willd.), kañiwa (*Chenopodium pallidicaule* Aellen) and other edible species known as "kiwicha", "achis", "milmi" or "coimi" (*Amaranthus caudatus* L). together formed an important part of the diet of prehistoric peoples in the highlands of the Andes, from Colombia to Argentina and Chile. Its use was common in the Andean regions until the beginning of the twentieth century, when countries in the region began to massively import wheat.

A Chenopodiacea, *Chenopodium nuttalliae*, known as "huauzontle", was also cultivated in the highlands of Mexico and is very similar to quinoa (Hunziker, 1952), although its cultivation area has now become very small.

Quinoa in pre-Colombian times

Important archaeological findings reveal that these species were formerly consumed in abundance. Towle (1961) mentions several archaeological findings of quinoa, consisting in terminal fruit-bearing branches and loose grains, found in different regions of Peru and in the coastal area of Arica, Chile.

Bollaert (1860) found quinoa seeds in ancient indigenous tombs in Tarapacá and Calama (Chile) and in the Colcha-qui-Diaguita region. Latcham (1936) found quinoa seed in an ancient indigenous burial ground in Tiltil (Chile) and a fair amount of seeds in Quillaga (Chile).

According to Nunez (1970), our understanding of how quinoa and potatoes were domesticated is incomplete, but on the basis of findings in northern Chile (Chinchorro complex), he notes that quinoa was used before 3000 B.C. Thanks to findings near Ayacucho, Peru, Uhle (1919) gives an even earlier date, 5000 B.C., for the beginning of domestication of this plant.

In 1586, Ulloa Mogollón speaks of quinoa being used in the province of Collaguas (Bolivia). As already mentioned, there is evidence that quinoa was widely cultivated in the valleys of northern Chile. In 1558, Cortés Hogea, on the first to visit the Island of Chiloé (Chile), found quinoa sowings on the island. In the Argentine territory, Pedro Sotelo (1583) refers to these crops in the Calchaquies Valley and in the surroundings of Córdoba.

With regard to quinoa crops further south, the Jesuit Father, Antonio Mechoni (1747), notes in his reports that "as far south as the shores of Lake Nahuel Huapi, the Araucanians cultivated this species".

Quinoa was widely cultivated in association with maize in northern Peru. Further south, it was widespread in Callejón de Huaylas as well as in the Mantaro Valley, where it was largely cultivated by the regional group Huancas and in the Ayacucho area by the Wari people (see ceremonial vessel).

Cieza de León (1560) reports that in southern Colombia, quinoa is also cultivated in the highlands between the cities of Pasto and Quito, and writes: "Very little or almost no maize is to be found in all these towns; because of the low temperatures only quinoa is to be found."

Pulgar Vidal (1954) notes that the Chibchas, as well as other tribes from the Altiplano Cundiboyacanse (Colombia), grew quinoa intensively. To explain the presence of quinoa in Ecuador, it has been suggested that the ancient inhabitants of Cuyumbe (the ruins of San Agustín in Huila, Colombia), who were in contact with the inhabitants of the Bogota plains, helped to spread quinoa towards the south of present-day Colombia. When they later migrated south of the continent, they took with them their seeds, including quinoa, sharing them with other nations.

The first Spaniard to mention quinoa cultivation in the New World is Pedro de Valdivia. In his report to Emperor Charles I in 1551 on crops in the vicinity of Concepción (Chile), he notes that the region "is abundant with all the bounties sowed by Indians for their food: maize, potatoes and quinoa". According to numerous sources, when the Spaniards set foot in the region they found "ccolcas" – barns – containing large quantities of quinoa grain, sufficient to feed them for several months.

Quinoa during the Colonial Period

Inca Garcilaso de la Vega, in his famous *Royal Commentaries*, notes the following about quinoa: "the second of the grains grown on the face of the earth gives what they call "quinoa" and it is known in Spanish as 'millet' or small rice: because the grain and colour are somewhat similar". The historian refers to the first export of quinoa grain to the Old World when he took samples of quinoa on his long boat journey to Spain but they failed to propagate since "they arrived dead".

However, considerable confusion prevailed as quinoa was not always identified with the *Chenopodium quinoa* species. At the time, the Spaniards associated quinoa with amaranth (*Amaranthius blitum* L.), which grew in Europe and thus probably received little attention all throughout the colonial era. Bernabe Cobo (1663) notes that quinoa is very similar to amaranth in the Iberian Peninsula.

Confusion arises when the eminent botanist Carolus Clusius, in his *Historia Plantarum Rarorium* (1601), presents the first illustration of a species he refers to as quinoa but which is actually an *Amaranthus caudatus* plant.

Quinoa has as many names as the number of regions or languages where it has been known. Quoted by Pulgar Vidal (1954), Robledo points out that the Chibchas (Colombia) called it "pasca", a word denoting "cooking pot or father's meal".

According to Pulgar Vidal, "suba" or "Supha" (Chibcha language) is the original name for quinoa in the Bogota area. Vidal links these terms to the Aymara word "hupha", still used in some parts of Bolivia. In the rest of the territory that is now Colombia, the Quechua name "quinoa" became widespread, while in Cundinamarca the indigenous name is "parca".

According to Latcham (1936), different names were given to quinoa in the Aymara language depending on their variety. Purple quinoa was called "cami", the best-liked white quinoa was called "ppfique", red quinoa was known as "kana llapi", yellow quinoa was referred to as "cchusllunca", another yellow variety was called "ccachu yusi" and wild quinoa was known as "isualla". However, Latcham confuses kañiwa and includes it among the varieties of quinoa, calling it "Cinderella quinoa" or "cañagua". Latcham adds that quinoa was grown in northern Chile and was known as "dahue" in the Atacameño language. Bertonio (1879) adds Aymara varieties, such as "aara", "ceallapi" and "vocali", which are the wild relatives of quinoa. He also mentions a variety called "cami hupa" that was between red and black.

The Origins of Quinoa

With regard to the origin of the domesticated species, in his studies of quinoas in the Puno and Cusco highlands, Toro (1964) links ancient cultivation and the origin of the domestication of quinoa with the current use of the words "kiuna" in Quechua and "jupha" or "jiura" in Aymara. He sees this as evidence that the Aymara and Quechua populations were the first to domesticate the plant.

Wilson (1990) notes that it is most likely that *Chenopodium hircimun*, widely distributed in the Andes, is amongst the progenitors of quinoa and has evolved to domesticate quinoa as we know it today. Mujica and Jacobsen (2006) point out that there are at least four species of *Chenopodium* related to quinoa and widely distributed in the southern Andes as relatives and progenitors of quinoa, and which have evolved and domesticated quinoa as we know it today. (*Chenopodium carnosolum*, *C. hircinum*, *C. incisum* and *C. petiolare*).

Figure 1. Samples of archaeological remains of quinoa compared with the current crop.
Figure 1 presents quinoas from different ages, proven via the carbon 14 method. It can be observed that the variable percentage of "ayaras" or "ajaras" seeds (wild black quinoa grain) steadily decreases in more recent samples.

Figure 1 presents quinoas from different ages, proven via the carbon 14 method. It can be observed that the variable percentage of "ayaras" or "ajaras" seeds (wild black quinoa grain) steadily decreases in more recent samples.

This domestication and selection process took centuries, and today the more recent varieties are recognized by their very low percentage of "ayaras" or quinoa with dark-coloured grains.

Toro (1964) links the age of cultivation and the origin of the domestication of quinoa with the current use of the words "kiuna" in Quechua and "jupha" or "jiura" in Aymara, taking this as proof that the Aymara and Quechua peoples were the first to domesticate this plant.

Contemporary Research

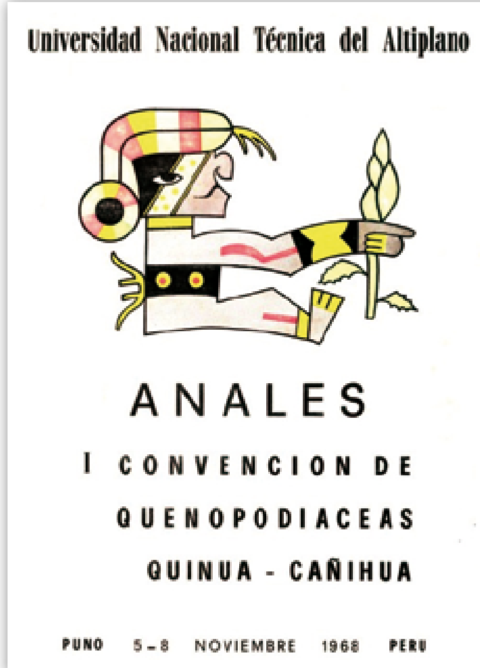
Contemporary research on these Andean grains dates back to the work carried out by the botanists Martin Cárdenas (1944, 1969) in Bolivia; Fortunato Herrera (1941) in Cusco, Peru, and the theses presented at faculties of agronomy, in particular in Cusco, but also in Puno, Peru, Quito and Riobamba in Ecuador, Pasto in Colombia, and Cochabamba in Bolivia.

One of the first events bringing together researchers from Bolivia and Peru was the organization of the First Convention of Chenopodiaceae that took place in Puno, organized by *Universidad del Altiplano* in 1968. This was an opportunity for the progress made by the Bolivian engineer and plant breeder, Humberto Gandarillas, in 1967 at the Patacamaya Experimental Station, Bolivia, to lay the foundations for guidelines for future research.

This convention was followed by a second convention held in Potosi in 1976, organized by the University Tomás Frías and the Inter-American Institute for Cooperation on Agriculture (IICA), with the participation of colleagues from countries including Argentina, Chile, Bolivia and Peru.

These conventions noted that not only these species were important in Andean agriculture, but that other species of tubers, roots and fruits also contribute to the human diet and should also be included in future conventions.

Ten years later, the first comprehensive book on growing quinoa and kañiwa was published in Bogota, Colombia (Tapia *et al.*, 1979). The study was sponsored by the International Development Research Centre (IDRC) of Canada, with the participation of IICA and input from specialists in Peru and Bolivia.



These twelve congresses on Andean Crops throughout the Andes, from Colombia to southern Chile and Argentina (1977–2006), represented an opportunity to present achievements as quinoa cultivation became increasingly renowned. Various projects were thus implemented, including the Agroindustrial Quinoa Project financed by the Simon Bolivar Fund of the Government of Venezuela in 1980, as well as various projects promoted by Andean governments with the cooperation of offices such as IICA, FAO, IDRC and CAN (Andean Community) and with the participation of regional universities and national research institutes.

Table 1. List of Congresses and Conventions on Andean Crops

Event	Location, year	Participants	Countries	Articles
I	Convention, Puno, 1968	41	2	20
II	Convention, Potosí, 1976	116	7	45
I	Congress, Ayacucho, 1977	122	6	39
II	Congress, Riobamba, 1980	754	25	
III	Congress, La Paz, 1982	159	6	88
IV	Congress, Pasto, 1984	101	9	63
V	Congress, Puno, 1986	699	7	133
VI	Congress, Quito, 1988	190	14	92
VII	Congress, La Paz, 1991	320	10	76
VIII	Congress, Valdivia, 1994	210	8	49
IX	Congress, Cusco, 1997	340	7	
X	Congress, Jujuy, 2001	285	6	52
XI	Congress, Cochabamba, 2003	290	7	64
XII	Congress, Quito, 2006	305	8	59

After an extensive tour of the Andes, Tapia (1996) distinguished five major groups of quinoa, based, in particular, on their ability to adapt to different agro-ecological conditions in the Andes:

- **Inter-Andean valleys quinoas**, in mesothermal zones.
- **Altiplano quinoas**, in the highlands north of Lake Titicaca between Peru and Bolivia and with a short growing season.
- **Salare quinoas**, on the salt flats in the southern highlands of Bolivia, comprising halophytes adapted to saline soils and with a larger grain size.
- **Coastal quinoas**, with dark-coloured grains and smaller in size, grown in the centre and in the south of Chile.
- **Yunga quinoas**, grown in the subtropical zone on the eastern slope of the Andes in Bolivia.

Table 2. Humidity and temperature requirements per types of quinoa as per agro-ecological zones. Tapia, 1996.

Agro-ecological group	Rainfall (in mm)	Minimum temperature
Inter-Andrean valleys Quinoas	700-1500	3 °C
Altiplano Quinoas	400- 800	0 °C
Salare Quinoas	250- 400	-1 °C
Coastal Quinoas	800-1500	5 °C
Yunga Quinoas	000-2000	11 °C

For centuries, farmers have obtained and cultivated traditional varieties in each of these quinoa cultivars, testing new selections in other environments with varying results. Gandarillas proposed the classification of quinoa according to the area of adaptation and morphotypes (Gandarillas, 1968).

More recently, Canahua *et al*. (2002) recognized the existence of up to six types of native quinoa grown in the highlands of Puno, based on farmers' knowledge of their agronomic characteristics, such as quality and use of the grain in the rural population's staple foods.

Figure 2: Quinoa cultivars on the Puno Highlands (photography by Mario E. Tapia)

Table 3: Native varieties of quinoa grown on the Puno Highlands. Canahua *et al*. 2002

Quinoa cultivar	Plant colour/Grain	Cold resistance	Main use	Secondary use
1. White, janko or yurac	White/white	Average	Broth or soup	Puree or pesque
2. Chulpi or hialinas	White/transparent	Fair	Broth	Puree
3. Witullas, coloreadas, Wariponcho	Red/red, purple	High	Kispiño	Meals, torrejas
4. Q´oitu	White or lead/lead-coloured, brown.	Fair	Torrejas	Meals
5. Pasancallas	Burst easily	High	mana	Meals
6. Cuchi willa	Red/black	High	chicha	Quispiño

This classification of the major types of quinoa is crucial for programming the use of varieties in planting systems depending on the agro-ecological zones and their agroclimatic conditions (Freere, Rijks and Rea, 1975).

The book published in 1989 by the United States National Academy of Science entitled "The Lost Crops of the Incas" also deals with quinoa and had a major impact in the scientific community. Risi (1994) analyses the importance of quinoa in the context of Andean husbandry systems, suggesting the need for an economic analysis to determine whether the production of quinoa in countries like Bolivia can be maintained. In fact, with the declaration of the International Year of Quinoa in 2013 and high demand for the product, quinoa prices have risen, making the crop highly profitable even with low yields.

Cooperation offered by the Government of Denmark has supported various research programmes on quinoa in Bolivia, Peru and Ecuador. Jacobsen (2013) established an assessment of quinoa varieties around the world.

Quinua around the World

In 1990, Latinreco, a company funded by Nestle, published the book entitled "*Quinua, hacia su cultivo comercial*", laying down a new vision of the potential of quinoa as an entrepreneurial crop (Wahli, 1990).

As part of the celebrations of the 500th anniversary of the arrival of Christopher Columbus in America in 1992, FAO published "Neglected crops: 1492 from a different perspective", including quinoa and other Andean grains as important food resources. FAO has also distributed an online publication updating knowledge on quinoa in 2003.

The scientific event that probably had the greatest impact on the dissemination of this crop was the First International Workshop on Quinoa held in Lima in 2001, followed by three World Congresses of Quinoa, the last of which (IV) was held in Ambato, Ecuador.

Table 4: World Congresses of Quinoa

I International Workshop on Quinoa	2001	Lima, Peru
II World Congress of Quinoa	2004	Arica, Chile
III World Congress of Quinoa	2010	Oruro, Bolivia
IV World Congress of Quinoa	2013	Ibarra, Ecuador

A sharp increase in the dissemination and consumption of quinoa was prompted in Peru by regional gastronomy and cooks adopting quinoa and other Andean crops to prepare various dishes, and by the recognition of its potential nutritional role in people's diet.

In the past two decades, quinoa has gone from being a regional crop that was consumed relatively little to becoming a very important pseudograin. It has ventured into new national and international markets, with increases in both annual per caput intake (especially in Andean countries) and grain export levels (e.g. in Bolivia). Today, export levels exceed USD70 million in Bolivia and USD25 million in Peru, as quinoa consumption gains popularity across the globe.

In each of these stages of research, quinoa has been recognized for its high genetic biodiversity and its ability to adapt to different environments. It is considered the "wonder grain" in global food and nutrition, leading to the proposal by the Government of Bolivia that the United Nations declare the celebration of the International Year of Quinoa in 2013.

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Section 1

Botanics, Domestication and Exchanges of Genetic Resources

CHAPTER 1.1

Botany, Phylogeny and Evolution

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Abstract

Quinoa (*Chenopodium quinoa*, 2n = 4x = 36) is an Andean seed crop belonging to a complex of allotetraploid taxa that includes wild, weedy and other domesticated forms from throughout the temperate and subtropical Americas. Quinoa was probably domesticated at ≥ 3 500 masl within the interior basin of Lake Titicaca. Consequently, the crop underwent prolonged selection in an environment that is extremely adverse with respect to abiotic stresses (with the exception of heat), but relatively mild in terms of biotic stresses. Subsequently, quinoa cultivation spread throughout the central and north-central Andean valleys and southwards into the Araucanian coastal region and adjacent Patagonia, diversifying into its five principal ecotypes: Altiplano, Salare, Inter-Andean valleys, Coastal and Yunga. Quinoa biodiversity suffered as a result of over 400 years of post-conquest neglect and cultural stigmatization. Recent evidence from *in situ* hybridization and phylogenetic studies employing molecular genetic markers and DNA sequencing, combined with previous reports involving isozyme and inter-taxa hybridization studies, confirms that allotetraploids can be considered a single biological species. This is of fundamental importance, because quinoa is on the brink of becoming an international commercial crop, targeted for large-scale production in lowland subtropical environments where disease and insect pest pressures are currently harbouring on alternate hosts (i.e. the cosmopolitan weeds *C. album* and *C. murale*). In anticipation of these threats, quinoa pathologists and breeders need to discover, transfer and deploy defensive alleles that should already be present in its sister taxa, particularly the ecologically diverse North American weed, *C. berlandieri*.

Introduction

Quinoa (*Chenopodium quinoa* Willd., 2n = 4x = 36) is a South American dicotyledonous crop plant whose seed has become an extremely popular food product in the last 30 years, particularly in Europe and North America, but also in the Andean region. This is in part the result of the increased popularity of vegetarian diets and the increasing number of diagnoses of dietary gluten intolerance or coeliac disorder, as well as increasing sociopolitical awareness of, and pride in, indigenous Andean culture and heritage in South America. Quinoa seed, when properly handled to remove the bitter saponins in the pericarp, has a mild flavour and can be consumed in many of the same ways as cereal grains – hence, quinoa's classification as a pseudocereal or pseudograin. However, compared with the highest strains of wheat, quinoa seed has a similar protein content, a more favourable amino acid profile and fewer glutinous seed proteins.

In addition to having favourable nutritional characteristics, the pool of *C. quinoa* germplasm includes halophytic and xerophytic ecotypes – most notably the high-quality 'Real' quinoas from the Salare region of the southern Altiplano. This area averages 150 mm of precipitation annually and lies at an altitude of ≥ 3 700 m asl. These extreme abiotic stress tolerance mechanisms are of interest to investigators seeking to increase crop production in arid, saline, highland and other marginal environments. However, quinoa varieties adapted to very high Andean elevations have had to acquire biotic resistance to a relatively narrow spectrum of insect pests, bacteria and fungi, most notably the downy mildew pathogen *Peronospora farinosa pv. chenopodii*. The same could be said for quinoa varieties from the geographically isolated lowland coastal region of Chile. With the onset of extensive quinoa production in new regions, and particularly in areas of the Eastern Hemisphere where it has close relatives that are widespread weeds – for example, *C. album, C. strictum* and *C. murale* – there is a significant threat that pathogens and pests in these related species will find large fields of genetically uniform (and unfortunately, susceptible) quinoa to be especially inviting hosts.

The main purpose of this chapter is to identify germplasm of greatest interest as primary and secondary genetic resources for quinoa improvement. There is also a review of evidence supporting the genetic relationships among quinoa and these relatives. Hopefully, this information will inspire international plant genetic resource conservation organizations to take notice of the importance of conserving quinoa and its closest wild relatives, and also provide guidance in existing and future collection and preservation efforts.

History and botanical background

LSouth American quinoa domestication has been driven by ancient and modern cultures along, and perhaps flanking, the Andes over a period of at least 5 000 years, such that today it includes forms ranging from semi-weedy types to high-yielding, high-quality commercial varieties (Jacobsen, 2003; Mujica, 2004). Andean quinoa diversity has been associated with five main ecotypes: Altiplano (Peru and Bolivia), Inter-Andean valleys (Colombia, Ecuador and Peru), Salare (Bolivia, Chile and Argentina), Yunga (Bolivia) and Coastal (Chile). The germplasm in each of these associated subcentres of diversity is commonly assumed to have descended from a central pool of domesticated landraces in the Lake Titicaca basin (Risi and Galwey, 1984).

Initially, the genetic diversity centre for quinoa was identified in the southern highlands of Bolivia (Gandarillas, 1979; Wilson, 1988). Subsequently, Christensen et al. (2007), using molecular approaches (SSR markers), suggested that the genetic diversity centre was the highland area between Peru and Bolivia (central Andean highlands). Their molecular data also revealed relatively limited diversity of quinoa germplasm from Ecuador and Argentina, though this could have been an artefact due to the small number of available accessions as well as the potential for severe historical bottlenecks related to limited *in situ* germplasm conservation in those areas. The data from Christensen *et al.* (2007) indicate that the most probable point of introduction for Ecuadorian accessions was the highland region of Peru–Bolivia, while the Argentinean strains originated in the Chilean highlands (north) and coastal/lowland zone (south). In addition, Christensen et al. (2007) highlighted the differences between accessions from coastal/lowland Chile and those from the northern highlands of Peru, confirming the hypothesis proposed by Wilson (1988) that quinoas from Chile are more similar to quinoas from the southern highlands of Bolivia.

Nevertheless, Fuentes *et al.* (2009a), assessing genetic diversity on a wide range of Chilean accessions using SSR markers, reported that Chilean coastal/lowland germplasm was much more genetically diverse than was previously believed. This finding was consistent with a cross-pollination system in the coastal/lowland quinoa fields with weedy populations of *C. album* and/or C. *hircinum* – most likely the latter, since the majority of *C. album* is hexaploid (2n = 6x = 54) and would therefore render sterile 5x progeny, while *C. hircinum* shares the same tetraploid genome as *C. quinoa*, agreeing well with the difficulty experienced by coastal/lowland quinoa breeders in obtaining pure new cultivars in south-central Chile (I. and E. von Baer, personal communication). Taken together, the recent genetic-based analyses consistently affirm that quinoa itself has existed until now as two distinct germplasm pools: Andean highland quinoa with its associated weed complex ("ajara" or "ashpa" quinoa, *C. quinoa* sp. *milleanum* Aellen, also referred to as *C. quinoa* var. *melanospermum* Hunziker); and central and southern Chilean coastal/lowland quinoa (*kinwa* or *dawe* to the Mapuche people living south of the Bio Bio River), representing a second centre of major quinoa diversity (Jellen *et al.*, 2011). However, the more recent microsatellite-based diversity data from northwest Argentina (Costa Tartara *et al.*, 2012) indicate the presence of a much wider diversity than previously known in quinoas from the Precordillera and subtropical eastern lowlands bordering on the Gran Chaco and Pampas. This study also highlighted likely patterns of ancient and modern quinoa germplasm movement in the Bolivia–Argentina–Chile region.

The recent molecular evidence suggests that genetic erosion – loss of genetic diversity – has been affected by at least four genetic bottleneck events (Jellen *et al.*, 2011; Fuentes *et al.*, 2012). The first and most severe occurred in the initial polyploidization step, when the two diploid ancestors of quinoa hybridized. The second event took place when quinoa was domesticated from its wild tetraploid relatives through long cycles of seed exchange and cultivation in new territories and climates. The third can be considered a sociological bottleneck, beginning more than 400 years ago during the Spanish conquest when quinoa was culturally stigmatized as food for the indigenous, rustic communities (Cusack, 1984). The recent history of quinoa suggests a fourth bottleneck event caused by human migration from rural zones of the High Andes to urban centres and the coca-growing regions of the eastern foothills, resulting in abandoned quinoa fields and quinoa germplasm loss (Fuentes *et al.*, 2012).

When quinoa was originally classified by Willdenow in 1797, it was assumed to be the only domesticated species of the genus from the New World. In 1917, other cultivated *Chenopodium* tetraploids were discovered in Mesoamerica (Wilson and Heiser, 1979). These plants were originally classified by Safford as *C. nuttaliae* and consisted of three different cultigens: *huauzontle*, an inflorescence vegetable; red chia (*chia roja*), a seed crop; and *quelite*, a semi-weedy form used as a leafy vegetable. They have been reclassified several times, including a period in which they were considered conspecific with quinoa. These forms are currently classified as part of the complex of *C. berlandieri,* commonly known as *C. berlandieri* var. *nuttaliae* (Wilson and Heiser, 1979). In addition to the *nuttaliae* cultigens, *C. berlandieri* includes an extinct North American domesticate, subsp. *jonesianum*, that is known by well-characterized remains in a number of archaeological sites from the Oak-Hickory Savanna/Woodland Belt and is hypothesized to have been supplanted as a crop during the first millennium A.D. by the northward-moving maize–bean–squash crop complex (Smith and Funk, 1985; Smith and Yarnell, 2009). Kistler and Shapiro (2011) analysed chloroplast DNA (cpDNA) sequences from ancient North American and modern Mexican domesticates, as well as wild samples of *C. berlandieri* from eastern and western North America. Their data demonstrated that the Eastern Woodlands strains were domesticated independently from the *nuttaliae* types in Mexico. Consequently, three independent domestications of the allotetraploid New World "quinoa complex" should now be widely acknowledged: one in the Eastern Woodlands of North America, a second in Mesoamerica, and a third (quinoa) in the Andean region (Kistler and Shapiro, 2011).

When genetic research on quinoa began systematically at the end of the 1970s, it was commonly assumed that quinoa had originated in South America from diploids that hybridized anciently in the Andean highlands. Candidate species included *C. pallidicaule* Aellen (Kañawa), *C. petiolar*e Kunth and *C. carnasolum* Moq., as well as tetraploid weed species from South America, such as *C. hircinum* Schard or *C. quinoa* var. *melanospermum* (Mujica and Jacobsen, 2000). An alternative hypothesis, originally raised by Wilson and Heiser (1979), was that quinoa descended from the tetraploid, *C. berlandieri*, in North America. However, when the Mexican complex of*C. berlandieri* was described, it was considered conspecific with quinoa. One popular hypothesis was that *C. quinoa* is descended from early tetraploids of *C. berlandieri* via *C. hircinum* and that the domesticated Mexican tetraploids are descended from *C. berlandieri* var. *sinuatum*. This hypothesis was supported by diverse studies based on morphological, experimental crosses, isozymes and genetic analysis (Heiser and Nelson, 1974; Wilson and Heiser, 1979; Wilson, 1980; Walters, 1988; Maughan *et al.*, 2006). If this hypothesis is correct, it implies that the wild North American tetraploid progenitor travelled to South America via human migration or, more likely, by long-distance bird dispersal (endozoochory), probably as *C. hircinum*, and was subsequently domesticated as quinoa (Wilson, 1990).

Archaeobotanical studies based on patterns of seed morphology and frequencies of *C. quinoa* and its associated weedy complex have shown interesting perspectives to support Wilson's hypothesis. Studies conducted by Bruno and Whitehead (2003) shed light on some processes contributing to the development of agricultural systems between 1500 B.C. and 100 A.D. in the southern Lake Titicaca basin (Bolivia). The results of this study suggested that during the Early Formative period, farmers maintained small gardens where the crop and weed species were both grown and harvested. However, around 800 B.C. the frequency of weedy seeds compared with quinoa seeds decreased drastically, revealing a significant change in crop management and use. This latter observation suggests that Middle Formative period farmers became more meticulous cultivators of quinoa, perhaps through weeding, careful seed selection and creation of formal fields for cultivation.

With the increasing number of *Chenopodium* genetic studies, data are progressively accumulating regarding the probable tetraploid and diploid ancestors of quinoa and the correct phylogeny of its genus. As mentioned below, evidence from cytogenetic studies using fluorescence *in situ* hybridization (FISH) with quinoa subgenome-specific repetitive sequence 18-24J indicates that this species has one genome in common with *C. berlandieri* and Eurasian *C. album* (Kolano *et al.*, 2011). Sequencing of rRNA genes previously verified the close relationship between *C. berlandieri* and *C. quinoa* (Maughan *et al.*, 2006). A series of ongoing recent studies involving comparative sequencing of low-copy nuclear genes (i.e. *SOS1, GLN-1, GBSSI, FTL2*) and chloroplast sequences (i.e. *trnH-psbA* spacer) indicate that quinoa's two diploid ancestors were from central North America and Eurasia, with the New World ancestor being the cytoplasm donor (E. Jellen, B. Walsh, E. Emshwiller and H. Storchova, personal communication).

Taxonomy

A trend has been observed among South American quinoa germplasm programmes to collect and preserve any and every wild or weedy taxon historically labelled as *Chenopodium* in the region – with little regard to the taxon's actual ability to serve as a gene source for quinoa improvement. For example, while the herb *paico* or *epazote* (*Dysphania ambrosioides*, formerly *Chenopodium ambrosioides*) has interesting medicinal and culinary properties, it is probably worthless as a quinoa genetic resource, given that the two taxa have differing base chromosome numbers of x = 8 versus x = 9 for *paico* and quinoa, respectively. Likewise, another South American species, traditionally classified as *C. incisum* but synonymous with Dys. graveolens, has the same base chromosome number as *paico*. Even collections of exotic *C. murale* (now *Chenopodiastrum murale*) are of dubious value in light of mounting evidence (below) that this is merely a distant relative of *C. quinoa.*

Fuentes-Bazan (2012a, b) performed two macro-scale molecular studies of the Chenopodiaceae and *Chenopodium* systematics using nuclear ITS and chloroplast *trnL-F* and *matK/trnK* sequences, and provided evidence supporting the division of this large and problematic genus into seven genera: *Chenopodium* (including the *C. quinoa* and *C. album* complexes); *Chenopodiastrum* (including *C. murale* and *C. hybridum*); *Oxybasis* (including *C. glaucum, C. rubrum* and *C. urbicum*); *Lipandra* (including *C. polyspermum*); *Blitum* (including *C. capitatum* and *C. bonus-henricus*); *Dysphania* (previously proposed by Mosyakin and Clemants in 1996, 2002 and 2008); and *Teloxys* (including *C. aristatum*). This taxonomic system tremendously simplifies the present discussion of *Chenopodium* taxonomy, as it is possible to focus closely on the species of greatest interest as potential genetic resources for improving *C. quinoa*. A summary of revised *Chenopodium* taxonomy is provided in Table 1.

Table 1. Revised species designations of *Chenopodium*, incorporating the proposals of Fuentes-Bazan *et al.* (2012a and b). Authoritative taxa as identified in the Integrated Taxonomic Information System (ITIS, www.itis.gov/index.html) are indicated in **bold**. The list does not include taxa previously reassigned to the genera *Dysphania* and *Teloxys*.

***Chenopodium* Taxon**	**Revised Designation**	**Habit**	**Origin**
acuminatum Willd.		Weed	Eurasia
albescens Small		Wild	N. America
album L.		Weed, domesticated	Eurasia
atripliciforme Murr.		Wild	Eurasia
atrovirens Rydb.		Wild	N. America
auricomiforme Murr. & Thell.		Wild	Australia
auricomum Lindl.		Wild	Australia
badachschianicum Tzelev	*Chenopodiastrum badachschanicum (Tzvelev) S. Fuentes, Uotila & Borsch*	Wild	Eurasia
berlandieri Moq.		Weed, wild, domesticated	N. America
bonus-henricus L.	*Blitum bonus-henricus (L.) Rchb.*	Weed, wild	Eurasia
borbasii Murr.		Weed	S. America
bryonifolium Bunge		Wild	Eurasia
bushianum Aell.		Weed, wild	N. America
californicum (S. Wats) S. Wats.	*Blitum californicum S. Watson*	Wild	N. America
capitatum (L.) Ambrosi	*Blitum capitatum L.*	Wild	N. America
carnosolum Moq.		Weed	S. America
chaldoranicum Rahimin. & Ghaemm.		Wild	Eurasia
chenopodioides (L.) Aell.	*Oxybasis chenopodioides (L.) S. Fuentes, Uotila & Borsch*	Weed	S. America
cordobense Aell.		Wild	S. America
crusoeanum Marticorena		Wild	Juan Fernandez
curvispicatum P.G. Wilson		Wild	Australia
cycloides A. Nels.		Wild	N. America
desertorum J.M. Black		Wild	Australia
desiccatum A. Nels.		Weed, wild	N. America
detestans T.W. Kirk		Weed, wild	New Zealand
erosum R. Br.		Wild	Australia
ficifolium Sm.		Weed, wild	Eurasia

***foggii* Wahl**		Wild	N. America
***foliosum* (Moench) Aschers.**	*Blitum virgatum* L.	Weed	Eurasia
***fremontii* S. Wats.**		Wild	N. America
frigidum Phil.		Weed, wild	S. America
giganteum D. Don		Weed, domesticated	Eurasia
***glaucum* L.**	*Oxybasis glauca* (L.) S. Fuentes, Uotila & Borsch	Weed, wild	Eurasia
gracilispicum H.W. Kung		Wild	Eurasia
***hians* Stand.**		Wild	N. America
***hircinum* Schrad.**		Weed	S. America
***humile* Hook.**	(Oxybasis rubra var. humile?)	Weed, wild	N. America
hybridum L.	*Chenopodiastrum hybridum* (L.) S. Fuentes, Uotila & Borsch	Wild	Eurasia
iljinii Gol.		Wild	Eurasia
***incanum* (S. Wats.) Heller**		Wild	N. America
karoi (Murr.) Aell.		Weed, wild	Eurasia
korshinskyi (Litv.) Minkw.	*Blitum korshinskyi* Litv.	Wild	Eurasia
***leptophyllum* (Moq.-Tand.) Nutt. Ex S. Wats.**		Wild	N. America
litwinowii (Paul.) Uotila	*Blitum litwinowii* (Paul.) S. Fuentes, Uotila & Borsch.	Wild	Eurasia
***macrospermum* Hook. f.**	*Oxybasis macrosperma* (Hook. f.) S. Fuentes, Uotila & Borsch	Weed, wild	S. America
***murale* L.**	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	Weed	Eurasia
***neomexicanum* Stand.**		Wild	N. America
***nevadense* Standl.**		Wild	N. America
nitrariaceum (F. Muell.) F. Muell. ex Benth.		Wild	Australia
novopokrovskyanum (Aell.) Uotila		Weed, wild	Eurasia
***oahuense* (Meyen) Aell.**		Wild	Hawaii
obscurum Aell.		Wild	S. America
***opulifolium* Schrad. ex Koch & Ziz**		Weed	Eurasia
***overi* Aell.**	*Blitum hastatum* Rydb.	Wild	N. America
***pallescens* Stand.**		Wild	N. America
***pallidicaule* Aell.**		Weed, domesticate	S. America
pamiricum Iljin		Wild	Eurasia
papulosum Moq.		Weed, wild	S. America

parodii Aell.		Wild	S. America
petiolare Kunth		Weed, wild	S. America
philippianum Aell.		Weed, wild	S. America
pilcomayense Aell.		Wild	S. America
polyspermum L.	*Lipandra polysperma (L.) S. Fuentes, Uotila & Borsch*	Weed	Eurasia
pratericola Rydb.		Wild	N. America
quinoa Willd.		Weed, domesticate	S. America
rubrum L.	*Oxybasis rubra (L.) S. Fuentes, Uotila & Borsch*	Weed	Eurasia
ruiz-lealii Aell.		Wild	S. America
salinum Stand.	(Oxybasis salina?)	Weed, wild	N. America
sancta-clarae Johow		Wild	Juan Fernandez
sancti-ambrosii Skottsb.		Wild	San Ambrosio & San Felix
scabricaule Speg.			
***standleyanum* Aell.**		Maleza, silvestre	América del norte
***strictum* Roth.**		Maleza	Eurasia
***subglabrum* (S. Wats.) A. Nels.**		Silvestre	América del norte
suecicum Murr.		Maleza	Eurasia
***urbicum* L.**	*Oxybasis urbica* (L.) S. Fuentes, Uotila & Borsch	Maleza	Eurasia
***vulvaria* L.**		Maleza	Eurasia
***watsonii* A. Nels.**		Maleza, silvestre	América del norte

Cytogenetics

Quinoa is an allotetraploid species with 2n = 4x = 36 chromosomes. As discussed above, mounting evidence points to *C. standleyanum* and *C. ficifolium* as the putative progenitor-diploids that donated the New World A- and Old World B-genomes, respectively, to the tetraploid complex that includes *C. quinoa*. These species presumably crossed in ancient times to produce a hybrid that subsequently became fertile when its chromosomes doubled – perhaps due to the mechanism of polysomaty (described below) in developing floral organs (Figure 1). The tetraploid chromosome number was observed in closely related weedy and domesticated forms of *C. berlandieri* and *C. hircinum* (Wilson, 1988c; Maughan *et al.*, 2006). The other cultivated South American chenopod, *C. pallidicaule*, is a diploid (2n = 2x = 18), while most of the Eurasian cultivated or semi-cultivated chenopods exhibited hexaploid chromosome numbers (2n = 6x = 54; *C. album, C. giganteum, C. formosanum* – Kolano *et al.*, 2012b). *Chenopodium* species have in general symmetrical karyotypes with small meta- or submetacentric chromosomes (Bhargava *et al.*, 2006; Palomino et al., 2008). Thus, it is very difficult to identify the chromosomes and study the genome organization, so cytogenetic characterization of *Chenopodium* karyotypes has been limited. A serious weakness of *C. quinoa* karyotype analysis is the paucity of chromosome markers. Until now only rRNA genes appear to be suitable cytological markers for quinoa chromosomes. The 35S RNA gene loci were placed in the terminal part of two chromosomes (Figure 2A arrows). The 5S rDNA were organized in two pairs of loci – one located in terminal position and the other in interstitial position in two different chromosome pairs (Figure 2A arrowheads; Maughan *et al.*, 2006). Similarly, only a few rRNA loci were mapped in the karyotype of *C. berlandieri,* which had one or two pairs of 35S rDNA loci and two or three pairs of 5S rDNA loci, depending on the accession. All rDNA loci were localized terminally in chromosomes (Maughan *et al.*, 2006). In *C. quinoa* and *C. berlandieri* karyotypes, 35S rDNA loci co-localized with GC-rich chromatin stained with chromomycin A3 (Kolano *et al.*, 2001; Kolano *et al.*, unpublished). Telomere sequences are the other functional tandem repeats in plant genomes. Quinoa and other studied chenopods have *Arabidopsis*-type telomere repeats located exclusively in the terminal position in each chromosome arm (Kolano, unpublished).

In quinoa chromosomes, the heterochromatin is located around the centromeres as was shown using C-banding (Figure 2B; Kolano *et al.*, unpublished). The pattern observed on C. quinoa chromosomes after C-banding resembled the signal distribution observed after fluorescent in situ hybridization (FISH) with the 12-13P clone, suggesting that the 12-13P sequence constituted a major part of the heterochromatin of *C. quinoa* (Figure 2C; Kolano *et al.*., 2011). This repetitive sequence showed partial homology to satellite DNA (pBC1447) detected near the centromere of *Beta corolliflor*a chromosomes (Gao *et al.*, 2000). Hybridization signals of 12-13P were observed in each chromosome of *C. quinoa*; however, the intensity of the FISH signals differed considerably among chromosomes, indicating that there are varying numbers of 12-13P repeats at each locus. The 12-13P sequence also hybridized to centromeric and pericentromeric chromosomal regions of related North American tetraploid *C. berlandieri* and to European accessions of hexaploid *C. album*; however, the relative intensity of hybridization signals was reduced in the latter (Kolano *et al.* 2011).

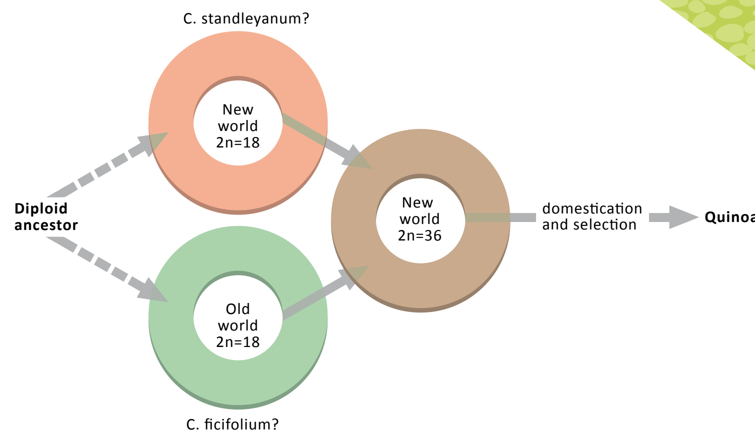

Figure 1: Schematic representation of the allopolyploidization event in the evolution of quinoa.

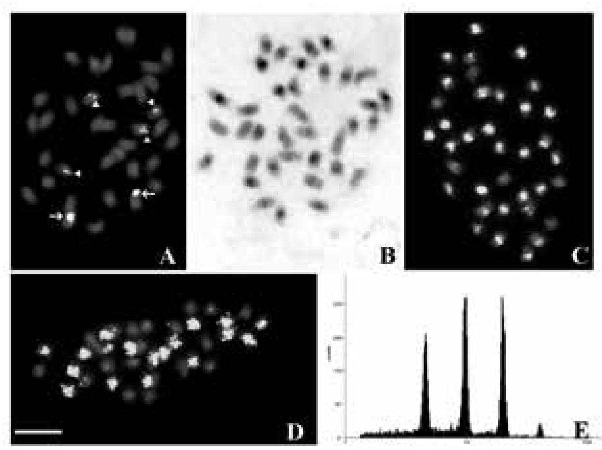

Figure 2: Localization of (A) 35S rDNA (arrows) and 5S rDNA loci (arrowheads) on metaphase chromosomes of quinoa. Metaphase plates of *C. quinoa* (B) after C-banding and after fluorescent *in situ hybridization with (C) 12-13P and (D) 18-24J as a DNA probe.(E) Typical flow cytometric histograms (log scale) of nuclei isolated from hypocotyl of 3 day-old quinoa seedling. Scale bar = 5* µm.

A pericentromeric localization was also detected for retrotransposons by performing FISH analysis with DNA probes for different reverse transcriptase-coding fragments of LTR retrotransposons. These studies indicated that both Ty1-*copia* and Ty3-*gypsy* retrotransposons were preferentially located in pericentromeric heterochromatin of quinoa chromosomes. Other dispersed repetitive sequences isolated and characterized in the quinoa genome were clones pTaq10 and 18-24J. These two clones showed dispersed chromosomal distribution; however, they were not homologous to known mobile elements. Hybridization signals of pTaqu10 were observed as small dots spread throughout all the chromosomes without a specific chromosome or subgenome distribution pattern (Kolano *et al.* 2008a). The pTaq10 repeat was present also in the *C. berlandieri* genome and it showed a similar dispersed chromosomal distribution – further evidence that these two species are related, especially considering that it was not detected in chromosomes of the European form of *C. album*. The second dispersed repeat, 24-18J, hybridized to 18 chromosomes (one subgenome) of *C. quinoa, C. berlandieri* and *C. album* (Figure 2D; Kolano *et al.*, 2011). These results support the hypothesis that *C. quinoa, C. berlandieri* and *C. album* share at least one common ancestor. Earlier studies based on 5S rDNA NTS sequences also support the hypothesis that C. berlandieri and C. quinoa are descend from at least one common diploid ancestor (Maughan et al., 2006). Comparison of the chromosomal distributions of 18-24J homologous and rDNA suggest that in these polyploid chenopods uniparental loss of 35S rDNA sequences took place. In all these three polyploids, the 35S rDNA loci were retained in the subgenome to which 18-24J abundantly hybridized (Kolano *et al.*, 2011).

Chenopodium species have a rather small genome size; however, in the species studied, the 1C nuclear DNA content revealed a 7.9-fold variation, ranging from 0.31 (diploid *C. aristatum* – now classified as *Teloxys aristata* as per Fuentes-Bazan *et al.,* 2012a, b) to 2.47 pg in hexaploid *C. album* (Bhargava et al., 2007; Palomino *et al.*, 2008). More recent reports estimate that quinoa has a haploid genome size (1C value) of approximately 1.48–1.62 pg (Bhargava *et al.*, 2007; Palomino et al., 2008; Kolano *et al.*, 2012a), and limited intraspecific genome size variation (5.9%) was demonstrated for this species (Kolano *et al.*, 2012b).

Another item of interest with regard to quinoa cytogenetics is that *C. quinoa* is a polysomatic plant (Kolano *et al.*, 2008b). Polysomaty was found in many organs of quinoa seedlings. Endopolyploid cells (cells with more than 4C DNA value) were observed in roots, hypocotyl and, to a lesser extent, in cotyledons. However they were not present in young leaves or the shoot apex (Figure 1E; Kolano *et al.*, 2008b). Polysomaty was also reported for *C. album* seedlings and other species from the Amaranthaceae family (e.g. *Beta vulgaris* and *Atriplex rosea*; Barow and Meister, 2003; Kolano *et al.* 2008b).

Crop wild relatives' conservation, breeding perspectives and conclusions

On the basis of accumulating data from molecular and cytogenetic studies, and in the light of the earlier genetic and hybridization research carried out by Wilson and co-workers, we contend that *C. quinoa* and its close allotetraploid relatives should no longer be considered, for practical purposes, as separate biological species. Quinoa breeders intending to adapt this crop for worldwide production will need to harness alleles for lowland subtropical- and temperate-zone biotic and heat stresses from the crop's sisters – quinoa's primary wild gene pool: *C. berlandieri* and *C. hircinum*. Even highland Andean quinoa breeders should seriously consider these lowland taxa as genetic resources in anticipation of a warming climate with heat stress and biotic stress organisms progressively moving closer to traditional quinoa production regions in the High Andes. In consideration of the global warming threat, Andean quinoa stakeholders would be wise to re-evaluate their adherence to restrictive international germplasm exchange policies that might impede their future ability to access heat- and biotic stress-tolerant *C. hircinum* germplasm from countries such as Argentina. The formalized expansion of the species concept of *C. quinoa* Willd. to encompass: *C. berlandieri berlandieri* (perhaps as *C. quinoa* subsp. *ancestrale*); *C. berlandieri nuttaliae* (as *C. quinoa* subsp. *mexicana*); extinct *C. berlandieri jonesianum* (as *C. quinoa* subsp. *jonesianum*); and *C. hircinum* (as *C. quinoa* subsp. *foetida*) could encourage such thinking.

Now that quinoa's diploid ancestor gene pools are being defined, it is interesting to contemplate how breeders might exploit these secondary genetic resources to their advantage in the future. The putative Old World ancestor, *C. ficifolium*, has a remarkably widespread distribution throughout temperate Eurasia and should therefore harbour immense diversity for genetic resistance to biotic stresses. It also represents a potential genetic "bridge" between quinoa and the tremendously diverse *C. album* 2x/4x/6x species complex, given that they share a set of chromosomes named the "B genome". This species complex is native or naturalized on all of the inhabited continents and major subtropical to temperate island groups.

The putative New World ancestor, *C. standleyanum*, is part of a complex of diploid taxa found in all temperate to subtropical environments of North America. This genetic similarity was confirmed through comparative sequencing of portions of various nuclear genes, such as *SOS1, GLN-1* and *GBSSI*, along with chloroplast regions including *TrnH-psbA* spacer, in the following diploid taxa: *C. atrovirens, C. desiccatum, C. fremontii, C. hians, C. incanum, C. leptophyllum, C. neomexicanum, C. pratericola* and *C. watsonii* (B. Walsh, E. Emshwiller, P. Maughan and E. Jellen, unpublished). A close genetic relationship was also detected between this group and two Andean diploids, *C. pallidicaule* (cultivated kaniwa) and wild *C. petiolare*. Considering this diversity, as in the case of C. ficifolium, there should be an abundance of allelic variation of breeding value for quinoa improvement in this gene pool. Possible traits of interest include: the free-threshing (utriculate) pericarp character of many of these taxa, including *C. standleyanum*; extreme sodium tolerance in *C. nevadense*; extreme drought tolerance in *C. desiccatum, C. hians, C. incanum, C. leptophyllum, C. petiolare* and *C. pratericola*; and extreme heat tolerance in several of these taxa from the Mojave and Sonoran deserts. These efforts were encouraged by observations made during several collecting trips, particularly in the southwest United States of America, where hybrid swarms between 4x *C. berlandieri* and diploids like *C. incanum* and *C. pratericola* were encountered along sandy arroyos in temperatures of ≥ 40°C in July and August. Quinoa breeders should be mindful of introgression strategies involving initial 4x quinoa X 2x diploid crosses, which restrict linkage drag to a single genome; these approaches were very successful in transferring genes from highly diverse diploids into allopolyploid crops like wheat (Cox *et al.*, 1991).

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CHAPTER: 1.2

Quinoa Molecular and Genomic Tools

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Abstract

A sophisticated toolbox of DNA-based genetic markers and genomic resources has been developed and is readily accessible to quinoa researchers. These genomic tools include thousands of characterized and mapped microsatellite and single nucleotide polymorphism markers, expressed sequence tag libraries, bacterial artificial chromosome libraries, and several immortalized recombinant inbred line populations, as well as second generation recombination linkage maps. Appropriate use of these resources should allow for the identification and cloning of genes of agronomic importance. Indeed, the tools necessary to identify quantitative trait loci (QTLs) through genetic linkage analysis are in place. Once marker-QTL associations have been identified, they should greatly accelerate the process of breeding elite cultivars through marker-assisted selection (MAS). The same tools should also facilitate the introgression of novel alleles into quinoa from wild relatives. It is noted that the utilization of marker-assisted breeding will greatly improve genetic gains without the incorporation of transgenic technology, an important consideration since many Andean countries have an unfavourable view of transgenic crops. Lastly, these genomic resources readily expand our ability to understand the diversity and evolutionary history of quinoa (and related taxa) and should be immediately utilized by national and regional institutions and breeding programmes to characterize and maintain quinoa germplasm banks – including the development of core breeding collections.

Plant breeding in the genomic era

The ability to produce inexpensive and high-throughput DNA sequence data, coupled with new computational advances in bioinformatics and statistical analysis, is dramatically changing the field of plant breeding. Plant breeding, once described as “an art and a science” is quickly adopting molecular tools that dramatically accelerate and improve the breeding process. Plant breeders of the major agricultural species (maize, soybean, cotton etc.) now routinely utilize molecular tools to accelerate the selection and improvement of complex traits in marker-assisted breeding programmes (Eathington et al., 2007). Fundamental to this change was the dramatic decrease in cost and time associated with developing the genotypic data necessary to make breeding decisions. State-of-the-art genotyping technologies easily produce tens of thousands of genotypic data points in less than 24 hours (e.g. Illumina GoldenGate™ and Fluidigm Dynamic Array IFC™). DNA genotyping efforts that previously took years to accomplish can now be easily delivered to the breeder in a cost-effective manner within the time frame of a breeding season. These dramatic changes in cost and speed provide the opportunity to apply these technologies to plant improvement of all crop species, including those with only regional status or minor significance in the world market, comprising quinoa. The utilization of molecular plant breeding methods not only allows plant breeders to work with highly complex traits, but is also essential for i) shortening the time necessary to tailor crops to meet new crop requirements, such as enhanced nutritional quality or agricultural changes necessitated by climate change, ii) facilitating the introgression of valuable traits from wild relatives into established crop species, and iii) shortening the time necessary to domesticate new crops from semi-wild plant species.

Molecular markers and genetic linkage maps

New advances in plant breeding utilize the tools of molecular genetics to accelerate selection of new varieties – increasing selection efficiency while reducing the time of varietal development. These methods utilize the selection of molecular markers, known to be linked to quantitative and qualitative traits of interest, to avoid the problems associated with the traditional method of selection based solely on phenotypes. The use of molecular markers to assist in trait selection is generally referred to as marker-assisted selection (MAS). Marker-assisted selection methods, including specific methodologies such as genome-wide association study (GWAS) applied directly to breeding populations (Kraakman *et al.*, 2004; Crossa *et al.*, 2007) and genomic selection (Jannink *et al.*, 2010; Windhausen *et al.*, 2012), are regularly utilized to enhance breeding efficiency in commercial and public plant breeding programmes (Eathington *et al.*, 2007; Moose *et al.*, 2008). The efficient use of these methodologies relies on access to numerous inexpensive, reliable and easily assayed molecular markers.

The first step towards the development of molecular markers for quinoa was the development of a genetic linkage map by Maughan et al. (2004). This map, which covered an estimated 60% of the genome, was based primarily on amplified fragment length polymorphism (AFLP). Unfortunately, the difficulties associated with AFLP marker technologies and the associated transfer of this technology to laboratories in the Developing World where quinoa is cultivated significantly limited the exploitation of these markers. The next step forward in quinoa marker development was the characterization of > 400 microsatellite markers (also called simple sequence repeat or SSR markers), reported by Mason*et al.* (2005) and Jarvis *et al.,* (2008). Microsatellites are short repeated nucleotide motifs, usually two to four base pairs in length, which are flanked by conserved sequences and occur ubiquitously throughout eukaryotic genomes (Tautz *et al.*, 1984). They are widely considered the genetic marker system of choice for taxonomic questions due to their characteristics of being highly reproducible, informative, locus-specific, multi-allelic and co-dominant (Morgante *et al.*, 1993). Because they are the most variable type of DNA sequence in eukaryotic genomes (Weber *et al.*, 1989), microsatellites have been extremely useful for determining taxonomic relationships among closely related individuals and assessing diversity within a species. While the initial cost of developing microsatellite markers is high, once developed, these PCR-based markers are inexpensive to use and require less technical expertise than other types of molecular markers (i.e. AFLP markers). These quinoa microsatellite markers have already been used to assess the genetic diversity among quinoa accessions within the USDA collection (Christensen *et al.*, 2007) and in efforts to genetically characterize Andean and Chilean germplasm. Indeed, these markers clearly show that the quinoa accessions can be broadly clustered into two main groups: one including accessions from the lowlands of Chile (Coastal ecotype), and the other comprising accessions from the Andean highlands (Altiplano ecotype) with origins in Peru, Bolivia, Ecuador, Argentina and extreme northeastern Chile. Fuentes *et al.,* (2009) developed a multiplex fluorescent set of microsatellites to study the genetic diversity patterns of northern and southern Chilean accessions. As expected, the accessions clustered into the two groups – Altiplano and Coastal. Interestingly, the Chilean Altiplano quinoas were genetically less diverse than the Chilean Coastal quinoas, suggesting a potential loss of genetic diversity in the commercial growing zones of Chile. Tartara *et al.,* (2012) studied the genetic structure of cultivated quinoa from northwest Argentina using 22 microsatellites. Aside from being underrepresented, northwest Argentina is also the southernmost point of quinoa distribution within the central Andes. The accessions showed a high level of genetic diversity which could be grouped into four regional ecogeographical groups, consistent with the geographic origin of the accessions: the transition region, characterized by high altitudes; puna, the highland plateau; eastern humid valleys; and dry valleys.

Single nucleotide polymorphisms (SNPs) are the most abundant type of DNA polymorphism found in eukaryotic genomes (Garg et al., 1999; Batley *et al.*, 2003), and are the marker of choice in marker-assisted plant breeding programmes (Batley *et al.*, 2007; Eathington et al., 2007). A single SNP can have four alleles, but most show only two and are regarded as bi-allelic. The high frequency of SNPs in plant genomes is well documented (Russell *et al*., 2004; Ossowski *et al.*, 2008), with actual SNP densities ranging dramatically depending on the species type (auto- or allogamous), the number and genetic diversity of the cultivars being assessed, and whether coding or non-coding regions are being considered. In quinoa, Cole *et al.,* (2005) identified 38 single-base changes and 13 insertions-deletions (indels) in 20 EST sequences analysed across five quinoa accessions, suggesting an average of 1 SNP per 462 bases and 1 indel per 1 812 bases. Maughan *et al.,* (2012) looked at SNP frequencies between pairs of parents of five mapping populations (Pop1, Pop39, Pop40, PopM3 and PopGO) and identified, on average, 1 SNP per 2 214 bp. It is noted that SNP frequencies are probably much higher than this estimate, as the parameters used to identify a sequence change as a true SNP were highly conservative (read coverage > 6X, minimum allele frequency > 20% and identity conservation = 100%). The highest number of SNPs were identified in PopM3, which is a cross between a Coastal Chilean ecotype (NL6) and Peruvian valley ecotype (0654).

The high frequency of SNPs offers the possibility to construct extremely dense genetic maps that are particularly valuable for map-based gene cloning efforts and for haplotype-based association studies. Maughan *et al.,* (2012) sequenced a genomic reduction quinoa library to identify 14 178 putative SNPs in five bi-parental quinoa populations. Genomic reduction, based on restriction-site conservation (GR-RSC), allows for the effective sampling of identical DNA fragments across individuals without a priori genome sequence information (Maughan *et al.*, 2009b). The incorporation of barcodes into specific DNA sequence fragments allows for the unambiguous assignment of fragments to specific samples in the sequence pool, thus enabling the identification of SNPs that will segregate in specific populations. When linked with second-generation sequencing, genomic reduction provides a cost-effective means to identify large numbers of high-confidence SNPs *en masse* with broad application across diverse genomes. Of the SNPs identified, transition mutations (A/G or C/T) were the most numerous, outnumbering transversions (A/T, C/A, G/C, G/T) by 1.6X margin, which was in accordance with the observation that transition SNPs are the most frequent SNP type reported in both plant and animal genomes (Zhang *et al.*, 2004; Morton *et al.*, 2006). Of the 14 178 SNPs identified, 511 were successfully converted into functional SNP assays using KBioscience's competitive allele-specific PCR genotyping chemistry (KASPar™). A diversity screen of 113 quinoa accessions using these 511 SNPs clearly revealed the two major quinoa subgroups. Minor allele frequency of the SNPs ranged from 0.02 to 0.50, with an average MAF of 0.28. Linkage mapping of the SNPs in two recombinant inbred line populations (KU-2 X 0654 and NL-6 X 0654) produced an integrated linkage map consisting of 29 linkage groups with 20 large linkage groups, spanning 1 404 cM with a marker density of 3.1 cM per SNP marker.

Expressed sequence tags and single nucleotide polymorphism (SNP) markers

Additional genomic resources for the improvement of quinoa are beginning to emerge and the usefulness of these resources for cloning genes of interest has been demonstrated. For example, expressed sequence tag (EST) data sets are beginning to be deposited in GenBank. EST sequences are partial sequences from transcribed cDNA sequences that reflect the genes being expressed in a given tissue type at a specific point of development. Made publically available, EST sequences greatly facilitate gene discovery efforts.

Collections of these sequences can also provide researchers with a rapid and cost-effective tool to analyse transcriptome changes using techniques such as microarray or RNA-seq analysis. Cole *et al.,* (2005) described the first set of 424 ESTs from developing seed and floral tissue (GenBank dbEST ID #GI47561370 - GI47561793). Of these sequences, 349 had significant homology to protein-encoding genes from other plant species. Putative functions related to metabolism, protein synthesis, development and so forth, have been assigned to many of these EST sequences. More recently, Raney *et al.,* (2013) reported the results of an RNA-seq transcriptome analysis of two quinoa accessions using four water treatments (field capacity to drought). cDNA libraries from root tissue samples for each variety × treatment combination were sequenced using Illumina Hi-Seq technology generating a *de novo* assembly of the quinoa root transcriptome consisting of 20 337 unique transcripts (all transcripts are publicly available from NCBI GenBank, SRA #SRR799899 and SRR799901). Gene expression analysis of the RNA-seq data identified 462 putative gene products that showed differential expression based on treatment, and 27 putative gene products differentially expressed based on variety × treatment, including significant expression differences in root tissue in response to increasing water stress. Differentially expressed genes were identified and bioinformatic methods were employed to implicate specific pathways putatively associated with water stress in quinoa.

An excellent example of the utility of these publically available EST libraries is the report of the cloning and analysis of the *11S* globulin seed storage protein (Balzotti *et al.*, 2008) and *Salt Overly Sensitive* (*SOS1*; Maughan *et al.,* 2009a) genes from quinoa. Balzotti cloned and described the two *11S* genes representing the two subgenomes of quinoa (an ancient allotetraploid). Identification and characterization of these genes provide important clues to understanding the high protein content and excellent balance of amino acids in quinoa grain. Maughan et al. utilized EST sequences and the available quinoa BAC library to clone and characterize two homoeologous *SOS1* loci (*cqSOS1A* and *cqSOS1B*) from quinoa, including full-length cDNA sequences, genomic sequences, relative expression levels, fluorescent *in situ* hybridization (FISH) analysis, and a phylogenetic analysis of *SOS1* genes from 13 plant taxa. Genomic sequence analysis of two BAC clones (98 357 bp and 132 770 bp) containing the homoeologous *SOS1* genes suggests possible conservation of synteny across the quinoa subgenomes. Salt tolerance is an agronomically important trait that affects plant species around the globe. Indeed, nearly one-third of all arable land worldwide is affected by soil salinity (Epstein and Bloom, 2005). *The Salt Overly Sensitive 1 (SOS1)* gene encodes a plasma membrane Na+/H+ antiporter that plays an important role in germination and growth of plants in saline environments. Morales *et al.,* (2011) also reported the development of primer information used for real-time expression of several additional salt tolerance genes, including *NHX1*, *TIP2* y *BADH*.

Quinoa bacterial artificial chromosome (BAC) library

Another important genomic tool that is available for quinoa is a 9X bacterial artificial chromosome library, consisting of 74 880 clones (Stevens *et al.*, 2005). The BAC library is available for public use in the Arizona Genomic Institute (AGI) at the University of Arizona, Tucson, Arizona, United States of America. The library was constructed using two restriction endonucleases, *BamHI* (26 880 clones) and *EcoRI* (48 000 clones) with an average clone insert size of 113 kb and 130 kb per insert, respectively. Approximately 1% of the clones lack inserts. The estimated coverage of the library is based on a calculated genome size of 967 Mbp. An average of 12.2 positive clones per probe were identified using 13 quinoa single-copy EST clones as probes of the high-density arrayed blots. Undoubtedly, the BAC library will continue to be an important resource for efforts to identify specific genes and the continued characterization of the quinoa genome, specifically for the construction of a quinoa physical map.

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CHAPTER: 1.3

Domestication and Prehistoric Distribution

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Abstract

This study reports on the current state of knowledge regarding the history of Chenopodium quinoa in four Andean countries: Argentina, Bolivia, Chile and Peru (Figure 1). The cultural environments in which quinoa was domesticated, adopted, exchanged and/or cultivated in ancient times, were reconstructed using archaeological data and, in particular, on the basis of archaeobotanical research by many specialists in these countries, as well as ethnohistorical sources and observations of the cultural continuities in communities that still produce quinoa using traditional methods.

The study begins with a review of the domestication of Chenopodium. It has been shown that the morphological features of archaeological seeds are the outcome of human manipulation over at least 3 000 years. This indicates that groups of hunter-gatherers in the Late Archaic Period (8000–3000 B.C.) in the Andean region subsisted on wild Chenopodium and applied selection, protection, treatment and transplantation processes that induced changes in its structure resulting in the characteristic features of domesticated quinoa.

The study then investigates archaeobotanical records from the Late Archaic to the Inca period. It outlines the distinctive morphological attributes in each region, the ecological conditions where quinoa was cultivated, the zones of origin and access routes, the various ways it was used, and quinoa's role in the sociopolitical processes of the time.

Finally, the study draws attention to the benefits (or necessity) of continuing regional research, optimizing methodologies and exchanging information and developments among researchers seeking answers to the many unsolved problems, including the presence of seeds not specifically identified as having traits that could match the early stages of the domestication process.

Introduction

Humans in the Americas began to domesticate the fauna and flora some 8 000 years ago. The Andean region was one of the most important centres for domestication, and quinoa (*Chenopodium quinoa* Willd.) was a primary crop. Since its domestication, quinoa has played a key role both in the livelihood and in the social and political systems of Andean societies. Archaeologists are gradually reconstituting this long history with a better understanding of how quinoa was used as a wild plant by hunters and gatherers, its domestication processes and its diffusion and diversification throughout the Andes.

While molecular genetics helps understand where quinoa was domesticated, archaeology provides essential chronological information about when it was domesticated and/or introduced and incorporated into farming systems (Zeder *et al.*, 2006). The settings reveal *why* quinoa was adopted and *what* role it played in the lives of the native peoples, from everyday cooking to rites, political festivities and community ceremonies.

Since the 1990s, archaeologists have developed fieldwork methods to recover seeds of ancient quinoa via flotation or dry screening, and laboratory tests have been done to recognize wild, domesticated and other varieties of quinoa (López *et al.*, in print). Quinoa plants bear fruits (achenes) in large numbers. The achenes structurally withstand natural processes (drying caused by extremely arid environments) and cultural processes (accidental or intentional charring), and quinoa seeds can, therefore, be recovered in a variety of settings in the Andes. The ubiquity of quinoa reflects its importance in the past, and the last 15 years have seen projects implemented in Argentina, Bolivia, Chile and Peru to understand its domestication and past consumption. This study summarizes the project findings published to date, fully aware of the amount of research that remains to be done, especially in those countries (e.g. Ecuador and Colombia) where quinoa growing once played a significant role but does no longer. In all cases, further archaeobotanical research is required.

Domestication of *Chenopodium quinoa*

It is not known exactly where and when *C. quinoa*, was domesticated, but it is certain that its domestication in South America was unrelated to that of Mexican (*Chenopodium berlandieri* spp. *nuttalliae) and North American* (*Chenopodium berlandieri* spp. *jonesianum*) (Kistler & Shapiro 2011; Wilson 1990). Its likely progenitors are C. hircinum, a lowland tetraploid, or some other extinct tetraploid ancestor in the Andes (Fuentes *et al.*, 2009; Wilson, 1990).

During the domestication process, a wide range of morphological changes occurred in the overall plant and in the fruit. Changes in the plant include infructescence compaction, loss of natural shatter mechanisms and uniform maturation of the fruit – all changes that facilitate production (Mujica *et al.*, 2001), although some varieties lack uniform maturation (Daniel Bertero, personal communication). The overriding archaeological evidence lies in the seeds, and the increase in their diameter is one of the most characteristic features. Other micromorphological features are observed, including reduction in thickness of the seed-coat or testa covering the embryo and the perisperm in the seed and preventing premature germination (Hugh Wilson, 1981). In wild populations, the seed-coat tends to be thick, somewhat hard and dark in colour. This prevents penetration of external elements that accelerate the development and growth of the embryo before full maturity, but also protects it from possible dehydration and insect attack. Selective domestication led to a reduction of seed-coat thickness via a genetically recessive trait which can only be maintained through human-made selection. Seed-coat reduction then led to morphological alteration of the fruit margins: wild forms have margins ranging from rounded to biconvex, while domesticated forms have truncated margins due to the flatter morphology of their adaxial and abaxial faces, the growth of cotyledons and the increased volume of the perisperm. Differences also developed in the seed-coat texture: domesticated seeds tend to have a smooth texture, i.e. without protuberances, while wild varieties have a reticulated testa structure (Bruno, 2006; Smith, 1992). Lastly, domesticated forms are characterized by light-coloured pigmentation due to less lignification in the epidermis, and wild-type fruits are dark in colour because of their hard, lignified epidermis (Wilson, 1981).

Molecular studies are currently underway in Argentina and Bolivia to better understand domestication, especially where it occurred and whether or not it was repeated. Initial findings led to the identification of four genetic groups: Altiplano, Dry Valleys, Eastern Humid Valleys, and Transition area (Curti *et al.*, 2012). The same SSR markers (microsatellites) were used to molecularly characterize populations from different Andean countries in South America, with the aim of ascertaining the links between origins and subsequent dissemination. The findings revealed germplasm clusters suggesting the presence of longitudinal corridors for the spread of quinoa throughout the Andes (Costa Tártara *et al.*, 2013).

Furthermore, these molecular studies have begun to investigate the domestication syndrome (changes that distinguish between a species and its wild ancestor species) for quinoa (Daniel Bertero, personal communication). Study of the ratio between testa thickness and loss of dormancy has shown, for example, that no such relationship exists for two germplasms (Ceccato, 2011). Nevertheless, more germplasm samples are needed to assert or invalidate this trait in a domesticated grain, observed in domesticated species, not only in the Andes but also in Mexico and North America.

As archaeologists, in this chapter we present data relative to the period when the first records of domesticated quinoa appeared and the beginning of the human–*Chenopodium* relationship in the Andes.

Figure 1: Andean region and geographic areas with archaeological finds of *C. quinoa*. 1a NWA (Argentina); 1b Cuyo (Argentina); 2a Northern Altiplano (Bolivia); 2b Central Altiplano (Bolivia); 2c Southern Altiplano (Bolivia); 3a North (Chile); 3b Coastal Centre and the Andes (Chile); 4 Peru.

Archaeological data on Pre-Hispanic distribution of *C. quinoa*

Herein is a summary of published papers containing data on the presence of quinoa in Argentina, Bolivia, Chile and Peru. We highlight the places where seeds have been found, the contexts in which they were used, and data pertaining to their age.The use of direct radiocarbon dating on the botanical material is essential to determine the age of the quinoa domesticated in the different regions. As explained below, contextual dating is utilized to date seeds at many archaeological sites in Argentina, Bolivia, Chile and Peru; this may be problematic and misleading when determining when quinoa was domesticated.

Argentina

In presenting the findings on *C. quinoa* in Argentina, the archaeological sites are covered by region. The first region encompasses northwest Argentina (hereafter NWA), with sites located in the provinces of Jujuy, Salta, Tucumán and Catamarca (Figure 1, sector 1a), and the second region covers Cuyo and includes the provinces of Mendoza and San Juan (Figure 1, sector 1b).

Northwest Argentina

The first record of *C. quinoa* in Argentina was presented by Hunziker (1943). It came from the archaeological site of Pampa Grande (Salta), which corresponds to the Precontact Period (500–700 A.D.). It comprises seeds recovered in a funerary setting together with other botanical remains. No taxonomic specifications were given concerning the *C. quinoa* variety found, but the author considers the seeds to be quinoa on the basis of a comparison with seeds cultivated in the Bolivian Yungas. Some specimens were identified only as *Chenopodium sp*., described as ajara (possibly in reference to the wild state). In this same work, Hunziker mentions quinoa seeds recovered in the past by Ambrosetti in Argentinean prehistoric tombs, but provides no further details.

In Salta, Muscio (2004) unearthed charred seeds of *Chenopodium sp*., morphologically similar to quinoa in Matancillas 2 (San Antonio de Los Cobres) and belonging to the early agropastoral groups. Meanwhile, Lennstrom (1992) and D'Altroy et al. (2000) also presented evidence of C*henopodium sp.* at sites in Valdez (1047–1288 A.D.), Puerta de la Paya (1470–1536 A.D.) and Potrero de Payogasta (1279–1660 A.D.). Although only the genus is specified, the seeds are considered domesticated on account of their morphological traits. Since they were ubiquitous, it was reported that this species was produced and consumed freely following the Inca conquest in the region. Lastly, Amuedo (2010) records the presence of quinoa at La Paya, Kipón, Mariscal, Ruiz de los Llanos and Tero, chronologically located in the Regional Developments Period (900–1450 A.D.).

In the province of Catamarca, in Antofagasta de la Sierra, Olivera (2006) discovered *Chenopodium sp.* in Cueva Cacao 1 (between 710 ± 60 and 870 ± 60 A.D.), while Rodríguez *et al.* (2006) presented evidence of *C. quinoa* in Punta de la Peña 4 (1190–1390 A.D.), where seeds, inflorescence branches and flowering stem were all taken from a hearth. These macroremains were recovered together with other domestic and wild plant species and are evidence of the high dietary consumption and, for the first time, the existence of the association *C. quinoa/Deyeuxia eminens* (the former is edible, the second was used as a tool for toasting the seeds). Furthermore, at Peñas Chichas 1.3, Aguirre (2007) recorded stems of this pseudocereal with signs of cutting – indication that it was harvested early in that region.

Caló (2010) identified *Chenopodium sp.* seeds from the archaeological site of Cardonal (Catamarca). On the basis of the morphological features of a group of seeds, Caló carried out a comparison with *C. quinoa*. It should be noted that, given the uncertainty of the morphometric features of the seeds, only the genus was identified and it was argued that it was consumed by the inhabitants of the southern sector of the Calchaquí Valley. Lastly, archaeological rescue excavations performed at the Las Champas site (Tinogasta) (1275–1435 A.D.) (Norma Ratto, personal communication) led to the recovery of seeds including *C. quinoa* var. *melanospermum* from a funerary context. The ajara showed signs of boiling or soaking in water and it is possible that ajara was consumed as part of a burial ritual – an indirect indication of quinoa cultivation in the region.

In Jujuy, charred grains of *C. quinoa* were recovered at the Finispatriae site (Rio Grande de San Juan) (800–1300 A.D.), on the border between Argentina and Bolivia. The archaeobotanical material was found in what was once a waste disposal area and, therefore, it can be deduced that quinoa was consumed by the inhabitants of the site (Nielsen et al., 2013).

Starch grains represent the earliest findings of this pseudocereal in northwest Argentina. Babot (2004) recovered microremains in milling instruments at the Los Viscos site (Catamarca) (320 ± 230 B.C. – 1130 ± 50 A.D. – Korstanje, 2005) and at Cueva de los Corrales (Tucumán) (50 B.C. – estimated chronology). Although only the genus was identified, with no differentiation between *Chenopodium* and *Amaranthus*, there was clearly a high level of consumption of pseudocereals by hunter-gatherer groups. Furthermore, in Antofagasta de la Sierra, Babot (2004) presented evidence of starch grains of *Chenopodium sp*. cf. *C. quinoa* in milling tools at Punta de la Peña 9.1 sites (520 ± 60 A.D.) and Quebrada Seca 3 (levels 2b [2] dated 2550 B.C., and 2b [3] dated 2750 B.C.). At the Morro Relincho site (Formative traits), Korstanje (2005) found small grains of quinoa-type starch in the sediment, inferring the possibility of quinoa production.

Cuyo

In San Juan, in the period of the Ansilta culture (around 500 B.C.), Lagiglia (2001) presented a list of places where quinoa was found, including Gruta de los Morrillos de Ansilta, Gruta Granero, Punta del Agua de los Morrillos, La Pintada and Gruta de Chacaycito. The seeds are merely referred to as "quinoa" and no genus or species is specified. In an analysis of the sites related to the Aguada culture (500–900 A.D.), Gambier (2002) mentions the presence of *C. quinoa* as a crop species, without specifying which particular sites supplied this evidence. Lagiglia (2005) later described individual contexts, as in the case of Gruta Los Morrillos, where quinoa was retrieved from a waste site in cave 1, while a "*C. quinoa loaf*" was recovered in cave 2, and human coprolites were found at a lower level with traces of this pseudocereal. At the Gruta Río Fierro site, 25 g of C. quinoa were found in a burial setting, while the recovery of C. quinoa var. quinoa and *C. quinoa* var. *melanospermum* was recorded at Gruta Río Salado.

Regarding Mendoza, the sites related to the Atuel II culture (around 300 B.C. until the Spanish incursion) contain a wide range of Andean pseudocereals. Hernandez *et al*. (1999–2000) presented the archaeobotanical records for the Agua de los Caballos 1 site (San Rafael), noting seeds of *Chenopodium sp.* In addition, Lagiglia (2001) presented evidence of several chronologically earlier sites, including Gruta del Indio del Rincón del Atuel (250 ± 70 B.C.), Cueva Pájaro Bobo de Ponontrehue (60 ± 70 B.C.) and Reparo de las Pinturas Rojas (390 ± 110 A.D.), with evidence of *C. quinoa* var. *quinoa* and *C. quinoa* var. *melanospermum*. No further details were provided concerning the sites and contexts, except for Gruta del Indio, where, years later, Lagiglia (2005) explained that *C. quinoa* was found in a basket made of pampas grass and reeds, noting that it had been in use in Cuyo for over 2 200 years. This site is mainly linked to funeral functions (Gil and Neme, 2010).

Castro and Tarragó (1992), on the basis of the presence of *C. quinoa* and other archaeobotanical remains at the San Juan and Mendoza sites, proposed the existence of socio-economic processes associated with the adoption of agriculture – similar to that which occurred in Chile and northwest Argentina during the Late Archaic Period.

Bolivia

PThe primary findings of *C. quinoa* are concentrated in three regions of the Bolivian Altiplano: north, particularly around Lake Titicaca (Figure 1, section 2a), centre, in the Lake Popóo and Oruro region (Figure 1, section 2b) and south, around the Salar de Uyuni (Figure 1, section 2c).

Northern Bolivian Altiplano – Lake Titicaca region.

Records in this region, mainly from the Taraco peninsula and the Tiwanaku Valley, come only from charred botanical macroremains. To date, they derive from various contexts spanning the Formative Period (1500 B.C. – 300 A.D.) and the Tiwanaku Period (300–1100 A.D.)

Kidder (1956) found charred remains of plants in niches of the “houses” of the Chiripa Mound, the most well-known Formative site. Towle (1961) later identified them as quinoa seeds. In the 1970s, Erickson (1976) analysed macrobotanical remains from Chiripa for his undergraduate thesis, and identified many seeds of the *Chenopodium* genus, including the *C. quinoa* species. Browman (1989) examined more samples and, given the differences in size of the *Chenopodium* seeds, proposed that the larger seeds (1–2 mm) were quinoa.

Archaeobotanical studies conducted since 1992 by Hastorf and her students in the Taraco Archaeological Project (PAT) have revealed high densities of several species of *Chenopodium* (Bruno, 2008; Langlie, 2008; Whitehead 2007). In a detailed analysis of the morphological features of the *Chenopodium* seeds – especially the seed-coat or testa – Bruno (2006) identified domesticated quinoa species and their wild counterpart, *C. quinoa* var. *melanospermum* (Bruno and Whitehead, 2003).

The Taraco Archaeological Project has obtained several direct radiocarbon dates for quinoa seeds from the Chiripa, Kala Uyuni, Sonaji and Kumi Kipa sites. The earliest are from Chiripa and Kala Uyuni (around 1500 B.C.) and the most recent are dated 400 A.D. in Kala Uyuni. All these studies reveal the presence of *Chenopodium* seeds alongside several other wild species, such as gramineae, legumes and malvaceae.

Research carried out by Bruno and Whitehead (2003) found that, during the Early Formative Period or Early and Middle Chiripa phase (1500–800 B.C.), agriculture was developed on a small scale, and quinoa – as well as ajara and black quinoa – was grown and harvested. In the Middle Formative Period (Chiripa, 800–200 B.C.), a significant decline in the archaeological presence of ajara began, indicating changes in the management (weed control, processing) and use of crops. This included its use in rituals, suggesting that quinoa was an important food crop. Studies of the various contexts in Taraco sites – from floors and niches in public and ceremonial structures to domestic waste sites – have shown that quinoa was both a household and a ritual food and had a role in social and political events during the Formative Period.

Studies conducted on sites associated with the earliest state in the region – Tiwanaku – demonstrate that quinoa continued to play an important part in small farms and in the diet of highland peoples at that time. The Wila Jawira project – led by Kolata and the first archaeobotanical studies from the urban site of Tiwanaku and other rural sites in the Tiwanaku Valley and Lukurmata – identified *Chenopodium* seeds in 93% of the samples analysed (Wright et al., 2003).

Schultz (2010) studied the Pirque Alto site (Cochabamba) – Formative Period (1800 B.C. – 300 A.D.) and Middle Horizon Period (600–1000 A.D.) – and recorded the presence of *C. quinoa*, indicating the cultigen's social and ideological significance.

Central Bolivian Altiplano

Langlie conducted a morphological study of *Chenopodium* seeds recovered from a hearth at the La Barca site (Langlie, 2008; Langlie *et al.*, 2011). La Barca is a Wankarani site from the Formative Period (1800 B.C. – 400 A.D.) in the department of Oruro. The seeds examined by Langlie were quite different from the domesticated and wild quinoa, observed in the Lake Titicaca region.. Although the seed-coat was relatively thin, its reticulated texture and biconvex margins were similar to wild black quinoa. Furthermore, it had a very prominent "beak", differentiating it from Titicaca quinoa. Langlie suggests that these seeds may be an early and distinct variety of domesticated quinoa developed in the Oruro region. A definitive identification has not been possible to date due to a lack of similar comparative samples. Nevertheless, their presence is indication of the diversity found in the early stages of quinoa domestication.

Southern Bolivian Altiplano

Research conducted by Nielsen and colleagues for the Southern Altiplano Archaeological Project (PAAS) recovered quinoa in different settings at numerous archaeological sites chronologically situated between 900 and 1550 A.D.

The residential areas of the elevated defensive sites (pukaras), Churupata (1285–1380 A.D.), Mallku Pukara (1310–1630 A.D.) and Pukara de Sedilla, provided carbonized seeds of *C. quinoa*. The seeds came from cooking stoves, and on the basis of the diagnostic traits of pre-consumption processing (Lopez et al., 2012), it can be inferred that they were used after saponin extraction and eaten as whole seed (boiled) and/or in soup.

Further archaeobotanical recovery was made in storage areas. These are located at the defensive sites of Laqaya (1236–1479 A.D.), Mallku Pukara (1310–1630 A.D.) and Jirira Vinto (1300–1400 A.D.), and in isolated places associated with Chinuil Vinto, Cueva del Diablo (1310–1460 A.D.), Lojo, Qhatinsho 1 (720–1630 A.D.), Oqhañitaiwaj and Paco Cueva farmlands. The identified species of *C. quinoa* include seeds and leaves, stems and infructescence rachis. With the exception of the Laqaya site, archaeological plant material was stored desiccated, and information is therefore available on taxonomic varieties and the various types of quinoa. At the Laqaya site, charred seeds were found in a chullpa stone tower located in the central plaza of the site. In addition to the individual seeds, which could be analysed, a mass of perisperm with grains and the remains of attached quinoa grainswas recovered. The mass had the morphology of a bowl, possibly used to extract seeds from the silo, and its negative mould remained after a fire in this part of the site. The archaeological context offers two possibilities as to why quinoa was stored in this tower in the plaza: for community consumption in the political commensalism system, or for protection by the embodiment of the Ayllu ancestor (represented by the chullpa tower) and use in community celebrations as part of the agricultural cycle (Lopez and Nielsen, 2012).

The presence of *C. quinoa* var*. quinoa* is reported at all sites near Salar de Uyuni, and, in addition, *C. quinoa* var*. melanospermum* is reported at Jirira Vinto, located at the foot of Cordillera Intersalar (north of Salar de Uyuni). Considering the agricultural systems in the two areas, it is believed that the presence of ajara in Jirira Vinto may be due to a production system in which it was not in competition with quinoa and was therefore tolerated, possibly maintained, and harvested for food consumption in times of scarcity (Lopez, 2012).

It has been established that *C. quinoa* var*. quinoa* was stored in two different stages of post-harvest processing. The first stage was bulk storage prior to saponin extraction, possibly with the intention of planting in the next cropping season. Based on fruit colour and diameter, the types recorded resemble quinoas known today as White or Yuraj Real Cashlala, Pasankalla, Pink or Puca, Orange and Black, depending on the key adopted (Lopez, 2012).The Purple and Toledo types may also be present, but they have not been accurately identified as their features overlap with other types (Lopez *et al*., 2012). The second stage involved grains after saponin extraction, stored ready for consumption. These grains reveal traces of parching, indicating that they were consumed as whole seed (boiled) and/or soup, and as pitu or toasted grain flour (Lopez, 2012). This points to consumption patterns similar to current practices. Pitu or pito is currently consumed both during the agricultural production stage (planting and harvesting), and during transportation of products to be sold (llama caravan trade). Toasted quinoa is consumed in agricultural fields at the end of the working day.

Chile

For archaeobotanical findings of quinoa, Chile is divided into northern Chile (Figure 1, sector 3a) and central Chile (Figure 1, sector 3b). Central Chile is then subdivided into mountains, valleys and coastline.

Northern Chile

The first findings of *C. quinoa* in Chile were revealed by Safford (1917, in Hunziker, 1943) who extracted whole plants of the species in Arica. Meanwhile, Uhle (1919) recovered quinoa from funerary contexts with mummified individuals from the Chinchorro culture. They were groups of fishermen, hunters and pre-agricultural gatherers who lived on the arid coastline before 3000 B.C. (Arriaza and Standen, 2002). However, there were no morphological descriptions to corroborate whether or not it was a domesticated species, although Uhle did suggest that this quinoa was the result of contact with the highlands. Recent archaeobotanical studies in coastal ravines demonstrate the presence of *C. quinoa* at Chomache 1 (1600–600 B.C.) (Núñez, 1986). Its presence is minimal, but is indication that it came from the lower valleys in the interior and from the highlands, where production was more feasible. These early pieces of evidence suggest that quinoa may have initially arrived on the coast from other areas (including the southern coast of Peru), since there are insufficient data to support local domestication or horticulture associated with early coastal developments (Vidal, 2007).

During the Formative Period (1000 B.C. – 500 A.D.), in the interior valleys of Tarapaca and the oases, the presence of high-elevation crops was detected on pampa sites, including charred quinoa seeds in the villages of Ramaditas and Guatacondo, with morphological traits ascribable to *C. quinoa* (Rivera *et al.*, 1995; Magdalena Garcia personal communication). In the early Gatchi phase (1200–350 B.C.), although not confirmed formally, seeds akin to the *Chenopodium* genus were recovered, but their charred state prevented the attribution of a more precise taxonomic category (Vidal, 2007). It has been suggested that it was a far more dynamic period for contact with trans-Andean areas, such as northwest Argentina and the southern highlands (Nunez *et al.*, 2002–2005).

South of Salar de Atacama, tin he Antofagasta region, Holden (1991) mentions the possible presence of a domestic variety of *Chenopodium* in coprolites at the Tulan 54 and 58 sites (1400–470 B.C.). The low ratio indicates a relative lack of importance in the inhabitants' diet. McRostie (2007) exercised greater caution, referring to charred specimens at Tulan 54 as cf. *C. quinoa*. They presented the morphometric traits of quinoa, but damage to the testa made it difficult to make a clear categorization. Among the microremains analysed, there is mention of "starch aggregates" similar to patterns in the Amaranthaceae family, together with other species corroborating the existence of links with trans-Andean areas and the highlands, as well as pointing to the likely involvement of outside groups with ritual elements.

In the highlands of Tarapaca, human occupancy in residential areas of the Huasco Sur sites, all from the Formative Period (900 B.C. – 900 A.D.), left traces of wild varieties of Amaranthaceae and, at just one of these sites, carbonized seeds of *C. quinoa* as the only cultivated species recorded (Magdalena Garcia and Alejandra Vidal, personal communication). Given the great heterogeneity of lifestyles at this time in the Tarapaca region, the absence of maize and other elements led the authors to propose that the Salar sector was a place of transition between the Pica Oasis and North Lípez, and it probably was not well connected with the Tarapaca valleys, lacking links with other sites in the Formative Period. Given the absence of suitable environmental conditions for cultivation, quinoa could have originated in Bolivia and then come from the precordillera ravines during the Late Intermediate Period. As for the Camiña 1 site (1250–1450 A.D.) – an extensive settlement with agglutinated structures in the Tarapaca region – there is evidence of quinoa which may have originated in the highlands, this time in a new socio-economic context with production on the platforms adjacent to the site (Garcia and Vidal, 2006).

It is, therefore, clear that there are insufficient data to ascertain the domestication of quinoa in these areas. Researchers share a consensus on transverse and longitudinal mobility and the exchange of products and goods between the highlands, inland low valleys, ravines and coastline since the Archaic Period. This may have increased during the Formative Period, including products for use in rituals, resulting in established cultivation in the Late Intermediate and Late Periods. It is possible that trans-Andean contacts and contacts with the highlands were instrumental in the process of adopting cultigens.

Quinoa played an important role in the rituals of the Incas. It was known as *chisiya mama* (mother grain) and was used in celebrations and offerings to mark the planting and harvesting of this valuable food. With the Inca incursion into Chilean territory (1440 A.D.), these ceremonial activities were introduced to the vanquished populations, also bringing improvements in infrastructures for cultivation, irrigation and storage.

Cordillera of Central Chile

In central Chile, the pre-Hispanic presence of *Chenopodium* has been confirmed at archaeological sites in the Andean foothills and mountains of the central valley and coast, between the basins of the Choapa and Maule rivers. Further south, there have been findings in the regions of Biobío, Araucanía, Los Lagos, and the islands of Chiloé, Mocha and Santa Maria. Accelerator mass spectrometry (AMS) was applied to charred quinoa seeds found on Santa Maria Island, and it is estimated that they were used during the period 1030–1460 A.D. (Massone *et al.*, 2012).

Chenopodium is the first plant resource with traces of human intervention found in central Chile. It dates back to the Archaic Period (3000–300 B.C.), when it was used by Andean hunter-gatherers, before the acquisition of maize by farming societies in the Early Period (from 300 B.C. to 1000–1200 A.D.). This has been established by stable isotope analysis (Falabella et al., 2008). In the high Andean region, opposite Santiago, there are two sites (2070 and 2500 m asl) with evidence of consumption of *Chenopodium*. Both are hunter-gatherer sites, without pottery, Late Archaic IV Period (Cornejo *et al.*, 1998), and they were temporarily occupied during thawing and snowless seasons from August/September to March/April. The El Plomo site (1460–1340 B.C.) has evidence of *Chenopodium* sp. cf. *C. quinoa*, with similar amounts of charred and other desiccated specimens. The desiccated specimens do not have a radicle, their diameters do not exceed 0.8–1 mm and their perisperm retain a natural ivory white colour with a truncated/rounded margin, no testa, and a prominent embryo (beak) (Planella *et al.*, 2011). *Chenopodium* cf. *C. quinoa* was recovered at Alero Las Morrenas 1 (1250–980 B.C., AMS direct dating with seeds). All specimens were carbonized, and taxonomic classification was, therefore, not possible in terms of variety or species. With diameters of up to 1.4 mm, most specimens featured radicles detached from the rest of the seed or the seed's extremity was swollen or bloated, probably due to carbonization (Planella *et al.*, 2005, 2011).

A cultigen domestication process in this mountainous area is quite unlikely due to the adverse weather conditions (restricting the possibility of farming practices) and the limited periods of human settlement. On the other hand, it has been suggested that the proximity to mountain passes on the eastern slope of the Andes favoured contacts and the exchange of goods, knowledge and innovations, including early cultigens or varieties of quinoa (Planella et al., 2011). Nevertheless, the dates given for the above-mentioned sites are prior to the dates obtained in Mendoza, Argentina, and are notoriously earlier than the dates reported on the coast and in the central valley.

Central Chile: valley and coastal areas

In the valleys of the coastal foothills of the Bernardo O'Higgins and Maule regions, and in scattered areas up to the Islands of Chiloe, *quinwa* or *dahue* (Mapuche ethnonym) is still grown today. In the O'Higgins region, there is pre-Hispanic archaeological evidence of *Chenopodium* at Early Ceramic Period sites (400–1000 A.D.) (Planella and Tagle, 1998; Tagle and Planella, 2002). In the carbonized macroremains (diameters of 1.3–1.8 mm), it was impossible to view the diagnostic attributes of the perisperm, which is always translucent or crystalline in Coastal ecotypes (Bertero, 2007) but rarely so in Andean varieties, with some exceptions – e.g. the humid valleys of northwest Argentina. For this reason it is not possible to determine the original diameter of the fruit. Quinoa growing today in the coastal region of central Chile presents characteristics or attributes associated with archaic traits linked to wild varieties (Wilson, 1988). It is, therefore, argued that the crop has remained in an original area of domestication (Bertero, 2007). This author and colleagues, with new contributions in their interdisciplinary line of research on quinoa, reinforce the hypothesis that central-southern Chile was an independent centre of domestication, in addition to the central Andes (Bertero *et al.*, 2013), and they support the proposal made by Planella and Tagle (2004) concerning local anthropogenic manipulation of quinoa in central Chile.

The earliest evidence of *Chenopodium* sp. in the valley was found in starches recovered in a milling instrument at the Lenka Franulic site of early potters groups (200 B.C. – 200 A.D.) (Tykot et al., 2009). Other early sites with evidence of Chenopodium are El Mercurio (120–150 A.D., Phase I) in the valley (Planella et al., 2010), and Las Brisas 3 (38 B.C. – 224 A.D.) on the coast (Rivas and Gonzalez, 2008).

Morphological analysis of archaeological specimens of *Chenopodium* is not straightforward at central valley and coastal sites. During the Early Ceramic Period, diameters range between 0.8 and 1.8 mm (Planella and Tagle, 1998; Tagle and Planella, 2002; Quiroz and Belmar, 2004). Larger sizes are not observed until the Late Intermediate Period (1040–1450 A.D.), under the Aconcagua culture (diameter 1.5–2 mm) (Planella 2005). A significant change in seed size of *Chenopodium* sp. (likened to quinoa, given its equatorial band), is also seen: from the most ancient levels at the Early Ceramic Lonquén site (100 B.C. – 900 A.D.) to the Late Intermediate El Cebollar site (815–1075 A.D.) (Quiroz and Belmar, 2004). These data point to an escalation in human–plant relations, possibly leading to tests and domestication procedures of *Chenopodium*. Belmar and Quiroz (2004) also noted changes in average sizes at Diaguitas culture sites in the semi-arid north, Chalinga and Illapel valleys, for specimens dated 1210–1520 A.D., distinguishing between the pre-Inca smaller diameter and Diaguita-Inca. During the Late Period and with the Inca occupation in the central area (Garceau *et al.*, 2010; Rossen et al., 2010; Martinez, 2012), diameters of about 2 mm are observed in numerous samples of ubiquitous charred macroremains.

Rossen *et al.* (2010) analysed the implications of the presence of quinoa, together with other local crops at the fortified site of Cerro Grande de la Compañía, under pre-Inca and Inca occupation (1310–1480 A.D.). *C. quinoa* is present in various contexts, which accounts for its selective storage in qollqas (separated from maize) and its use in residential areas. Archaeological records of quinoa (and of maize) in pre-Inca regional sites increase with the introduction of new mechanisms for intensifying cultivation, and with its increased use in the diet and in the political-ceremonial sphere.

In the central Andean area of Chile, the sporadic presence of quinoa has only emerged in waste sites of human settlements. In contrast, in the coastal foothill valleys and "secano" lands, cultivation of the Coastal variety has long been a traditional activity, from the chronological depth indicated or even earlier (200 B.C. – 200 A.D), with quinoa one of a group of cultigens associated with maize, pumpkin, squash and bean (Planella and Tagle, 1998, 2004).

Peru (Figure 1 sector 4)

En In 1880, Wittmack and Rochebrune first reported the discovery of quinoa in archaeological excavations. They uncovered fruits, leaves and even *C. quinoa* flour in funerary contexts at Ancón (Hunziker, 1943). Early studies by Uhle (1919) and MacNeish (1969, in Lumbreras, 2003) in Ayacucho record seeds identified as domesticated quinoa at a very early date (5500–5000 B.C.). However, subsequent direct dating of the archaeobotanical material (e.g. beans) in the same context gave later dates, suggesting that it is unlikely that the quinoa found was as ancient as initially thought (Bowman *et al.*, 2005).

Dillehay *et al.* (2007) present evidence of *Chenopodium* at the Nanchoc Valley sites (northwestern Peru) (Figure 1, sector 4), which, given their association with a dated hearth, are placed chronologically between 5500–6000 B.C. The specimens are charred and dry and their identification as *C.*

quinoa cannot be confirmed due to the presence of grooves in the seed, distinguishing them from herbarium specimens.

Pearsall (1980, 1989) measured *Chenopodium* seeds from Pachamachay and Panaulauca Cuevas (around 3000 B.C.) in Junín – sites representing the shift from hunter-gatherers to farmers and herders – and suggested, on the basis of their size (0.75–1.00 mm), that these seeds were domesticated. Nordstrom (1990) examined seeds from Panalauca and from Pancan and confirmed that the seeds had thin and smooth testas, meaning that they were indeed domesticated. The seeds came from contexts dated 3000–700 B.C. Pearsall (2008) proposed that quinoa cultivation may have started in approximately 3000 B.C.

In the Andes, west of Lake Titicaca, Eisentraut (1998) studied archaeobotanical samples of Late Archaic–Early Formative (5000–1000 B.C.) sites at Quelcatani, and Formative (1500–800 B.C.) sites at Camata. Among several wild species, domesticated and wild quinoa (black quinoa) seeds were identified. Although some domesticated seeds came from a layer associated with the Late Archaic Period, direct dating of a quinoa seed indicated the Early Formative period (740 ± 50 B.C.). Furthermore, Murray (2005) identified *Chenopodium* grains at the Jiskairumoko site as from the Late Archaic Period (around 3400–2000 B.C.). However, direct dating indicated the Formative Period (Mark Aldenderfer, personal communication). Nevertheless, due to the presence of domesticated seeds at various Formative sites on the north coast (Rosen, 2010), the central Andes and Lake Titicaca basin, we can speculate that the domestication process began before 3000 B.C.

D'Altroy and Hastorf (1984) studied Inca storage structures (qollqas) in Mantaro Valley and revealed the presence of Chenopodium sp., considered as quinoa, together with other plant products and ceramic pots – an indication as to how seeds were stored during this time. In their study, the authors identified the different storage methods used (only maize, only quinoa, or all crops together: maize, quinoa, poroto or beans). In subsequent archaeological research spanning the Wanka periods (beginning around 1000 A.D.), Hastorf (1990, 2002), studied the organization of groups inhabiting the region and how they organized the extraction of resources in the Andes. After identifying *Chenopodium* sp. as possible quinoa in Mantaro Valley, Hastorf inferred its consumption and production with other crops. She assessed changes in settlement patterns associated with quinoa production, noting that production increased when settlements moved to regions at higher altitudes, and decreased in the other direction. She also noted that, unlike maize, quinoa does not reveal differences in consumption between the elite and the workers in society. Thus, Hastorf concludes that, while maize was the main focus of Inca production, other resources – depending on the productive areas – were equally important.

Conclusions

In this study of four distinct geographical and cultural areas in the Andes, both differences and convergences emerge in the search to understand the history of quinoa, its economic significance and its social, ritual and political implications.

Archaeological studies explain how wild species of *Chenopodium* were consumed by hunters and gatherers in the Archaic Period (8000–3000 B.C.) in Peru, Argentina and Chile. Although there is no direct evidence of their activities, these populations initiated the changes that led to the domestication of quinoa. While many gaps remain to be filled in order to determine when and where quinoa was domesticated, available data suggest that domestication occurred in the centre-south Andes before 3000 B.C. Indeed, domesticated seeds have been found in these countries dating back to this period, and direct radiocarbon dating places archaeological quinoa at around 2000 B.C. in the Andes of central Chile.

In addition to place of origin and/or domestication, each area had its own particular cultural niche where the acquisition and use of this pseudocereal took place. Once domesticated, quinoa became a subsistence crop for societies from the Formative Period through to the Inca Empire. There are quinoa remains in domestic settings, evidence that it was an everyday part of the diet, but also in contexts associated with rituals, funeral and politics, where it will have been consumed in community events. Although it appears that it lost ground to maize in political contexts in Tiwanaku (Goldstein, 2003) and Inka states, quinoa continued to play a significant role in the diets of communities in cold and arid regions.

Recent research, at local and regional level, in addition to the discovery and identification of varieties never before reported, opens up new perspectives for the exchange of knowledge and reassessment of the role of *Chenopodium* in feeding native peoples. Furthermore, these studies support the continued cultivation of quinoa and promote its increasing acceptance in Western diets.

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CHAPTER 1.4

THE DYNAMICS OF THE GLOBAL EXPANSION OF QUINOA GROWING IN VIEW OF ITS HIGH BIODIVERSITY

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Abstract

Quinoa (*Chenopodium quinua* Willd.) was first domesticated in Andean countries over 5 000 years ago. Following the Spanish conquest, quinoa was rejected and scorned as "Indian food". Its potential was rediscovered during the second half of the twentieth century and, since then, the number of countries growing quinoa has risen from 6 to 13, while 23 other countries are in the process of actively experimenting before launching field production in the near future. Another 20 countries are planning to plant quinoa for the first time in 2014. The organization of research has had a powerful impact, creating links and strategic partnerships between countries as is the case of the worldwide CIP/DANIDA programme in the 1990s or, more recently, with trials conducted by the European project SWUP-MED around the Mediterranean Sea. By networking researchers around the world, countries form partnerships based on affinities. One example is the United Kingdom which has established special contacts with India, Australia, China and Nepal. Today, experimentation centres are to be found in new countries that did not previously import quinoa. Although most publications of scientific findings are based on studies carried out in the Andean countries (Bolivia and Peru in particular), research is spreading around the world with studies conducted in new areas such as virology, dietetics, or quinoa processing for uses other than food. South American countries must now face global competition for the enhancement of quinoa varieties and must reflect upon possible competition between countries to access new markets. This is why some of them have already adopted the plant variety certificate (COV) system to protect their improved varieties, or they are in the process of applying for certificates. This paves the way for the conservation of plant genetic resources through recognition of local farmers' varieties and their use in future enhancement programmes.

Introduction

The genus *Chenopodium* (Chenopodiaceae) includes some 150 species which are mostly annual herbaceous plants occupying large areas of America, Asia and Europe, although some are also perennial and arborescent. The genus is cosmopolitan, meaning that it can adapt to any environment in the world, but it is concentrated in temperate and subtropical regions. Because of its great ecological plasticity and hardiness, the genus has given rise to a large number of species through a long process of adaptation and diversification in order to survive in environments with major biophysical constraints. As a result, most of its species are major constituents of arid and/or saline environments. Today, cultivated *Chenopodium*

– especially *C. quinoa* – are gaining importance due to the excellent quality of their proteins (good balance of all amino acids) and their high content of a variety of minerals and vitamins (Vegas-Gálvez *et al.*, 2010). Their potential contribution to global food security was recognized in the declaration of the International Year of Quinoa (IYQ, 2013) (Small, 2013). Furthermore, quinoa represents an alternative as a new crop in response to global changes (Jacobsen, 2003; National Academy of Sciences, 1975; Schlick and Bubenheim, 1996). For example, the increased salinization levels on farmlands due to intensified conventional farming since the 1960s leads to a decline in agricultural production, followed by the abandonment of degraded land depending on its location. Quinoa's tolerance to saline soils offers an alternative, not only in terms of recovery of these lands, but also by producing food of high nutritional value. In view of climate deterioration within the framework of global change, quinoa's resistance to drought raises expectations for those regions that are strongly impacted by these factors.

Domesticated by farmers in the Andean countries, scorned during the Spanish invasion and appreciated around the world today, the history of quinoa is both rich and complex. As for all domesticated crops, the history of quinoa and its diversity is directly linked to human activities (Bermejo and León, 1994). The last 60 years have seen major advances in quinoa expansion and experimentation, in its enhancement and adaptation to various environments around the world. This chapter seeks to explain how the quinoa cultivation area has spread from 6 to 56 countries and why today, in 2014, nearly 20 more countries wish to try.

Interest in quinoa (*Chenopodium quinoa* Willd.) goes back a long way and it should be recognized that quinoa is not the sole species of the genus *Chenopodium* – on the contrary, there are very close relationships between the various species of this genus. Current specificities tend to relate to its rapidly increasing spread worldwide since the 1970s as grain for consumers in the northern hemisphere, with the aim of introducing it as a new crop on all continents. The dynamics existing today affect the balance that had been established between producers and consumers from 1990 to 2010. Thus, the global spread of quinoa creates new outlooks for many countries, but at the same time profoundly disrupts the balance, as it is necessary to maintain sustainable production, while enabling Andean countries to cope with the upsurge in international demand (Bazile, 2014; Jacobsen, 2011, 2012; Winkel *et al.*). In addition, new relationships are being forged between countries – not only to trade grain, but also to establish rules and regulations on access to quinoa seeds. The current tensions concerning the flow of genetic resources and quinoa seeds necessitate international dialogue and global governance, so that countries can adjust to the environmental shift already underway and modify the agricultural model.

Although informal research networks in the past drew attention to quinoa growing, resulting in its experimentation in various parts of the world, greater transparency is now required to adapt activities to international legal regulations which acknowledge the Andean people's role in creating and maintaining quinoa biodiversity.

The conclusion of this chapter shows that, in facing the challenges of learning about the quinoa plant, its origin and evolutionary dynamics, its adaptation and enhancement, it is essential for farmers, researchers and policy-makers from around the world to exchange information, in order to be able to move forward, together with all those stakeholders seeking to capitalize on this plant.

Globalization of quinoa: a historical fact

It is important to make an in-depth examination of the ancient origins of the worldwide distribution of the genus *Chenopodium* and the diversity of its species, in order to properly understand the current development of cultivated quinoa. It is a historical fact that the use of *Chenopodium* leaves and seeds for human consumption is not exclusive to the Andean region. A species of Chenopodiaceae (classified as *Chenopodium album*) was cultivated in the Himalayas a long time ago at altitudes of 1 500–3 000 m asl (Hooker, 1885; 1952; Partap, 1982). When Stewart (1869) described the complete flora of the Punjab region in northern India, the presence of three groups of Chenopodium in the area studied was mentioned:

- *Chenopodium álbum* album L. was a common weed in the plains and also appeared at altitudes of 2 600–4 100 m asl in the Ladack region, where the plant was sometimes used as a "pot herb" or in soup.

- *Chenopodium murale* L. was present on the plains where it was also consumed as a pot herb.
- *Chenopodium sp.* belonged to a complex of two species (*C. album* and *C. quinoa*) grown in the Himalayan regions of Punjab, and more precisely at high altitudes (1 700–2 700 m asl) in the Ravi River basin, as well as higher up in Kashmir and Ladack. The plant was cultivated for its leaves and used as a pot herb, but these Chenopodium species were mainly grown for their grains, which were considered superior to buckwheat (Singh and Thomas, 1978).

Stewart's document is of considerable historical value in understanding the phylogenic relations caused by contact, in a certain period, between species of the genus *Chenopodium*. Moreover, since plant selection is guided by the intended uses, the same line of reasoning is adopted by peoples in both the Andean mountains and the Himalayas. Ethnobotanical studies by Partap and Kapoor (1985a) show that the group of *Chenopodium* grains used in the Himalayas was a minor subsistence food crop for many isolated hill communities in the middle Himalayan range. It has been consumed in various forms since time immemorial and its consumption is part of the people's eating habits in isolated hill communities. The authors describe it as a summer crop cultivated in mixed cropping systems (finger millet, rice, potatoes, maize and beans) (Partap and Kapoor, 1987b).

The analysis carried out by Partap and Kapoor (1985b) of the Himalayan Chenopodiaceae consumed as grains shows that they were considered domesticated forms of *Chenopodium album* L. Given the great morphological diversity, the authors selected four varieties recognized by local farmers to perform an agromorphological analysis (Partap and Upadhya, 1987b). Three of the four cultivars (black, brown and red) had a similar morphology and only their seed polymorphism differed. The findings produced sufficient evidence to be able to classify them as the domesticated *C. album* L. species. The type of polymorphism found in these cultivars is further indication of their close relationship with the non-domesticated form of *C. album* L. The authors presented the fourth cultivar as being very different from the others, expressing doubts about their close taxonomic relationships with C. *album* L. and *C. quinoa* Willd.

Nevertheless, Stewart's publication also bears witness quinoa's early role in globalization, given the international grain trade already existing at the time:

"Within the last year, considerable stir has been made by correspondents of the Agri-Horticultural Society of India, regarding the introduction into the Himalaya of the C. quinua *Willd. of the Andes; and the Society made arrangements to get a supply of seed, which has arrived and been distributed. The original proposition appears to have been made in ignorance the fact that a* C. *is cultivated extensively in the Himalaya, and there seems reason to doubt if very much would be gained from the introduction of the quinoa in these mountains, where cereals are cultivated to quite as high elevations as men can occupy throughout the year."* (Stewart, 1869)

The same *Chenopodium album* L. species prevalent throughout the geographical area delineated as Eurasia (Uotila, 1978) is now regarded as a European cosmopolitan weed (see Chapter 6.11) for cereals, although it was once a secondary crop and part of the human diet according to prehistoric human remains found in Tollund (Denmark) and Cheshire (United Kingdom) (Helbaek 1950, 1954, 1958, 1960; Rowley-Conwy, 1982, 2000; Rowley-Conwy and Stokes, 2002). Although there is evidence that *Chenopodium album* L. was an important crop in Europe –via a domesticated form that was also found in the Himalayas – researchers and enhancers have focused their efforts on Europe to adapt *Chenopodium quinoa* Willd., a tropical species (Galwey, 1989, 1993; Risi and Galwey, 1984, 1989, 1991; Jacobsen, 1997), to temperate climates. In plant breeding programmes, cultivation of this crop from the Andes highlands was considered suited to the relatively low temperatures of regions in northern Europe (e.g. United Kingdom and Denmark). This reasoning was based on the analysis of *C. album* as a wild species from which it would be difficult to obtain a crop. Today, this vision may be revised to make better use of the genetic resources available and the adaptability of *C. album.*

Furthermore, a similar species – *Chenopodium berlandieri* subsp. *Nuttaliae* – is consumed in Mexico. Considered a wild species in the United States of

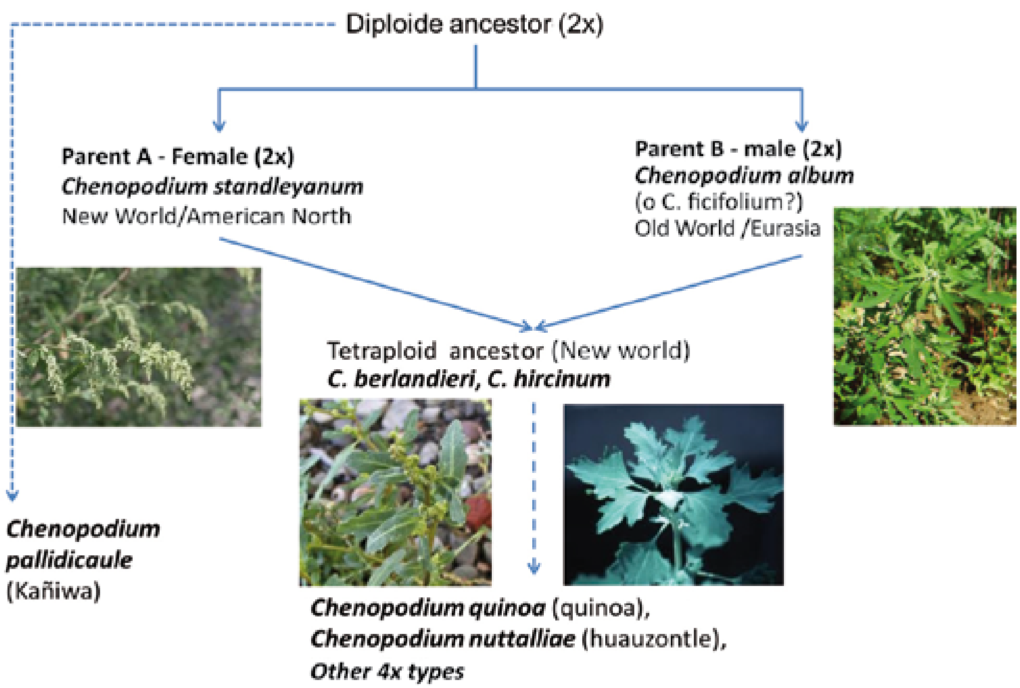

Figure 1. The origin of modern quinoa (*adapted from* Jellen and Maughan, 2013)

America, *C. berlandieri* is being studied for its potential for crossing with *C. quinoa* so as to withstand high temperatures. Without going into more detail about the entire genus, it may be noted that cultivated *Chenopodium* are becoming increasingly important. *Chenopodium quinoa*, which offers a wide adaptability to many harsh environments, as it is resistant to salt and drought tolerant, shares its food niche with two closely related species, cañihua (*Chenopodium pallidicaule*) and huazontle (*Chenopodium nuttaliae*), which are also used in human nutrition (Wilson and Heiser, 1979).

A study of quinoa phylogeny highlights various species of the genus Chenopodium, some of which are of economic importance:

- *Chenopodium quinua* (2n = 36) used as a grain crop;
- *Chenopodium pallidicaule* (2n = 18) and *Chenopodium berlandieri* subsp*. nuttalliae* (2n = 36) used for both grains and vegetables;
- *Chenopodium album* (2n = 18,36,54) used mainly as a leaf vegetable and forage crop;
- Some types from the Himalayas (*C. album* and *C. quinua*)) grown for their grains and leaves.

Chenopodium species are well known for their uses in cooking (see chapter 3.4), but there are also medical applications (see chapters 3.5 and 3.6).

There are four stages in the complex process of creating quinoa from its various wild ancestors (Heiser and Nelson, 1974; Nelson, 1968; Wilson, 1990), explaining not only its domestication, highlighting the key milestones in its history, and giving insight into the genetic aspects of its evolutionary dynamics (Pearsall, 1992). The first stage in the life of quinoa was when the two diploid ancestors hybridized to create the first form of wild quinoa. This is how a female relative, *Chenopodium standleyanum* from temperate America, was crossed with a male relative, *Chenopodium album* from Eurasia (another theory proposes *C. ficifolium*) through a natural hybridization process engendering its tetraploid ancestor in the New World (Figure 1). *C. berlandieri* and *C. hircinum* are tetraploid forms derived from the tetraploid ancestor enabling domestication of the ancestor of modern-day quinoa and generating the second stage of its evolution (Jellen and Maughan, 2013).

A first "bottleneck" in the genetic diversity of quinoa may have occurred when the two diploid ancestors hybridized to form wild quinoa. A second bottleneck may have occurred when quinoa was domesticated from tetraploid wild ancestors (Fuentes, Maughan and Jellen, 2009). This could explain quinoa's constant ability to be crossed with other tetraploid species (Wilson and Manhart, 1993) and to exist in multiple forms. The importance of this second bottleneck is directly contingent upon the first bottleneck. This implies the presence of a relatively small degree of genetic diversity suitable for sharing with compatible wild relatives across the board (Fuentes *et al.*, 2009).

Seed exchanges and circulation of quinoa in Latin America have generated five ecotypes associated with subcentres of diversity (Fuentes *et al.*, 2012). Nevertheless, this third stage of species diversification after local domestication around Lake Titicaca came to an end following the Spanish conquest for several reasons: loss of interest in the product which was viewed as "food for Indians"; the Catholic Church's rejection of its use as a drink in cultural ceremonies (Mudai); changes in dietary habits due to schooling; and new agricultural modernization policies adopted to impose the Spanish Crown's authority (Bazile and Negrete, 2009; Bazile and Thomet, 2013; Thomet and Bazile, 2013). The soaring demand for quinoa in the 1990s brought the fourth stage of its evolutionary dynamics and its current dissemination around the world (Bazile, Fuentes and Mujica, 2013).

Importance of quinoa biodiversity for its worldwide distribution

The ancient process of quinoa domestication was developed by leveraging the species' diverse genetic resources. They adapted to different geographical areas with specific environmental contexts, determining the overall survivability of quinoa, and creating multiple forms within the same species throughout the ages. Due to the special adaptations of quinoa in different zones in the Andes, five ecotypes are recognized: Inter-Andean valleys (Colombia, Ecuador and Peru); Altiplano (northern highlands in Peru and Bolivia); Yunga (Bolivia); Salare (salt flats or southern highlands in Bolivia, Chile and Argentina); and Coastal (coastal or sea level areas in central and southern Chile, extending to at least the Island of Chiloe) (Fuentes *et al*., 2012; Risi and Galwey, 1984).

Quinoa has soared remarkably since the 1980s due to increasing regional and international demand. It remains a staple product in Andean countries and is increasingly appreciated in North America and Europe for its dietary properties, organic farming and fair trade principles. To meet demand, production has more than doubled in Bolivia, the second largest producer after Peru, while Chile has taken initiatives to develop and capitalize on this marginal crop. Quinoa has also attracted the interest of researchers in Europe and North America for its nutritional characteristics and resistance to adverse factors, and there have been several attempts since the 1980s to introduce quinoa at high latitudes (Lopez-Garcia, 2007; NRC, 1989). The problem is understanding what can be grown in temperate environments? Early attempts systematically failed using materials from Peru and Bolivia (latitudes near Ecuador) which did not reach maturity during the summer at high latitudes. The requirements for temperate agriculture are present in the Coastal quinoa ecotype accessions from southern and central Chile.

Global recognition since 1973

The United States of America has shown interest in quinoa grain since 1948. A pioneering crop experiment using seeds from Chile was carried out in southern Colorado in the early 1970s (Johnson and Croissant, 1985). Although two Andean countries, Bolivia and Peru, currently account for most of global quinoa production, cultivation really has been spreading across all continents since the 1980s (Figures 1 and 2). The United States of America conducted quinoa experiments on a large scale for the first time in southern Colorado and then gradually extended trials to other states (Cranshow *et al.*, 1990; Kephart, Murray and Auld, 1990; Oelke *et al.*, 1990; Tobin, 1995). In Canada, quinoa is grown in the Saskatchewan and Ontario lowlands (traditionally occupied by grasslands or wheat cultivation), and it is estimated that the whole of North America accounts for nearly 10% of world quinoa production. In the United States of America, quinoa tests are currently been carried out on the Northwest Pacific coast using materials from Chile; results are very promising. However, although these developments appear significant at first sight, they are negligible when compared to the total volume actually sold in the United States of America and which is still imported from South America.

Quinoa was first introduced in Europe (see Chapter 6.11) in 1978 using Chilean germplasm (University of Concepción, Chile), which had been collected, selected and tested by Colin Leakey in Cambridge (United Kingdom) and in the Loire Valley (France). This Chilean germplasm – together with the Andean germplasm collected in 1982 by Galwey and Risi – laid the foundations for the breeding programme carried out at the University of Cambridge and directed by Nick Galwey (Fleming and Galwey, 1995; Galwey, 1989, 1993) (Figure 3). From Cambridge, quinoa then spread to Denmark, the Netherlands and other European countries (Risi and Galway, 1991). In the United Kingdom, quinoa is used as a cover crop and is planted separately or mixed with rapeseed. In Denmark, quinoa is widely recognized and used by people allergic to gluten and this could become a specific market segment.

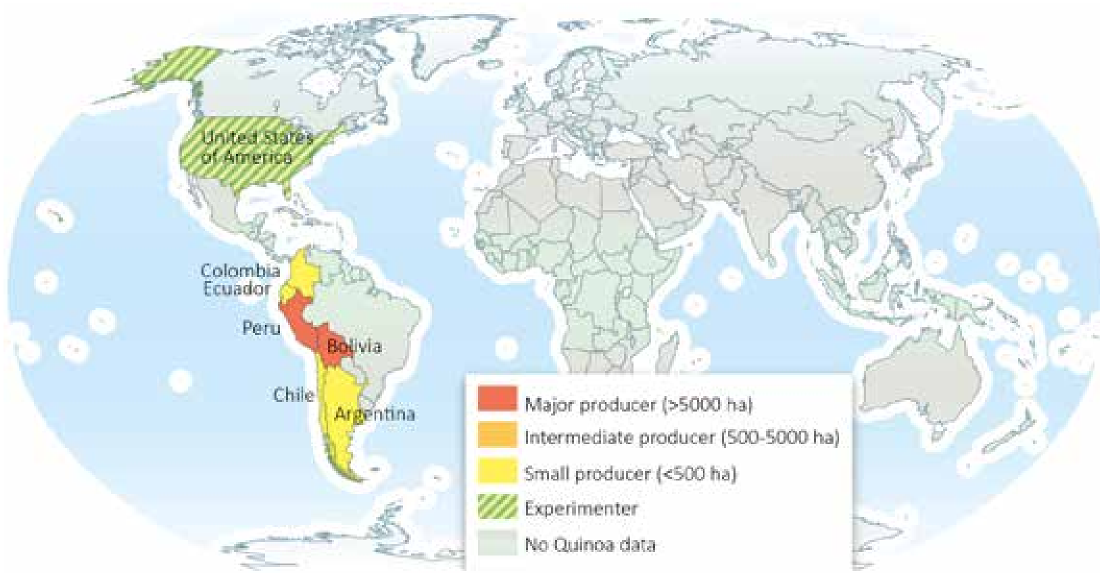

Figure 2. Quinoa worldwide development in 1973

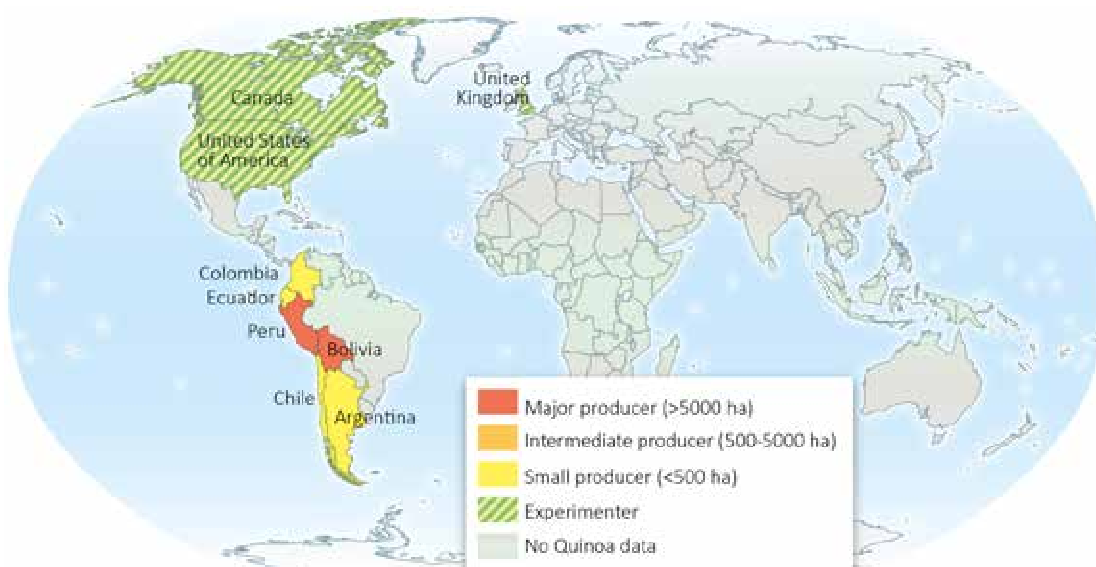

Figure 3. Quinoa worldwide development in 1983

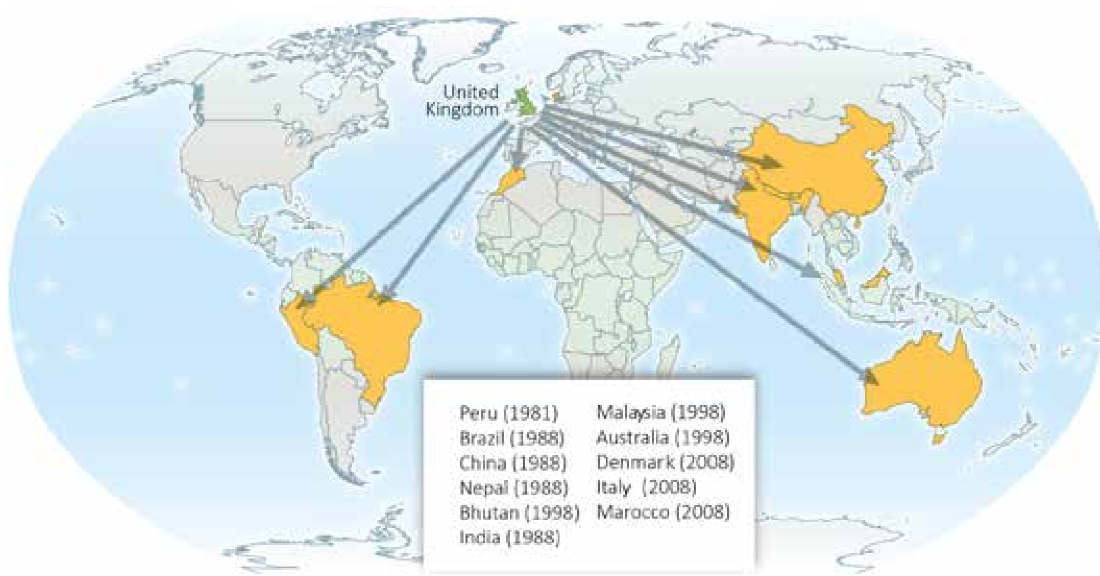

Figure 4. Collaboration with Cambridge, United Kingdom, to begin testing quinoa (since 1981)

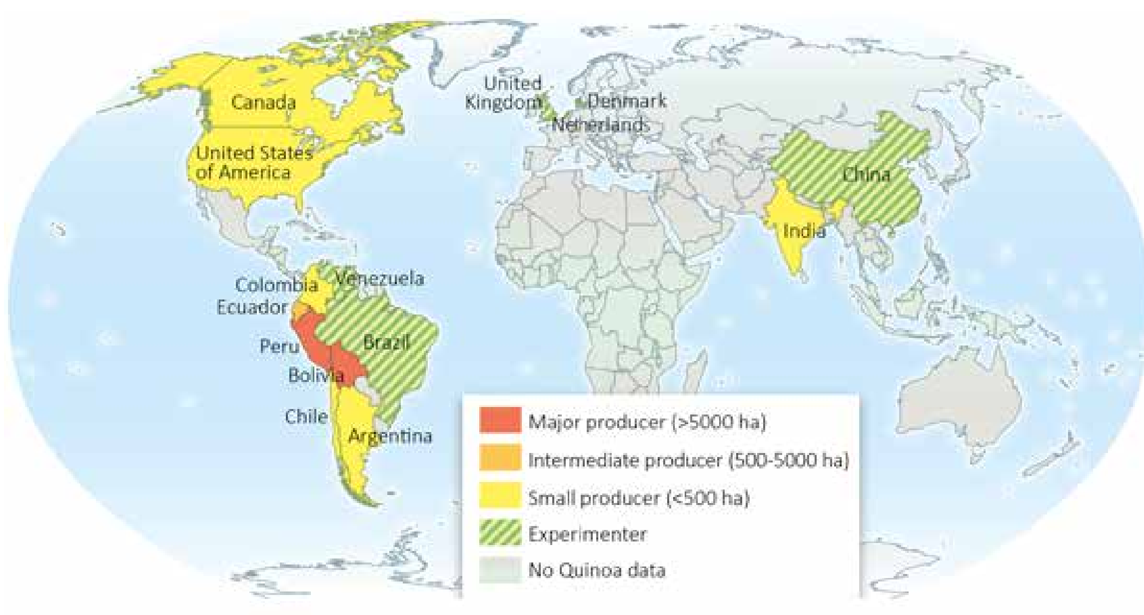

Figure 5. Quinoa worldwide development in 1993

Tests around the world in the 1990s and 2000s

From its point of entry in Europe, Cambridge (United Kingdom), quinoa then spread to Denmark, the Netherlands and many other countries (Gesinski, 2008; Jacobsen, 1997, 2003). During this period, experiments also began in Brazil and Asia (India and China) (Bhargava *et al.*, 2006) (Figure 4).

In 1993, the European Union launched a project with field trials in England (United Kingdom), Denmark, the Netherlands and Italy, as well as laboratory tests in Scotland (United Kingdom) and France (Figure 5). However, the project that began in 1996 as a joint venture between the Danish International Development Agency (DANIDA) and the International Potato Center (CIP) in Peru (Mujica *et al.*, 1998, 2001) was certainly the most important in the

Figure 6. Collaboration with CIP-DANIDA (FAO-Univ. Puno): American and European Test of Quinoa (1996-98)

Figure 7. Quinoa worldwide development in 2003

1990s and underlies the global expansion of quinoa. Through this first network of international cooperation to promote quinoa, field trials were set up in other countries such as Sweden, Poland, Czech Republic, Austria, Germany, Italy and Greece (Iliadis *et al.*, 1997). All these countries have expressed interest in quinoa experimentation and most of them participated in the American and European Test of Quinoa (Figure 6), organized by the Food and Agriculture Organization of the United Nations (FAO) and coordinated by the National University of the Altiplano (Puno, Peru), and the CIP-DANIDA project. The aim of this project was to learn the art of quinoa and perform multiple experiments at international level. This initiative strengthened the research network and increased the number of research centres working on quinoa in both developing and developed countries.

Denmark and the Netherlands have both since expressed their interest in breeding quinoa for various environments (Jacobsen *et al.*, 1994). They created the first European variety, 'Carmen', and research is now seeking to reduce the level of saponin on the basis of the sweet variety, 'Atlas'. The University of Copenhagen is also developing new quinoa tests as quinoa breeding becomes increasingly important (Figure 7). Other scientific collaborative efforts were fostered recently during the SWUP-MED project (2008–2012) "Sustainable water use securing food production in dry areas of the Mediterranean region". This project is the last major step in the spread of quinoa and brings together numerous partners from countries in the European Union (Italy, Portugal, United Kingdom, the Netherlands and Denmark) and in the Mediterranean (Turkey, Morocco, Egypt, the Syrian Arab Republic) (Benlhabib, 2006; Pulvento *et al.*, 2012) (Figure 8).

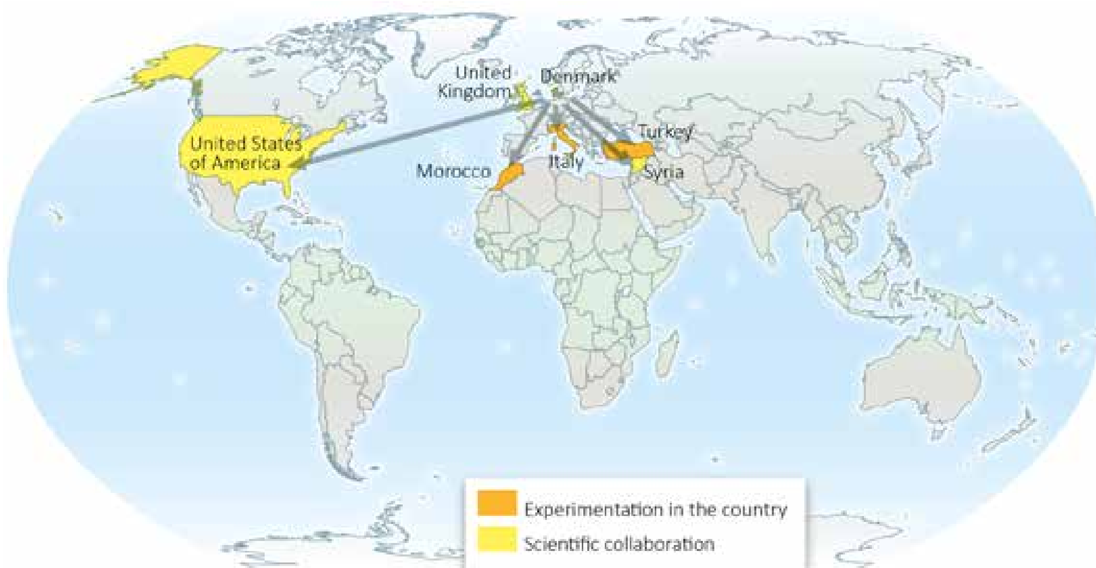

Figure 8. Collaboration with the University of Copenhagen to initiate quinoa testing in the SWUP-MED Project (UE: 2008–2012)

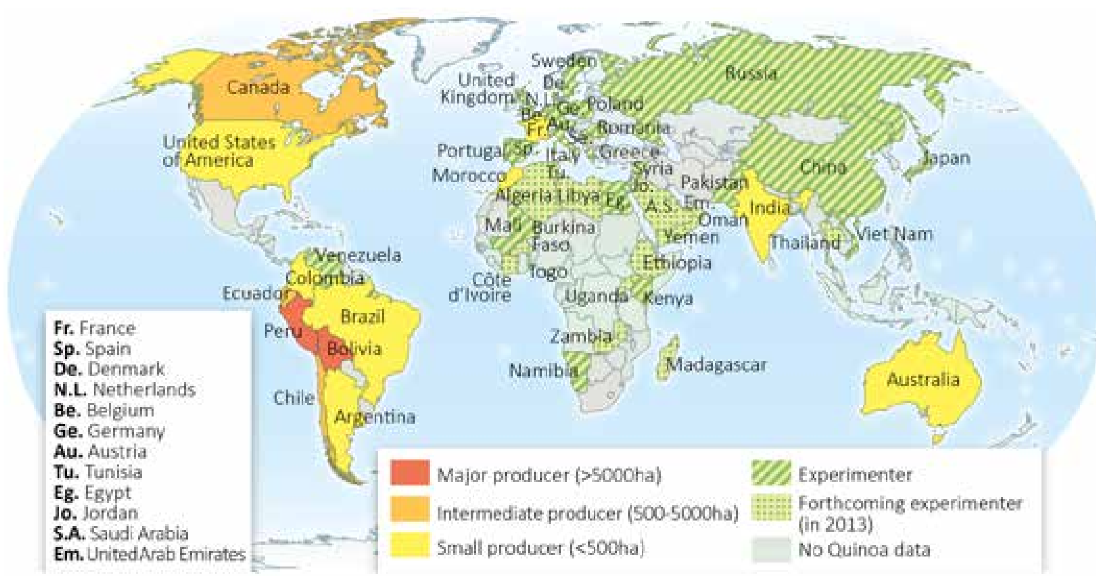

Figure 9. Quinoa worldwide development in 2013

Outlook since the International Year of Quinoa IYQ 2013

The early stages of expansion revealed interest among importing countries and consumers in adapting quinoa to their environments, for example, in the United States of America, Canada, France, the United Kingdom and the Netherlands. Another stage in the global spread of quinoa has begun in recent years as part of a response to global climate change and the salinization of agricultural land. Expansion has spread to the Asian continent in India (Barghava *et al.*, 2006), Pakistan (Munir, 2011) and China, followed by Australia and countries around the Mediterranean Sea and in North Africa.

We are now entering another phase of quinoa development and a turning point prompted by the fact that new producing countries are no longer consuming countries and/or traditional importers (Figure 9). The current wave of quinoa development is linked to the great adaptability of quinoa given its high genetic diversity, its resistance to drought and salt tolerance, its high nutritional value ensuring food security for local populations, and its ability to generate new sources of income for farmers.

The expansion of quinoa cultivation continues, with more than 20 countries on the lookout for seeds with which to experiment this year.

With each stage in the worldwide spread of quinoa, the number of research centres studying the crop and carrying out experiments has increased. International cooperation has generated many different projects, and research stations have been set up around the world, yet remain largely unknown because they were operational only during project implementation.

An analysis of scientific publications over the past 30 years highlights five subjects of particular importance to researchers (Bazile, 2013a):

- Nutrition and dietetics (gluten or saponins)
- Agronomy
- Botany and plant physiology
- Food biotechnology
- Biochemistry

There are very few publications dealing with policies, especially in consideration of the fact that the challenges to biodiversity conservation are increasingly entrusted to international laws governing access to and use and exchange of genetic resources and/or seeds.

The worldwide spread of quinoa is built on strong relationships between institutions that share their genetic material both formally, via legal provisions (Material Transfer Agreements – MTAs), or informally, via research networks. The largest collection of quinoa is still in the hands of the Andean countries (see Chapter 1.5). However, many countries have created their own collections: the red triangles on the map in Figure 10 show 19 non-Andean countries.

A significant number of countries have also developed new certified varieties and have set up a plant variety certificate system (COV in the UPOV system under the 1978 or 1991 Act). Most collections were established prior to the Convention on Biological Diversity in Rio de Janeiro (1992) which provides for the sovereign rights of states over their genetic resources. This means that these countries can develop new varieties with this germplasm without having to refer to the accession's country of origin (see Chapter 1.6). Certain quinoa-breeding countries have applied for plant variety certificates (Israel, Denmark, the United Kingdom, the Netherlands, Canada, Peru and Chile), but a new plant variety certificate is also being assessed at the request of Israel (Figure 11).

The Nagoya Protocol (adopted in Japan in 2010) is an international agreement that aims to share the benefits of using genetic resources in a fair and equitable manner, and to support the conservation of biological diversity and the sustainable use of its components. This begs the question as to how this is relevant to Andean countries in the case of quinoa.

Agriculture has always been based on access to and exchange of seeds, never on the exclusive principles seen today with property rights extended to cover living organisms. It is impossible to classify agrobiodiversity within a grid (private – public, individual – collective) on the basis of the number of interactions in connection with the circulation of seeds. Maintaining agricultural biodiversity requires active and continuous management. *In situ* conservation

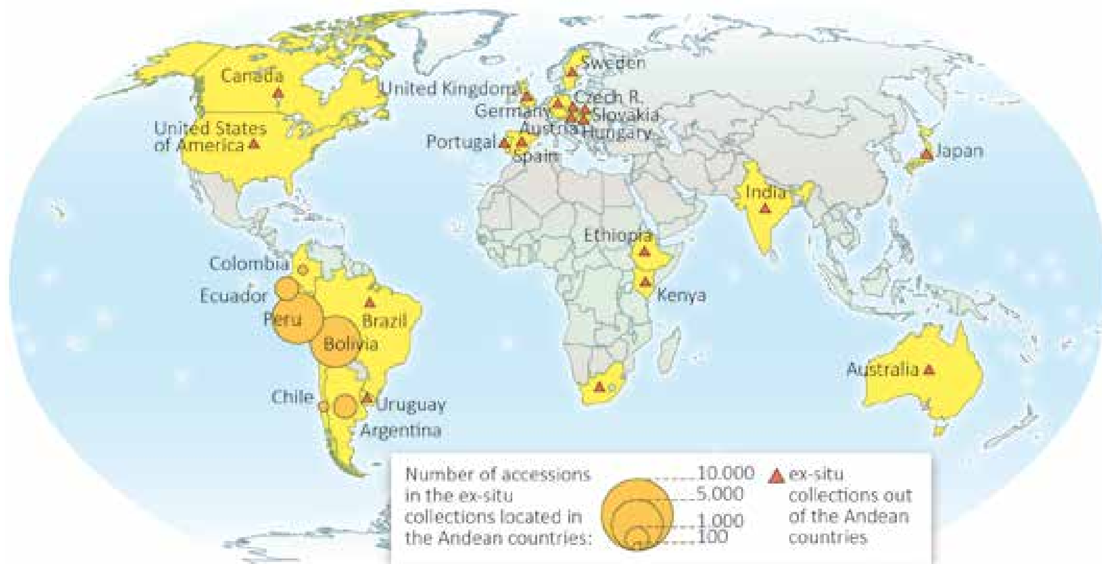

Figure 10. Worldwide Distribution of Quinoa Genetic Resources (*ex situ*) in 2013

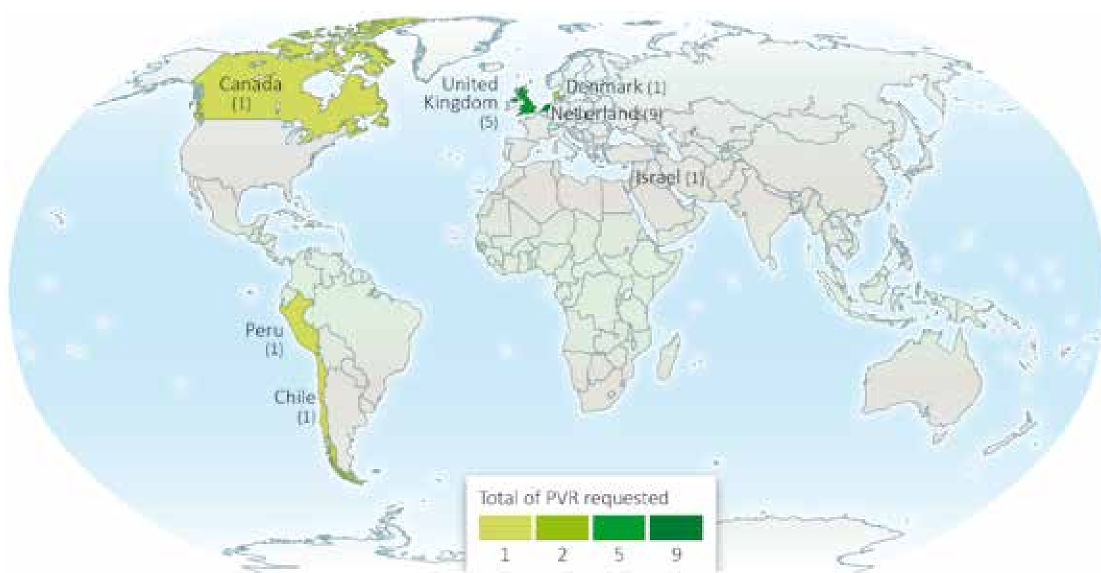

Figure 11. Number of quinoa varieties protected by plant variety certificates according to country of breeding in 2013

in farmers' fields encourages the co-evolution of peasant varieties of quinoa in response to the factors in that environment, generating a continuous momentum of quinoa biodiversity, with the species adapting to changes as they occur.

Conclusion

The wide genetic diversity of quinoa has made it possible to adapt cultivation to different types of soils, particularly saline soils and environments with extremely variable conditions in terms of humidity, altitude and temperature. This hardiness and adaptability is a major advantage in the context of climate change and salinization of agricultural land worldwide (Ruiz *et al.*, 2013). The spread of quinoa around the world is built on strong relationships between institutions sharing their genetic material.

However, the potential role of quinoa biodiversity in the world is based on farmer or peasant varieties that have been maintained via agro-ecological practices developed mainly through family farming (Altieri, 1992). The promotion of quinoa through enhanced varieties, standardized to comply with norms on seeds or to "simplify" farming practices linked to intensified conventional agriculture, will not generate the same resilience in response to the global change faced today. It is, therefore, necessary to maintain quinoa biodiversity – an assertion recognized and valued by organic farming (Bazile, 2014). The dynamics of the global expansion of quinoa cultivation may constitute a threat to farmers if generated with a narrow genetic base.

Thus, irrespective of the possibilities offered by the quinoa chain for the development of territories around the world, several questions arise with regard to the extension of cultivation outside the Andean countries, as promoted by the International Year of Quinoa (Bazile, 2013b). This minor crop may become widespread, but how can fair and equitable compensation (to use the terms of the Nagoya Protocol) be guaranteed for the selection process performed over generations by farmers in the Andean countries? Furthermore, how can this be achieved without prompting a decline in agrobiodiversity in the new producing countries?

We are now at the end of 2013 (International Year of Quinoa). Since the Rio Summit meeting in 1992, several international treaties have been signed on the management of plant genetic resources (CBD, Nagoya, UPOV, ITPGRFA, CAN, TLC etc.). There are many questions about and challenges for the future of quinoa. They need to be discussed in depth, involving all stakeholders and countries in the debate for the benefit of quinoa cultivation and of the farmers who earn their livelihood with quinoa.

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CHAPTER: 1.5

QUINOA GENETIC RESOURCES AND *EX SITU* CONSERVATION

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a potentially strategic crop that plays a vital role in food security and sovereignty. It makes an important contribution to the staple needs of the population and is part of the ancestral and cultural heritage of Andean countries. Its wide varietal diversity constitutes an extraordinarily valuable gene pool: wide range of colours of plant, inflorescence and seed; varying crop cycle duration; high nutrient and agro-industrial value; and high saponin content of grains. Thanks to its extraordinary genetic diversity, the crop is very adaptable to different agro-ecological conditions (soils, rainfall, temperature and altitude) and is tolerant to frost, drought and salinity. Worldwide, 16 422 accessions of quinoa and its wild relatives (*C. quinoa, C. album, C. berlandieri, C. hircinum, C. petiolare, C. murale* and *Chenopodium* sp.) are conserved in 59 genebanks distributed in 30 countries. Genebanks in the Andean region conserve more than 88% of the crop's accessions. Despite this immense diversity, it is not currently used to the full. The grain and processed products available on market are derived from a small set of landraces, which means that the genetic potential is underutilized. In general, countries do not have clear policies on the *ex situ* conservation of quinoa germplasm collections. Within countries with the greatest diversity, genebanks are poorly linked; and between different countries, the links are even worse.

Each genebank operates according to the goals of the institution, often reflecting individual interests of researchers rather than a strategy planned to complement the national programme. This chapter

reviews collections of quinoa germplasm in different countries, particularly in the Andean region, the distribution of its genetic variability and a description of the infrastructure and facilities used for its conservation. Information is also provided on characterization and evaluation, procedures for regeneration and multiplication and the documentation systems adopted. Lastly, the links between *in situ* and *ex situ* conservation are discussed.

Introduction

Over the past four decades, the number of *ex situ* germplasm collections has notably increased as a result of the worldwide effort to conserve PGRFA (plant genetic resources for food and agriculture) resources. These collections are maintained under very different conditions, depending on national or international policies, institutional environment, available expertise, facilities and budgets, and on the level of national and international cooperation (Engels and Visser, 2003). According to the Second Report on the State of PGRFA (FAO, 2010), the total quantity of samples stored ex situ throughout the world has increased by approximately 20% (1.4 million) since 1996, amounting to 7.4 million accessions stored in 1 750 genebanks. This increase in the number of accessions and diversity means that the highest international conservation standards must be adopted to handle the collections.

Setting up a genebank is no guarantee that a country's plant genetic resources will be conserved, or that the collections will be handled in accordance with proper conservation standards. These issues were highlighted in the first and the second report on the state of world's PGRFA (FAO, 1996; FAO, 2010). Genebanks are essential for the food security and sovereignty of a nation. They are part of a country's ancestral and cultural heritage are the responsibility of government and of society as a whole. Conservation therefore requires institutional support – the sustained provision of financial resources and availability of specialized staff with the equipment necessary to maintain germplasm collections and carry out conservation activities.

According to Engels and Visser (2003), increasing attention is being devoted to the regeneration of germplasm from a collection, given the possibility of genetic erosion over time when a bank is not properly managed. The maintenance and regeneration of collections thus involve rising costs. The economic management of a genebank involves the allocation of budgets to specific operations on the basis of an internal consensus regarding the costs involved and the genebank strategy.

The management of genebanks has often developed without proper planning. Furthermore, local germplasm management conditions vary enormously, resulting in a range of different management approaches and experiences. This is the case despite international efforts to standardize the management of genebanks, particularly for seed collections (FAO/IPGRI, 1994; Engel and Visser, 2003; Rao *et al*., 2007; FAO, 2013).

Quinoa genebanks and collections in the world

Quinoa seed has been classified as behaving in an "orthodox" manner (Ellis *et al.*, 1988). In other words, its viability can be maintained in a predictable manner within a controlled range of environmental conditions by reducing the seed temperature and moisture content (Ellis and Roberts, 1980). Ex situ conservation of quinoa is carried out in genebanks that use these seed properties to achieve the maximum storage time with minimum physiological activity and minimum loss of viability. Genebanks represent an efficient solution with a low cost-benefit ratio for quinoa seed conservation. A large quantity of seed samples may be stored in a relatively small space (Leon-Lobos *et al.*, 2010). Genebank management includes a series of stages and procedures that require staff trained in seed processing and regular checking of seed viability (FAO, 2013).

The FAO Second Report on the State of the World's PGRFA states that at international level there are 16 263 accessions of the genus *Chenopodium* (FAO, 2010), including quinoa (*Chenopodium quinoa* Willd.), qañiwa, qañawa or qañawi (*C. pallidicaule Aellen*), paico orepazote (*C. ambrosoides* L.) and other wild relatives of quinoa.

On the basis of recently updated information on *ex situ* collections of quinoa and its wild relatives, carried out with the support of FAO, Bioversity International and experts working with quinoa collections, it is estimated that the number of accessions of *Chenopodium quinoa* (see chapter 1.1), *C. album,*

Figure 1. Countries that conserve quinoa germplasm collections

C. berlandieri, C. hircinum, C. petiolare, C. murale and *Chenopodium* sp. conserved worldwide is 16 422 (Annex 1).

Thirty countries throughout the world conserve quinoa and its wild relatives in 59 genebanks (Figure 1). These are: 10 countries in the Americas (Argentina, Bolivia, Brazil, Canada, Colombia, Chile, Ecuador, the United States of America, Peru and Uruguay), 11 in Europe (Germany, Austria, Slovakia, Spain, Hungary, the Czech Republic, Portugal, the United Kingdom, Sweden, Turkey and Romania), 5 in Africa (Ethiopia, Kenya, Lesotho, Zambia and South Africa) and 3 in Asia (India, Japan and Jordan) and Australia (Annex 1).

Among the Andean countries, Bolivia and Peru are those that retain the greatest diversity, followed by Ecuador, Argentina and Chile. Among the remaining 25 countries in the world, Germany has 987 accessions, India 294, the United States of America 229 and Japan 191 accessions of quinoa and its wild relatives (Figure 2 and Annex 1).

Genebanks have been implemented in the Andean region since the mid-twentieth century. Management and conservation are preside over by agricultural institutions and universities, for example in Argentina, Bolivia, Colombia, Chile, Ecuador and Peru. Of the 16 422 accessions conserved worldwide, 14 502 (88%) are conserved in genebanks within the Andean region.

In **Bolivia** six genebanks conserve 6 721 quinoa accessions (Figure 3 and Annex 1). They are located in the *Centro Toralapa* (Toralapa Centre) run by INIAF (*Instituto Nacional de Innovación Agropecuaria y Forestal* – National Institute of Agricultural and Forestry Innovation), in the *Estación Experimental Choquenaira* (Choquenaira Experimental Station) run by UMSA (*Universidad Mayor de San Andrés* – Major University of San Andrés), in the *Centro de Investigaciónen Biotecnología y Recursos Fitogenéticos* (Biotechnology and Plant Genetic Resource Research Centre) run by UTO (*Universidad Técnica de Oruro* – Oruro Technical University), in the *Unidad Académica Tiahuanacu* (Tiahuanacu Educational Unit) run by UCB (Universidad Católica Boliviana – Bolivian Catholic University), in the *Centro Experimental Kallutaca* (Kallutaca Experimental Centre) run by UPEA (Universidad Pública de El Alto – El Alto Public University), and in the *Centro de Investigación y Promoción Comunal* (Municipal Research and Promotion Centre – CIPROCOM). The quinoa germplasm with the highest number of accessions managed by INIAF with 3 178 accessions is the National Quinoa Germplasm Collection; it is followed by the UTO and UMSA collections which have, respectively, 1 780 and 1 370 accessions (FAO WIEWS, 2013).

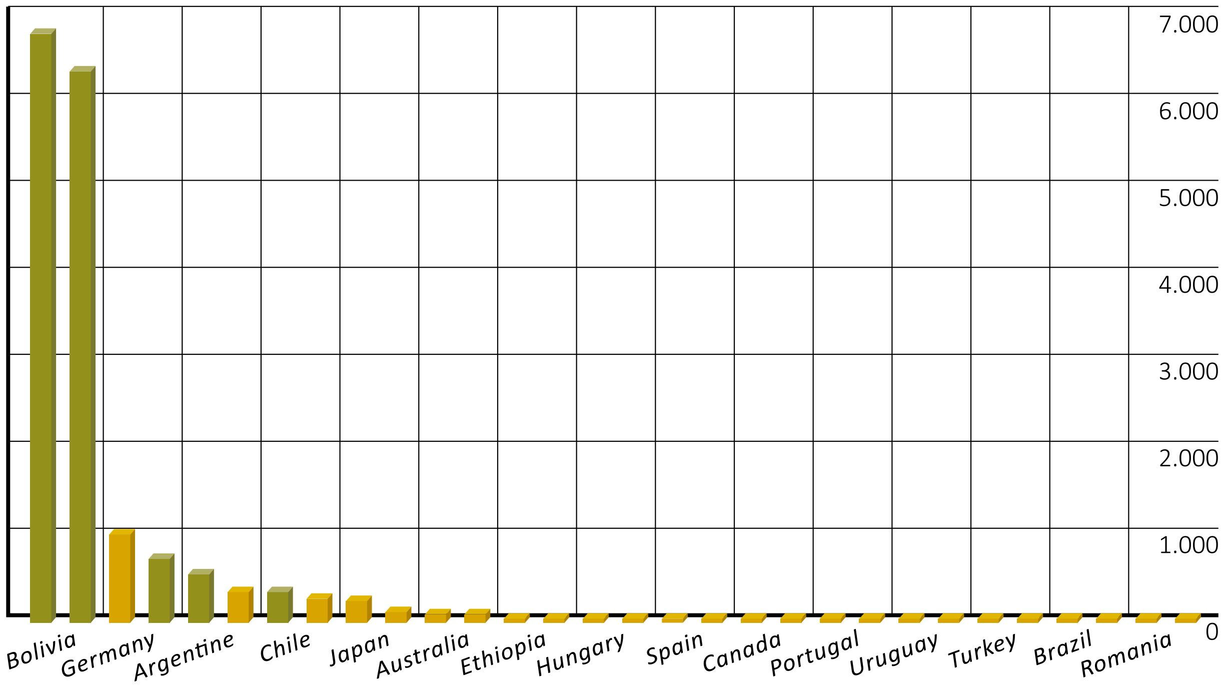

Figure 2. Number of quinoa accessions preserved throughout the world

In **Peru**, eight genebanks conserve 6 302 quinoa accessions (Annex 1). The genebanks are located in experimental stations run by INIA (*Instituto Nacional de Investigación Agropecuaria* - National Institute of Agricultural Research) in Illpa (Puno), Andenes (Cusco), Canaán (Ayacucho), Santa Ana (Huancayo) and Baños del Inca (Cajamarca), and in the *Universidad Agraria La Molina* (La Molina Agricultural University) of Lima, the *Universidad Nacional de San Antonio Abad* (San Antonio Abad National University) of Cusco and the *Universidad Nacional del Altiplano* (National University of the *Altiplano*) of Puno (Mujica, 1992; Bonifacio *et al*., 2004; Bravo and Catacora, 2010; Gómez and Eguiluz, 2011). The collections with the highest number of accessions are the *Universidad Nacional Agraria La Molina*, the *Universidad Nacional del Altiplano* and INIA in Puno with 2 089, 1 910 and 1 029 accessions, respectively (FAO WIEWS, 2013).

In **Argentina**, the national genebank conservation network holds a total of 492 quinoa accessions (Annex 1) conserved in the base genebank run by INTA (*Instituto Nacional de Investigaciones Agropecuaria* - National Institute of Agricultural Research) and partly duplicated in the active genebank of Northwest Argentina and the genebank of *Consulta* (Argentina MNII - *Mecanismo Nacional de Intercambio de Información* [National Information Exchange Mechanism], 2013; FAO WIEWS, 2013). This collection is the outcome of joint efforts by the Agricultural Faculty of UBA (*Universidad de Buenos Aires* - University of Buenos Aires) and INTA.

In **Ecuador**, 673 quinoa accessions are conserved by the National Department of Plant Genetic Resources and Biotechnology in the *Estación Experimental de Santa Catalina* (Santa Catalina experimental station) run by INIAP (*Instituto Nacional de Investigaciones Agropecuarias* - National Institute of Agricultural Research) (Ecuador MNII, 2013; FAO WIEWS, 2013; Peralta, 2006).

In **Colombia** the genebank run by the *Corporación Colombiana de Investigación Agropecuaria* (Colombian Agricultural Research Corporation) in Tibaitatá conserves 28 accessions (FAO WIEWS, 2013).

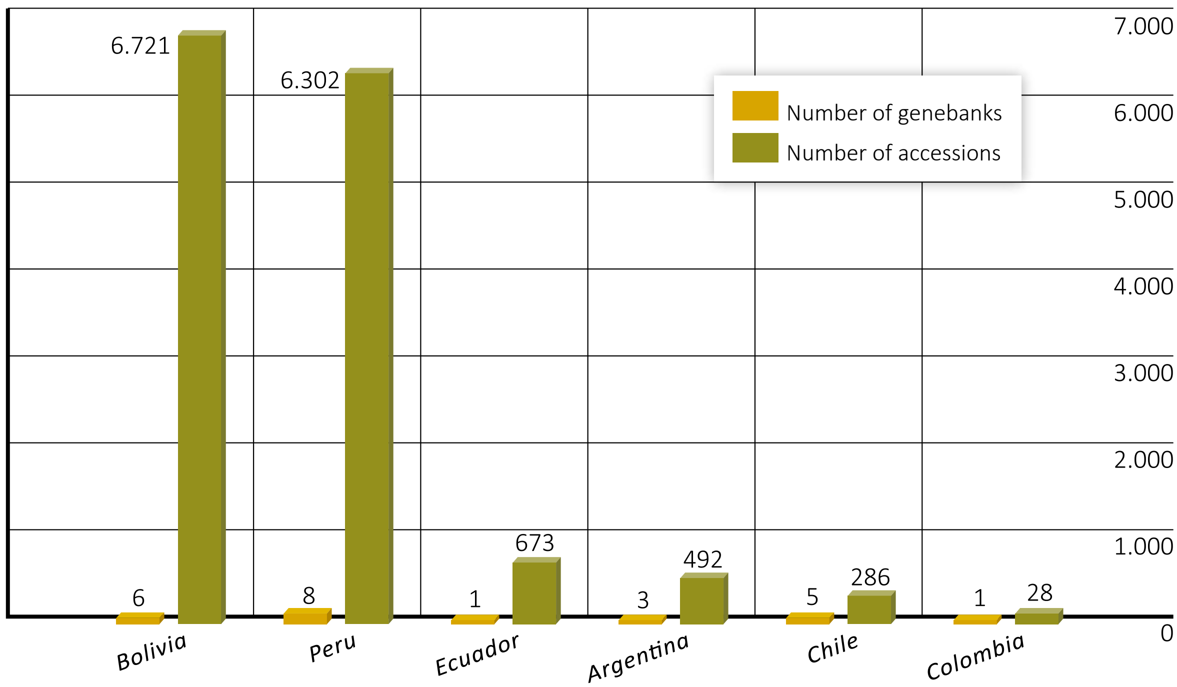

Figura 3. Number of accessions and genebanks conserving quinoa germplasm in countries of the Andean region

Of the 286 accessions conserved in **Chile** (Annex 1), 203 are stored in the base genebank of the *Centro Experimental Vicuña* - INIA (*Instituto de Investigaciones Agropecuarias* - Agricultural Research Institute), and the rest in the genebank of the Faculty of Agricultural Sciences of UACH (*Universidad Austral de Chile* - University of Southern Chile), in the active genebank of the *Centro Regional de Investigación Carillanca* (Carillanca Regional Research Centre) - INIA, in UNAP (*Universidad Arturo Prat* - Arturo Prat University), and in the Baer genebank (Barriga *et al.*, 1994; Salazar *et al.*, 2006; Madrid *et al.*, 2011; Chile MNII, 2013; FAO WIEWS, 2013). Figure 4 indicates the geographical location of the 26 genebanks in South America that store quinoa. Twenty-four of these banks belong to countries in the Andean region.

Distribution of the geographical origin of quinoa collections conserved *ex situ*

It is essential to have access to proper information on quinoa distribution, because it is considered a potential and staple resource for national and global food security. By analysing the (available) passport information held by the banks, it is possible to establish an approximate representation of crop distribution and determine the areas of influence of each one, establishing where more in-depth action is required.

According to studies carried out with the **Bolivia** national collection (Rojas, 2002; Rojas *et al.*, 2010), the geographical origin of the collection is distributed from 15°42'S (Omasuyos province, department of La Paz) to 21°57'S (M. Omiste province, department of Potosí), and from 64°19'W (Tomina province, department of Chuquisa to 69° 09' W (Manco Kapac province, department of La Paz). It is found at altitudes of and 2 400–4 200 m asl. (Figure 5).

The national quinoa collection in Bolivia houses a large number of accessions. A total of 3 178 are currently conserved, including cultivated and wild accessions collected between 1965 and 2008 in Altiplano communities and Inter-Andean valleys in the country in the departments of La Paz, Oruro, Potosí, Cochabamba, Chuquisaca and Tarija. The collection also includes germplasm from Peru, Ecuador, Colombia, Argentina, Chile, Mexico, the United States of America, Denmark, the Netherlands and the

Figure 4. Geographical location of the 26 genebanks in South America that store quinoa. Twenty-four of these banks belong to countries in the Andean region.

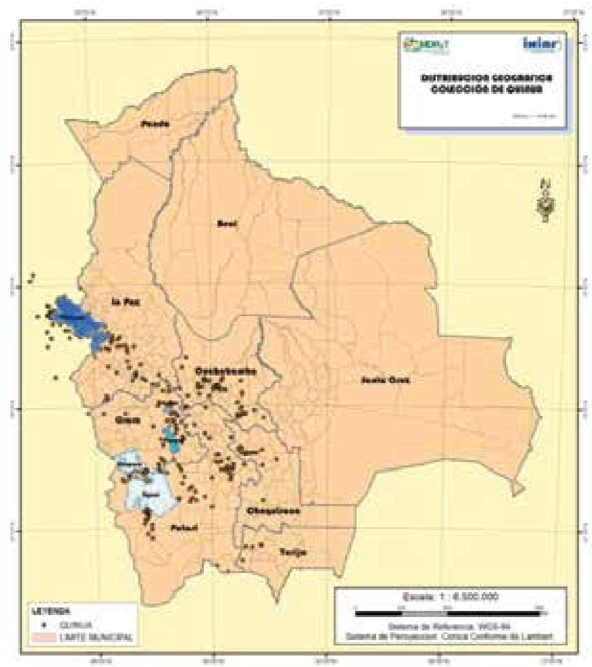

Figure 5. Distribution of quinoa germplasm conserved in INIAF, Bolivia

United Kingdom (Rojas *et al*., 2010a; Rojas *et al*., 2009).

Figure 5 shows that most accessions collected in Bolivia come from the Altiplano region, mainly in areas adjacent to the road that leads from Lake Titicaca, La Paz, Oruro, Challapata and Uyuni, in the case of the southern Altiplano, and also in the areas of Salinas de Garci Mendoza, Daniel Campos and Lipez. In the Inter-Andean valley region there is, on the other hand, a greater concentration of accessions from Cochabamba, Chuquisaca and Potosí than from Tarija.

In **Peru**, examination of the quinoa accessions stored in the seed collections of the *Universidad Nacional Agraria La Molina* and the *Universidad Nacional del Altiplano*, reveals that the distribution is mainly focused in the Inter-Andean valleys and mountains. Quinoa accessions have been collected from the Inter-Andean valleys at 2 200–3 500 m asl, mainly in the departments of Cajamarca, Ancash, Junín, Ayacucho, Huancavelica, Arequipa, Apurímac and Cusco. In the mountains, the accessions come from altitudes of 3 600–4 050 m asl, from the departments of Huancavelica, Arequipa, Apurímac, Cusco and Puno.

Of the 2 089 accessions held in the UNALM quinoa collection, 69.78% are from the department of Puno, 13.19% from the department of Cusco, 7.19% from the department of Apurímac and 6.28% from the department of Ancash. The four departments account for more than 96% of the total number of accessions stored in the university (Figure 6).

In **Chile**, quinoa accessions stored in the Intihuasi CRI (INIA Regional Centre) base genebank come from three main areas in the country (Figure 7). In the north, accessions are from the municipality of Colchane in the region of Tarapacá, and from the provinces of Elqui and Limarí in the region of Coquimbo. In the centre, accessions are mainly from the coast of the Libertador General Bernardo O'Higgins region. In the south, accessions are from the regions of Araucanía and Los Lagos (Madrid, 2011).

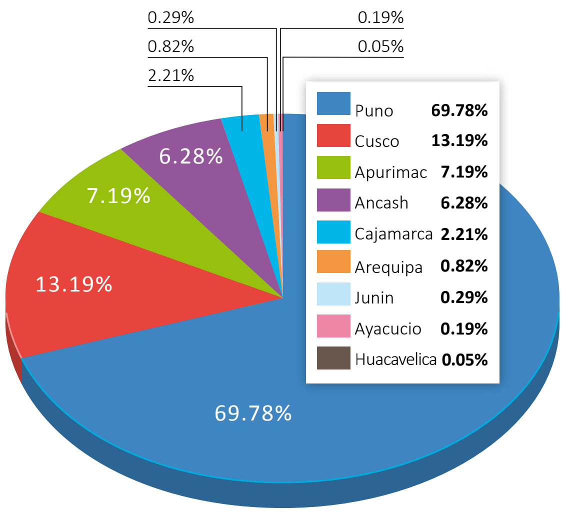

Figure 6. Distribution of quinoa germplasm stored in the *Universidad Nacional Agraria La Molina* of Peru by department.

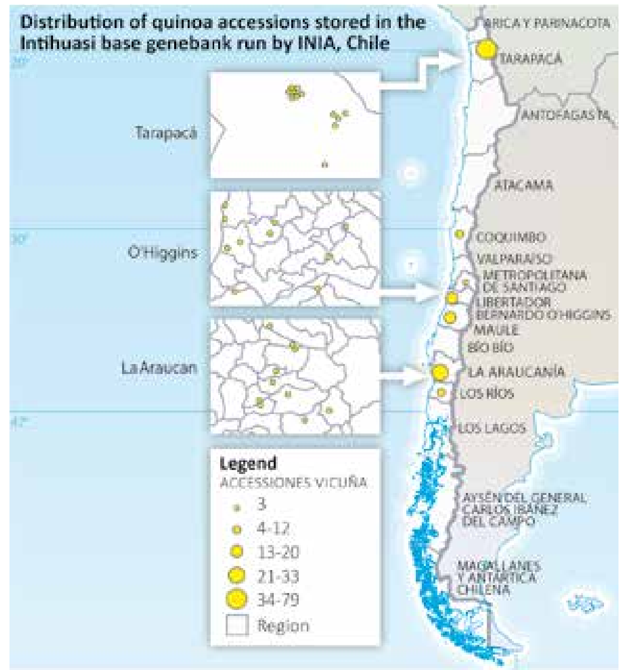

Figure 7. Distribution of quinoa accessions stored in the Intihuasi base genebank run by INIA, Chile (according to Madrid, 2011)

Characteristics of conservation infrastructure

The storage room equipment is vital for preventing the rapid decline of quinoa seed viability and a reduction in germination percentage. The location and characteristics of the storage facilities where accessions are conserved in Bolivia, Peru and Chile are described below.

In **Bolivia** the national collection of Andean grains is located in the Toralapa experimental station run by INIAF (17°31'S, 65°41'W; 3 430 m asl), 73 km from the city of Cochabamba, on the old road to Santa Cruz.

This genebank has a storage room, a laboratory and a sample conditioning room. The storage room measures 72 m², its walls are made of brick and windowless, they are fully lined with expanded polystyrene and the floor is ceramic. The average temperature in the storage room is 15°C and the humidity is 40%. A dehumidifier system is used to remove moisture from the room.

The conditioning room measures 20 m². Here, seeds are prepared for laboratory analysis and their size is checked. In the adjacent laboratory, which measures 16 m², the biological quality of seeds is analysed (germination, plant health, moisture content etc.) before the accession is placed in storage.

Due to the climatic characteristics of the genebank location and the conditions of the storage room, it is only possible to carry out short- and medium-term conservation under natural conditions.

This type of storage has been used since the beginning of the Bolivian quinoa collection. Plastic storage containers, 0.4–2 mm thick, with a double lid and 1 000 g capacity, are used. These containers are able to withstand temperatures of 8–20°C and relative humidity of 15–60%. They are well designed for short- to medium-term storage (IPGRI, 1996). Under these conditions, accessions may be stored and conserved for approximately 20 years, depending on the genetic material (Figure 8).

In order to implement long-term quinoa conservation in Bolivia, research began in 2002 to test the use of silica gel and Borax for drying seeds, but the results failed to achieve the moisture levels as

Figure 8. Storage room and laboratory for the processing and germination of seeds in the Andean grains genebank run by INIAF in Bolivia

recommended in the Genebank Standards (FAO/IPGRI, 1994). This was mainly due to the nature of the small-scale prototypes that were built for this purpose (Rojas and Camargo, 2002).

In the subsequent year of research, it became possible to establish a protocol for implementation of long-term storage (Rojas and Camargo, 2003), in accordance with international standards (FAO/IPGRI, 1994), and long-term conservation began with 247 quinoa accessions comprising the "core collection" (Rojas, 2010).

This work represents the first experience of long-term conservation with Bolivian quinoa germplasm. The samples are 5 g per accession and the seed moisture content is 3–7%. These samples vacuum-packed and hermetically sealed in aluminium pouches and conserved at 20°C. After 5 years of storage (2008), the first monitoring operation was carried out on long-term stored seed. The results were encouraging, because the germination percentage remained stable at 90–98% (an improvement on initial germination percentages).

In **Peru** the main genebanks storing quinoa contain areas and/or rooms prepared for conservation but without cooling equipment. The areas are generally kept closed; temperature and humidity are low, as is typical of the climatic conditions in places located at > 3 000 m asl. This means that the genetic material can be conserved naturally for long periods.

In the *Universidad Nacional Agraria La Molina* (La Molina National Agricultural University), the genebank is situated in two locations: one in San Lorenzo in the department of Junín, at 3 200 m asl (under natural conditions typical of the locality); and the other on the La Molina campus, where two cold chambers are available with a capacity of 19 m³ with dehumidifiers and temperature gauges. In this case, the accessions are stored at temperatures of 4–5°C and 60–70% relative humidity.

The genebank of the *Universidad Nacional del Altiplano de Puno* (Puno Altiplano National University) is located in the Camacani Research and Production Centre in Platería – Puno (15°56'41"S, 69°51'30"W; 3 824 m asl). The genebank run by INIA (Puno) is located in the Illpa Experimental Station (15°40'55''S, 70°04'29''W; 3 815 m asl) (Bravo *et al*., 2010).

The Puno INIA genebank offers short- and medium-term storage for the quinoa collection at room temperature (Bravo *et al*., 2010). On the UNALM campus, on the other hand, storage is short term in both cool chambers and naturally cooled areas, because the collections are active and continually added to and assessed. Plastic or glass containers are used to store the seeds in both banks.

In **Chile**, the quinoa collections are stored in four banks. The base genebank of the Vicuña Experimental Centre (INIA) is located in the region of Coquimbo. This contains a 330-m² storage chamber under controlled conditions that operates at – 18°C and

20% balanced relative humidity, and uses sealed containers. It has the capacity to store 50 000 seed samples. The Carillanca CRI active genebank (INIA – *Instituto de Investigaciones Agropecuarias* [Agricultural Research Institute]) is located in Temuco (region of Araucanía). It contains a storage chamber that operates at -5°C and 40–45% relative humidity, and uses sealed containers (Salazar *et al*., 2006; León-Lobos *et al*., 2012; Madrid *et al*., 2011).

The *Universidad Arturo Prat* genebank is located in Iquique (region of Tarapacá), where the seeds are stored at 4°C. The Baer genebank is located in *Fundo 'El Hualle'* ('El Hualle' estate) (region of Araucanía). The seeds are stored in a dark environment and at room temperature and humidity; this form of storage does not allow the seeds to be kept in a good condition for subsequent germination (Salazar *et al*., 2006; Madrid *et al*., 2011).

Progress in the characterization and evaluation of quinoa

Characterization and evaluation are employed to describe the qualitative and quantitative characteristics of accessions. On the basis of these characteristics, it is possible to differentiate and discriminate between accessions, determine their potential utility, build core collections and identify duplicates in the collection. The characteristics, combined with passport data, constitute essential information for each accession. On this basis, it is possible to establish regional, national and international databases, networks and platforms to share the information.

In **Bolivia**, the national quinoa germplasm collection has been in existence for over 40 years. During this time, characterization and evaluation have focused, in particular, on agromorphological analysis. In 1985, the first catalogue of quinoa conserved in the genebank was published by the Patacamaya Experimental Station (Espindola and Saravia, 1985). The second edition, which was published in 2001 (Rojas *et al*., 2001), described the genetic variability of 2 701 quinoa accessions through 59 qualitative and quantitative variables. Although the information was recorded on the basis of a "Quinoa descriptors", published in 1981 by IBPGR (now Bioversity International). The catalogue reports information on many more variables which have been identified in various papers published since the 1980s.

A new "Quinoa descriptors" was subsequently proposed, validated by researchers from Ecuador, Peru and Bolivia (Rojas *et al*., 2003). The document was revised by more than 50 experts from 40 organizations in 10 countries, and served as a basis for publishing an updated list of "Descriptors for quinoa and wild relatives" (Bioversity International *et al*., 2013). It should be emphasized that the wild relatives of quinoa were included in this revised version.

In 2001, work started on evaluating the nutritional value and agro-industrial variables. Information was recorded on 555 quinoa accessions with the aim of increasing their use in the production of quinoa-based processed products. Work was also carried out on the molecular characterization of most quinoa accessions (Veramendi *et al*., 2013). The most notable results are set out below, grouped on the basis of certain parameters and according to the number of accessions evaluated (Bioversity International *et al*., 2013; Rojas and Pinto, 2013).

Agromorphological variables

The morphological and agricultural variability of quinoa germplasm observed phenotypically during the crop cycle was studied in Bolivia. The parameters of some variables of interest are given below (Rojas, 2003; Rojas *et al*., 2009; Rojas and Pinto, 2013; Bioversity International *et al*., 2013).

Growth habit. Although branching and growth habit are influenced by sowing density, four different growth habits could be identified in the quinoa collection (Figure 9).

The architecture of quinoa plants is very variable – at both varietal and intrapopulation level. This hinders the adaptation and/or design of harvest mechanization prototypes and makes other cultivation work very labour-intensive. For this reason, it is important to work and select varieties taking into account the growth habit. For example "habit 1" (corresponding to plants that do not develop branches) and "habit 2" (with branches to the bottom third) could be very well suited to mechanized harvesting. "Habit 3" generally corresponds to plants of the Inter-Andean valleys, whose plant architecture makes them a possible alternative for use as forage while their genes could contribute to crop expansion areas in valleys and places with higher rainfall (Rojas and Pinto, 2013).

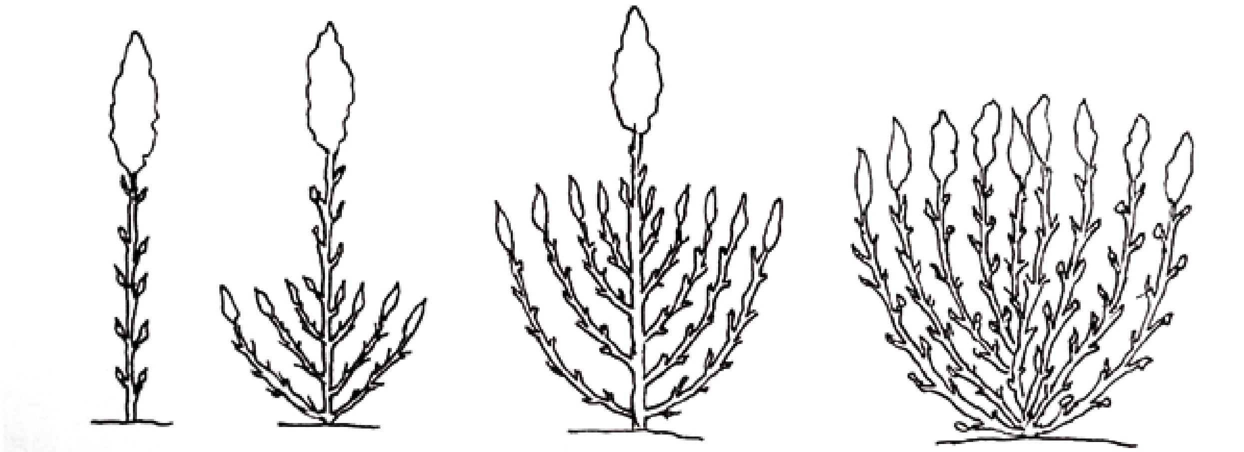

Figura 9. Quinoa growth habits: 1 Simple, 2 Branched to bottom third, 3 Branched to second third and 4 Branched with main panicle undefined (Rojas and Pinto, 2013; Bioversity International *et al*., 2013)

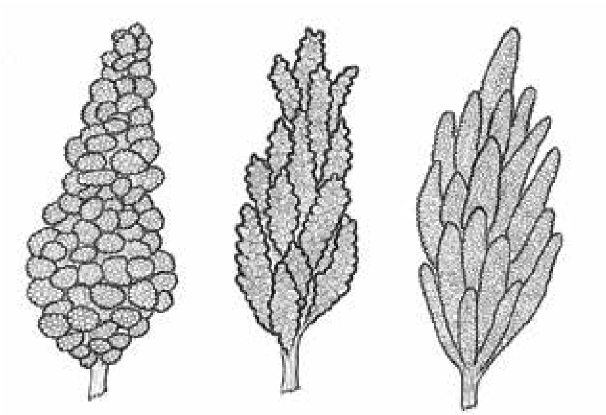

Figure 10. 1 Glomerulate, 2 Intermediate and 3 Amarantiform (Rojas and Pinto, 2013; Bioversity International et al., 2013)

Figure 11. Panicle shapes: amarantiform (left) and glomerulate (right)

Plant colour. Between the stages of "panicle emergence" and "start of flowering", four colours are expressed that are typical of the quinoa crop: green, purple, mixture and red. As the grain forms and physiological maturity is reached, the quinoa plants nevertheless display different colours and colour combinations: white, cream, yellow, orange, pink, red, purple, coffee, grey, black, mixtures and wild green.

Panicle shape and density. Three panicle shapes are observed: "amarantiform", when the glomerules are inserted directly in the secondary axis and have an elongated shape; "glomerulate", when the glomerules are inserted in the glomerulate axes and are globose in shape; and "intermediate", when the panicles express both amarantiform and glomerulate traits (Rojas and Pinto, 2013). The panicle may also be lax (loose) or compact – a characteristic determined by the length of the secondary axes and pedicels. It is compact when both are short (Figure 10).

Grain colour and shape. When quinoa grains reach physiological maturity, they display a wide range of colours, including: white, cream, yellow, orange, pink, red, purple, light coffee, dark coffee, greenish coffee and black. A total of 66 grain colours have been characterized in the Bolivian national quinoa collection (Cayoja, 1996).

There are four quinoa grain shapes (Figure 12). The cylindrical and lenticular shapes (determined by the appearance of the endosperm) of these grains

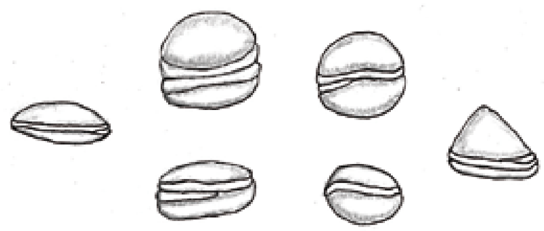

Figure 12. Quinoa grain shapes (from left to right) : 1 lenticular, 2 cylindrical, 3 ellipsoid and 4 conical (Rojas and Pinto, 2013; Bioversity International *et al*., 2013)

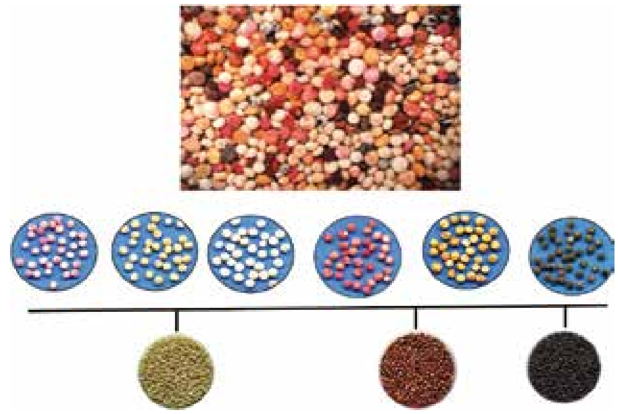

Figure 13. Diversity of quinoa grain shapes, sizes and colours compared with the three commercial colours

means that they can be satisfactorily used to make products that, due to their amylose and amylopectin content, can easily be used to produce custards, puddings and instant sauces. Similarly, depending on the starch grain size, they can also be used for the production of popped or puffed grains (Rojas and Pinto, 2013).

Figure 13 shows a wide diversity of quinoa grain shapes, sizes and colours. When the product is purchased in markets and fairs, however, consumers differentiate between three colours: white quinoa, coffee-coloured quinoa (known on the international market as "red quinoa") and black quinoa.

Quinoa grains are characterized by a particular feature: after desaponification, they assume three commercial colours. Mixtures of quinoa varieties are consumed and there is indirect underutilization of the crop's genetic potential. Quinoa consumption in both Andean countries and export countries corresponds to the raw material; farmers and companies normally mix a set of varieties in order to satisfy market demand in terms of volume.

Grain diameter. Grain diameter ranges from 1.36 to 2.66 mm; there is sufficient variability to imply it could be exploited through genetic improvement (Rojas, 2003). Small-grained quinoa varieties come mainly from the northern Altiplano and the Inter-Andean valleys, the large-grained accessions mainly originate in the Intersalare areas of Uyuni and Coipasa, corresponding to the southern Altiplano in Bolivia.

According to IBNORCA (2007), the quinoa grain may be classified into four categories according to its diameter: "extra large" (> 2.20 mm); "large" (1.75–2.20 mm); "medium" (1.35–1.75 mm); and "small" (< 1.35 mm). The "extra large" category includes 'Quinoa Real', whose main characteristic is the large size of its grains, making it very desirable on the international market. 'Quinoa Real' originates in Bolivia.

Its quality and reputation are exclusively due to the geographical environment in which it is produced, including the natural and human factors typical of the southern Altiplano (Rojas and Pinto, 2013).

Crop cycle. Some accessions reach physiological maturity within 119 days, while others take up to 220 days to mature (Table 1). This characteristic depends on the genotype. Quinoas of the Inter-Andean valleys are later than those of the Altiplano. The wide range of variation in the crop cycle is encouraging in terms of adapting the crop to variable weather conditions and climate change.

Grain yield per plant. Yields as high as 250 g per plant have been recorded. This variable also depends on the genotype and variables believed to contribute to yield, such as stem diameter, plant height, panicle length and diameter, and grain diameter.

Variables of nutritional and agro-industrial value

A summary of statistical parameters estimated for each characteristic of the nutritional and agro-industrial value of quinoa is given in Table 2. These are expressed on a dry basis (Rojas and Pinto, 2006; Rojas *et al*., 2007; Rojas and Pinto, 2008). The ac-

Table 1. Statistical parameters of central trend and dispersion for quantitative characteristics of Bolivian quinoa germplasm

Component	Minimum	Maximum	Mean	SD
Flower bud (days)	38	95	51.72	5.66
50% flowering (days)	60	145	93,5	12.04
Physiological maturity (days)	119	209	176.89	19.79
Harvest index	0.06	0.87	0.4	0.12
Stem diameter (mm)	10.16	26.26	17.12	2.66
Panicle length (cm)	15.4	62.8	37.41	8.09
Panicle diameter (cm)	2.86	19.42	6.85	1.66
Plant height (cm)	54	174.2	110,84	17.51
Grain diameter (mm)	1.36	2.66	1.96	0.23
100-g weight (g)	0.12	0.6	0.27	0.08
Saponin content (cc)	0	10.88	3.16	3.02

SD = Standard deviation; Source: Rojas (2003)

cessions show wide variability for most characteristics studied, which is a sign of the genetic potential of the quinoa germplasm.

The amount of protein ranges from 10.21% to 18.39% (Table 2). These values are wider than the range of 11.6–14.96% reported by Morón (1999), quoted by Jacobsen and Sherwood (2002). Although the quantity of protein is a basic aspect, the quality is specific and depends on the essential amino acid content. The quality of quinoa protein is higher than that of protein in cereals.

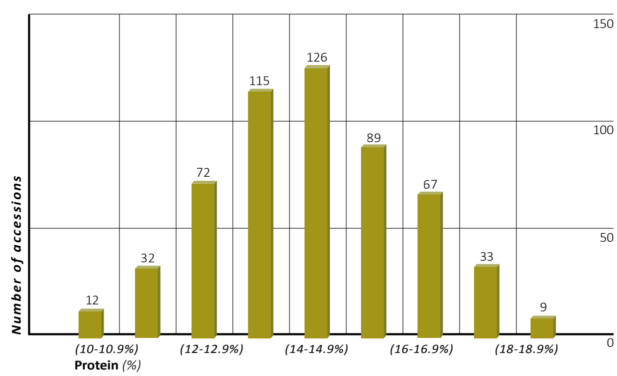

Figure 14. Variation in the protein content of 555 quinoa accessions

Figure 14 shows the distribution of protein content variation frequencies in part of the Bolivian quinoa collection. It can be seen that in most quinoa accessions, the protein content ranges from 12% to 16.9%, while in a small group of accessions (42), the content fluctuates between 17% and 18.9%. This latter group constitutes an important source of genes for promoting the development of products with high protein content.

In these accessions, the fat content ranges from 2.05% to 10.88% and averages 6.39% (Table 2). The upper range of these results is higher than the range of 1.8–9.3% described by βo (1991) and Morón (1999), quoted by Jacobsen and Sherwood (2002), who reported that the fat content of quinoa is high value due to the high percentage of unsaturated fatty acids. It is hoped that these quinoa values will be useful for obtaining fine vegetable oils for culinary and cosmetic use.

Genetic variation in starch granule size ranges from 1 to 28 µ. This variable makes it possible to provide agro-industrial guidelines for producing different mixtures with cereals and legumes in order to establish the functional character of quinoa. The starch granule needs to be small to facilitate the texturizing process. When the starch granule is small, it is easier to insufflate, as the spaces between the

Table 2. Characteristics of nutritional and agro-industrial value and simple statistics for Bolivian quinoa **germplasm** (n = 555 accessions)

Component	Minimum	Maximum	Mean	SD
Protein (%)	10.21	18.39	14.33	1.69
Fat (%)	2.05	10.88	6.46	1.05
Fibre (%)	3.46	9.68	7.01	1.19
Ash (%)	2.12	5.21	3.63	0.50
Carbohydrates (%)	52.31	72.98	58.96	3.40
Energy (kcal/100 g)	312.92	401.27	353.36	13.11
Starch grain (μ)*	1	28	4.47	3.25
Invert sugar (%)*	10	35	16.89	3.69
Fused water (%)*	16	66	28.92	7.34

Standard deviation; analysis performed by LAYSAA (Laboratorio de Análisis y Servicios de Asesoramiento en Alimentos Test Laboratory and Food Advisory Services), Cochabamba, Bolivia; *(n = 266) Source: Rojas and Pinto (2013)

granules allow for larger quantities of air to be introduced and exchanged, permitting a higher generation of air bubbles (Rojas *et al*., 2007).

The content of inverted sugars ranges from 10% to 35%. This variable expresses the quantity of sugar that initiates fermentation by unfolding or inversion; in other words, it can be used to determine the quality of carbohydrates. This parameter also permits quinoa to be classified as a food product appropriate for diabetics. The optimum percentage of inverted sugar is ≥ 25%. The accessions analysed comply with this requirement and can be used in mixtures with flour to produce bread, cereals etc. (provided all the external saponin is removed from the grain).

The variable “percentage of fused water” ranges from 16% to 66%. It measures the capacity of the starch to absorb water when making pasta, bread and other baked goods. The ideal value for this parameter for industrial application is ≥ 50%. In view of this characteristic, quinoa germplasm also constitutes an important source of genes for developing this product type.

Allowing for the concept of “genetic diversity” in the production of processed products will ensure that the genetic potential of quinoa is used in an appropriate manner. It is possible to select and obtain: varieties with higher protein percentages (≥ 18%) suitable for more attractive products; varieties with small starch granule diameters (≤ 3 μ) ideal for excellent, homogeneous popped/puffed grains; and varieties with stable percentages of amylase and amylopectin for the production of custard desserts, jellies, instant sauces and noodles. This immense range of ways to enjoy and use quinoa goes hand in hand with the conservation and use of genetic diversity.

Molecular characterization

In the Bolivian collection, between 2004 and 2008, it was possible to characterize 86% (2 701 accessions) of the germplasm, allowing the genetic fingerprinting of each quinoa accession. The information generated can therefore be used to group and differentiate accessions which are similar at molecular level. Seventeen microsatellite primers and ISSR markers were used for the typing. The polymorphic information content (PIC) for the quinoa collection showed values of between 0.73 and 0.95 with an average of 0.84; all the markers were found to be highly polymorphic (Veramendi *et al*., 2013). The microsatellites, QAAT074, QAAT076 and QAAT022, were found to be the most polymorphic and their values were higher than those reported by Mason *et al*. (2005) and Maughan *et al*. (2004).

In **Peru**, the 2 089 accessions in the *Universidad Nacional Agraria La Molina* were characterized and evaluated, using the IBPGR quinoa descriptors (1981). The groups of characteristics are described below:

Morphological variables

Table 3 shows the most significant morphological characteristics recorded in the UNALM quinoa collection, which allows all morphological characteristics of variants to be identified. The plant tissue colour chart prepared by the Royal Horticultural Society of the United Kingdom was used to record the colours. In the grain, colours were evaluated in the quinoa pericarp (fruit-coat) and episperm (seed-coat).

The variables evaluated include: flowering, corresponding to the number of days from seedling emergence from the soil to 50% of plants with the first flower; maturation, corresponding to the number of days from seedling emergence until 50% of plants have dry stems and hard pasty grains; plant height, measured from the soil surface to the apex of mature inflorescences and expressed in centimetres.

The evaluation generally showed a predominance of earlier, shorter accessions from the Altiplano (Puno) and a greater predominance of later, taller accessions in other representative locations of the Inter-Andean valleys (Gómez and Eguiluz, 2011). When accessions were grouped by geographical origin (Table 4), no clear pattern of differentiation was identified between locations, considering the descriptors of plant height and days to flowering and maturation. This could be the result of the exchange of accessions between experimental stations and farmers.

The scale proposed by Solveig and Ames (2000) was used to evaluate the reaction of the germplasm to mildew, which is the most important disease of quinoa, caused by the fungus *Peronospora variabilis*. Table 5 shows an overview of the reactions of accessions to mildew under conditions in Valle de Mantaro – Junín, considered an area with a high incidence of the disease. This collection did not show total or qualitative resistance to the fungus. A selection was therefore carried out for partial or

Table 3. Variation in the morphological characteristics of the quinoa collection held by *Universidad Nacional Agraria La Molina (UNALM)*

Morphological characteristics	Peru (UNALM) ***
Leaf colour before flowering	Green, purple, mixture, red
Colour of leaf axils	Green, purple, red, pink
Colour of stem striae	Yellow, green, purple, pink, red
Colour of inflorescence at physiological maturity	Greenish-yellow, yellow, yellow-orange, orange, orange-red, red, red-purple, purple, purple-violet, violet, violet-blue, white, grey-white, yellow-white, white-orange, grey-yellow, grey-orange, grey-red, grey-purple, grey-green, grey-brown, brown, grey, black
Shape of inflorescence	Amarantiform, glomerulate and intermediate
Density of inflorescence	Compact, intermediate, lax
Colour of pericarp - seeds (fruit-coat)	yellow, yellow-orange, orange, orange-red, red, red-purple, white, white-yellow, white-orange, grey-yellow, grey-orange, grey-red, grey-purple, grey-green, grey- brown, brown, grey, black
Colour of episperm-seeds (seed-coat)	yellow, yellow-orange, orange, red-purple, purple, white, white-yellow, white-orange, white-grey, grey-yellow, grey-orange, grey-purple, brown, black

Source: Gómez and Eguiluz (2011)

Table 4. Range of variation in the agronomic characteristics of plant height, days to flowering and grain maturation in the *Universidad Nacional Agraria La Molina* (UNALM) quinoa collection.

Locations	N° of accessions	Height (cm)	Flowering (days)	Maturation (days)
Ancash	131	90 – 240	70 - 115	170 - 215
Apurímac	140	125 – 240	58 -110	170 - 210
Arequipa	17	64 – 140	85 - 115	170 - 220
Ayacucho	4	89 – 126	60 - 65	160 - 180
Cajamarca	46	77 – 165	55 - 110	150 - 215
Cusco	275	52 – 176	50 - 115	140 - 200
Junín- Huancavelica	6	75 – 141	68 - 80	160 - 190
Puno 2	1434	36 – 185	50 - 98	115 -185
Puno 1	138	75 – 205	46 - 80	130 - 175

Source: Gómez and Eguiluz (2011)

Table 5. Range of variation in response to the presence of mildew (Peronospora variabilis) for 2 089 quinoa accessions under conditions in the Valle del Mantaro - Junín, Peru.

Departments	Number of accessions evaluated	Range of variation (%)	Number of accessions evaluated with partial resistance
Puno	1466	10 - 100	74
Cajamarca	39	40 - 90	0
Arequipa	17	80 – 90	0
Ancash	131	20 - 90	14
Junín	6	30 - 60	1
Huancavelica	1	30	1
Ayacucho	4	40- 60	0
Apurimac	150	20 - 90	18
Cusco	275	30 - 90	12
Total	2089	10 - 100	120

Source: Gómez and Eguiluz (2011) *Grain quality variables.*

quantitative resistance, which made it possible to identify some accessions worth including in the improvement programme based on percentage severity and reproductive development of the pathogen.

Of the 2 089 accessions from the UNALM germplasm collection, 953 were characterized according to grain size, protein and saponin content.

For the grain size, the accessions were graded on the basis of grain size using meshes containing perforations with diameters of 1.4 mm (small grains), 1.7 mm (medium-sized grains) and 2.2 mm (large grains).

The procedure used to evaluate saponin was developed on the basis of a proposal by Koziol (1990), modified by Balsamo (2002). Koziol (1990) established 0.11% (wet basis) as the threshold for the detection of bitterness caused by saponins in quinoa. Quinoa accessions containing less saponin may therefore be considered sweet (0.7 cm foam height), while very bitter quinoas exceed a foam height of 6.6 cm – the equivalent of 1.69% (dry basis) of saponin.

Table 6 shows the information generated on these three descriptors associated with quality. The quinoas were grouped into sweet (0) and bitter (1) on the basis of their saponin content (Gómez and Eguiluz, 2011).

The *Universidad Nacional del Altiplano* in Puno has characterized 1 029 accessions using eight phenotypic descriptors (stem colour, days to flowering, type of inflorescence, inflorescence colour and length, plant height, biomass and grain yield). Based on these characteristics, a "core collection" was built up comprising 103 accessions containing native ecotypes and varieties representing a large proportion of variation in the germplasm collection (Ortiz *et al*., 1988).

The Puno INIA characterized 536 quinoa accessions (68%) by applying the descriptors of plant colour, type of inflorescence, frost damage and grain yield. The results show that the plant colour (green, pink and purple) was observed in 149 accessions. The predominant inflorescence type is glomerulate with 380 accessions, 21 of which amarantiform and 135 intermediate. A total of 91 frost-tolerant accessions were identified. Grain yield varies widely (Bravo *et al*., 2010).

In **Chile**, there is currently an agronomic characterization for 28 accessions from the UNAP collection, using 11 morphological and productivity descriptors, (Fuentes and Bhargava, 2011). This characterization was carried out at low altitude, in the Canchines Experimental Station run by UNAP (20°26.562'S, 69°32.197'W; 1 005 m asl), near Iquique. The quinoa collection of the INIA base genebank, on the other hand, was regenerated and characterized in 2013 and the information is currently being processed (P. León-Lobos, unpublished data).

This involved evaluation of the genetic diversity of the 28 UNAP accessions plus 31 accessions from low altitude areas from different genebanks, using microsatellite markers (Fuentes *et al*., 2009). This study succeeded in detecting 150 alleles among the quinoa accessions evaluated, with an average of 7.5 alleles per locus. Based on an analysis of the main components, it was possible to separate the accessions into two separate groups: one containing accessions from the Chilean Altiplano (Salare ecotype); the other containing accessions from low altitude coastal areas (Coastal ecotype).

Procedures for regenerating and multiplying accessions

In general, even though the seeds are stored under optimum conditions, there is a decline over time in terms of quantity (due to use and distribution) and germination rate. According to Jaramillo and Baena (2000), aim of achieving an optimum seed sample size is known as "multiplication", while the aim of restoring viability is known as "regeneration or rejuvenation". This routine procedure is part of the process of managing a genebank: when accessions

Table 6. Occurrence of seeds germinated from INIAF quinoa accessions, Bolivia.

Locations	**Nº of accessions**	**Size** (diameter mm)	**Protein** (%)	**Saponin** (0=sweet 1= bitter)
Apurímac	145	1.2-1.7	10.3 -16.7	0 -1
Ayacucho	3	1.4	13.1-13.9	1
Cajamarca	12	1.4 -1.7	13.2-14.9	0 -1
Ancash	127	1.2 -2.2	10.3-16.5	0 -1
Cusco	133	1.4 -1.7	13.3 -18.6	0 -1
Junín	3	1.4	14.1-14.3	0 -1
Puno 1	138	1.4-1.7	7-24.4	0 -1
Puno 2 Bitter	220	1.4 -2.2	7.9 -23.7	1
Puno 2 Sweet	172	1.4 -1.7	7.1 - 23.2	0

fall below a threshold of quality (FAO, 2013) and quantity, they must be regenerated and multiplied.

In the INIAF collection in **Bolivia**, prior to regeneration, monitoring is carried out by means of germination tests to establish the seed germination rate, following the procedures established by ISTA (1993). The latest seed germination tests were carried out from 2010 to 2012 and involved 2 675 accessions. The aim was to monitor the behaviour of quinoa accessions and plan the germplasm regeneration on the basis of the results. In 2010, 200 accessions were analysed: for 31% of accessions, the germination rates were ≤ 80; for 69% the germination rates exceeded 80% (Table 7).

During 2011 and 2012, 2 475 accessions were analysed, and it was observed that the germination rates were ≤ 80 for 70.11% (in 2011) and 79.40% (in 2012) of the accessions; the germination rates were > 80% in 20.89% (2011) and 20.60% (2012) of accessions (Table 7). These results were used to plan the regeneration process, taking into account also the areas of origin of the accessions. As far as the seed quantity is concerned, it has been calculated that 60 g of quinoa is the minimum quantity that can be used as a parameter for the multiplication operation (Rojas and Bonifacio, 2001).

In the UNALM genebank in **Peru**, seed generation is carried out every 4–5 years. This period was calculated taking into account the effect of storage conditions on the viability of quinoa, which very easily loses its viability as a result of the climatic conditions under which it is stored.

The accessions are multiplied on the La Molina campus (located in mountainous conditions at

Table 7. Occurrence of seeds germinated from INIAF quinoa accessions, Bolivia.

		Seeds germinated					
Year	**Country**	**≤ 80 (%)**		**> 80 (%)**		**Total**	
		Accessions	**%**	**Accessions**	**%**	**Accessions**	**%**
2010	04 Bolivia	62	31.00	138	69.00	200	100.00
2011	02 Ecuador	5	62.50	3	37.50	8	100.00
	03 Peru	192	55.81	152	44.19	344	100.00
	04 Bolivia	617	75.89	196	24.11	813	100.00
	05 Chile	9	60.00	6	40.00	15	100.00
	06 Argentina	9	90.00	1	10.00	10	100.00
	07 Mexico	1	50.00	1	50.00	2	100.00
	11 No data	18	78.26	5	21.74	23	100.00
	Unidentified	3	100.00	0	0.00	3	100.00
	Total	**854**	**70.11**	**364**	**29.89**	**1218**	**100.00**
2012	02 Ecuador	8	61.54	5	38.46	13	100.00
	03 Peru	85	76.58	26	23.42	111	100.00
	04 Bolivia	891	79.84	225	20.16	1116	100.00
	05 Chile	1	100.00	0	0.00	1	100.00
	07 Mexico	1	50.00	1	50.00	2	100.00
	08 No data	2	100.00	0	0.00	2	100.00
	09 No datao	1	50.00	1	50.00	2	100.00
	10 No data	2	100.00	0	0.00	2	100.00
	Unidentified	7	87.50	1	12.50	8	100.00
	Total	**998**	**79.40**	**259**	**20.60**	**1257**	**100.00**
Total		**1914**	**71.55**	**761**	**28.45**	**2675**	**100.00**

3 200 m asl) in small groups to facilitate isolation. The main aims of multiplication are: to increase seed quantity for subsequent adaptation and yield studies in different locations; and to proceed further with quality studies, sometimes including destructive tests.

Care is taken to avoid genetic and physical contamination of accessions. Sowing for regeneration and/or multiplication is carried out in small, manageable groups that are interspersed with accessions of amaranth, corn, oats or rye; when necessary, the seeds are cultivated in complete isolation.

Documentation systems applied in the management of quinoa germplasm

The process of recording, organizing and analysing conservation data is known as documentation. It is essential for identifying the germplasm and making decisions with regards to its management. The value of the germplasm increases as more is known about it – hence the importance of ensuring it is well documented (Jaramillo and Baena, 2000).

The likelihood of accessions being used increases in direct proportion to the availability of information describing their characteristics and genetic potential. An accession cannot be identified as such if no information is available. For this reason, it is important to document the information in a systematic manner, including the maximum amount of possible detail.

In the INIAF quinoa collection in **Bolivia**, germplasm information is documented using one manual system and one electronic system. The data sets in which the quinoa germplasm information is organized are as follows: a) passport and collection data; b) characterization and evaluation data; and c) management data.

The electronic system is organized in different databases. In the pcGRIN system, provided by IPGRI (Hoogendijk and Franco, 1999), 2 701 quinoa accessions are documented with the following information: passport data, personnel data, geographical data, taxonomic data and characterization and evaluation data (Rojas and Quispe, 2001).

The information is organized using Microsoft Excel into double-entry tables; the database is interactive with an information flow structure, supported by pivot tables and menus for quick reference. Descriptive statistics of inventory, passport and viability data are generated, making it possible to make practical decisions (Figure 15).

Lastly, progress has been made with the DBGermo system, developed by INTA in Argentina. It organizes information on passport, characterization and evaluation data for the INIAF quinoa germplasm collection.

In **Peru**, the quinoa germplasm collection held by the *Universidad Nacional Agraria La Molina* has set up a database based on the quinoa passport and descriptive data published by IBPGR (1981). The program NTSYS Spc2.1 (Numerical Taxonomy System) is applied for the statistical analysis of information.

In **Chile**, institutions managing genebanks and working collections record the information manually and using computers (through the use of electronic aids such as Excel spreadsheets). INIA genebanks implement the Grin-Global database to curate their collections. INIA quinoa collection passport data are entered in this IT system and may be consulted online.

Experiences and links with *in situ* conservation work

The Andes is one of the most important mountain ranges in the world. In this ecoregion containing many special niches and a large number of plant associations, it has been possible for wild and cultivated quinoa to develop great genetic diversity. The plant is still found under natural conditions and growing as a crop in the fields of Andean farmers.

In the Andean region, it is possible to find agro-ecological areas housing quinoa with significant diversity and variability, displaying individual characteristics in terms of botanical, agronomic and crop adaptation traits. These areas have developed their own production systems based on the different individual agro-ecological conditions: Salare, Altiplano, Inter-Andean valleys, Coastal and Yunga (Lescano, 1989; Tapia, 1990; Rojas and Pinto, 2013).

In situ conservation is defined as the maintenance of crop genetic resources in their natural habitat and

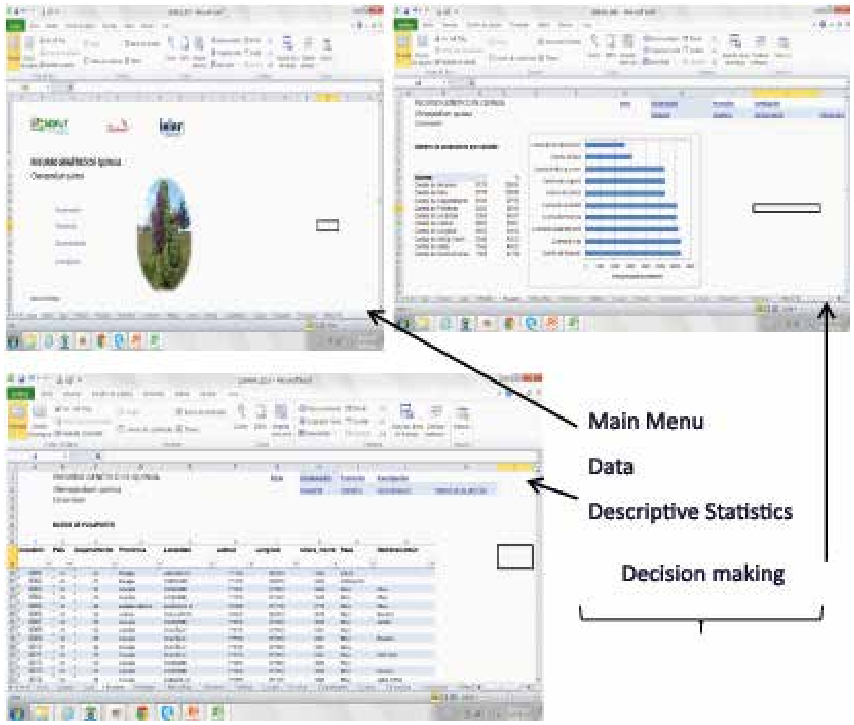

Figure 15. Microsoft Excel database of the INIAF quinoa collection, Bolivia

wild forms (Oldfield and Alcorn, 1987; Brush, 1991; Friis-Hansen, 1994). Traditional systems of cultivation – the *chacras* or farms – by means of which farmers traditionally conserve crop diversity, are also considered local spaces for *in situ* conservation of plant genetic resources for food and agriculture.

Traditional crop fields are a "mine of germplasm", where traditional varieties are maintained and where nature does its work of natural selection in conjunction with peasant farming traditions of seed propagation. Traditional knowledge is a key component of present-day agricultural biodiversity, and rural communities are responsible for its existence and evolution. Many factor, including knowledge of crops, use of food, associated culinary arts, agricultural management technologies and infrastructure, and local weather, are as important as the genetic resources themselves.

In this form of *in situ* conservation, farming families play an important role, with a number of interacting external and internal factors determining whether or not they will decide to continue planting a particular variety (landrace) and/or crop. These local dynamics occur in areas that are home to a wide diversity of crops and varieties, and where the ongoing management by families of the different local varieties will confer an evolutionary trend of adaptation to environmental, social and economic conditions by the planted materials.

While *ex situ* conservation is a model operating through genebanks that have been built up from biological material collected during prospecting operations conducted *in situ* and on farms, it is very unlikely that genebanks will contain the same material present *in situ* for various reasons (Wood and Lenne, 1997).

Local diversity is constantly evolving and accessions delivered to genebanks reflect a snapshot or image of a situation at a particular time or period. On the other hand, the same methodology has not always been applied when collecting samples from different quinoa-growing areas in widely varying geographical locations. As a result, genebanks do not fully reflect the variability present in a given region or country (Madrid *et al*., 2011).
The great genetic diversity of quinoa comes from wide geographical diversity backed by a variety of farming practices and systems (Bazile and Negrete, 2009; Fuentes *et al*., 2012). The perception and scale of this diversity must be considered in order

to appreciate the genetic diversity of quinoa and to support the maintenance of crop diversity for *in situ* conservation (Louafi *et al*., 2013), in particular through networks promoting and generating this biodiversity (Santonieri *et al*., 2011).

Support initiatives have been developed throughout the Andes to promote *in situ* conservation of quinoa. For example, in **Bolivia**, the first *in situ* conservation work with quinoa began in the area surrounding Lake Titicaca in 2002. It involved the study of varieties kept locally in traditional management systems. The results showed a reduction of up to 70% in locally conserved diversity compared with the diversity safeguarded in the genebank (Pinto *et al*., 2006; Pinto *et al*., 2007; Rojas *et al*., 2003b). Subsequently, preliminary findings from case studies showed that internal and external factors influenced families when deciding whether or not to continue planting quinoa varieties (Alanoca *et al*., 2004).

As part of the *ex situ–in situ* relationship, annual participative assessment studies have been performed using quinoa since 2003, including genebank material and local varieties. Seed diversity fairs were organized to promote the diversified use of quinoa (Pinto *et al*., 2010). Visits by farmers to genebanks were promoted, and genebank staff were encouraged to participate in various rural and urban fairs. In this way "community quinoa and cañahua genebanks" were set up within the framework of the National System of Genetic Resources for Food and Agriculture (SINARGEAA) and were implemented in the communities of Antarani, Patarani, Coromata Media and Rosapata near Lake Titicaca (Rojas *et al*., 2012).

In 2011, a network of "farmer custodians" was established, and "community genebanks" have since been implemented in eight communities (Cachilaya, Coromata Media, Antaquira, Pucamaya, Erbenkalla, Rosa Pata, Corqueamaya and Suruquiña) near Lake Titicaca as part of a strategy for the participative documentation and monitoring of agricultural biodiversity and traditional knowledge. This experience is conducted with an agricultural biodiversity approach and focuses its efforts on understanding and observing inter- and intraspecific diversity of crops useful for food, medicine and other applications. It also includes the development of a new method involving a red list for cultivated species (Padulosi *et al*., 2012).

The quinoa collection that INIAF is in charge of is linked to two microcentres of the area surrounding Lake Titicaca, located in the community of Cachilaya (province of Los Andes) and the community of Titijoni (province of Ingavi) in the northern Altiplano of La Paz (Figure 16). *In situ* conservation work is carried out in these microcentres, including monitoring and characterization of the genetic diversity of crops and varieties kept by families, taking into account local dynamics and interactions with the surroundings.

In situ conservation in **Peru** is mainly carried out

Figure 16. Microcentres: Titijoni (left) and Cachilaya (right), department of La Paz, Bolivia

in Puno, through the annual cultivation of quinoa in *Aynokas*or areas, where it shares space with its wild relatives. Traditional management practices ensure a food supply for family and community and effectively manage crop diversity, pests and diseases, thanks mainly to the adoption of a rotation system and the cultivation of the crop at different altitudes (Ichuta and Artiaga, 1986).

Mujica and Jacobsen (2000) reported the presence of systems where quinoa and its wild relatives are preserved under different names, such as *mandas* and *laymes*. Wild relatives are also found growing in isolation on the edges of fields or in places considered sacred (House of the Gentiles or *Phiru*). These species are prized by farmers as food (leaves consumed as a vegetable, or grain consumed roasted), for their medicinal value or for use in ancient rituals, especially in periods of climatic adversity.

Conclusions

Quinoa plant genetic resources are essential for food and nutrition security and sovereignty of peoples and they make a significant contribution to the basic needs of humanity. They are part of countries' ancestral and cultural heritage, especially the countries of the Andean region; their conservation and sustainable use are therefore the responsibility of society as a whole.

In Andean countries, policies for conservation of plant genetic resources are on the whole unclear. This is particularly the case for the *ex situ* conservation of quinoa germplasm collections. Bank activities are determined by the objectives or interests of the institution in charge and are often based on the individual interests of researchers. They should receive priority in budget allocation, because these resources must be handed down from one generation to the next as they have a vital role in supporting the very existence of the human species.

The genetic diversity of quinoa preserved *ex situ in different countries is relatively large considering the number of accessions in the collections and their ecogeographical origin.* More than 88% of this diversity is located in genebanks in the Andean region. Although this concentration could promote use of the resources, in reality, the extent of use of collections is inadequate and way below potential.

Despite the effort made, not all genebanks in the Andean region conserving quinoa have optimal storage conditions to ensure medium- and long-term preservation of germplasm. Technologies must be adopted to optimize the efficient and safe conservation of quinoa collections. It is important to rationalize the resources invested to maintain the collections and meet international standards for germplasm management.

Efforts must be made to develop or adapt protocols and procedures to optimize the management of quinoa collections. Management of the bank must also been streamlined: increasing the use of germplasm; creating links between genebanks; and making connections with potential users of conserved germplasm.

In general, the databases where the information generated by the banks is stored are off limits to bank staff, and there is no online access. This means that data are only circulated through technical reports, scientific publications and sometimes through germplasm catalogues. With the exception of INIA (Chile), there are no public Web sites with minimal information on the quinoa accessions conserved in genebanks.

There are a limited number of initiatives linking the activities of quinoa banks with *in situ* conservation work undertaken by farm families. It is important for *ex situ* and *in situ* work to be complementary, because the disadvantages of one are offset by the advantages of the other: the material preserved *in situ*, in particular, contains genes that are important for improvement.

It is necessary to develop protocols and/or lists of *in situ* descriptors to record agrobiodiversity managed in traditional farming systems, and to involve members of the community in carrying out this work in conjunction with local stakeholders, such as municipalities and other organizations.

In Bolivia INIAF is spearheading the drive to establish a national genetic resources system, with the participation of the various stakeholders in the country working with *ex situ* and *in situ* conservation, including farmers' organizations.

Annex 1. Details of countries and institutions in the world which maintain *ex situ* collections of quinoa (*Chenopodium quinoa, C. album, C. berlandieri, C. hircinum, C. petiolare, C. murale* and *Chenopodium* sp.).

N°	Countries	N° total of accessions	Code WIEWS	Institution	Acronym	Accessions by Institution
1	Bolivia	6721	BOL138	Instituto Nacional de Innovación Agropecuaria y Forestal - INIAF	BNGA	3178
			BOL094	Facultad de Agronomía UMSA	FA-UMSA	1370*
			BOL100	Facultad de Ciencias Agrícolas, Pecuarias y Veterinaria	FCAP-UTO	1780
			BOL318	Unidad Académica Campesina Tiahuanacu – Universidad Católica Boliviana San Pablo	UACT-UCB	257*
			BOL319	Carrera de Ingeniería Agronómica – Universidad Pública de El Alto	CIA-UPEA	136*
			BOL107	Centro de Investigación y Producción Comunal IRPANI	CIPROCOMI	262
2	Peru	6302	PER859	Estación Experimental Agraria Illpa, Banco Base Quinua	INIA-BB Quinua	1910
			PER014	Estación Experimental Agraria Illpa	INIA-EEA.ILL	789
			PER030	Estación Experimental Agraria Andenes	INIA-EEA.A	700
			PER012	Estación Experimental Agraria Baños del Inca	INIA-EEA.BI.	235
			PER041	Estación Experimental Canaán	INIA-EEC	123
			PER029	Estación Experimental Agraria Santa Ana	INIA-EEA.SA.	63
			PER002	Universidad Nacional Agraria La Molina	UNALM	2089
			PER007	Universidad Nacional del Altiplano	UNA	1873
			PER027	Universidad Nacional San Antonio Abad del Cusco	UNSAAC/CICA	430
3	Ecuador	673	ECU023	Departamento Nacional de Recursos Fitogenéticos y Biotecnología	DENAREF	673
4	Argentina	492	ARG1191; ARG1342	Facultad de Agronomía, Universidad de Buenos Aires; Banco Base de Germoplasma, Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuaria	UBA-FA; BBC-INTA	492
			ARG1349	Banco Activo de Germoplasma del Noroeste Argentino (NOA)	BGNOA	40
			ARG1350	Banco Activo de Germoplasma de La Consulta	BGLACONSULTA	15
5	Chile	286	CHL028	Banco Base INIA Inihuasi	INIA INTIH	203
			CHL004	Centro Regional de Investigación INIA Carillanca	INIA CARI	84
			CHL003	Facultad de Ciencias Agrarias Universidad Austral de Chile	IPSV - UACH	15
			CHL142	Universidad Arturo Prat de Iquique	UNAP	31
			CHL006	Ingrid Van Baer de Temuco		91
6	Colombia	28	COL029	Centro de Investigación de La Selva, Corporación Colombiana de Investigación	CORPOICA	28
7	Germany	987	DEU146	Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research	IPK	984
			DEU109	Greenhouse for Tropical Crops, Institute for Production and Nutrition of World Crops, Kassel University	GHK	3
8	India	294	IND001	National Bureau of Plant Genetic Resources	NBPGR	193
			IND032	Regional Station Shimla, NBPGR	NBPGR	98
			IND414	CSK HP Krishi Vishvavidyalaya, Palampur	CSK HPKV	3

N°	Countries	N° total of accessions	Code WIEWS	Institution	Acronym	Accessions by Institution
9	USA	229	USA020	North Central Regional Plant Introduction Station, USDA-ARS, NCRPIS	NC7	229
10	Japan	191	JPN003	Department of Genetic Resources I, National Institute of Agrobiological Sciences	NIAS	191
11	United Kingdom	65	GBR016	Genetic Resources Unit, Institute of Biological, Environmental & Rural Sciences, Aberystwyth University	IBERS-GRU	23
			GBR004	Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place	RBG	42
12	Australia	36	AUS048	Australian Tropical Crops & Forages Genetic Resources Centre	ATCFC	27
			AUS006	Australian Medicago Genetic Resources Centre, South Australian Research and Development Institute	AMGRC	9
13	Ethiopia	20	ETH013	International Livestock Research Institute	ILRI-Ethiopia	20
14	South Africa	19	ZAF001	Division of Plant and Seed Control, Department of Agriculture, Technical Service	PREPSC	5
			ZAF064	RSA Plant Genetic Resources Centre	PGRC	14
15	Hungary	17	HUN003	Institute for Agrobotany	RCA	17
16	Slovakia	15	SVK001	Plant Production Research Center Piestany	SVKPIEST	15
17	Spain	9	ESP003	Comunidad de Madrid. Universidad Politécnica de Madrid. Escuela Técnica Superior de Ingenieros Agrónomos. Banco de Germoplasma	UPM-BGV	7
			ESP004	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Centro Nacional de Recursos Fitogenéticos	INIA-CRF	1
			ESP109	Junta de Castilla y León. Instituto Tecnológico Agrario de Castilla y León. Centro de Investigación de Zamadueñas	ITACYL	1
18	Kenya	6	KEN015	National Genebank of Kenya, Crop Plant Genetic Resources Centre - Muguga	KARI-NGBK	6
19	Canada	5	CAN004	Plant Gene Resources of Canada, Saskatoon Research Centre, Agriculture and Agri-Food Canada	PGRC	5
20	Austria	5	AUT001	AGES Linz - Austrian Agency for Health and Food Safety / Seed Collection	BVAL	3
			AUT025	Office of the Styrian Regional Government, Department for Plant Health and Special Crops	WIEWS	2
21	Portugal	4	PRT102	Banco de Germoplasma - Universidade da Madeira	ISOPlexis	3
			PRT018	Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomía	ISA	1
22	Czech Republic	3	CZE122	Genebank Department, Division of Genetics and Plant Breeding, Research Institute of Crop Production	RICP	3
23	Uruguay	3	URY003	INIA La Estanzuela	INIA LE	3
24	Zambia	3	ZMB030	SADC Plant Genetic Resources Centre	SRGB	3
25	Turkey	3	TUR001	Plant Genetic Resources Department	AARI	3
26	Lesotho	2	LSO002	Department of Agricultural Research		2
27	Brazil	1	BRA003	Embrapa Recursos Genéticos e Biotecnología	CENARGEN	1
28	Sweden	1	SWE054	Nordic Genetic Resource Center	NORDGEN	1
29	Romania	1	ROM007	Suceava Genebank	BRGV	1
30	Jordan	1	JOR006	National Centre for Agricultural Research and Technology Transfer	NCARTT	1
Total		**16422**		**59 banks**		**18787**

Source: Prepared with information from WIEWS 2013 and with the collaboration of experts working with ex situ collections of quinoa

* Data reported directly by the institution and not reflected in WIEWS

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CHAPTER: 1.6.

Quinoa and the exchange of genetic resources: improving the regulation systems

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Abstract

As proposed by FAO, the General Assembly of the United Nations declared 2013 as the International Year of Quinoa (IYQ), highlighting the potential role of quinoa's biodiversity in contributing to global food security, given its high nutritional value and tremendous potential to adapt to different agro-climatic conditions. The declaration recognizes the role of the Andean communities in creating this biodiversity and conserving numerous local varieties of quinoa. The cultivation of quinoa on other continents will continue to expand in the coming years, and there will be an increasingly widespread distribution of systems of intellectual property rights (IPR) governing varieties or genes. It is, therefore, essential to recognize the contribution made by the Andean communities, applying measures to guarantee the fair and equitable sharing of the benefits derived from the use of quinoa's genetic resources and associated traditional knowledge. This chapter addresses these issues.

Four main targets can be identified: recognition of the Andean identity of quinoa's genetic resources and the associated traditional knowledge; conservation of the components of biological diversity and ecosystems; sustainable and effective use of quinoa's genetic resources in order to encourage innovation; fair and equitable sharing of the benefits derived from the use of these resources and associated traditional knowledge.

The existing international frameworks do not address these issues in a satisfactory way. The CBD and the Nagoya Protocol regulate bilateral access and benefit-sharing. However, quinoa's genetic resources are transboundary and for decades they have been disseminated outside the Andean zone.

The International Treaty on Plant Genetic Resources for Food and Agriculture (Treaty) addresses these various objectives but does not cover the many different non-agricultural and non-food uses of quinoa (medicinal applications, cosmetics etc.). It also fails to address adequately (at least so far) the in situ conservation dimension — a critical aspect for the fair and equitable sharing of benefits derived from the use of quinoa with the Andean populations. Intellectual property rights, even those that are sui generis, including plant variety protection (PVP) certificates, geographical indications and collective trademarks, mainly focus on encouraging innovation. They are temporary (of limited duration and validity) and are not recognized by all countries. IPR mechanisms do not address the conservation of genetic resources and alternative solutions are required. Soft laws instruments such as the FAO's Globally Important Agricultural Heritage Systems (GIAHS) and biocultural landscapes deals with in situ conservation for the protection of agrarian systems that enrich biodiversity. Nevertheless, they are unsuitable for dealing with ex situ biodiversity conservation and the fair and equitable sharing of the benefits derived from the use of genetic resources. Although the Open Source Seed Initiative seems to provide an interesting alternative mechanism for the multiple stakeholders involved in different stages of production, selection, diffusion and conservation, it lacks a legal structure which would allow it to protect the exchange of genetic material and prevent misapropriation. However, these systems do not include wild crop relatives. They focus predominantly on varieties of the cultivated species.

Consequently, there is no single legal framework capable at this stage of dealing simultaneously and globally with the four areas identified. The gaps in each of these instruments are an incentive for improvement. Solutions still need to be developed to better harmonize the different existing legal frameworks and soft laws mechanisms and/or create new complementary ones. The rapid spread of quinoa at global level provides an opportunity to consider the implications of the current regulatory instruments for genetic resources so that they can be improved and implemented anew.

Introduction

At present, just 12% of the main crop species cultivated provide 75% of our food. Of these, wheat, rice and maize provide 60% of the calories consumed in the world (FAO, 2010).

All countries are now increasingly interdependent for meeting their food and agricultural requirements. It should be noted that over the past 10 000 years, since the emergence of agriculture, the world's agrarian societies have created and developed agricultural plant genetic resources in five main centres of origin: the Near East (barley and wheat); southern Asia (rice); Africa (millet and sorghum); Central America (maize); and South America (potato and quinoa) (Bazile, 2012).

The history of the domestication of cultivated plants that has led to their world expansion goes back a long way and is linked to several periods of agricultural development (Bazile, Fuentes and Mujica, 2013). The genetic resources of cultivated plants have been collected and exchanged locally or via human migration for over 10 000 years. These species are now cultivated on vast areas of land throughout the world. Furthermore, they are considered the main crops for agricultural production and world food security.

The genetic resources of the main crop species have been and continue to be the focus of major plant breeding research associated with processes of ex situ conservation. In the case of "secondary" food species, the creation of genetic diversity occurs via a continuous process in the field. Farmers are constantly looking to introduce new genetic material in order to avoid low productivity of their own varieties reproduced each year. Low quality observed in seeds is often resulting in a decrease in productivity due to the degeneration of the genetic material caused by cross-pollination with other local varieties.

To guarantee these dynamics of change, the dissemination of plant genetic resources is based on principles of free access and distribution. In the light of recent advances in biotechnology, intellectual property rights (primarily patents) are being extended to the genetic resources of living organisms based on principles of ownership and exclusivity. This defines the standards governing the movement of improved varieties produced by both private and public breeders of new plant varieties (Bazile, 2011).

In this context, the case of quinoa is highlighted. It is a crop located in Andean countries, which is spreading to numerous countries across all the continents and has the potential to become a main crop in world agriculture (Galwey, 1993; Jacobsen, 2003; NRC, 1989). At global level, the rapid expansion of the areas where it is grown led FAO to declare 2013 as the International Year of Quinoa. It is rare for a crop of regional status, and considered as minor crop, to obtain such world recognition. This situation must be emphasized.

The evident change in status of this species, which was domesticated on the shores of Lake Titicaca, may provide a model for examining and analysing the current legal regulatory frameworks for genetic resources.

In fact, potato (Solanum tuberosum sp.) ranks fourth among the world's main food crops. Like quinoa, potato originates from the Andes, in the Lake Titicaca basin, where it was first cultivated over 8 000 years ago.

Andean farmers had access to a large wild population from which they were able to select and improve the first specimens, which thousands of years later have produced the tremendous diversity of potato species and varieties known today.

The genetic diversity of Solanum tuberosum is divided into two subspecies: the first, andigena, is adapted to a photoperiod of 12 hours of sunlight and is mainly cultivated in the Andean region; the second, tuberosum, is grown throughout the world. The tuberosum subspecies will have developed from andigena, which was introduced in Europe long ago and gradually adapted to the Northern Hemisphere with its longer days.

Nowadays, approximately 5 000 local potato varieties are grown in the Andes. The new potato varieties are cultivated mostly in Asia and Europe and currently account for over 80% of world production (Alary et al., 2009). Europeans are the world's biggest potato consumers – 85 kg per person in 2009 (FAOSTAT).

Although the evolution of potato's global distribution took place in a different period, it could shed light on the trajectory of quinoa's current spread across the world. When examining the existing legal regulatory frameworks, potato could be a useful reference to determine whether or not these processes will be repeated. This historical insight provides the opportunity to see how new legal regulatory frameworks can be applied to genetic resources (Trommetter, 2001, 2012).

At present, industrialized countries – with industrial farming – have the majority of intellectual property rights or legal protection for new plant varieties or so-called improved varieties. This asymmetry with developing countries is due in part to the differences in access and research capacity with regard to new biotechnology for plant breeding. In EU countries, there are over 1 600 varieties of potato registered in the European catalogue and 16 481 plant variety protection (PVP) certificates have been deposited in the International Union for the Protection of New Varieties of Plants (UPOV). At global level, there are now 20 PVPs for new varieties of quinoa, of which 16 were obtained in Denmark and the Netherlands.

Introduced by the Spanish to Europe in the sixteenth century, potato went from being just a few tubers to becoming an essential food for countries in northern Europe in the eighteenth century. Unfortunately, mildew developed as a result of the monoculture of a small number of potato varieties. This situation led to the great famine of the nineteenth century (1846–1851), causing the loss of 25% of the Irish population in 10 years.

Even today, the strategies for disseminating new plant varieties or so-called improved varieties depend on a limited genetic base (to respect uniformity – one of the criteria required for a new PVP or for registration in a catalogue of plant varieties). This situation creates considerable risks related to potential diseases, epidemics and the spread of pests. These risks are exacerbated by the fact that all the improved varieties come from a small number of parent plants (as with potato when it was introduced and cultivated in Ireland).

In the twenty-first century, the agro-industry's continued research on the potato provides an insight into the growing dynamics of improvement and legal protection of new varieties relating to this sector. Similar dynamics are also likely to apply to quinoa in the short term. However, regardless of the intellectual property rights for genetic resources, discussion should be extended to the agricultural models to which the IPR apply: industrial farming versus family farming. This raises broad questions about genetic resources in relation to other criteria, such as identity, equity, *in situ* conservation and innovation for new plant varieties.

Those who promoted the 2013 declaration for the International Year of Quinoa, including FAO, expect to see a global expansion in the areas cultivated, with an immediate increase in demand for seed from other countries wishing to promote processes of genetic engineering and/or varietal improvement of quinoa. The IYQ keeps quinoa in the spotlight, making it possible to reflect on other alternative legal frameworks, without having to use the standard conventional framework for intellectual and industrial property rights. The case of quinoa provides insight into the case of a cross-border genetic resource, whose uses have recently extended beyond the agricultural and food sector. Until now, the legal framework of industrialized countries has dictated at international level, limiting the driving force behind alternative legal frameworks. Before the signing of the Convention for Biological Diversity (Rio de Janeiro, 1992), the global dissemination of genetic resources, in theory, made the CBD proposal ineffective in terms of a bilateral framework for the negotiation of genetic resources with sovereign states relating to the existing biodiversity on their territory. In this context, alternatives are required for cross-border links to assess whether regional and international levels of negotiation would facilitate or hinder the process in relation to the specific situations or issues at stake.

Quinoa: issues to consider that go beyond food and agriculture

The International Year of Quinoa: a new lease of life for global expansion

In July 2011, the United Nations General Assembly declared 2013 as the International Year of Quinoa following the proposal presented to FAO in Rome by the Plurinational State of Bolivia. The declaration brought recognition to the role that this plant can play in world food security. According to the FAO Resolution 15/2011, approved at the United Nations General Assembly in New York in December 2011, the declaration of the IYQ highlights the quality of quinoa as a natural food of high nutritional value and the importance of the role played by the Andean peoples in the creation and conservation of quinoa biodiversity. In addition, it emphasizes the importance of traditional knowledge and agricultural practices that respect and conserve nature.

On this basis, the declaration of the IYQ underlines the fact that, in 2013, world attention should focus on the role that quinoa's genetic diversity can play in terms of world food security and the eradication of extreme poverty and hunger, thus contributing to the Millennium Development Goals – MDGs (PROINPA, 2011).

At global level, the crop started to spread across all the continents in the 1980s, although two Andean countries, Bolivia and Peru are still the main quinoa producers (see Chapter 1.5) (Giuliani *et al.*, 2012). In the 1980s, the United States of America introduced the crop first in the south of Colorado, and then in other states. Canada grows quinoa on the plains in Saskatchewan and Ontario. According to estimates, Canada and USA produce around 10% of the world's quinoa – that is probably more than Ecuador, which had until now been considered the world's third largest producer country.

In the 1990s, FAO-RLC (FAO Regional Office for Latin America and the Caribbean) defined one of its institutional priorities as: the exchange of plant genetic resources from diverse "underutilized" Andean food species that are considered suitable for production in different ecosystems in North America and Europe. In this context, the promotion, exchange and dissemination of quinoa's plant genetic material took the form of an experiment known as the American and European Test of Quinoa. Many countries from all over the world took part in the experiment through research networks that included national research institutes and universities (Mujica *et al.*, 2001).

In Europe, quinoa is grown particularly in the United Kingdom, Sweden, Denmark, the Netherlands, Italy and France. In Asia, it is cultivated in the Himalayas, on the plains in north India and Pakistan where yields are promising. In Brazil, it is being grown experimentally as a cover crop in the Amazon Basin. In Africa, specifically in Kenya, it has also been grown experimentally for many years. More recently, it has been cultivated in Mali, where the plant has been introduced to reduce hunger and poverty.

With the quinoa boom in the 1990s and the impetus from FAO, the crop continues to expand, particularly in the Mediterranean region. Given the multiple exchanges and diverse uses of quinoa, the implementation of standards to regulate the movement of its genetic resources is complex, also because of the plant's tremendous ecological rusticity and plasticity (Ruiz *et al*., 2013).

A biodiverse plant with a great capacity to adap

Quinoa (*Chenopodium quinoa* Willd.), is an annual plant that originates from the Andes in South America. Its domestication is thought to have begun around 7 000 years ago with the continuous selection of the characteristics of individual plants from one generation to another. Selection criteria were linked to crop practices, as well as to organoleptic qualities for consumption among the diverse populations in distinct territories (Mujica, 2004). This broad process of selection and improvement from generation to generation led to a multitude of local varieties; dehiscence was suppressed and priority was given to increased seed size and adaptation to local environmental conditions (Bazile, Fuentes and Mujica, 2013; and see Chapter 1.4).

Despite the standardization process, with the loss of alleles during selection, even now cultivated quinoa exhibits a wide range of colours on different parts of the plant. The grains may differ in terms of stem type, panicle shape, rate of productivity, tolerance to abiotic stresses (drought, salinity) and disease resistance (Fuentes and Bhargava, 2011; Ruiz-Carrasco *et al*., 2011).

The diversity of quinoa on the South American continent is associated with five major ecotypes (Bazile, Fuentes and Mujica, 2013): Altiplano (Peru and Bolivia); Inter-Andean valleys (Peru, Ecuador, Colombia); Salare (Bolivia, Chile, Argentina); Yunga (Bolivia); and Coastal (Chile). All these ecotypes originate from the same region of primary domestication located near Lake Titicaca. In addition, eachone can be associated to a subcentre of diversity (Risi and Galwey, 1984; Fuentes, Bazile *et al*., 2012).

Many generations of farmers have been involved in this vast quinoa selection process, which explains its tremendous genetic diversity today. Its broad genetic diversity enables it to adapt to different ecological environments (highlands, valleys, mountains, salty zones etc.), different types of soil (in particular, saline soils), and places characterized by wide ranges in humidity (40 to 90%), altitude (0 to 4 800 masl) and temperature (-8° to +38°C). This capacity to adapt constitutes an advantage in today's context of climate change and salinization of agricultural land.

Quinoa's rusticity (its capacity to resist extreme biotic and abiotic stresses) and ecological plasticity are central to its potential in terms of developing cultivation in other parts of the world. These factors are even more relevant today, when measures to adapt to climate change must be promoted. Quinoa's great biodiversity means that it has capacities of adaptation and resistance and can, therefore, be grown in agri-ecological systems requiring lower levels of inputs. This coincides with the health requirements for its use in medicine, cosmetics and food. At present, quinoa is known primarily for its nutritional qualities, because it contains proteins (all the essential amino acids), minerals, vitamins, linoleic acid (omega-3) and amylases, and it is gluten-free. However, quinoa is also used in farming as an animal feed, as a cover crop or as an intercrop to stop the cycle of certain parasites. The uses of quinoa as a detergent, in cosmetics and medicine are less well known. Nonetheless, all its uses must be taken into account for the implementation of a legal framework to regulate movement and exchange of and access to quinoa's genetic resources on a global scale (see Chapters 3.4 and 3.5).

Agricultural systems with diverse legal frameworks

For a long time, Andean populations were in charge of quinoa production. In fact, when the Spaniards arrived, one way of making the Andean peoples submit was to impose a cereal-based diet. That is how quinoa was displaced and devalued, and its production confined to the Andean peasant communities. The Mapuche in southern Chile (Thomet *et al*., 2010) and the Andean communities in Peru are a good example.

Until recently, quinoa was considered food of the Andean peoples. It gained worldwide recognition in the 1970s, and was particularly appreciated by vegetarians for its dietary characteristics. For a long time it was classified as a subsistence crop, which explains why the Andean communities conserved a diverse range of traditional agricultural practices, because they could not combine them with a conventional agricultural model. This agri-ecological model is the most appropriate in a fragile environment subject to major abiotic constraints.

In Andean countries, most areas where quinoa is cultivated use traditional varieties, also known as peasant varieties or landraces. The Andean peasants focus on groups of varieties made up of heterogeneous plant populations. This means that they can cope strategically with different biotic and abiotic risks, by alternating individuals in a population (or landrace) on an annual basis. Seeds are home produced and the most resistant individuals are selected in the field for the next generation (seeds for the following year). This makes quinoa management dynamic and able to face risks and adapt to environmental, economic, social and political changes.

Traditional peasant management of the quinoa genetic resource pool contributes to the dynamic adaptation of quinoa varieties. These are the same varieties that have evolved continuously in relation to their ecosystems. Taking into account the characteristics of quinoa cultivation, the joint evolution of varieties and their environments can also include some results of crosses with quinoa's wild relatives growing near the cultivated plots. The networks of traditional seed exchange – seed paths – and the knowledge networks associated with the varieties have made it possible to build and maintain peasant innovation processes. This can now be seen in quinoa's huge genetic diversity (Aleman, 2009; Fuentes *et al*., 2012; Thomet *et al*., 2010).

The boom in global demand for quinoa in the 1990s led to the emergence of an intensive agricultural model and the use of only a few so-called improved varieties. Research on varieties shifted to the field of agronomic research (private and/or public) for the development of pure lines, hybrids etc., all of which had an increasingly narrow genetic base. Until then, the improvement of quinoa varieties had been based on three techniques: traditional massal selection, controlled crossing between genotypes and the development of commercial hybrids. The main objectives of the research in Andean countries were increased yields and improved disease resistance, gradually extending to include adaptation to the photoperiod (latitude), temperature and altitude found in countries outside the Andean zone.

Although various countries have signed international agreements, the transposition of these texts into national legislation differs from one country to another, depending on the agricultural policies implemented previously. Despite this, agricultural research remains public in Andean countries. Consequently, the new varieties obtained are not subject to intellectual property rights when they are released on the market. There is one exception: a case in Chile, where the quinoa variety 'Regalona', the fruit of private research (Semillas Baer), was protected by a PVP in order to protect the rights of the private breeder.

The current use of biotechnology in plant improvement via assisted selection, involving the use of molecular markers or genes of interest (resistance, chemical components, nutrients etc.), is in danger of modifying research and the legal frameworks for the regulation and protection of future quinoa varieties. The use of genes from wild quinoa relatives (for example, from *Chenopodium hircinum* or *C. album*) is considered the next step in creating new varieties that are part of strategies of adaptation to climate change (drought tolerance and soil salinity).

Over the last 40 years, different varieties of quinoa have been developed in Peru, Bolivia, Chile and Argentina, as well as in the United States of America, Brazil, Denmark, the United Kingdom, the Netherlands, and India etc. All these varieties come from the same initial pool of quinoa genetic resources linked to the domestication of the species in the Andes. They are "cross-border" resources, because the area of origin of the domesticated species covers several countries sharing these genetic resources. It is important to note that the movement of quinoa's genetic resources began long before the signing of the Convention on Biological Diversity (Rio, 1992). The CBD establishes principles and standards for the movement of genetic resources and, in general, recognizes that states have sovereignty over their biodiversity. The collections of quinoa germplasm are now spread throughout the world (see Chapters 1.4. and 1.5.). Even though the largest collections are in Andean countries (Bolivia, Peru, Argentina, Ecuador, Chile and Colombia), over 20 countries across the world conserve quinoa genetic resources in their ex situ genebanks. These include: South Africa, Germany, Australia, Austria, Brazil, Canada, Slovakia, Spain, United States of America, Ethiopia, Hungary, India, Japan, Kenya, Portugal, Czech Republic, United Kingdom, Sweden, Turkey and Uruguay. They share information with international systems such as FAO.

Since the Convention on Biological Diversity, more stringent legal frameworks have been created for access to genetic resources through bilateral contracts and material transfer agreements (MTA). The main objective is to guarantee the traceability of genetic resources and define the rights and responsibilities of each party in the exchange. Monitoring research on the adaptation of quinoa in different cropping contexts outside the Andes (e.g. ongoing improvement of varieties in future quinoa-producing countries) and seed multiplication raise numerous issues concerning systems for the management of genetic resources. Legal frameworks and regulations for the movement of quinoa's genetic resources need to recognize the role of the Andean peoples, who were involved in the varietal improvement long before these innovative processes. The objective is to avoid appropriation or limited access to quinoa's genetic resources, as was the case with the patent registered by the University of Colorado (subsequently abandoned due to international pressure). The patent was for the male sterility of quinoa discovered in the Andean quinoa populations conserved in the United States of America and known as 'Apelawa'.

The research to improve quinoa varieties has focused mainly in use of quinoa in food and agriculture. However, major research is underway on the by-products of quinoa as part of programmes to reduce cancer, obesity and diabetes or to find different ways of adding value to saponins etc.

Issues to consider for genetic resource management

DFor more than 500 years, varieties of potato have been part of food security strategies in many countries outside the Andes, the hub of its domestication. This is the result of the global dissemination of plant material domesticated and selected by the Andean peoples over thousands of years. The potatoexperience highlights the fact that the Andean peoples have received no benefits or significant recognition for having shared this improved plant material, which has since spread throughout the world. New species introduced into Andean countries do not offer comparative advantages to the local populations.

The current huge demand for quinoa has generated a boom in consumption, primarily in industrialized countries (some of which are new quinoa producers). This situation has brought changes to the agricultural systems in the Andes. In contrast to what happened a few centuries ago with the potato, the Andean populations are now active stakeholders in defending the recognition of their contributions to the improvement of quinoa varieties and the conservation of its genetic resources. They also want to be recognized stakeholders in world trade.

International treaties recognize the sovereignty of states with regard to their genetic resources and the contribution made by indigenous communities to their conservation. They set out the principles to promote the fair and equitable sharing of the benefits derived from the utilization of these genetic resources, which are available to all the countries in the world. At present, those seeking to spread quinoa cultivation are supporting experimental agronomic campaigns in many countries outside the Andean zone. It is, therefore, of the utmost importance to analyse how dissemination programmes can ensure a return (fair and equitable sharing of the use of quinoa's genetic resources) for the Andean communities and states as laid down in international agreements (CBD/Nagoya, ITPGRFA). This also includes an analysis of the systems of intellectual property rights in force (patents, PVP certificates).

The UN declaration of 2013 as the International Year of Quinoa emphasizes the role of the Andean peoples in creating and conserving the biodiversity of quinoa. In this context, considering the current global boom in quinoa, several issues are raised: Will promotion simultaneously guarantee the Andean peoples the fair and equitable sharing of the benefits derived from the use of quinoa's genetic

Table 1: Characterization of the issues related to plant genetic resource management

Identity	Recognize the traditional ways of life of interest for the conservation of biodiversity and the sustainable utilization of its genetic resources. Respect, conserve and maintain the knowledge, innovations and practices of indigenous and local communities.
Conservation	*Ex situ* conservation: conservation of the elements that constitute biological diversity outside their natural environment. *In situ* conservation: conservation of the ecosystems and natural habitats, maintenance and reconstitution of viable populations of the species in their natural environment and, in the case of domesticated and cultivated species, in the environment where their distinct characteristics were developed.
Mobilization Sustainable utilization	Facilitate the exchange of genetic resources. Encourage different forms of innovation and synergy between formal and traditional systems for utilization and adding value to genetic resources. Encourage an evolutionary dynamic for genetic resources to increase the capacities of adaptation to cope with global changes (resilience).
Equity	Draw up equitable rules for access to genetic resources. Draw up equitable conditions for sharing the benefits derived from the utilization of genetic resources at stakeholder and country level. Increase the capacities for exchanging information and accessing technology for the equitable utilization of genetic resources between countries and stakeholders with different capacities.

resources? How should quinoa's genetic resources be conserved *in situ* and *ex situ* to avoid their genetic erosion? What mechanisms should be set up for the fair and equitable exchange of quinoa's genetic resources? How can such exchanges contribute to the recognition of the Andean populations and to the processes of conservation used by them for quinoa's genetic resources? To what extent exisitng regulatory frameworks make it possible to enrich quinoa's genetic heritage?

Many different issues are at stake with regard to the legal frameworks regulating the movement of quinoa's genetic resources. The existing regulatory frameworks should be examined to determine how they contribute to quinoa's genetic resources in terms of: conservation (i*n/ex situ*), the identity of the Andean communities (cultural recognition) and the potential mobilization of the resources (exchange, innovation, formal/informal). Table 1 outlines a proposal for characterizing the different issues and serves as a guide throughout this chapter for analysing the advantages and disadvantages of the legal regulatory frameworks currently in effect and assessing which other regulatory frameworks could be outlined to bridge the gaps in the existing ones.

Are the legal frameworks adapted to the diverse aspects of quinoa's genetic resource management?

In the light of global concern about the depletion of biological diversity resulting from human activity, an international regime composed of several instruments was set up to guarantee the sustainable utilization and management of biological resources.

Genetic resources, which are biological resources, are genetic material of real or potential value to humanity. The majority of agricultural genetic resources, including quinoa's genetic resources, are mainly regulated by the CBD and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA/FAO 2001, http://www.plant-treaty.org/). ITPGRFA governs the genetic resources of the main food crops, listed in its Appendix 1.

The principles of fair and equitable benefit-sharing proposed by the CBD

LThe Convention on Biological Diversity (CBD) adopted within the framework of the 1992 Earth Summit in Rio de Janeiro (http://www.cbd.int/) recognizes the sovereignty of states and acknowledges that states are responsible for the conservation of their biological diversity and for the sustainable utilization of their biological resources. Consequently, states should establish national strategies for the conservation of their biological diversity, and provide a framework for bilateral arrangments relating to their biological resources.

The practices involved in accessing and exchanging genetic resources are regulated via private law agreements on sharing the benefits derived from contractual bilateral agreements between a provider and a recipient.

This solution is based on Coase's theory of externalities (Coase, 1974): the market does not confer a value on diversity for individuals and society, so in parallel, no person can be easily excluded from its use (consequently there is no incentive for an individual to pay the costs of access to this diversity). Therefore, a negotiation between private parties, via the establishment of a contract granting property rights for genetic material, is considered an effective method for reflecting the value of genetic diversity. In addition, direct or indirect monetary incentives are established, linked to the sharing of benefits derived from the use of the genetic diversity.

Nonetheless, there is still considerable uncertainty surrounding the value of the material at the time of access to genetic resources, as well as a lack of legal security in the event of non-compliance by one of the parties. As a consequence, these contracts are embedded within national legislation and are part of a wider range of legal mechanisms or agreements seeking to limit opportunistic behaviour (Dedeurwaerdere, 2004). These mechanisms include, *inter alia*, standard contracts, mechanisms to monitor and enforce contractual obligations (e.g. disclosure of origin of genetic resources or certification of origin) and prior informed consent of the indigenous local populations.

Nonetheless, even when part of national legislation, the contractual approach for regulating access to genetic resources and sharing the benefits of their use is not sufficient to achieve broader related societal objectives such as social equity and conservation and sustainable use (Dedeurwaerdere, 2004; Goëschl and Swanson, 2002). In effect, the combination of (hierarchical) public regulations and monetary incentives applied in these contracts fails to account for the diversity and complexity of the stakeholders' actual motivations in exchanging genetic resources. These regulations do not properly reflect the needs of the wide range of actors involved in the use and exchange of genetic resources. In fact, they are only efficient for the category of users and uses that are most responsive to monetary incentives. The exchange of genetic resources actually responds to a more complex set of motivations, including societal motivation (global public objectives, such as increasing knowledge, conserving biodiversity or reducing hunger) and more basic social motivation (such as reputation, reciprocity). In fact, stakeholders' surveys (Dedeurwaerdere *et al.*, 2012) tend to demonstrate that striving for notoriety (by virtue of material quality, information exchanged or publications) and/or reciprocity (exchange of information between stakeholders) are among the principle motives for the conservation and exchange of genetic resources.

Furthermore, even supposing that economic incentives work properly, they will never allow for sufficient investment to maintain and exchange genetic resources, because the value of most of these resources is and will remain unknown for years.

Lastly, in certain cases, using monetary incentives for all types of exchange of genetic resources can be counterproductive. The introduction of market values can be a disincentive for contributing to the collective effort to conserve the genetic resources within local communities. Introducing monetary-based approaches where it does not exist could generate mistrust and suspicion ("crowding-out" effect, described by Frey and Jegen, 2001). In other words, the emergence of a contract can undermine the cooperative or collective practices required for genetic resource conservation.

Clearly, these problems seem to be even more acute in the case of plant genetic resources for food and agriculture, including quinoa genetic resources. Going back to the identity dimension of our analytical framework, Article 8j of the CBD explicitly covers this dimension, recognizing *knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity*. Nonetheless, in the CBD, the question of to exactly what extent this would be applied is left to the responsibility of the states. In the case of quinoa, the question of local identity is linked to the Aymara, Quechua and Mapuche cultures but development policies ultimately depend on the national perspective, which may or may not recognize these local groups in genetic resource management. (It may also lead to other broader debates not directly related to genetic resource management. In such context, it would inevitably be difficult to implement the CBD.

With regard to conservation, the CBD applies to all genetic resources, without exception. The specificities of agricultural genetic resources/plant genetic resources useful for food and agriculture were not taken into account. One of the main criticisms of the CBD (and the Nagoya Protocol) is that the mechanisms for access and fair and equitable sharing of benefits derived from the use of genetic resources are loosely linked to conservation. This should be factored into national strategies. Nonetheless, the situation for plant genetic resources for food and agriculture often appears to be secondary in national strategies, especially in the case of genetic resources from wild biodiversity (crop wild relatives).

The challenges relating to innovation (derived from?) genetic resources make the implementation of national strategies even more difficult. However, the CBD framework ensures full control of access to quinoa's genetic resources. Furthermore, it could be consolidated by the implementation of national strategies, with the support of national authorities responsible for access to and traceability of genetic resources. In this context, the rights and responsibilities of the parties are more explicit. Conversely, in the context of bilateral contractual relationships between states, the supplier country could easily block access to its genetic resources and effectively prohibit all possibility of innovation. In the case of research processes to improve and obtain new plant varieties, the exchange of genetic resources is and should be recurrent. Consequently, bilateral contractual frameworks for access to these genetic resources can be cumbersome, in addition to generating high transaction costs.

The incremental nature of the innovation process on genetic resources for food and agriculture makes it particularly difficult to adopt a bilateral and case-by-case approach, in terms of both access and sharing the benefits derived from the use of genetic resources (Schloen et al., 2011). Besides, in the case of quinoa, its genetic resources were circulating between stakeholders and countries long before any ABS measures were in place. There are currently collections of genetic resources of quinoa in different places in the world. From a strictly legal point of view, the exchange processes for these genetic resources (obtained before the CBD, 1992) could be conducted legally, without involving the countries of origin (the zones where quinoa was domesticated) in the exchange.

Furthermore, a relatively high number of products (not necessarily all marketable) can be derived from the utilization of plant genetic resources for food and agriculture. Many of these could be elaborated or developed from multiple genetic resources. Each genetic resource, taken individually, can contribute to the final product at different levels and at different points in time. The task of monitoring the separate contribution of each genetic resource and determining the benefits to be shared in relation to its individual contribution, on the basis of the terms and conditions specified in a bilateral contract for each genetic resource, could prove to be extremely complicated (Schloen *et al.*, 2011).

Despite the different limits identified, the legal framework established by the CBD is now compulsory for processes involving the prospection and collection of new genetic resources of quinoa. This limits the potential cases of biopiracy associated with the collection of new genetic material for agricultural, pharmaceutical, medical and cosmetic purposes under development for quinoa. Nonetheless, this legal framework is ineffective when genetic material is accessed from germplasm collections located outside Andean countries.

The legal framework for intellectual property rights (WIPO–WTO)

- Patents (TRIPS) versus PVP (UPOV)

The legal framework for intellectual property rights (IPR) for living organisms is based on financial incentives that aim to encourage biological innovations. By providing legal protection mechanisms for inventions based on genetic diversity, intellectual property should encourage the use of quinoa's genetic resources. As mentioned in relation to the different concepts relating to the status of genetic resources, the agricultural sector is characterized by the coexistence of at least two intellectual property systems: patents and plant variety protection (PVP). Both systems are promoted at international level by the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) of the World Trade Organization (WTO) and by the International Union for the Protection of New Varieties of Plants (UPOV). The latter advocates a *sui generis* system adapted to the self-reproductive and evolutionary nature of plant genetic material. A product derived from innovation, i.e. a new plant variety, is a genetic resource in itself. A balance must be found between protecting innovation and limiting access to genetic resources. This balance is central to the UPOV and does exist in the form of exemption for research. Thus, the genetic resources of a new plant variety protected by plant variety protection (PVP) can legally be used for research purposes.

The UPOV system also provides better legal security than the patent system: a product can have numerous patents, while a new plant variety is protected by a single PVP (Dutfield, 2011). There are far more disputes in the patent system than in the PVP system, and "patent thickets" arise – intricate problems of patents dependent on other patents (Shapiro, 2000; Heller and Eisenberg, 1998), monopolization of patents or inadvertent violations of patents.

In general, with the exception of a limited number of countries (including the United States of America), the patent system is not used to protect new plant varieties directly. It is used to protect biotechnological inventions, such as genetic sequencing or procedures that constitute the basis of plant breeding. Irrespective of the technical differences between the two systems, the intellectual property system for genetic resources reveals fundamental problems that have been and are the subject of debate in numerous publications. One of the main criticisms directly related to the problem of the conservation and use of genetic resources is, in fact, that intellectual property rights only intervene at the very end of the genetic resource value chain. Consequently, they only function effectively as an incentive mechanism for new plant varieties or plant material for which the value is already known (even partially) either from available data on characterization or assessment. Thus, intellectual property rights provide far too few incentives for the exchange of most components of genetic diversity found *ex situ* (even less for those *in situ*, where the value of genetic diversity is still unknown at the time of its accession) (Swanson and Goëschl, 2000; Goëschl and Swanson, 2002).

In addition, intellectual property rights are not an effective incentive for innovation and research – either in cases of low demand, or for countries lagging behind in the scientific advances of cutting edge innovation. Such countries are unable to benefit from the advantages of legal protection provided by intellectual property rights. Lastly, as with the effects of exclusion (crowding-out), described earlier for access and benefit-sharing mechanisms, the introduction of economic incentives can negatively impact the exchange of genetic material or information during pre-competitive phases. These situations – "anticommons" – can negatively impact cooperative and altruistic behaviour (Heller and Eisenberg, 1998; Cassier, 2002). All these problems are exacerbated in the agricultural sector, as agricultural innovation is about coordinating research between many different stakeholders rather than a question of individual incentives.

If the private seed sector manages to function well thanks to individual incentives, it should be noted that the private sector depends directly and indirectly on public research institutions and their studies of genetic diversity. In the public sector, financial incentives do exist, but they by no means represent all the existing motivation factors behind the exchange and use of genetic diversity. Similarly, those who defend local community rights or rights relating to the traditional knowledge associated with biological diversity, recognize, first and foremost, the existence of collective rights that govern access, exchange and use of seeds and genetic resources. They are not restricted to a framework of individual rights, as in the case of intellectual property rights.

- Geographical indications and collective trademarks

Geographical indications and collective trademarks are also part of a system of intellectual protection or, more specifically, industrial protection.

In the agricultural sector, *in situ* exploration, for both biological material and local knowledge associated with biodiversity resources, generally serves the purpose of enhancing *ex situ* collections (defining the characteristics and legal status of the plants collected). It should therefore be asked what role geographical indications can play (e.g. protected geographical indications or designations of origin) to promote the conservation of genetic resources, or maintain and protect local knowledge. Geographical indications denote that a product originates from its place of production. In the case of plant selection, this makes it possible to add value to a variety, not only in relation to its geographical origin but also its genetic identity. For example, the aim of a plant variety protection (uniform, distinct and stable variety, close to varieties from pure lines) is to obtain a phenotype independent of local ecological conditions.

Geographical indications reveal the characteristics of a product. These are determined by the specificities present when the geographical indication is developed and include: geology, soil, topography, climate and human factors (current techniques and/or traditional knowledge). A geographical indication can also refer to cropping practices or processing practices that affect the quality of the product and contribute to its distinguishing features and reputation. Consequently, there is a link between the product and the geographical environment, making it possible to distinguish the product from those originating from other regions.

Geographical indications are also part of the TRIPS of the WTO. Each member state is free to define appropriate mechanisms for implementation within the national legislation. Some countries, such as the United States of America and South Africa, have not adopted national standards for the protection of geographical indications, but use other mechanisms, including consumer protection, trademarks or fraud control (passing off) (Kalinda, 2010).

Geographical indications are used for products of specific geographical origin, with qualities and/or a reputation derived from that place of origin. In general, a geographical indication states the name of the product's place of origin. Geographical indications include the "appellation of origin". This is a special type of geographical indication used for products with specific qualities that must be exclusively or essentially from the product's geographical context of production or processing. The regulation of geographical indications for processing products must be approved and should also be subject to control by nationally accredited organizations. For example, in Bolivia, the designation of origin "Quinoa Real" from the southern altiplano of Bolivia has existed since 2002 and was also recognized in the administrative resolution N°18 (on 23/07/2002) of the governmental intellectual property organization (SENAPI).

The Lisbon Agreement for the Protection of Appellations of Origin and their International Registration made it possible to obtain protection for an appellation of origin specified in all the contracting parts of the agreement, following a single international registration procedure. Currently, 28 countries are party to the Lisbon Agreement, and Peru is at present the only country from the Andean region. At regional level in Latin America, the Andean Community (CAN) also protects appellations of origin in its member countries via the Common Intellectual Property Regime outlined in Decision 489 – CAN.

The duration and cost of protection vary from one country to another, and it is often necessary to obtain the geographical indication in the country of origin. This hinders innovation aimed at improving product quality; changes in the regulations could lead to an improvement in practices and quality.

A trademark is a distinctive symbol enabling consumers to distinguish the geographical origin or characteristics of a product.

A collective trademark belongs to an association. Its members – companies, producers, public institutions or cooperatives – define rules to guarantee that the product meets certain quality requirements or has specific characteristics (WTIP, 2013).

A collective trademark should be protected independently in each country or group of countries seeking protection (e.g. Peru or the EU, where common protection exists). A trademark encourages stakeholders to innovate in order to improve product quality and represents progress. A trademark is more dynamic than a geographical indication. It ensures more effective value added for products, as it recognizes the specificities that add value to these products. Nonetheless, trademarks do not protect genetic resources.

Today, no intellectual property rights protect genetic resources and guarantee the fair and equitable sharing of benefits derived from their use. What is more, high costs are entailed both in registering intellectual property rights and in maintaining rights over time.

- National or regional catalogues

In France at present, for a plant variety to be authorized and put on the market, it must be registered in a catalogue of varieties and satisfy criteria of distinctness, uniformity and stability (DUS). It must also demonstrate that it has an adequate value for cultivation and use (VCU). The new variety must exceed commercially available varieties for certain criteria. The DUS criteria are the same as those for PVP and are an intellectual property right for seeds.

In West Africa (including Mali), a catalogue of plant varieties exists, comprising also newly obtained plants and local varieties (populations). In many countries, registering a variety in the catalogue is not a precondition for selling and/or using the seed (including in the United States of America).

Finally, if a country decides that to commercialize and/or use a variety, it may be registered in a national catalogue. But, once registrered in such catalogue this does not mean that it automatically qualifies for DUS/VCU as a prerequisite for registration. Some catalogues (e.g. in West Africa) have adopted less stringent requirements that in DUS/VCU.

Assuming that a harvest can be sold – i.e. that a market exists – a new variety can be registered in a specific catalogue (catalogue for the conservation of varieties in the European Union) of conservation varieties, i.e. primitive races and agricultural varieties naturally adapted to local and regional conditions or threatened by genetic erosion. This catalogue was created with the aim of conserving local and traditional varieties (genetic resources and associated knowledge) in view of their genetic resource heritage.This catalogue limits varietal improvement a priori (improvement goes against conservation), unless the new plant variety satisfies the DUS and VCU requirements for registration in the "official catalogue of plant species and varieties." In this case, France has a particularly strict legal framework.

In conclusion, the analysis of the framework for intellectual property rights and of the application of intellectual property for innovation (especially for new plant varieties), highlights the asymmetry between countries in terms of their research capacities and access to global research results. The ongoing development of new quinoa varieties depends on access to and management of cutting edge biotechnologies used to obtain new plant varieties. However, a country which has access to the scientific capacity for obtaining new varieties also has the financial means to protect varietal innovations. The cost of a PVP or patent application is a constraint for some countries.

Finally, intellectual property rights in relation to genetic resources go beyond the legal framework of seed production for agriculture, because new uses in medicine and cosmetics are being developed. Thus, DUS should be considered not only in terms of the characterization of functions, but also for the resulting transformations (UPOV 91, TRIPS patents).

Can FAO's The Treaty address all the situations arising linked to quinoa

Sustainable use and conservation of plant genetic resources for food and agriculture are a common concern for countries across the world. This is because all countries depend primarily on the exchange of plant genetic resources from other areas. Concern about the continuous depletion of these resources calls for specific measures that take into account the special nature of these resources.

The development of The Treaty is a direct response to this call for a specific solution. In harmony with the CBD, it aims to achieve the conservation and sustainable use of plant genetic resources and the fair and equitable sharing of the benefits derived from their utilization for sustainable agriculture and food security. While these objectives apply to all plant genetic resources for food and agriculture, the principal tool –the Multilateral System for Access and Benefit-Sharing (MLS) – only applies to a list of cultivated species registered in Annex 1 of ITPGRFA and in which quinoa is not to date included.

ITPGRFA as a pluralistic legal framework

Given the limitations of the CBD's legal framework for access and benefit-sharing, the sector of plant genetic resources for food and agriculture (PGRFA) developed alternative mechanisms that are better adapted to the specific nature of PGRFA and the way they are used in research and development.

Considering the various aspects of PGRFA (diversity created by man, importance of diversity intraspecies for improvement, greater interdependence between countries, constant need for new varieties, importance of food security etc.), a collective management mechanism has been designed to enable access to these resources and to ensure the fair and equitable sharing of benefits derived from their use. The MLS indeed pools at global level genetic material coming from contracting parties (i.e. state governments), international and regional institutions, and natural and legal persons. They all agreed on the same contractual obligations for any transfer of material coming from the MLS: the Standard Material Transfer Agreement (SMTA). The objective of the standardized access and benefit-sharing provisions is to reduce transaction costs that would occur if access and benefit-sharing were subject to bilateral negotiations rather than to a multilaterally agreed standard agreement. The system also reduces the costs of redistribution by dissociating distribution of benefits from individual supplier countries. It also highlights the non-monetary aspects of the benefits generated, which are often expressed independently of the fact that a product may or may not be on the market.

The Treaty adopts a "global commons" approach rather than a bilateral approach (Halewood et al., 2012). This international collective approach is nevertheless compatible with a vision of genetic resources as private goods. Genetic resources conserved privately are free to be included in the Multilateral System and the private appropriation of plant genetic resources from the MLS is still possible (via a patent), although sanctioned by a fee. The fee is designed to sanction the breaking of the facilitated access logic agreed collectively within the MLS. The fees are allocated to a general global fund for the benefit of all signatory parties.

The Treaty is far from limited to the Multilateral System. Other provisions are equally important in the context of this paper. Article 9 deals with farmers' rights. It recognizes past and present contributions of local communities and farmers to improve and conserve plant genetic resources, and it encourages the protection of traditional knowledge relevant to plant genetic resources for food and agriculture. However, implementation is limited by the fact that it remains the responsibility of states to implement this provision. While limited in practice, the proclamation of farmers' rights does acknowledge the legitimacy of the existence of a form of management in which plant genetic resources are not considered to be a private good or a public good (national or international), rather a common good shared by farmers of the world.

The effective implementation of this right generates problems and, despite some local initiatives, there is little support from states (Andersen, 2008). However, the Treaty is currently the only treaty that proposes a pluralistic legal framework, recognizing the legitimacy (despite the immense difficulties involved in its effective implementation) of the different concepts involved in relation to the status and management of genetic resources.

However, the fragile balance achieved by the Treaty remains imperfect. The treaty's various components are being implemented by countries at different rates, and there is a perception of inequity for some signatory parties. If facilitated access to genetic resources (promoted by the Treaty) is crucial for the agricultural and food sector, one of the main inequities perceived is that not all countries can benefit in the same way from facilitated access to PGRFA. Whether it is justifiable or not, greater and exclusive emphasis on ex situ conservation is perceived by many to mainly serve the interests of industrialized countries and of stakeholders that are more developed in terms of biotechnology. This situation is exacerbated by the fact that the effective use of plant genetic resources obtained from the MLS for commercial purposes only requires minimal monetary compensation, which – depending on the type of protection applied to the innovation – may even be voluntary. The voluntary and compulsory compensation payments are allocated to an international fund.

Advantages and limitations of including quinoa in Annex 1 of the Treaty

The species *Chenopodium quinua* is currently absent from Annex 1 of the Treaty. Proposing its inclusion in the list is no easy task, partly because of its specific characteristics, which are linked to its original geographical distribution, the current distribution of its genetic resources, its different uses etc. Therefore, an in-depth analysis of the advantages and disadvantages of its inclusion would help identify the various situations arising. Perception varies, depending on the specific interests of the different stakeholder groups in relation to the species' genetic resources.

Advantages:

- Quinoa collections are spread in different countries throughout the world and international exchanges occur largely outside the Andean countries. The MLS may be a way to recover some kind of control on quinoa's genetic resources for which they de facto lost control on.
- Such an international legal framework makes biopiracy more difficult or at least more risky. Including quinoa in the Treaty's MLS could be an efficient defensive measure to avoid the misappropriation of genetic resources.
- The Treaty allows the benefit-sharing fund to be open to developing projects to characterize phenotypes, or to participative breeding programmes for quinoa varieties (participatory plant breeding – PPB). Projects developed at regional or global level may yield collective benefits and generate new sources of financing.

Limits:

- Despite the undeniable advantages of the Treaty, it does have certain limits and cannot respond to all the situations that arise with regard to quinoa's genetic resources. This is largely due to the fact that quinoa is a species with multiple uses. Little is known about the exchange of quinoa's genetic resources for non-agricultural and non-food purposes (e.g. pharmaceutical and/or cosmetic). These activities are not regulated under The Treaty.
- Quinoa's countries of origin may have difficulty understanding and, consequently, agreeing with the implementation of the Treaty. For this reason, they are opposed to quinoa's inclusion in Annex 1, especially considering the small amounts of money available in the benefit-sharing fund. Focusing on the financial dimension – rather than on the non-monetary compensation or advantages and the benefits derived from respecting the Treaty requirements – is somehow contradictory from a practical point of view. However, it is a strong political argument undermining the treaty.
- The loose interest in the implementation of Article 6 (sustainable use of genetic resources) and Article 9 (farmers' rights) of the Treaty, which are particularly adapted and relevant for promoting the sustainable use of quinoa, may be a source of frustration for some stakeholders. Although not directly related to the MLS, the lack of progress in these areas means less support for the inclusion of quinoa in Annex 1. Obviously, the Treaty is still a relatively new instrument, and further developments are still to come. However, these articles do not have the same operational character and power as Articles 10–13 concerning the MLS.
- The MLS is particularly adapted for genetic material conserved ex situ in national or international seed banks; it is less adapted for the exchange of material conserved in situ and for genetic material developed in plant breeding centres.
- Regardless of the Treaty's operational dimension, unless there is a major and drastic change in the treaty, the challenges posed by strong intellectual property rights will remain outside the scope of the treaty and will need to be addressed by other international legal texts.

In conclusion, two main issues are fundamental to the inclusion of quinoa in the MLS: the recognition of quinoa as a cultivated species, as well as its wild relatives, and their role in its evolutionary dynamics; and the industrial use (for medicinal and/or cosmetic purposes) of quinoa.

Although The Treaty proposes a more pluralistic and better adapted framework than the CBD, it does not address all the challenges in relation to the management of quinoa's genetic resources. Important issues for quinoa's countries of origin – e.g. recognition of the Andean communities and sharing the benefits derived from the utilization of quinoa – remain to be properly addressed.

Other alternatives

Following this preliminary analysis of the existing legal frameworks, the question of "inaction" must also be raised in order to compare this analysis with the case of potato and its genetic resources (for example, to date, neither Bolivia nor Ecuador, both UPOV member countries, have any PVPs).

Various aspects of genetic resource management have been taken into account in the legal frameworks provided by the CBD, the Treaty, the TRIPS and UPOV conventions. Nevertheless, this context raises questions: can the current legal frameworks be improved or can their implementation effectively take into account the diverse situations not addressed until now? If not, which alternative legal frameworks would deal with these situations better?

Improving the current legal frameworks

- The Convention on Biological Diversity CBD

As previously mentioned, the CBD provides a global legal framework (in terms of application). The recently adopted Nagoya Protocol provides a precise legal framework capable of responding to some of the challenges identified concerning quinoa's genetic resources. The modalities of exchange and innovation, and the importance of ex situ collections, mean that this legal framework is not sufficiently adapted for its current application.

Articles 10 and 11 of the Nagoya Protocol outline potential changes that could be of interest in the case of quinoa. Article 10 concerns cases where the sovereignty of genetic resources is unclear or difficult to deal with. It obliges the parties to examine the need for and the modalities of a global multilateral benefit-sharing mechanism to ensure the fair and equitable sharing of the benefits derived from the use of genetic resources and the associated traditional knowledge. It applies to cross-border situations or cases in which it is not possible to reach an agreement or obtain prior informed consent. In such situations, member states should examine the need for and the modalities of a global multilateral benefit-sharing mechanism.

A multilateral mechanism could help avoid the excessive costs of monitoring and traceability, and its scope could be either broad or narrow. A broad interpretation addresses the question of the temporal or geographical scope of the Nagoya Protocol (Dedeurwaerdere *et al*., 2012). In a narrow interpretation, the multilateral mechanism covers the genetic resources of the centres of origin and those of unknown status, and even encompasses genetic resources in ex situ collections in place before the CBD came into force (Buck and Hamilton, 2011).

As with The Treaty, it is important to highlight that, in accordance with the multilateral mechanism, the benefits to be shared should be used to promote and implement processes geared to the conservation of biological diversity and the sustainable use of its component parts on a global scale. This means that benefits are not shared with the supplier country or countries, a situation that may prevent some countries from adopting this type of mechanism.

Article 11 envisages collaboration when the same genetic resources are located *in situ* in the territory of more than one member country. Unfortunately, as in the case of Article 10, the language is vague and poorly defined. There is no precise definition for "similar genetic resources". In the framework of common scientific research projects, the case of the same genetic resource from two countries would only occur in the case of plants (characterized by high genetic stability), and not microbial strains (most strains of the same species are not exactly the same or the slight genetic differences generate different properties because of the relatively small size of a microbe's genome) or animals (different individuals of a race). Consequently, the article probably has a very limited field of application in relation to agreements on access for research purposes.

In addition, questions relating to benefit-sharing in cross-border situations remain unanswered. If the same rule applies as in Article 10, it may not be considered worthwhile applying it in the case of quinoa.

- The Treaty, Articles 6 and 9

The Treaty member countries are faced with the challenge of successfully promoting the sustainable use of PGRFA. This involves equitable policies for maintenance of agro-ecosystem diversity, agro-ecological research, maintenance of a broad genetic base, participative plant breeding, and promotion of underused crops to reduce genetic erosion and increase food production at global level.

The responsibility of member countries is emphasized: to protect and promote farmers' rights via the sharing of benefits derived from the use of PGRFA, protect traditional knowledge linked to PGRFA, participate in the adoption of decisions on conservation and the sustainable use of PGRFA, and guarantee farmers the right to exchange and sell their varieties.

One of the key elements of The Treaty is the sustainable use of plant genetic resources, as specified in Article 6. This article applies to all plant genetic resources and not only those from the species listed in Annex 1. However, all the the Treaty signatory parties pledge to implement the provisions required to achieve these objectives, without delegating the responsibility solely to the states, as in the case of Article 9, "Farmers' Rights", or Article 5.1, "Conservation".

Article 6 resumes *de facto* the key topics described in the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture, adopted at the 1996 Leipzig Conference.

These specificities on the sustainable utilization of plant genetic resources should make it easier to implement in those states party to the agreement – in contrast to Article 9 on farmers' rights, which is generally a subject of major debate in negotiations, at both national and international levels.

However, in practice, Articles 6 and 9 are frequently associated with paragraph 9.3, related to the rights that farmers have to save, use, exchange and sell farm-saved seed/propagating material, subject to national law. This article clearly follows on from paragraph 6.2, which promotes the maintenance of agricultural systems that conserve diversified genetic resources in a sustainable way. The analysis of the objectives of Articles 6 and 9 emphasizes the need for discussion to review and adapt the standards for the diffusion of varieties and selection strategies, while leaving room for a participative breeding framework.

It is also necessary to examine the protection of traditional knowledge linked to the promotion of the use of local varieties and underutilized species. The benefit-sharing measures are general, and their application depends on the definition adopted for the fair and equitable sharing of the benefits derived from their utilization. A purely commercial approach based on economic interests creates the risk of introducing subsidy mechanisms for the conservation of local varieties. Consequently, fair and equitable benefit-sharing should investigate mechanisms of implementation that promote the non-economic benefits of the sustainable utilization of agricultural biodiversity. In this way, farmers' access to genetic resources could be facilitated and extended. In addition, processes could be implemented to support farmers to exchange and mutually enrich their strategies for breeding/varietal creation, taking into account, above all, their needs and their participation in the innovation process. In this context, the diverse existing legal frameworks (CBD, ITPGRFA, UPOV, TRIPS, regional and national legislation), as well as participative breeding processes, could serve as a basis for reflection at global level.

- Recognition of traditional varieties apart from PVPs and patents

In this analysis, it is important to underline the case of the EU, particularly France, where intellectual property rights are not necessarily linked to an authorization for marketing, but rather to the right to prohibit. Likewise, the case may arise when a variety is authorized to be put on the market but may not be protected by intellectual property rights. Consequently, if intellectual property rights are applied on their own, it is not possible to control all the issues relative to the management of genetic resources and the seed sector. In this case, "complementary" rights should be assessed, for example, the right to introduce a variety on the market with a single authorization. Analysing these aspects is important and particularly useful for understanding the utilization, exchange and, above all, sale of traditional and local seeds (most of which do not comply with DUS criteria and do not have a sufficiently high VCU).

In France and in the majority of EU countries, a seed from a plant variety that is not registered in the official catalogue cannot be sold or exchanged. However, it is possible to sell the harvest derived from the utilization of varieties of seeds not registered in the national catalogue. Varieties for conservation are exempt because they have their own catalogue, although their uses are limited (see previous point). What would be the consequences if a similar system became more widespread? What would the risks be for farmers who only use their own seeds from traditional varieties (with no exchange and no marketing) or who become dependent on national or transnational seed companies?

At national or regional level, should a legal framework be defined for licences to market agricultural inputs, including seeds? In this legal framework, what should the criteria be for authorization or prohibition? The objective is to develop licensing strategies as a function of the varieties actually utilized in countries and which are adapted to the varieties developed in the country. This means that all the stakeholders involved (interested parties) should contribute to the development of these strategies (both farmer breeders and seed and processing companies). In this legal framework, the case of biopesticides in Europe is enlightening: in terms of the criteria of homologation, biopesticides are less effective than their chemical substitutes. Consequently, they are authorized as supplements. This decision may be considered “not fully satisfactory” yet it provides authorization.

It should not be permitted to consider traditional varieties as supplementary in relation to a set standard for the new plant varieties registered in the catalogue. It undermines the perception of local and traditional varieties obtained by farmers and/or their organizations.

Various negotiations are underway at CBD and WIPO–WTO level in relation to the use of traditional varieties in breeding programmes in order to: guarantee the traceability of exchanges of biological material; and implement the certification of origin and a process of disclosure of origin for biological material at the time of application for intellectual property rights and, particularly, at the time of application for patents. However, the application of these certificates in the seed sector could be complicated because there are multiple crosses, which means that transaction costs would grow exponentially. The alternative is to recognize the knowledge that farmers have of traditional and other varieties, as suggested in the previous section within the framework of the Nagoya Protocol and IPGRFA.

The different options available to countries for the management of the relationship between traditional seeds and seeds from new plant varieties include: defining the licences for market sale; and defining the conditions of seed utilization and exchange. However, the choice of these different types of licensing will have an impact on agricultural production in the country in question and on the possible methods of selection and development of new plant varieties. The interests at stake in relation to licensing for market sale and certification, therefore, concern numerous materials and multiple uses. Following the EU example, there are at least seven types of seeds: protected varieties registered in the catalogue; varieties registered in the catalogue that are not protected; old varieties no longer registered in the catalogue; traditional varieties registered in the catalogue of conservation varieties; traditional varieties not registered in the catalogue of conservation varieties; seeds from protected farm varieties; and seeds from farm varieties that are not protected and are registered in the catalogue.

For each of these varietal types, there are many possible options that are mutually inclusive in terms of access and utilization:

Can they be marketed? Is registration in the catalogue required or not? Who can market them? In France, for example, only the owners or suppliers of varieties registered in the catalogue can market them. A farmer cannot sell any variety that he has improved if it is not registered in the catalogue.

What are the conditions to ensure seed exchange between farmers? In France, a country that has a legal framework, one of the most limiting factors for farmers is that the exchange of seeds is prohibited, regardless of whether they are protected, unprotected, traditional or local or whatever!

Who can improve plant varieties and with what material? *A priori*, the entire world can improve a plant using existing seeds, including those protected by a PVP. However, the utilization of an improved variety is limited. The improved variety has to be registered in the catalogue in order to be marketed. Otherwise, the variety can only be used by plant breeders and cannot be given to other farmers (even free of charge), except for the purpose of developing new plant varieties.

What conditions are required in order to be able to produce and utilize seeds from a farm? Can they be utilized for profit or not for profit? At global level, the conditions for farm seed production have become increasingly difficult in recent years. The EU and France, for example, opted for the hard line of the 1991 UPOV Convention that stipulates the obligation to pay profits to breeders and also prevents farmers from exchanging seeds, regardless of the type of seeds. Even within the programmes to develop new plant varieties, these requirements apply to all seeds/varieties and at all levels, from farmer to private sector. In the framework of a breeding programme, conservation varieties can be used as inputs. The level of investment for developing new plant varieties (empirical or using state of the art biotechnology) will depend on the levels of return on the investment and then on market size and/or the existence of public subsidies to promote them.

For farmers, the possibility of selecting seeds and developing new varieties is essential and has been essential for thousands of years. This analysis raises the following questions:

Who is the selection for? Is it for oneself or for a group?

Why select? Given the absence of a commercial variety adapted to a specific niche market demand, the applicants lack the financial means to buy seeds and expensive agricultural inputs etc.

How will the selection be organized? What if I improve or obtain new varieties just for myself? If I exchange with my neighbours or mobilize other stakeholders, from public or private research for breeding purposes, do I depend on a framework linked to the values of private marketing or to social values of innovation? In this context, participative breeding is an appropriate model to link public and private stakeholders (primarily farmers) and to share technology in the field of genetics and molecular biology.

When plant varieties are selected by and/or with farmers, different aspects should be considered. Does selection involve public research or not? Do criteria for commercialization and varietal exchange apply? These criteria relate to farmer breeders, rather than to seeds from previous farm harvests or obtained from reproducing their own seeds.

Farmers select on their own or within the framework of a participative programme if the available varieties are not suitable, either because they are too fragile or because they are poorly adapted to their objectives. Selection is generally governed by the user (or users). However, there are budgetary constraints. Selection should not be too expensive in relation to expected future profits. The organization of selection for and with farmers depends on the farmers' objectives and the national institutional constraints. In general, farmers involved in this process are not geared towards the international market. In the case of participative breeding, the work to obtain new "improved" varieties from traditional varieties is conducted within a clearly defined framework. The farmers may face high investment costs: cost-sharing within the framework of a participative breeding programme; time spent by the farmer; and mobilization of plots for the project. The compulsory registration in a catalogue and the strict conditions of registration mean that at present, in France, varieties that are modified in participative breeding projects do not comply with the criteria for approval. Consequently, they cannot be sold or, in theory, exchanged.

In the EU, some aspects of flexibility have been identified, particularly in Germany, where farmers' clubs have gained recognition. There are also similar initiatives in France (Moÿ, 2010). Club members have access to varieties developed "collectively" (this leads to a new area of analysis), a return on the common assets of a club. As a result, this analysis can make reference to the club's common varieties, which are developed collectively and with collective rights. Thus, implementing a registry of this type is conceivable at global level (FAO, ICRISAT etc.). It would make it possible to identify the varieties developed in these projects and their characteristics. This would bring them institutional recognition without necessarily providing legal protection.

Alternative soft law regulatory frameworks for protecting genetic resources

Some alternative solutions can also be implemented or promoted by groups of stakeholders (farmers, rural communities, public or private researchers, cooperatives, processors, traders, consumers etc.) involved in the use and exchange of genetic resources and in adding value to the products obtained from these resources.

The impact of these solutions depends on many factors, including the involvement of numerous stakeholders on a large scale and recognition by other stakeholders. In fact, certain proposals are sometimes blocked because it seems that they cannot be applied at global level: they lack the mechanisms for political influence to obtain recognition; they reveal gaps at legal level; they do not include all the issues relating to the management of genetic resources or traditional knowledge, which makes them ill-adapted to these specific cases.

- Globally Important Agriculture Heritage Systems – GIAHS (FAO–UNESCO)

Globally Important Agriculture Heritage Systems (GIAHS) seek to promote and conserve specific ecosystems and agricultural landscapes that have been shaped over time by different generations of local inhabitants (farmers, herders, fishermen etc.), who have developed original practices and techniques adapted to the local contexts and still used today. These systems take into account the numerous and complex interactions between species and the human practices that contribute to the development and maintenance of agricultural and associated biodiversity.

GIAHS within the UNESCO World Heritage framework has brought recognition to the sites identified, both for the resources conserved and the associated practices, thus revealing the importance of agrobiodiversity for the creation and maintenance of these agricultural landscapes. However, this recognition is not a tool for legal protection linked to the management of plant genetic resources. This recognition attributes a value to a defined geographical area, which in turn enables the promotion or development of agrotourism in these territories. In order to attribute a value to these systems within a sustainable production process, recognition gives a level of protection similar to that provided by geographical indications or collective frameworks, with the aim of obtaining world agricultural heritage identity in the different markets. As previously mentioned, this recognition does not provide protection for the basic agricultural varieties in relation to the agricultural practices that have developed with the history of agrarian societies. Consequently, basic plant genetic resources are not taken into consideration.

- Biocultural landscapes

In line with the GIAHS approach, the UNESCO World Heritage Treaty in 1992 enabled the recognition and protection of **cultural landscapes** that are created by interaction between humans and the environment, and which are an expression of the broad and intimate relationship that people have with their environment (UNESCO, 2013). Some cultural landscapes are linked to specific techniques of land use that guarantee and maintain biological diversity. Others are linked to beliefs, artistic practices and established customs that bear testimony to man's exceptional spiritual relationship with nature.

UNESCO promotes three categories of cultural landscape:

- Landscapes that are essentially evolving are those that have a social role and can be subdivided into two categories: living landscapes that continue to evolve; and relic landscapes, where evolutionary processes are non-existent.
- Associated cultural landscapes that result from the association of cultural, artistic or religious phenomena associated with the environment.
- Landscapes that are clearly defined and created voluntarily by man, such as parks and gardens.

The protection of cultural landscapes makes it possible to develop new sustainable land-use techniques, improving the natural values of the landscape. Therefore, they are useful for the conservation of biodiversity.

Consequently, in the case of quinoa, cultural landscapes are integrated with agro-ecosystems to varying degrees. Therefore, cultural landscapes interact directly with human practices in relation to the use and in situ conservation of quinoa's genetic resources and the traditional knowledge linked to the resources of biodiversity. On the other hand, they do not depend on processes of protection, valorization and the fair and equitable sharing of the utilization of these resources and knowledge. Thus, cultural landscapes constitute a tool adapted to the partial conservation in situ of quinoa's genetic diversity.

They do not constitute tools to conserve quinoa's genetic diversity in its entirety, nor to guarantee fair and equitable benefit-sharing with the countries of origin of these genetic resources. Nonetheless, these systems encourage recognition of the identity of the human practices developed in relation to specific environmental conditions, and promote values (sociocultural) that are distinct from purely monetary values.

Recognition of cultural landscapes (hence, of agroecosystems) ensures maintenance of the agrobiodiversity developed by farmers who adopted sustainable agricultural management practices over time, and guarantees the in situ conservation of quinoa's genetic resources. Nevertheless, cultural landscapes dedicated to conservation should be open to new knowledge and techniques and to the exchange of genetic resources.

- Open source seed licences

The open source seed licence (OSSL [3]) is the direct transposition to the seed sector of a concept initially developed for computer programmes. According to the OSSL concept, plant varieties and seeds are considered common goods in the public domain to be shared free of intellectual property rights.

Primarily, this system incorporates the varieties derived from participative and/or traditional breeding with a broad genetic base. These are well adapted in terms of their environment and the potential effects of global climate change. They include the traditional quinoa varieties cultivated in the Andean zone.

In an OSSL, the varieties mentioned do not have to comply with requirements of novelty, distinction, uniformity and stability, since they are not in the classic circuit of intellectual protection via a PVP, patent or regulation through registration in an official catalogue of cultivated varieties (Deibel, 2013).

The OSSL is complemented by the concept of "copyleft" ***[4]***, which prevents a third party from appropriating the initial variety after a slightly modification, and on top of that, OSSL maintains the improved modified variety in the system covered by the same rights and regulations (Kloppenburg, 2010).

The promoters of OSSL also propose a licence or model contract in which the beneficiaries agree to provide some free seeds produced from the variety acquired under the scheme. A licence is signed and information on all the cropping practices used is made public. The basis of integrating the copyleft concept also requires that the genetic improvements obtained should be made public. Lastly, by virtue of this licence or contract, the main objective of which is to free up access to varietal genetic resources, the contracting parties agree not to use the seeds to produce genetically modified organisms (GMOs).

Some people also propose associating the OSSL with the philosophy of open/free data in order to promote and preserve the traditional knowledge associated with traditional or modern varieties and to enable free access to the genetic sequences of these varieties to avoid patent applications. However, this scheme also has its weaknesses. Mechanisms should be developed to protect the OSSL from patent registration for specific functions in relation to plants' genes.

If the OSSL is to function properly, a wide seed exchange network must be created to encourage open exchanges between local communities, so that farmers, researchers and other stakeholders involved in varietal improvement can have access and work using open source licences.

In conclusion, the OSSL and the concepts mentioned encourage the free circulation of traditional and/or modern varieties to ensure continued innovation and improvement. Consequently, the OSSL could be an important tool for preventing a third party from appropriating a variety through a patent or PVP.

It is, therefore, an open framework that simultaneously promotes production, seed reproduction and innovation. Consequently, it can be associated with the protection of know-how and knowledge relating to the genetic materials that are freely accessible.

It is important to note that the genetic resources from the wild relatives of cultivated quinoa, as well as the traditional knowledge associated with the agricultural practices in the public domain, are included in the CBD's regulatory framework. In this regard, the OSSL only partially includes raw genetic resources and very few wild genetic resources.

Lastly, as in other systems, it is difficult to guarantee the monitoring/traceability of exchanges and the future utilization of quinoa's genetic resources to ensure that the OSSL functions properly and achieves its fundamental objectives.

Conclusions

Questioning the management of genetic resources based on the case of quinoa involves an examination of the diverse situations that arise from: the geographical origin of the genetic resources shared between various countries; the current dynamics of the global expansion of quinoa cultivation; and its multiple potential uses.

The current situation relating to genetic resources – under state sovereignty since the adoption of the CBD in 1992 – provides a specific legal framework for access and exchange that have a strong impact on use and innovation.

The main conclusion drawn from this comparative analysis is that, at the moment, there is no single existing legal framework perfectly covering all the issues related to the genetic resources and their sustainable management. This calls for an examination of the complementarity of existing legal frameworks, their potential overlaps and the possibilities of harmonization for the future.

Different regulatory instruments apply at different levels (local and international), for different purposes (genetic resources, varieties and seeds, landscapes, agricultural by-products etc.). The aim of this paper was to reflect on how the different issues at stake can be integrated, taking into account the limitations of these regulatory instruments.

An analysis of the norms and regulations related to genetic resources in the agricultural sector, particularly in the case of quinoa, involves identification of the various systems for food security.

The changing conditions for access to seeds and the options available to make the seed sector more effective and adapted to agriculture's diverse requirements will, inevitably, also depend on national public policies for developing an effective seed market capable of meeting the challenges of the international year of quinoa. This includes primarily: recognizing the work of the Andean peoples in the selection and conservation of local quinoa varieties; and maintaining and adding value to quinoa's biodiversity for the benefit of world food security and poverty reduction.

Inevitably, this process of reflection will involve in-depth dialogue between all the stakeholders (managers, users or legislators) involved in managing quinoa's genetic resources. No single solution is adapted to all the situations that arise. Thus, the stakeholders will have to either consider a new legal regulatory framework based on existing ones, or develop a completely new framework, based on compromise, with the aim of integrating the diverse points of view concerning the management of quinoa's genetic resources.

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[1] ADPIC, Part II, third paragraph relates to Geographical Indications.

[2] For example, some countries, like the United States of America or South Africa have no specific legislation for protecting geographic designations, the same that then pass for other mechanisms, like consumer protection, brands or the fight against falsifacation, through the usurpation of a designation (passing off: Kalinda, 2010).

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[4] *Copyleft* is a general method to make a programme free (or other kind of work). The requirement is that all the modified versions and related extensions are also free. Concept created by Richard Stallman in 1983.

CHAPTER 1.7.

Voluntary payments for the conservation of quinoa diversity: exploring the role of payments for ecosystem services in the Andes

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Abstract

Quinoa farming in the Altiplano has become more market oriented compared with its traditional role as a subsistence crop. This has resulted in the increasing marginalization of many quinoa landraces and therefore in a loss of quinoa diversity. From an economics perspective, the maintenance of socially desirable levels of agrobiodiversity requires the implementation of mechanisms that provide farmers with the incentive to conserve these quinoa landraces. Payments for Environmental Services (PES) are such mechanisms, but they have yet to be developed in the context of agrobiodiversity conservation. The aim of this chapter is therefore to analyse the potential of payments for agrobiodiversity conservation services (PACS) instruments, in particular in the context of quinoa farming. The impact of different PES-type approaches on quinoa diversity conservation (cost-effectiveness) is analysed together with their interactions with collective action. In particular, the chapter focuses on two types of reward system: competitive tenders and fixed-price payments. Key issues addressed relate to the design of these PES and the effect of context on their effectiveness. Experiments were run in two study sites – a Peruvian site where farming systems are mainly subsistence-based and a Bolivian site where farming systems are more commercialized – to permit a comparison across market contexts. The results show the following:

- The conservation goals and targeting rules chosen for the competitive tender significantly condition the scheme's performance and as such cost-effectiveness and equity trade-offs.
- The way a fixed-price payment is designed impacts farmers' behaviour and therefore the conservation outcome.

[1] *"The findings, interpretations, and conclusions expressed in this paper are entirely those of the authors. They do not necessarily represent the views of the International Bank for Reconstruction and Development/ World Bank and its affiliated organizations, or those of the Executive Directors of the World Bank or the governments they represent".*

- Fixed-price payments have different impacts depending on the market context and on existing collective action institutions.

Introduction

The tenth meeting of the Conference of the Parties of the Convention of Biological Diversity (CBD) held in Nagoya, Japan, in 2010 adopted a new Strategic Plan for Biodiversity including the 20 Aichi Biodiversity Targets, one of which (Target 13) highlights the importance of conserving biodiversity in agricultural landscapes (http://www.cbd.int/sp/targets). Agricultural biodiversity (henceforth agrobiodiversity) is the basis of human survival and well-being, contributing importantly to sustainable agriculture, food security and a wide range of ecosystem services.

Despite this, diversity at ecosystem, species and genetic level is increasingly and more rapidly lost from many production systems throughout the world leading to genetic erosion and vulnerability (among others, FAO, 2009). As farming systems become more commercialized and industrialized, also as a result of the green revolution, agro-ecosystems are increasingly characterized by a high level of intensification with low levels of diversity (Thrupp, 2000; Jackson *et al.*, 2007). This is mainly because a wide range of local plant and animal genetic resources (PAGR) are being replaced by a small number of commercially profitable ones, as markets tend to create a bias towards the latter by not fully capturing the total economic value of agrobiodiversity due to the public good characteristics of many of its services (Drucker *et al.*, 2005; Narloch *et al.*, 2011a).

Peasants in the Andes have a long history of farming, reaching back almost 7 000 years, and they are used to managing great diversity in traditional food crops, such as maize, potatoes and quinoa. Nevertheless, decreasing use of some traditional crops has been observed in the region (Velasquez-Milla, 2011). There is growing demand for minor crops from developing countries, as consumers in industrialized countries seek to satisfy specific tastes, improve nutrition or contribute to rural development. For example, quinoa is being heralded as an organic fair-trade crop, thus gaining popularity among consumers in the Western world. Quinoa farming on the Altiplano has therefore become more market oriented compared with its traditional role as a subsistence crop. This has resulted in the increasing marginalization of many quinoa landraces and an ongoing loss of traditional agricultural knowledge (Canahua *et al.*, 2002; Laguna, 2002; Rojas *et al.*, 2004, 2009).

The loss of agrobiodiversity is expected to have far reaching consequences, especially for the livelihoods of poor indigenous farming communities (Gruère *et al.*, 2009). Such communities play a key role in the conservation of species, varieties or breeds with unique adaptive traits (e.g. disease resistance, drought tolerance) bred over thousands of years of domestication across a wide range of environments. At the same time, agrobiodiversity conservation and use provide a mixture of benefits: private benefits to the farmer (e.g. through their largely private direct use values); local public benefits to the farming community (e.g. through their indirect use values, such as contributing to risk management, agro-ecosystem resilience, maintenance of soil and water quality, maintenance of indigenous knowledge and sociocultural practices); and national and global public benefits (e.g. maintenance of evolutionary processes and option values, as well as non-use values such as existence values). Insurance values play a role in contributing to ecological stability and resilience (Baumgärtner, 2007), while option values permit the maintenance of material resources and knowledge (Bellon, 2008). As markets capture only a part of the value of these resources, thus underestimating their true worth (Gruère *et al.*, 2009), distortions result where any trade-offs that must be made between growth and biodiversity conservation tend to favour the former, regardless of the increasing scarcity of the latter (Pearce and Moran, 1994; Drucker, 2007).

Agrobiodiversity and collective action

El The impure public goods nature of agrobiodiverse resources has led many poor farming communities to make use of institutions of collective action in order to manage PAGR and complementary inputs (e.g. land) collectively (Eyzaguirre and Dennis, 2007). Ostrom (1990) has shown that, under certain conditions, rural communities are able to self-organize in order to manage natural resources for reaching common goals; while, among others, Nagarajan et al. (2008) provide a specific example of how the collective efforts of producer groups have had a positive impact on minor millet conservation in India.

In the agro-ecological context of the Andean Altiplano, an interesting example of collective action can be found associated with the traditional crop rotation practices undertaken on community lands, known as *Suyo*. A group of farmers - sometimes the whole community - decides collectively which plots within a communal land area are to be planted with a certain crop species. Each farmer then individually manages his/her piece of land according to these group-level decisions (Canahua *et al.*, 2002). In support of these strong collective action institutions, communities in the Altiplano have developed complementary ways of co-managing their farming systems, for example through the exchange of labour, germplasm and agricultural equipment (VSF, 2009). Furthermore, many farmers have arranged to receive technical assistance and to participate in markets.

Instruments to conserve quinoa's biodiversity: the Payments for Environmental Services

It may, however, be questioned whether collective action institutions are enough to confront the loss of quinoa biodiversity induced by the growing global demand for a narrow set of quinoa varieties. From an economics perspective, the maintenance of socially desirable levels of agrobiodiversity requires that, where significant public good values exist, these should be recognized and mechanisms put in place to permit the "capture" of those values by the farmers who incur the conservation costs. Such mechanisms would provide farmers with the incentive to conserve that which benefits wider society. While Payment for Environmental Services (PES) schemes are one such mechanism and have been hailed by some observers as "arguably, the most promising innovation in conservation since Rio 1992" (Wunder, 2005), there has been almost no explicit consideration of PES in the context of agrobiodiversity conservation and only limited consideration of indigenous farmer contexts (Narloch *et al.*, 2011a).

Such so-called "payments for agrobiodiversity conservation services" (PACS), a subcategory of agriculture-related PES (see Narloch *et al.*, 2011a), would seek to tackle market price distortions associated with the public good characteristics of genetic diver-sity. This may be achieved through the use of (monetary or in kind) reward mechanisms in order to increase the private benefits from local PAGR so as to sustain their on-farm utilization. It is hypothesized that the rewards associated with PACS instruments can be designed in such a way as to create incen-tives to act collectively in order to contribute to the conservation goal and receive rewards. By contrast, it is also possible that PACS schemes, if not appro-priately designed, could undermine existing institu-tions of collective action in poor farming communi-ties. PES schemes should therefore be studied and designed carefully prior to implementation in order to be ecologically effective, economically efficient and socially fair.

Research focus

This chapter analyses the impact of different PES-type approaches on quinoa agrobiodiversity conservation (cost-effectiveness) and explores their interactions with collective action. In particular, it focuses on two types of reward system: competitive conservation tenders and fixed payments. It reports the results from two experimental studies conducted with Andean farmers between 2010 and 2012. In the first study, a conservation tender through which community-based groups (CBGs) applied for conservation contracts by defining their participation conditions (including the required payment level) is implemented to provide incentives to farmers to protect agrobiodiversity. In the second, framed field experiments in which farmers make hypothetical decisions with regard to the cultivation of different crop varieties are conducted to test the effectiveness of different types of fixed-price payments for agrobiodiversity conservation.

Key issues addressed in this chapter relate to the design of PES as well as to the effect of the context on PES' effectiveness. In particular, it focuses on the following:

- How should tender schemes be designed to provide farmers with incentives to act collectively and undertake public good agrobiodiversity conservation activities?
- Should fixed payments (rewards) be collective or individual? And how should collective payments be shared within communities?
- Do rewards have the same impact in different contexts?

Experiments were run in two study sites – a Peruvian site where farming systems are mainly subsistence-based and a Bolivian site where farming systems are more commercialized – to permit a comparison across market contexts (both sites are shown in Figure 1).

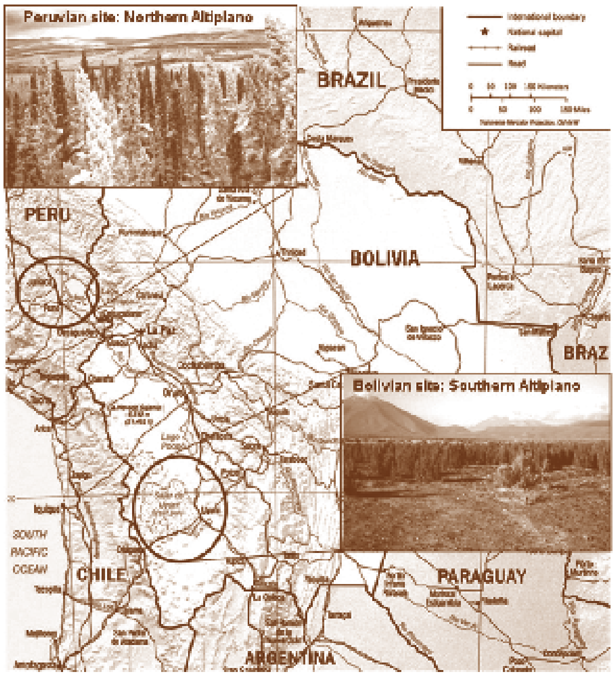

Figure 1: Bolivian and Peruvian study sites on the Andean Altiplano - *Source: Narloch (2011) based on map from US CIA (2006)*

Competitive tenders to conserve agrobiodiversity

This section investigates the potential of competitive tenders for conserving quinoa biodiversity using framed field experiments. In particular, it focuses on: (i) What should be targeted and how does targeting impact equity? (ii) What type of pricing should be used (discriminatory vs uniform)?

Background on competitive tenders

Competitive tenders are used to award conservation contracts to those land users who can provide conservation services at the least cost and thus require lower compensation payments. These sorts of reverse tender are a means by which to tackle the existence of information asymmetries (Ferraro, 2008). Due to the competitive process, the scope for rent-seeking behaviour is limited, as farmers have an incentive to bid for contracts close to their real opportunity costs (Latacz-Lohmann and van der Hamsvoort, 1997).

Conservation tenders in general have proven to be more efficient in generating conservation services than fixed-price programmes, whereby a uniform price is offered for a pre-defined conservation activity. That said, the transaction costs of running conservation tenders can be relatively high, since the conservation agency has to coordinate invitation, bidding, selection, contracting, verification and delivery of payments to a number – of possibly dispersed – land users. However, dealing with groups of land users can reduce transaction costs and foster self-organization skills in communities. Moreover, group-level approaches may be more appropriate in contexts where land use is based on customary rights established on community lands accessible to a larger group of land users.

There are a growing number of conservation tender examples through which farmers apply for compensation payments for setting land aside for conservation purposes (Latacz-Lohmann and Schilizzi, 2005). Despite the potential in terms of informing the targeting of conservation payments by estimating possible environmental and social outcomes *ex ante*, there is very limited application of tender approaches in developing countries as part of PES programmes (Jack *et al.*, 2009). This section presents the results of a tender implemented in the Andes and provides insights into the design of conservation tenders.

Project implementation

A group-level agrobiodiversity conservation tender process to award PACS contracts and payments to farming community-based groups (CBGs) was implemented. It was based on a first-price (i.e. CBGs could only prepare one offer) and sealed-bid (i.e. CBGs would not know about competing offers) reverse procurement tender (Latacz-Lohmann and Schilizzi, 2005) approach. Representatives from 18 Bolivian and 20 Peruvian CBGs were invited to submit proposals for the conservation of previously identified priority landraces. The invited CBGs came from four Bolivian and five Peruvian districts, covering different zones within the two study sites. Whereas in the Bolivian tender the focus was on communities with a long history of quinoa farming, in the Peruvian tender quinoa-based production groups were invited.

Between March and May 2010, those CBGs interested in participating in the tender were assisted by local extension experts in the preparation of their bids following a consultative process with the CBG representatives. The final bid offer included (for each of the chosen priority landraces): (i) the total conservation area; (ii) the number of farmers to take part in the conservation activity; and (iii) the bid price per conservation land unit. CBGs were also asked to define their preferred participation mode, choosing between accepting conservation contracts only if all their landrace bid offers were selected (conditional participation) and accepting conservation contracts for any of the landraces from which their bid offers were selected (partial participation).

The CBGs were advised that payments would be made in kind and representatives could freely choose their in-kind payment type, such as agricultural equipment, inputs (e.g. seeds), and construction or school materials. Participating CBGs were informed that winners would be selected on the basis of "bid value", i.e. those who could offer the greatest conservation service in terms of area and farmer numbers per conservation cost. Bids were received from 13 Peruvian CBGs and from 12 Bolivian CBGs.

Targeting and design of the tender

The targeting of payments determines distributional outcomes, i.e. who gets how much for what. Many authors have highlighted the potential of PES as a multipurpose instrument, with their design guided by different motivations, such as reducing poverty and local inequities (e.g. Grieg-Gran *et al.*, 2005). Yet, as it is widely argued that PES's primary emphasis should be on conservation goals (Wunder, 2007), it may be that socially desirable goals need to be traded off or even that existing inequities are exacerbated (Corbera *et al.*, 2007a, b). Nonetheless, targeting payments on efficiency grounds only, while ignoring fairness considerations in the distribution of payments, may erode the legitimacy and sustainability of such interventions, so that social and conservation goals are intertwined (Pascual *et al.*, 2010; Muradian *et al.*, 2010). Consequently, equity considerations are extremely relevant in the growing application of PES in communities that share strong norms of fairness. Drawing on data from the agrobiodiversity conservation tenders, a number of targeting approaches are assessed with regard to their cost-effectiveness in terms of different conservation goals, as well as their equity impact.

Narloch *et al.* (2011b) ranked the bids with regard to their cost-effectiveness in terms of three conservation goals: (i) cultivated land *area* under a specific priority landrace as a proxy for the seed production and maintenance of genetic diversity in the field; (ii) the number of *farmers* conserving such landraces as proxy for the maintenance of local agricultural knowledge and cultural traditions; and (iii) the number of participating *CBGs* as proxy for the maintenance of informal seed exchange networks and, hence, gene flow across communities. Combined rankings were also considered, with a weighting of 40% for ranks from (i), 40% for ranks from (ii) and 20% for ranks from (iii) found to reach the best compromise solution of balancing conservation area, farmers and CBGs.

For the selection of bid offers, an iterative process was followed for each of the targeting approaches under consideration, whereby the highest ranked bids per landrace were selected, while seeking to distribute the conservation funds as equally as possible among the landraces, until no further bids could be selected without exceeding the budget of USD4 000. This selection process can be subject to alternative targeting rules, which can incorporate different equity principles as explained in Narloch (2011). Firstly, a discriminatory pricing rule may be applied, whereby the payment per land unit equals the indicated bid price, which may vary between CBGs. In line with a proportionality principle, groups would be compensated for the costs they incur under the conservation programme. Second, a uniform pricing rule may be applied, whereby every selected CBG would receive a payment according to the highest accepted bid price per landrace (Ferraro, 2008). Such a non-discrimination principle would be relevant where local resource users consider it as highly unfair when different payments are made for the provision of conservation areas of the same size.

Whereas these two approaches assume partial participation resulting from an assessment of bid offers on a landrace-by-landrace basis, a third approach may be applied, accounting for the preferred participation mode defined by the CBGs. Where CBGs indicated conditional participation conditions, their bid offers for the different landraces must be assessed as a package. Such an approach may be considered to concur with procedural fairness principles, whereby groups set their own participation conditions.

The combination of the aforementioned four conservation goals and three targeting rules results in a set of 12 targeting approaches. As conservation area, number of farmers and number of CBGs serve as delivery proxies for the provision of specific conservation services (as explained above), they all measure cost-effectiveness in terms of their underlying conservation goals (Narloch *et al.*, 2011b). At the same time, the targeted number of farmers represents collectivism as grounded in a common goods principle, while the number of targeted CBGs would measure the inclusiveness of the scheme in a context in which CBGs have incurred time and effort to prepare their bids and thus may find it unfair to not receive any compensation at all (Narloch, 2011: chapter 7). In addition, the Gini index measures the inequality in the distribution of the payments. Therefore, the four performance indicator variables used represent three different cost-effectiveness criteria and three equity principles.

Results

Results from the bid offers received identify significant cost-effectiveness trade-offs between alternative agrobiodiversity conservation goals and their associated conservation services. There appears to be a non-complementary relationship between maximizing the conservation area and the number of conserving farmers, since area-based targeting approaches would result in significantly smaller numbers of farmers and vice versa. Neither area-based nor farmer-based targeting would be closely connected with maximizing the number of targeted groups. Optimizing cost-effectiveness with regard to the conservation area or number of farmers would also be associated with a highly unequal distribution of payments. Further trade-offs can also be identified when taking fairness considerations into account, namely those between efficiency and equity (Narloch *et al.*, 2011b).

Overall, it seems that targeting rules accounting for uniform pricing or preferred participation mode underperform compared with discriminatory pricing rules. This is because the latter two pose a binding constraint on the targeting of payments, whereby generally fewer CBGs (and thus fewer farmers) would be targeted, so that payments are distributed highly unequally and smaller conservation areas are attainable, as explained by Narloch (2011: chapter 7). This would imply that non-discriminatory or procedural fairness principles would need to be traded off against cost-effectiveness, as well as equity principles based on collectivism, inclusiveness and equality (Narloch, 2011).

As a result, the conservation goals and targeting rules taken into account in the targeting process would significantly condition the scheme's performance and, as such, cost-effectiveness and equity trade-offs. Targeting approaches based on combined goals and discriminatory pricing rules (reflecting proportionality principles) would not only result in the most equal distribution of payments and relatively high number of CBGs and farmers taking part in the conservation activities, but would also only be related to only very modest efficiency losses in terms of conservation area.

Fixed-price payments to conserve agrobiodiversity

This section investigates the potential of fixed rewards for conserving quinoa biodiversity using framed field experiments. In particular, it focuses on: (i) whether different contexts impact the rewards' effectiveness; and (ii) the effect of each type of reward on conservation, collective action and how they interact with social preferences.

Background on field experiments

It has been widely acknowledged that resource users often do not behave in an economically rational way when they face social dilemmas, so that neoclassical theory predicting purely selfish behaviour fails and scholars need to look beyond *homo-economicus* (Gintis, 2000; Henrich *et al.*, 2001; Anderies *et al.*, 2011). Behavioural economists have applied framed, game-theory-based experiments in which participants make hypothetical decisions in the face of different pay-off scenarios. A framed field experiment is a conventional laboratory experiment with the relevant subject pool, undertaken within a field context in the resource task or information set available to the subjects (Harrison and List, 2004). The experimental data provide insights into social preferences for individual and group benefits and thus into behavioural dynamics.

In particular, the application of framed field experiments can provide valuable insights into the multiple layers (individual, group and incentive level) relevant to understanding collective action in conservation (Cárdenas and Ostrom, 2004) and the pathways through which conservation behaviour is affected by external institutions. To learn about people's preferences and decision-making in real resource and group contexts, research in real field contexts is needed (Cárdenas, 2000; Velez *et al.*, 2010). There is a growing body of literature analysing cooperative behaviour in the management of natural resources in field-framed experiments conducted in developing countries (Cárdenas and Carpenter, 2008), but no application in the context of agrobiodiversity conservation.

Experimental design and protocol

Two series of field experimental games took place in Peru and Bolivia between 2010 and 2012. The phase I games were held between February and April 2010. The games were constructed to analyse the impact of two reward systems on conservation and their interaction with farmers' social preferences. The results led to a second series of experiments in Peru in September 2012 (phase II games), which aimed to study both the robustness of the previous results and a third type of reward to better understand how rewards can lead to collective action. The full experimental design and results are reported in Narloch *et al.* (2012) and Midler *et al.* (2012).

It is in this context that an (impure) public good game was framed around decisions between different quinoa varieties (Narloch, 2011). Each participant was part of a group of four players and had a number of fixed land units (4). Over 12 rounds, participants decided how many land units to allocate to the conservation of a threatened variety. As market prices for such varieties are lower than for commercial varieties, the farmer incurs private conservation costs equivalent to 10 points. Yet, the cultivation of the threatened variety is associated with public conservation benefits (4 points) that accrue to every group member once a certain threshold is reached (in this case defined as the group altogether conserving seven land units). Six rounds of a baseline game were played, before introducing economic incentives for conservation and playing six additional rounds with one of the following:

- An individual reward: each farmer receives 4 points more for each land unit allocated to conservation.
- A collective egalitarian reward: each farmer receives 1 point more for each land unit allocated to conservation in the group if the threshold is reached. This reward corresponds to a group reward shared equally within the group, with no consideration of individual effort.
- A collective proportional reward: each farmer receives 4 points more for each land unit allocated to conservation if the collective threshold is reached. This reward corresponds to a group reward shared proportionally on the basis of individual efforts. Both collective rewards therefore differ in the way they are shared among farmers.

The amount of each reward was determined so that they were equivalent from a budget point of view.

The social optimum, i.e. where the group's total benefits are maximized, is reached when all the group members allocate all their land units towards conservation. However, a social dilemma arises from the participants' private incentive not to conserve and instead to free ride on the others. Only when expecting the group peers to conserve a certain number of land units would it be rational to conserve one or two land units and allow the threshold to just be reached (thereby moving from no public benefits to a situation where everyone receives 4 × 7 points in public benefits). With external rewards, the set of optimal private strategies would include the conservation of more land units depending on the expectations of others' behaviour, but there would be no dominant strategy allowing the social optimum to be reached. The effectiveness of the reward and communication was then assessed by analysing the change in level of conservation between phase I and phase II.

Four experimental game sessions were organized in Bolivia and 14 in Peru. Each session was organized

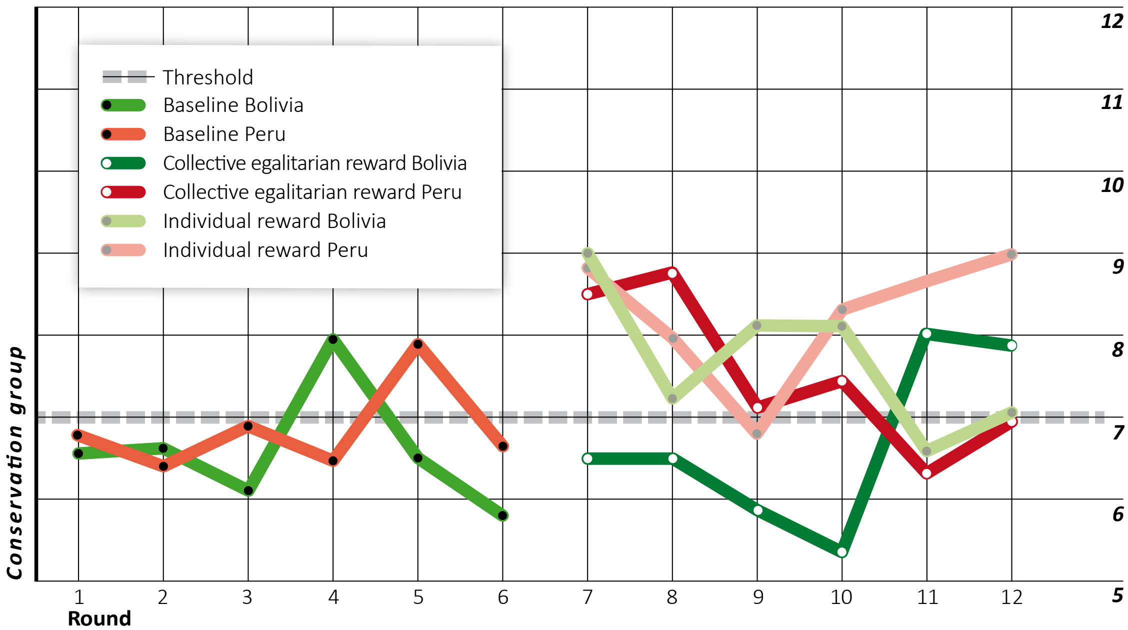

Figure 2: Average group contribution over the 12 rounds, differentiated by country and type of reward

with 16–20 participants from quinoa-based farm households in the same (or neighbouring) communities, which were selected from different zones within the two study sites so as to maximize the representativeness of the sample. Following the experimental game, a brief survey with questions about household demographics, quinoa farming, organizational affiliation and informal connections with other households was completed.

Results

Effect of the context on conservation level

Figure 2 shows the average group contribution over the 12 rounds, differentiated by country and type of reward (only individual and collective egalitarian). It should be noted that the first six rounds are the baseline game - identical in every experimental game in each community, regardless of the treatment that followed.

The behaviour observed in the games indicates that farmers are willing to conserve a certain share of their land units and do cooperate for conservation purposes, as can be seen in Figure 2. Interestingly, collective action appears to result in conservation levels that are close to the defined thresholds – here of seven land units. Generally, it appears that, in terms of conservation, the Peruvian groups outperform their counterparts in Bolivia.

Further empirical analyses (Narloch *et al.*, 2012; Narloch, 2011: chapter 5) provide strong evidence for market orientation significantly decreasing the likelihood of growing non-commercial quinoa varieties in both sites. This supports the hypothesis that growing commercialization results in lower agrobiodiversity conservation levels. Agricultural networks, however, seem to play only a very limited role in conservation decisions. Interestingly, in contexts where collective action institutions are weakened, as in the Bolivian site, the safeguarding of non-commercial varieties seems to be mainly driven by those farmers who still interact in more pro-social environments and follow social norms such as altruism and reciprocity. In contexts where collective action is more robust, as in the Peruvian site, farmers who value the safeguarding of threatened resources per se appear to play a key role in agrobiodiversity conservation.

Also it seems that the impact of both types of reward depends strongly on the context. In the Bolivian site, collective rewards do not seem to have any effect on conservation behaviour, whereas individual rewards create a conservation-enhancing effect in different ways. In the Peruvian site, where farming is more subsistence-based and collective action institutions are more developed, collective rewards seem to directly increase conservation. Possibly, in contexts where collective action is still relatively engrained, group-level payments provide stronger economic incentives to enhance conservation.

Effect of the type of reward

Figure 3 shows the average group contribution over the 12 rounds in Peru, differentiated by the type of reward.

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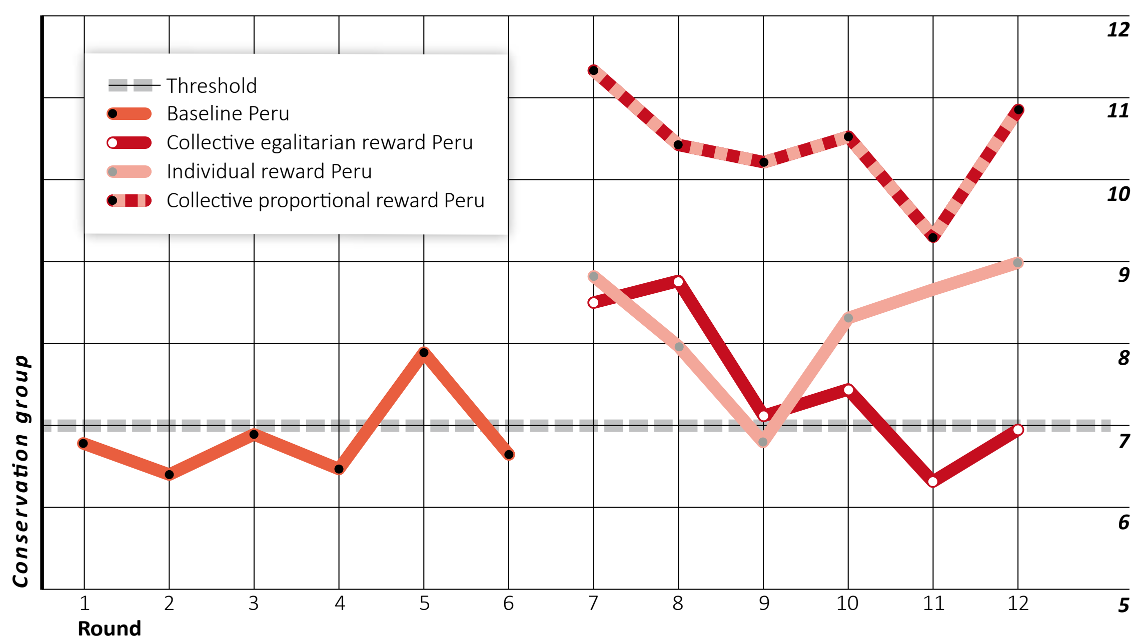

Figure 3: Average group contribution over the 12 rounds in Peru, differentiated by the type of reward

economic incentives to enhance conservation.

Effect of the type of reward

Figure 3 shows the average group contribution over the 12 rounds in Peru, differentiated by the type of reward.

As can be seen in Figure 3, introducing a reward always results in an immediate increase in conservation level. This increase is higher with the proportional reward than with either the individual or the egalitarian one. As the three rewards would involve the same cost per conservation unit for policy-makers, collective proportional rewards seem to be more cost-effective than both other payments at the levels chosen in this design.

The proportional reward combines features from both the individual and the egalitarian reward. First, it is given only when the group collectively reaches the ecological threshold, contrary to the individual one. The fact that individual farmers need to self-organize to become eligible for group level rewards may in itself foster collective action through enhancing bonding and linking social capital. As a result, both collective rewards provide an additional incentive for collective action that the individual reward does not. Second, the proportional reward is based on individual efforts while the egalitarian reward is given to all farmers independently of their conservation efforts. The latter may therefore increase the incentives for free-riding as one can receive the reward without actually conserving. Post-experiment focus groups to understand farmers' decisions during the game suggested that participants did not like the egalitarian reward because their peers could "take advantage of their own efforts without doing something themselves". Farmers may therefore have increased their conservation level less with the egalitarian reward than with the proportional one because they anticipated this free-riding behaviour. (Narloch *et al.* [2012] provide more details on the interactions between rewards and farmers' social preferences.)

To summarize, the way rewards are implemented (collectively or individually) and the way collective rewards are shared within a community may have a strong impact on their effectiveness. The above findings suggest that collective proportional rewards perform best to increase quinoa biodiversity conservation in the Peruvian context. Nevertheless, results also show that the context (marked oriented vs subsistence level farming) and existing collective action institutions may affect the way a reward impacts farmers' conservation behaviour. Also, it is worth noting that PES schemes with collective reward systems may also generate additional socio-economic benefits. There may be reduced transaction costs from working with groups, as opposed to individuals, and cost-saving may then be directed towards higher collective reward levels, which could result in different social dynamics.

Conclusions

The above findings reveal both the potential and the complexity of making PES work for agrobiodiversity conservation in an effective, efficient and equitable way. As is argued in this paper, such incentive mechanisms may draw on a "domesticated" version of payment for ecosystem services (PES). These PACS schemes can also generate rewards for farmers not only for undertaking conservation activities *per se* but also for supporting status monitoring and PACS scheme monitoring and verification services, thereby allowing poor farmers to diversify their livelihood options.

Carefully designing PACS may therefore be the key to effective agrobiodiversity conservation, in particular protection of ancestral varieties of quinoa. With the growing implementation of PES schemes in the field, there is also an urgent need for site-specific research in order to widen the understanding of the ways external reward systems may affect existing resource management practices given various market and group contexts.

Given that, generally, threatened PAGR are located in disadvantaged and remote rural areas in developing countries, the above PACS-based framework may prove to be a useful part of rural development packages and a useful potential tool for policy-makers. Under such circumstances, PACS schemes would need to be designed in a way that takes fairness considerations on board in order not to undermine the long-term legitimacy of such programmes and thus their robustness. As Bowles (2008) notes, "good policies are those that support socially valued ends not only by harnessing selfish preferences to public ends but also by evoking, cultivating, and empowering public-spirited motives." Consequently, before PACS schemes are adopted, a careful assessment should be undertaken of existing social preferences that are of relevance to the success of formal institutions brought from outside the community. Participatory approaches may also be needed during the process to guarantee farmers' involvement in conservation. This is apparent as the experimental game findings indicate that we cannot generally assume that external reward mechanisms would unequivocally provide resource users with the incentives to increase their conservation efforts. Clearly, different reward systems influence different types of resource users in different and complex ways, and thus may differ in their effectiveness depending on the market context.

Acknowledgements

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Section 2

Agronomic and Ecological Aspects

CHAPTER: 2.1

Environmental control of development

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Abstract

The duration of development stages is one of the key determining factors of the adaptation of a species, conditioning adjustment to the growing season, the distribution of photoassimilates, water and nutrient absorption and lastly, the yield achieved. Four factors affect the progression of quinoa development: temperature, photoperiod, hydric status and radiation; the last two variables have been barely analysed in terms of its impact on development and there is documented genetic variability regarding sensitivity for the first two. Temperature is the environmental factor with the highest relative impact on duration of development. Sensitivity to temperature was evaluated for the time to visible floral buds and leaf appearance rate; variability for both variables is associated with characteristics of the original environments, being higher in environments with limited water and low temperatures, indicating that adaptation to short growing seasons is expressed through higher earlyness, partly offset by a higher leaf appearance rate, whilst most late genotypes are found in more humid and warmer environments. Quinoa behaves as a short-day plant and the higher photoperiod sensitivity is expressed in valley genotypes, grown between Argentina and Colombia. At the opposite extreme, those in the southern Altiplano, including Bolivia and north-western Argentina, together with Chilean sea-level genotypes, show little or no sensitivity to this factor in respect of time to flowering. Photoperiod sensitivity is manifested from the early stages of development up to advanced stages of grain filling; there is also variability in the duration of the sensitive period.

1. Environmental control of development and intraspecific variability in sensitivity to environmental factors

Optimizing productivity implies adjusting ontogenesis (the sequence of development stages) in such a way that the crop explores the best environmental conditions (e.g.: favourable temperatures or proper availability of water) and when the unfavourable conditions are unavoidable, minimizing their coincidence with the more vulnerable stages of the crop. Therefore and unsurprisingly, phenology (the influence of environment on ontogenesis) is a most important factor in determining genotype adaptation (Lawn e Imrie, 1994). Ontogenesis can be adapted to the environment through two ways: by breeding through manipulation of the genes that cause sensitivity to the environment or through management of sowing dates and sites (Richards, 1989).

The previous paragraph stresses the importance of variation in duration of development as a key aspect of the adaptation of crops to the environment (Evans, 1993), and this is also valid for quinoa. Knowledge of the environmental factors that regulate duration of development of crops constitutes a key element for predicting their agronomical behaviour and yield in an area of known climate regime (Miralles et al., 2001). The most relevant environmental factors in controlling crop development are temperature and photoperiod, and their relative importance depends on the sensitivity of the plant in each phase (Hall, 2001).

2. Importance of knowledge about development control in quinoa

In a crop cycle, we can distinguish between separate periods characterized by the initiation of specific organs and the pattern of distribution of photo-assimilates. These periods are known as phases or stages, where a phase can be defined as the period spanning two clearly-identifiable development events. These events are often observable at the meristematic level (in the apical or axillary meristem, depending on the crop) and involve changes in organ generation and photo-assimilate distribution. The most important events in the life cycle of an annual crop are: emergence, floral initiation, flowering (usually identified as the date of anthesis, i.e. the appearance of anthers) and physiological maturity. These events are used to determine three major development phases: (i) vegetative phase (between emergence and floral initiation), (ii) reproductive phase (between floral initiation and flowering) and (iii) maturity or grain-filling phase (between flowering and physiological maturity) (Ritchie, 1991). These phases can also be broken down into sub-phases.

Since certain scales used to characterise crop development are based on phenomena such as the appearance of leaves, while others are based on changes in the activity level of apical meristems, a distinction has been made between phasic and morphological development (Ritchie, 1991). The first involves changes in growth stages (succession of phases) and the second refers to the onset and end of the generation of organs within the life cycle of a plant (e.g. the time between the appearance of two leaves).

The duration of the cycle or specific stages of development is one of the most important variables to explain genotype-by-environment interaction patterns for yield (Bertero *et al.*, 2004) or genetic variability in quinoa germplasm collections (Ortiz et al., 1998, Rojas 2003, Curti *et al.*, 2012). The BLUPS - estimators of genotypic effects - for sowing-maturity duration showed a strong positive association with yield (R2=0.88) and total above-ground biomass (R2=0.87) and negative association with the harvest index (HI, proportion of above-ground biomass in grain, R2=0.7) in a network of experiments conducted in the inter-tropical zone (Bertero *et al.*, 2004). When this analysis was conducted by environmental group (environments that have a similar impact on the behaviour of genotypes in terms of yield), the duration of development showed higher variation and better association with yield components in colder environments, while duration were shorter with less variation in high temperature and short-day tropical environments (e.g. Brasilia, Brasil and Gia Loc, Vietnam, Bertero et al., 2004). The genetic component (genotype/ genotype-by-environment, G/GxA) have a relatively high weight for duration of development (1.69) vs. 0.25, 0.89, 0.44 and 0.0026 for yield, grain weight, biomass and Harvest Index respectively, indicating better hereditability of these traits and the possibility of responding to selection (Bertero et al, 2004); with even higher weights for evaluations made in more delimited geographical regions such as North-Western Argentina (R. Curti, com. pers.). On the other hand, the time to floral initiation, 50% a anthesis and maturity have the highest weight in explaining genetic variance and discrimination on the first axis of the main components analysis (which explains 30% of the total systematic variance) for a collection of 1,512 accessions in Bolivia, explaining 78, 87.5 and 56% of the variance, respectively (Rojas, 2003). Similar results were obtained for the Peruvian (Ortiz *et al.,* 1988) and Argentinian collection (Curti *et al.*, 2012). An interesting aspect of this variability is the tenuous association found between phase durations, which suggests that it could be independently manipulated (Risi and Galwey, 1989).

The duration and sequence of developmental phases are the most relevant parameters in controlling the time-dependent dynamics of leaf area generation. For instance, the number of leaves on the main stem is determined at anthesis (Bertero *et al.*, 1999a, Ruiz and Bertero, 2008), the leaf area on the main stem around anthesis and those on branches during the flowering period (Ruiz and Bertero, 2008). While there is a strong coordination between phasic development and morphology, there is no unique relationship, with genotypes that can continue generating leaf area for a longer period after anthesis, with a lesser relative reduction of the leaf area compared with genotypes of similar precocity, and of interest regarding genotypes selection for short crop seasons (Ruíz and Bertero, 2008). The association between the duration of development phases and the start and interruption date (from lowest to highest) in photo-assimilate distribution to quinoa panicle and stems was quantified (Bertero and Ruiz, 2010). Like other crops (e.g. González *et al.*, 2003), active stem growth starts earlier than that of panicles in quinoa, possibly conditioning the competition between these two organs. This information was subsequently used to define the moments for applying growth regulators to enhance photo-assimilate distribution and yield (Gómez *et al.*, 2011). The distinction between developmental stages allowed the identification of the flowering period (between 1st anthesis and end of flowering) as the most important in determining the number of grains in Chilean quinoa genotypes (Bertero and Ruiz, 2008).

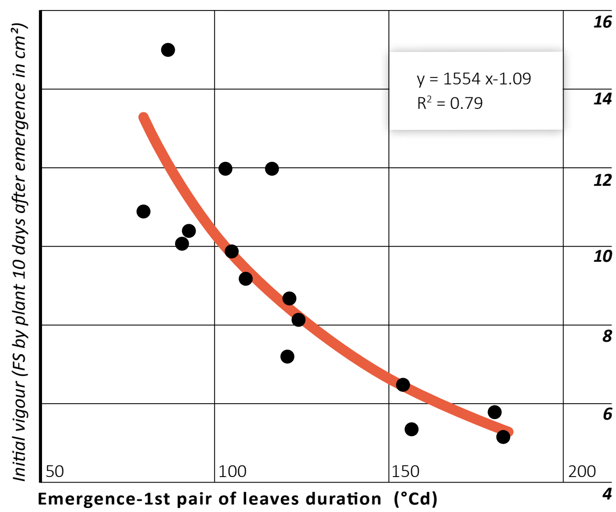

Figure 1: Association between the duration of the emergence-appearance of the first pair of green leaves (in °Cd, bT = 2 °C) stage and initial vigour, measured as the leaf area (FS) by plant 10 days after emergence, for 15 genotypes grown in temperate climates.
Source: Bertero, unpublished data.

3. Development scales

LDevelopment scales are important for quantifying the effect of environment, the association of development with the generation of yield or crop management (identification of periods of tolerance to frost or drought, application of herbicides or periods of tolerance to weeds or application of fertilisers). There are various scales to study quinoa development; and a few are described below. Flores (1977) defined five sub-periods between sowing and physiological maturity separated by four events: emergence, appearance of the first pair of true leaves (marking the onset of leaf area generation), appearance of inflorescence and anthesis. The duration of the second sub-period (emergence-appearance of first pair of leaves) has an average duration of approximately 160 °Cd (base temperature (Tb) = 2 °C) and is used to model the appearance of leaves (Bertero, 2001a). The length of this sub-period shows a close association with early vigour (ability to cover the ground and quickly reach a high growth rate), important for genotype selection (Bertero, 2001b and unpublished data) (Figure 1).

Jacobsen and Stölen (1993) proposed a development scale involving 23 stages, the most relevant events of which are panicle formation, anthesis, floral dehiscence, fruit set and maturity. Unlike other scales, this one includes combinations of development and growth aspects (e.g. the time when a specific panicle width is reached). Bertero *et al.* (1996), based on apical meristem scale observations using stereomicroscope and scanning electron microscopy, generated a development scale that distinguishes between amarantiform (7 stages) and glomerulate type (8 stages) panicles.

When the proposed scores were analyzed using a thermal time scale (Tb= 3.7 and 6.4 °C for the amarantiform and glomerulate scale respectively), they were distributed at regular intervals between stages. In a subsequent analysis Bertero *et al.* (1999a) proposed a division into four sub-periods for the emergence-anthesis period named: Vg (between emergence and floral initiation), Rp1 (between floral initiation and the end of leaf primordia initiation in the apical meristem), Rp2 (between the end of Rp1 and differentiation of stigmatic branches in the apical meristem (G7 on the scale of Bertero et al., 1996)), and Rp3, between the end of Rp2 and anthesis. More than 50% of total leaf primordia were initiated during Rp1. Mujica *et al.* (2001) proposed a scale based on 12 stages for the American and European Quinoa Trial. Lastly, Bertero and Ruiz (2008) used a scale based on external characters (non invasive) and distinguished four phases: emergence-visible floral bud (VFB), visible floral bud-anthesis, anthesis-end of flowering and end of flowering-maturity for the identification of the critical period for yield generation. Variations in a few aspects of these scales were used by García Cárdenas (2003) and Geerts (2008). The lack of precision in the description of the events of the various scales, or the lack of availability of information in easily accessible publications, poses difficulty in establishing analogies between scales (e.g. for determining whether, for example, the stages inflorescence appearance (Flores, 1977), panicle formation (Jacobsen and Stölen, 1993) and VFB (Bertero and Ruiz, 2008) correspond to the same event).

4. Response of phasic development to environmental factors

Quinoa is a plant with a short-day quantitative response to photoperiod (Sívori, 1945, Fuller, 1949)); this implies that duration of some development stages is longer when plants are grown during longer days, but reach flowering in all the range of photoperiods explored. Furthermore, the duration of development is sensitive to temperature and these two factors interact to determine its duration under field conditions (Bertero *et al.*, 1999b). This chapter analyzes existing knowledge for all assessed stages, using the Bertero and Ruiz scale (2008), due to its greater simplicity. Existing knowledge for the sowing-emergence period is analyzed in detail

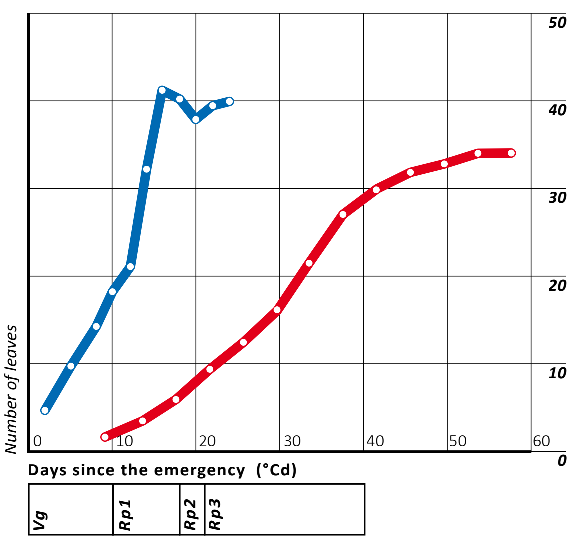

Figure 2: Association between the duration of sub-periods in development (Bertero *et al.*, 1999a) and leaf initiation and appearance (cv. Kanckcolla, Peruvian Altiplano). The pictures correspond to the following stages: G0 (vegetative), G1 (early reproductive), G3 (start of differentiation of the terminal flower) and G7 (start of differentiation of stigmatic branches) for a glomerulate type panicle. Adapted from Bertero *et al.* (1996) and Bertero *et al.* (1999a)

in chapter 2.6, hence the treatment of only a few aspects in this chapter.

The duration of the photoperiod-sensitive periods were analyzed in a few genotypes. The juvenile phase (period after emergence when the crop is not in condition to allow the detection and response to changes in photoperiod) shows variability between genotypes and this is associated with the latitude of origin of the genotypes (longer duration at lower latitudes), varying between 0 and 9 days for plants growing under a temperature of 21°C (Bertero *et al.*, 1999b). This is in contrasted with an estimate of 16 days at 16°C for variety Real according to Christiansen *et al*. (2010).

Expressed by a common base temperature of 3°C (Bertero, 2003) this would imply a duration of 208°Cd, against a maximum of 162° Cd estimated by the Colombian variety Nariño, and by ~ 80 °Cd, according to the equation proposed by Bertero (2003) which links the duration of the juvenile phase with the latitude of origin of a variety. It's possible that this difference is due to the response variables used, floral initiation in the first and anthesis in the second work. The end of the period of sensitivity to photoperiod is less known. Christiansen *et al.* (2010) found variation in the duration of grain filling as a consequence of plant transfers between photoperiods, but the sensitivity period to transfers ends ~ 25 days after sowing (Figure 2 of the article quoted for the Real variety), before the start of anthesis. In other experiments (Bertero *et al.*, 1999a, Píriz *et al.*, 2002), quinoa displayed the capacity to respond to photoperiod changes effected after flowering, and this period appears to stretch at least between 10 and 15 days after anthesis, as observed upon analysing the impact of plants transfer between photoperiods after anthesis on grain filling rate.

A first look at genotypic variation in sensitivity to the environment is shown by Figure 4. It shows the response of development rate (sowing-maturity days -1) to temperature for four genotypic groups identified based on their GxA interaction patterns for yield (Bertero *et al.*, 2004). Rhis figure includes results of field experiments carried out in tropical environments, but the average photoperiod showed little variation between environments (~1 h), so that much of the presented variation is attributable to the effect of temperature.

4.1 Sowing-emergence

Jacobsen and Bach (1998) studied the influence of temperature on germination rate in a Chilean origin variety selected in Denmark. They identified a Tb of 3 °C and an optimum temperature (associated with the maximum development rate) of between 30 and 35 °C. The seeds achieve 100% germination within 30 ° Cd (Thermal time units), which implies that under high temperatures and adequate humidity all the seeds will germinate within approximately one day. Bois et al. (2006), studied the variability in response to temperature in 10 Bolivian cultivars and detected variation in Tb and time of up to 50% of the germination, Tb varied between 0.24 and -1.97 °C, several degrees lower than the figure reported by Jacobsen and Bach (1998). Interestingly, lower temperatures seem to characterize cultivars originally from colder and drier climates (example, the Bolivian Altiplano compared with the south of Chile).

The variation between cultivars is more obvious when seeds were incubated at 2 °C, in that environment, the time to 50% germination (T50) varied between 45 and 67 hrs. Quinoa can be grown at the end of winter in southern Bolivia (Joffre and Acho, 2008) that is why the impact of this variation on the crop's ability to adapt to lower temperatures deserves to be explored.

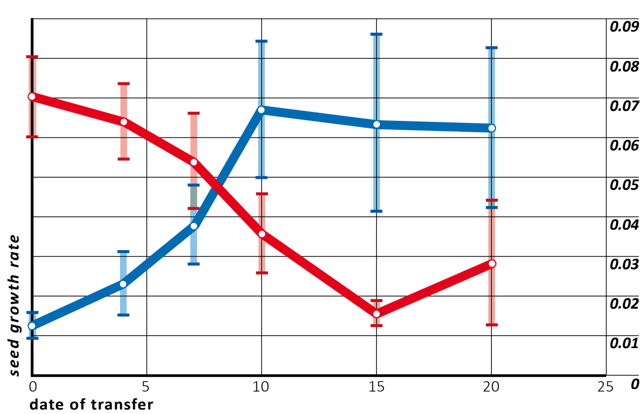

Figure 3: Effect of transfers between photoperiods (from 16 to 10.25 (◆) and from 10.25 to 16 hrs (■) a regular intervals from anthesis, on the rate of increase in grain volume (mm d^{-1}) measured as changes in the maximum diameter of seeds. The plants grew under a temperature of 25 °C in a greenhouse with controlled temperature and photoperiod and natural radiation. Blanca de Junín cultivar (Inter-Andean valleys of Peru, more details on this experimental procedure in Bertero *et al.*, 1999a)

Higher Tb values were obtained in a comparison of four Bolivian quinoa genotypes (Boero *et al.*, 2000) but in this case the same ratios were estimated using polynomial type relationships, unlike the linear relationships used in the usual approximations. An example of this variability is observed in Figure 5,

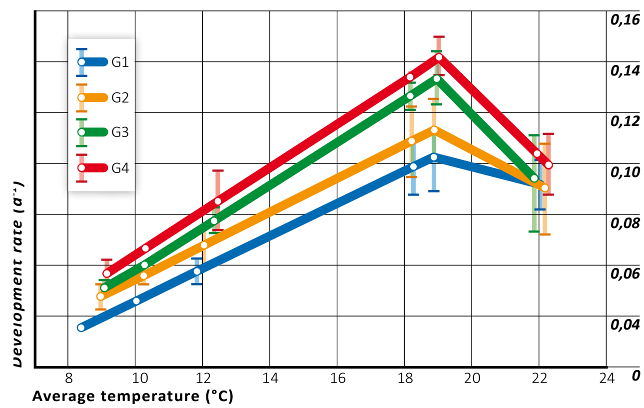

Figure 4: Association between the average development rate (d-1) measured by genotypic group for the sowing - maturity period in five cropping environments included in the American and European Quinoa Trial (Mujica *et al.*, 2001). The symbols correspond to: genotypes natives to the Inter-Andean Valleys (G1), Peruvian Altiplano (G2), Bolivian Altiplano (G3) and Sea level (central and southern part of Chile, G4) according to Bertero *et al.* (2004).

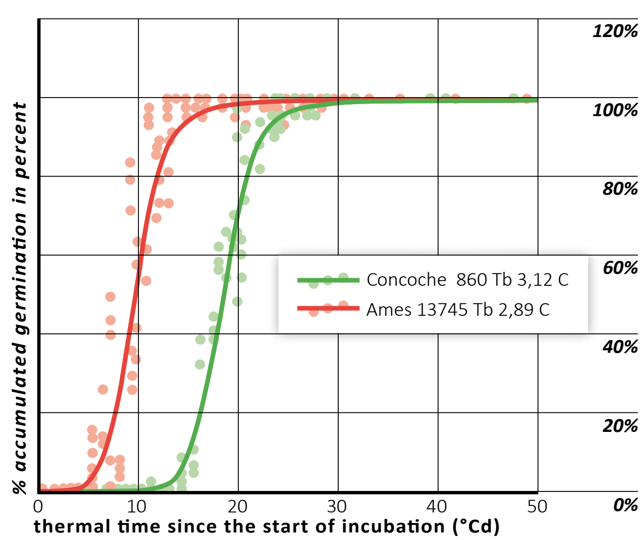

Figure 5: Progression in accumulated germination as a function of thermal time since the start of incubation (°Cd, see base temperature values in the diagram) for two contrasting response genotypes: Concoche, native of Valdivia, Chile, and AMES 13745, native of Bolivia (Christensen *et al.*, 2007). The data corresponds to seeds incubated at 6, 11, 15 and 19 °C.

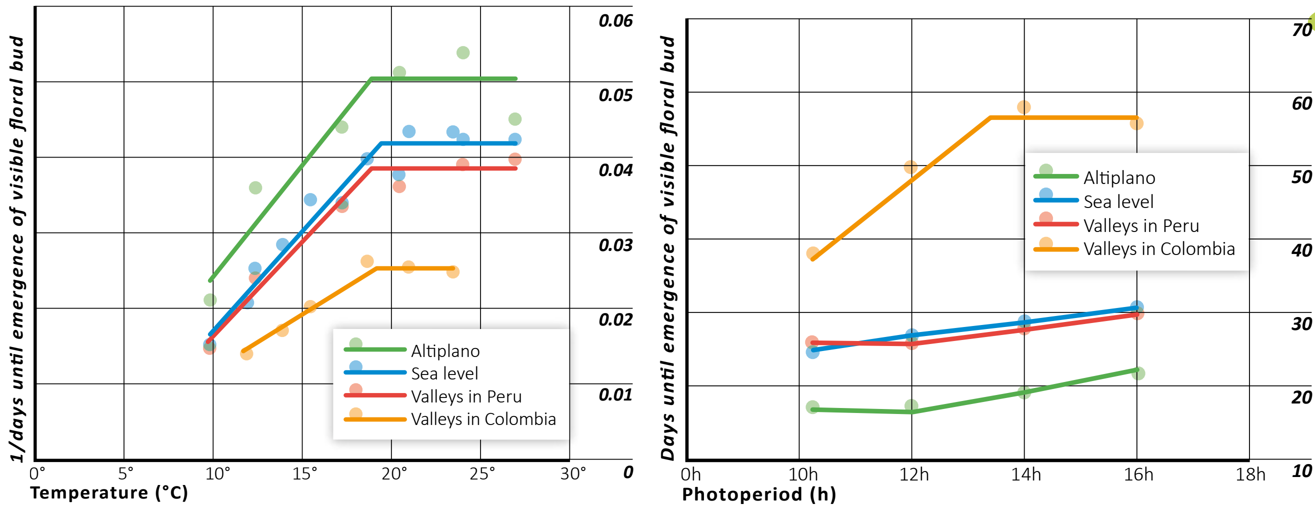

Figure 6: Variability in the response of development rate to temperature (a) and of duration from emergence to visible floral bud (VFB, b) to photoperiod, in four genotypes representative of the range of responses to these factors, evaluated under controlled conditions. The represented genotypes are: Nariño (◆), Colombia, Inter-Andean Valleys), Amarilla de Maranganí (■), Peru, Inter-Andean Valleys), Blanca de Julí (◆) Peru, Altiplano) and Baer (■) Chile, sea level). The response to temperature was analyzed for a photoperiod of 10.25 hours and that to photoperiod for a temperature of 21 °C. All the genotypes show a maximum development rate at a temperature of ~20 °C, whereas in the response to photoperiod, a threshold photoperiod of ~12 h could be observed for Blanca de Juli and a critical photoperiod of ~14 h for Nariño. More details on the experimental procedure in Bertero *et al.*, (1999b)

which compares the germination dynamic between two contrasting response genotypes, one Chilean and the other Bolivian. In this example T50 varied between ~ 10 and 18 °Cd, between 1 and 2/3 of values estimated by Jacobsen and Bach (1998) using similar Tbs.

4.2 Emergence-visible floral buds

This phase includes the Vg and Rp1 stages (Bertero *et al.*, 1999a) and therefore the entire leaf primordia initiation period, both stages are affected by the length of the day. The quantity of primodia, but not its initiation rate (primordial day $^{-1}$) varied between photoperiods in the two genotypes that were analyzed (Bertero *et al.*, 1999a).

Regarding genetic variability for duration of this stage two parameters that characterize responses to temperature and photoperiod, the basic vegetative phase (BVP), estimator of the temperature sensitivity (1/BVP) and photoperiod sensitivity (PS) are the most useful for explaining the differences between genotypes (Figure 6).

The BVP is the minimum duration of the phase, found under short days in short-day plants, whilst photoperiod sensitivity is the change in the duration of a phase per unit of change in photoperiod, expressed in °Cd for variable temperature conditions or in days h^{-1} for constant temperatures. Both parameters changed through a latitude gradient: a tropical cultivar (Nariño, from Colombia) displayed longer BVP duration and higher PS values (700 °Cd and 65 ° Cd h^{-1} (Tb = 1.5 ° C)) and the lowest values were observed in cultivar Baer (380 ° Cd and 12 ° Cd h^{-1} (Tb = 3.4 ° C)) from southern Chile (Bertero, 2003). Lower BVP and PS values were observed in the early flowering cultivars from the Peruvian and Bolivian Altiplanos, as an adaptation to the short vegetative period experimented in these environments. Unlike other stages (see anthesis-physiological maturity below), such as grain filling, the effects of temperature and photoperiod can be regarded as independent (Bertero *et al.*, 1999b).

4.3 Visible floral bud-anthesis.

This phase is also affected by photoperiod, sometimes directly or as by photoperiods experimented in the previous phases (Bertero *et al.*, 1999a). This in turn has an impact on the dynamic of leaf appearance. Although the number of primordia is determined at the onset of this stage, the number of leaves expanded from Rp2 until the end of the leaf appearance period changes with photoperiod, through modifications in the proportion of primordia which expand to form leaves (Bertero, 2003).

Photoperiod sensitivity is greater in this stage than in the previous one (Bertero *et al*., 1999a) and is reflected in the range of variation shown in Fig. 7 (from insensitivity to more than three times the maximum value estimated by the same combination of genotypes for the emergence –VFB phase). A regression adjusted to the relationship between photoperiod sensitivity and latitude of origin for the 0-20 °S range allowed the estimate of an slope of -11.1 °Cd h^{-1} lat^{-1}, compared with -1.5 °Cd h^{-1} lat^{-1} for the emergence period-VFB (Bertero, 2003). Preliminary evidence suggests that pollen viability might be reduced through the effect of photoperiod (less under long days, unpublished data).

4.4 Anthesis-physiological maturity

Perhaps the most decisive limitation to phenological adaptation to non-tropical environments is linked to photoperiod sensitivity and the temperature experienced during seed filling. A temperature x photoperiod interaction affects seed filling, which is strongly inhibited by the combination of long days and high temperatures (Bertero *et al.*, 1999a). While some Andean cultivars can be grown and matured in high latitudes (Carmen, 1984, Risi and Galwey, 1991), only limited by the duration of the growing season, seed production is strongly inhibited in mean latitudes when flowering occurs under long days and high temperatures. In the American and European Quinoa Trial (Mujica *et al.*, 2001) all temperate environments were excluded from the analysis due to the fact that the cultivars adapted to the tropics produced a large amount of plant biomass but little or no grains (Bertero *et al.*, 2004; Correa Tedesco, 2005). An interesting point for the adaptation to temperate climates is that this inhibition does not appear to occur, or has a lesser impact (Christiansen *et al.*, 2010) on sea level and some highland cultivars, which can be cultivated in these environments.

High temperatures also appear to explain the poor adaptation of varieties from the Chilean and Bolivian Altiplano, cultivated at an altitude of around 2,500 m in Colorado, USA., even though they performed well at 2,800 m in other locations in the same State (Johnson and McCamant, 1986). Making even more

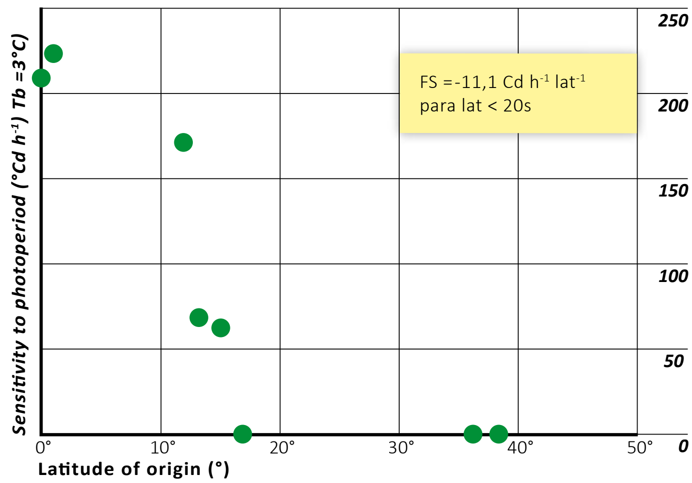

Figure 7: Association between photoperiod sensitivity (PS) for the VFB-anthesis phase and the latitude of origin of the genotypes (same as included in Bertero et al., 1999b). PS was estimated for plants growing in different sowing dates in Buenos Aires, Argentina, in the 10-14.4 h range of average photoperiod per phase for each planting date. PS is expressed in °Cd h^{-1}, for a Tb = 3 °C.

complex the interaction between photoperiod and temperature during seed filling, plants grown under short days before flowering present less inhibition for photoperiod during seed filling than those from long days (Bertero et al., 1999a, Bertero et al, 2002) (Fig. 8). An additional aspect of the effect of photoperiod on grain filling is delayed senescence (Bertero *et al.*, 2002, Christiansen *et al.*, 2010) possibly a consequence of alterations in the source-sink relationship linked to the inhibition of photoassimilate partitioning (and nitrogen?) to the grain (Fig. 8). This is manifested as a stay-green behaviour which does not generate an advantage in terms of grain weight or yield, since the latter is inhibited. The difference between the sample (S) and F2 (extension of photoperiod from anthesis to maturity) appears to be associated with differences in time to physiological maturity between these treatments, while the leaves shown in F1 (extension from floral bud to maturity) correspond to plants which, shortly after the beginning of samplings, were shaded by new leaves which continued to appear on the main stem, and the acceleration observed in senescence can be interpreted as a consequence of this shading. With respect to S, senescence was faster than in F2, associated with differences in maturity date. For both F2 and S, at physiological maturity senescence is associated with the start of a significant drop in SPAD values (Fig. 8). Plants under the treat-

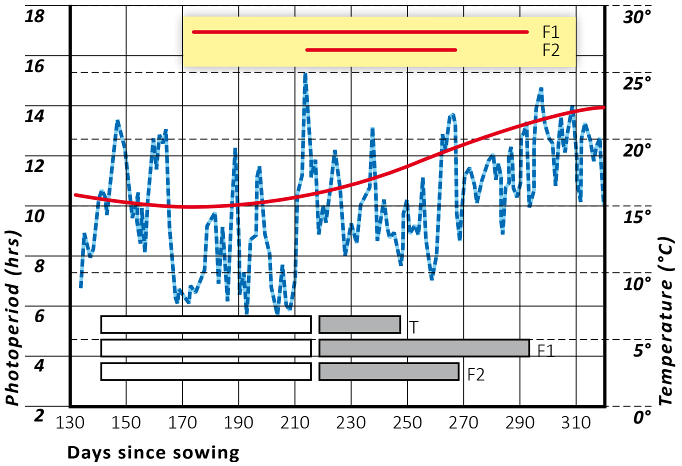

Figure 8: Effect of the manipulation of photoperiod under field conditions (Faculty of Agronomy, University of Buenos Aires) on the duration of development stages. The clear horizontal bars correspond to the duration of the emergence-anthesis period, while the dark ones correspond to anthesis-physiological maturity. Treatments are: plants grown under natural photoperiod (T), photoperiod extension from VFB to maturity (F1) and from anthesis to maturity (F2). The upper horizontal line indicates the duration of the photoperiod extension treatments (16 hrs), the dotted line to temperature averages over ten day periods and the rest to the progression of natural photoperiod (calculated according to Charles-Edwards et al., 1986). Sajama cultivar (Bolivian Altiplano. F1 does not cause changes in the time to flowering, indicating the insensitivity of this stage of the genotype to photoperiod).

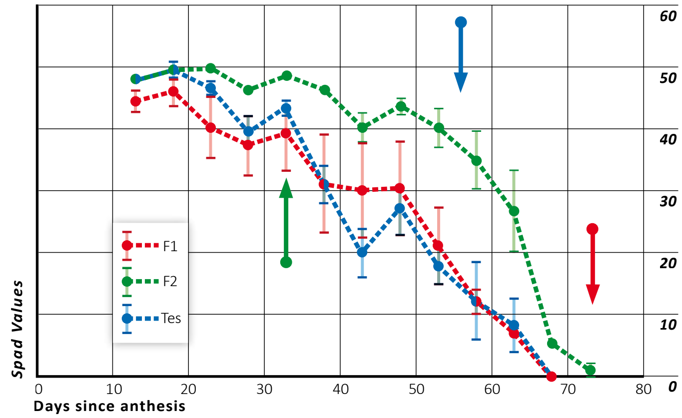

Figure 9: Effect of photoperiod on the chlorophyll content (SPAD arbitrary units, Minolta 1989) during grain filling, leaf No.13. The arrows indicate the time of physiological maturity, and the treatments are the same as for Fig. 7. Cultivar Sajama.

ment of photoperiod extension never mature, the stems stay green and growth of new ramifications from the inflorescence can be observed (Christiansen *et al.*, 2010).

5. Response of morphological development to environmental factors

Other development processes are those linked to the appearance of leaves. The leaf appearance rate (day leaves-1) is affected by temperature and photoperiod in quinoa, even though the effects of temperature are more relevant in relative terms (Bertero et al., 2000) (Figure 9).

The variation in phyllochron (thermal period between the appearance of two successive leaves on the main stem, in ° Cd) shows a similar pattern to that of time to flowering: late flowering plants are also those with a higher phyllocron (and therefore, lower leaf appearance rate), while the opposite is observed in Altiplano and Southern Chile accessions. This indicates that, in short season environments, as in the Altiplano (Geerts *et al.*, 2006) the genotypes flower in less thermal time, but this reduction in available time can be partly offset.

By the production of a higher number of leaves per time unit than varieties from warmer and more humid environments. An interesting fact is that the phyllocron is shorter (25%) in nine varieties selected in the Bolivian highlands compared to a traditional landrace variety (Bois *et al.*, 2006), perhaps a consequence of the selection for a higher crop growth rate and biomass production.

However, as a general rule, early flowering plants pay a cost in terms of yield potential due to the shorter available time to capture resources (above and below ground) as indicated by the positive association between crop biomass and cycle length (Bertero et al., 2004). The accumulation of biomass is also a function of changes in crop growth rate however, and the lower phyllocron could lead to a faster generation of leaf area, greater interception of radiation and growth, which would allow the design of cultivars that achieve similar biomass values with shorter cycles or high biomass values with similar cycles, as proposed for maize (Padilla and Otegui, 2005). The partial superposition between leaf appearance and reproductive development, mentioned previously, is also an interesting option

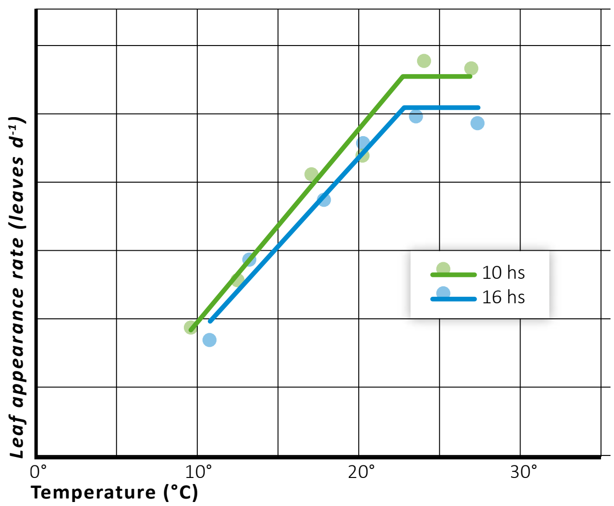

Figure 10: Response of leaf appearance rate (leaves day -1) to temperature and photoperiod, cv. Amarilla de Maranganí (Cuzco valleys, Peru). The data correspond to experiments conducted under controlled conditions in two photoperiods (10.25 and 16 hrs) and in the range between 10-27 °C in greenhouses under natural radiation. The estimated Tb is 3.1 °C, the optimum is 23 °C, and phyllocron responds to the equation: phyllocron (°Cd)=15 + 0.29 x photoperiod. More details on the experimental procedure in Bertero et al., (2000).

and, in fact, in quinoa, the generation of leaf area and panicle growth are partially simultaneous (Ruiz and Bertero, 2008).

6. Other factors

Another factor that appears to play a key role in development control, at least for varieties from the Bolivian Altiplano, is water scarcity. Geerts et al. (2008) reported 30 (from 65 to 95) days of delay in the time until the first anthesis with an increase in water deficit, while a similar stress can accelerate maturity if it occurs during seed development. This discovery has several implications. Extended dry periods can occur during the growing season coinciding with flowering and the filling of seeds in this milieu (García *et al.*, 2007). Flowering is more sensitive to stress (García, 2003), and also part of the critical period for yield determinination (Bertero and Ruiz, 2008, Mignone and Bertero, 2008); postponing flowering could act as an escape mechanism if this means exposing flowering to a condition of more favourable water availability after the stress.

An additional factor of complexity is the effect of radiation on duration of development and the appearance rate of leaves (Bertero et al., 1999b, Bertero 2001a). When models generated under controlled conditions were used to predict the time to VFB and the leaf appearance rate under field conditions, a systematic underestimation of both variables was detected when simulating the behaviour of crops growing at high temperatures. One of the differences between conditions was that under controlled conditions, a plateau is reached in development rate for temperature values ~ at 20 °C, which was not observed in the field (Bertero *et al.*, 1999b); and this “saturation of the rate of increase in development rate” is associated with lower incident radiation under controlled conditions (see Figure 3 in Bertero et al., 1999b). Based on this, a hypothesis is proposed that, in the presence of high temperature conditions, radiation is a limiting factor in the acceleration of development rate, in a manner equivalent to source limitations when analyzing carbon demand for growth processes (Borrás et al., 2004). When these variables were simulated assuming a single linear relationship between development rate and temperature, without a plateau, the systematic differences between observed values and predictions were eliminated, and greater prediction accuracy was achieved. An additional confirmation of this hypothesis was the analysis of the relationship between time to VFB and incident radiation (generated through different planting dates in a greenhouse under high temperature

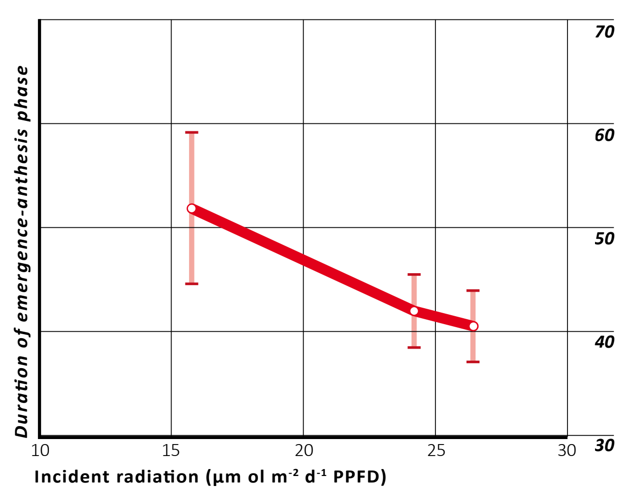

Figure 11: Association between incident radiation (mol PPFD m-2 d-1) and duration of the emergence-anthesis phase, for plants growing under constant temperature and photoperiod (27°and 16 hrs, respectively) in a greenhouse. Cultivar Sajama

and constant photoperiod) (Fig. 10) which possibly explains the apparent long day response found in Chilean quinoa genotypes when photoperiod was reduced through shading (Tejeda et al. 2007, Urbina *et al.* 2010).

Concluding remarks

The results presented in this chapter highlight the complexity of environmental control of quinoa development. The most studied factors are photoperiod, followed by temperature, covering a range of genotypic variation, while water deficits and radiation have only been partially studied in a few genotypes (and then for a few development stages). Given that the impact of factors such as water deficit and radiation are usually associated with growth process, we can speculate that the availability of nutrients will also affect the phenology of quinoa. We are yet to know the mechanism through which these last factors affect development. Among the affected phases, grain filling appears as the most critical in affecting latitudinal adaptation, as it may be strongly inhibited by high temperatures and/or long days. Experiments simulating the duration of phases and leaf appearance in field conditions have been reasonably successful (e.g. Bertero *et al.*, 1999b, Bertero, 2001, Geerts *et al.*, 2008, Lebonvallet, 2008), even though grain filling requires a better understanding to succeed in precisely simulating it. All this available information can be useful for taking decisions about crop management, adaptation to new environments or genetic improvement, decisions which so far have been taken empirically. High genetic control (G/GxE) of the duration of development may result in a high success rate for selection and management. The genetic control of quinoa development is yet to be addressed and represents the next chapter in quinoa development studies.

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CHAPTER 2.2.

Seed physiology and response to germination conditions

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Abstract

This chapter brings together knowledge of the germination and storage behaviour of quinoa seeds in relation to three general aspects: germination response to different factors and in situations of stress; tolerance to pre-harvest sprouting and dormancy control; and the dynamics of ageing and potential longevity of seeds in storage. Quinoa seeds demonstrate the capacity to germinate at temperatures around zero degrees and show tolerance to brief exposure to freezing in some cases. In general, accessions from saline and arid zones are more tolerant to water stress and salinity, as a result of their adaptation. Nonetheless, the distinctiveness of the ionic and osmotic components of salinity has revealed diverse responses and levels of tolerance in accessions of different origins. Pre-harvest sprouting limits the expansion of quinoa cultivation to humid regions. The study of the germination behaviour in accessions with dormancy made it possible to determine the effect of different factors: environmental (temperature and photoperiod), hormonal (ABA and GA3) and structural (coat thickness), on the level of dormancy of quinoa seeds during development, ripening and storage. Quinoa seeds have the capacity to tolerate water loss and maintain viability, recovering vital functions when rehydrated. The kinetics of the reactions of deterioration leading to a loss in viability are largely determined by the degree of water mobility in multilayers. The reactions of deterioration include lipid peroxidation and the formation of compounds from the Maillard reaction. There are references to the differences in storage tolerance for different cultivars, although these are inconclusive in terms of the link between longevity and the characteristics of the regions of origin.

Introduction

Successful crop establishment requires timely sowing of quality seeds (high viability and germination capacity) in adequate environmental and soil conditions to ensure the rapid and uniform emergence of seedlings. The period between sowing and seedling establishment is particularly vulnerable to stress (Carter and Chesson, 1996; Bennett, 2004). For good crop adaptation in regions that differ from traditional areas, the factors affecting the seed germination process must be taken into account. Given quinoa's tolerance to aridity, low temperatures and salinity, knowing the germination response when seeds are subjected to these conditions will enable the implementation of more appropriate management practices for sowing in these conditions, as well as for choosing the most adapted varieties and sources.

In terms of germination capacity, seeds should ideally germinate rapidly and unhindered at the time of sowing. However, depending on the environmental conditions, high germination capacity in physiologically ripe seeds of unharvested panicles can indicate a high risk of pre-harvest sprouting and consequent seed deterioration (Paulsen and Auld, 2004; Gubler *et al.*, 2005; Kermode, 2005). In temperate regions of cultivation, with high ambient humidity or high probability of rain during seed ripening, dormancy is a desirable characteristic, reducing germination capacity at the pre-harvest stage (Bertero and Benech-Arnold, 2000; Bertero *et al.*, 2001). This characteristic can be used in breeding programmes to adapt quinoa to humid environments. However, if this incapacity to germinate continues over time, it may become a problem at the time of sowing. Therefore, dormancy control should be studied carefully in order to anticipate the impact that environmental conditions will have on the level and rate of dormancy release (Ceccato *et al*., 2011).

Furthermore, correct seed storage will ensure the maintenance of seed viability at the time of sowing. Longevity is the time that a seed remains viable in specific storage conditions, and it depends on the initial quality of the seed, the humidity and temperature conditions during storage, and the rate of ageing characteristic of the species (Ellis and Roberts, 1980). There is considerable variation in ageing rate and progress between quinoa varieties (Castellión, 2008; López Fernández, 2008). Therefore, it is important to understand the mechanisms involved in the process of seed deterioration and ageing, as well as the characteristics they depend on and which determine the differences in storage behaviour. Understanding this process will help improve storage conditions and optimize the quality of the seeds used for sowing.

This chapter brings together knowledge of germination and storage behaviour of quinoa seeds in relation to three general aspects: germination response to different factors and in situations of stress; tolerance to pre-harvest sprouting and dormancy control as an alternative for adaptation to humid environments; and the dynamics of ageing and potential longevity of seeds in storage conditions.

1. Response to conditions of germination

1.1 Effect of temperature

Bois *et al*. (2006) indicate that the optimum temperature for maximum germination in quinoa, at which germination reaches 100%, occurs between 18° and 23°C. For the cultivar 'Olav' (selected in Denmark from Chilean germplasm), the optimum temperature for maximum final germination is 15°–20°C, whereas for the germination rate it was approximately 30°Cd (degree days), with a base temperature of 3°C. The thermal time requirement of 30°Cd for visible radicle protrusion in cv. 'Olav' indicates a rapid response to temperature (Jacobsen and Bach, 1998), although much shorter durations were estimated for genotypes of different origins, such as the Altiplano (Chapter 2.5).

Low temperatures can induce total inhibition in a number of germinating seeds due to embryo death, as described by Rosa *et al.* (2004). This occurs because protein synthesis and activation is affected and the seed's reserves have started to deteriorate (Bove *et al.*, 2001). Seeds from two Salare (salt flats) accessions from the northern Altiplano in Chile ('Roja' and 'Amarilla') were exposed to freezing at different thermal thresholds (0°, -2° and -4°C) in the three germination phases; subsequently, their percentage germination rate was assessed at 20°C. Exposure to freezing during phases I and II (imbibition and metabolic preparation) considerably reduced the percentage of germination in both accessions, from almost 80% to over 95% less than the control at -4°C during phase I (Delfino, 2008). This may be because the application of low temperatures during the imbibition period (4 hours) freezes the water in the tissues, affecting the embryo and ultimately killing the seed (Delouche, 2002). During phase III (radicle emergence), the effect was less marked, although there was clearly a difference between the accessions. The accession 'Amarilla' was more affected than 'Roja' (40% versus 15% reduction in germination, for the thresholds of 0° and -2°C). In turn, both were more affected when they were exposed to -4°C (50% and 25% reduction in germination for the 'Amarilla' and 'Roja' accessions, respectively) (Delfino, 2008). According to Boero *et al.* (2000), reduced germination in the field is mainly due to the large thermal variations that occur in the early hours of the day, when temperatures virtually reach freezing point, and later in the day, when the air or the first centimetre of soil can reach 40°C.

1.2 Effect of water stress

Water supply during germination is essential for the process to be completed (Johnston *et al.*, 1999). Water stress can be triggered by lack of water but also by low temperatures or high salinity. The more difficult it is for a seed to absorb water from the surrounding environment, the longer it takes to reorganize the membranes and develop metabolic processes (Tarquis and Bradford, 1992; Soeda *et al.*, 2005). The lower the osmotic potential of the environment simulating saline stress, the longer it takes to complete phase II of germination (Jeller *et al.*, 2003).

The exposure of quinoa seeds from one Salare accession ('Amarilla') and from a Coastal accession from the southern coast of Chile ('Hueque') to a low osmotic potential in the incubation medium, affected the germination process, as shown in Figure 1 (Moncada, 2009). Solutions of PEG 8000 at low osmotic potential slowed down seed imbibition for both accessions. For the Salare accession, the water contents were significantly different ($p \leq 0.05$) for the seeds imbibed with distilled water and those with an osmotic solution of 0.5, -1, -1.5 and -2 MPa of PEG 8000. Remarkably, in the Coastal accession, the water content of seeds imbibed in solutions of -0.5 MPa was statistically equal to that of the seeds imbibed in distilled water ($p \leq 0.05$). Significant differences ($p \leq 0.05$) were observed between these treatments and those with -1, -1.5 and -2 MPa. Imbibition was slower with the -2 MPa treatment, reaching only 20% humidity in the seeds in 24 hours of hydration. The imbibition kinetics of seeds from the Coastal accession ('Hueque') were more affected by the lower osmotic potentials. For these seeds, a 60% decrease in the relative water content was observed in comparison to distilled water and -2 MPa, while for the Salare accession ('Amarilla') this difference was only 40% at the end of the assessment period (Moncada, 2009). These results

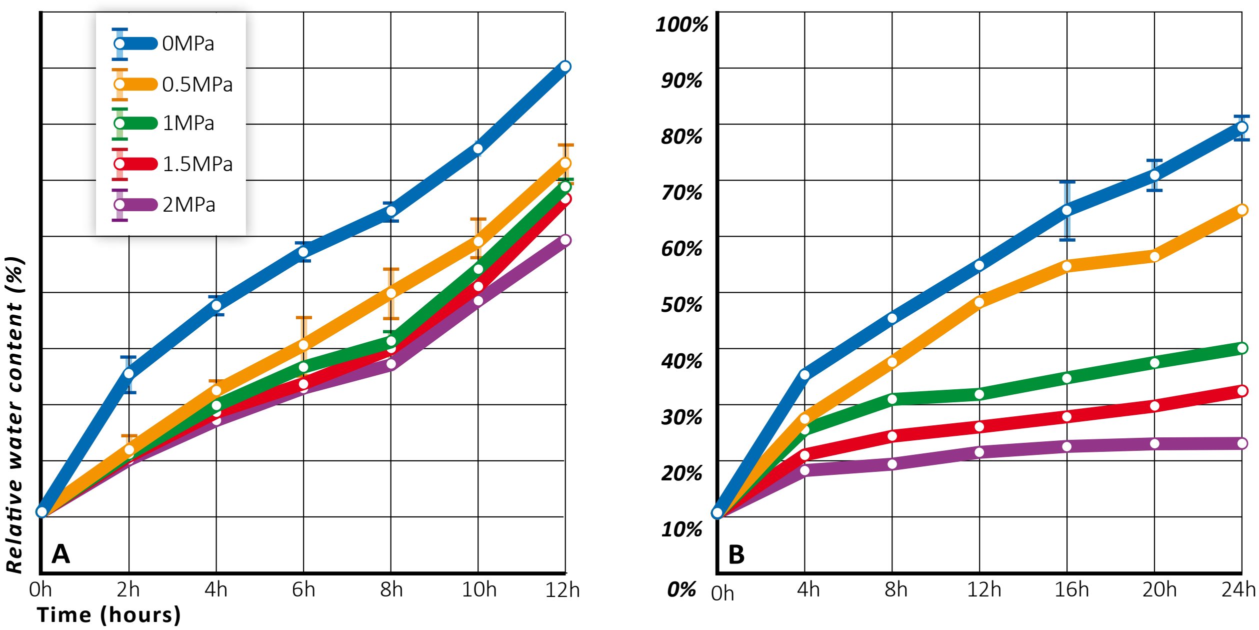

Figure 1: Kinetics of imbibition of quinoa seeds from Salare accessions: 'Amarilla' (A) and Coastal: 'Hueque' (B) treated with PEG 8000 at different osmotic potentials. Values correspond to the average of five repetitions. Vertical bars indicate the standard error (±). Different letters indicate significant differences (p 0.05) between the curves.
Source: Moncada, 2009.

correspond to the figures obtained by Delatorre (2008), who recorded a reduction in the percentage of germination for the 'Hueque' and 'Amarilla' accessions of 50% and 26.2%, respectively, after 24 hours of hydration in a solution of PEG 8000 at -1.4 MPa.

1.3 Effect of salinity

The decrease in seed germination caused by salinity results from the combined action of two types of stress factors: the water deficit produced by the osmotic effect of the salt in the soil solution, also called "osmotic drought", and the toxicity as a result of the excessive influx of ions, such as Cl- and Na+ into the tissues (Munns *et al.*, 1995; Zhu, 2003). Delatorre and Pinto (2009) assessed the influence of saline stress and its components (osmotic and ionic factors) during the germination of accessions of quinoa grown in the arid and saline zone of the Altiplano or Salare ('Amarilla' and 'Roja'), and the south coast ('Pucura' and 'Hueque') of Chile, with conditions of high humidity and no soil salinity. The seeds were treated with different concentrations of saline solutions (0, 0.2, 0.4, 0.8 and 1.2 M NaCl). The osmotic effect was determined by incubating the seeds in an isotonic solution of polyethylene glycol (PEG 8000) with an osmotic concentration equivalent to each saline solution. The ionic effect in the reduced final germination was calculated for divergence in relation to the control. The treatments without salt reached 100% germination at 25°C for all the accessions. By applying 0.5 M NaCl, final germination decreased by 53% for the accession 'Amarilla' (Salare), which was more resistant, and by 89.9% for 'Hueque' (Coastal), the most sensitive. The components of the saline stress (osmotic and ionic) had different degrees of influence on quinoa germination, depending on the accession. Thus, the accession 'Amarilla' was the least affected by both factors, particularly the ionic factor (27%), which had a greater impact on 'Pucura' and 'Roja', as well as on 'Hueque', although the latter was more affected by the osmotic factor (50%). According to Delatorre (2008), a delay in the germination process is another difference observed in the treatments with salt. The imbibition of coastal accessions is normally slower and they are more affected by environmental salinity during the imbibition process. During germination in saline conditions, carbohydrate mobilization is also activated. Bewley and Black (1994) indicate that the mobilization of carbohydrate reserves starts once the radicle has emerged. However, in embryo tissue, and particularly in quinoa, this occurs before the testa rupture (Prego *et al.*, 1998). This high consumption of reserves is noteworthy in Coastal accessions ('Hueque') after 24 hours of incubation in a saline solution (0.4M NaCl), correlated with higher respiration rates, while in Salare accessions ('Amarilla') there is greater starch availability, which is demonstrated by lower consumption (Delatorre, 2008).

Although the cultivars from northern Chile generally demonstrate high saline tolerance, as with the ecotypes from other latitudes (Koyro and Eisa, 2008), exceptions have been observed in the physiology of adult plants (Orsini *et al.*, 2011; Ruiz-Carrasco *et al.*, 2011) and in their seed germination (Cortés-Bugueño and Navarro-Honores, 2010). Some local ecotypes in the central zone of Chile show surprisingly high tolerance to ionic salinity for NaCl. This is attributed to the fact that in some coastal regions of central Chile, high tides flow into the river mouths carrying salt, and as levels rise on ancestral quinoa croplands, the soils become more saline. Therefore, farmers have inadvertently developed greater resistance to salinity. In these coastal zones, farmers even modify the riverbanks to make evaporation pools and collect the dry residues in order to sell sea salt. In central southern Chile, the yields obtained from these seeds are equivalent to those in the southern zone (2 tonnes/ha) and higher than those in the northern zone (< 1 tonne/ha) (Martínez *et al.*, 2007).

Chilo *et al.* (2009) studied the combined effect of temperature (5°, 10° and 20°C) and salinity (0, 0.1, 0.2, 0.3 and 0.4 M of NaCl) for the varieties 'Cica' and 'Real' collected in Salta, Argentina. The rapidity and later the final percentage rate of seed germination were affected in the environment with a reduced temperature and a treatment of increased salinity. This combination of effects completely inhibited germination at a temperature of 5°C and in solutions of 0.3 M and 0.2 M of NaCl for 'Cica' and 'Real', respectively, demonstrating the high tolerance and suitability for cultivation in arid and semi-arid valleys.

2. Tolerance to pre-harvest sprouting and dormancy control

Pre-harvest sprouting is one of the problems limiting the expansion of quinoa cultivation to humid regions. In the humid Argentine Pampas, conditions of high relative humidity or prolonged rainfall are common at any time of year. When these conditions coincide with the germination capacity of grains (grains with no dormancy), the seeds sprout on the mother plant. Sprouting is a phenomenon that occurs frequently in different regions of the world. It causes economic losses due to a reduction in yields, in quality for industry and/or in viability of harvested seeds, which may even result in total loss. Dormancy is a seed characteristic that can be used in processes to breed or adapt a species to a specific zone with the aim of increasing tolerance to pre-harvest sprouting. Dormancy is understood as the internal status of the seed that prevents germination in water, thermal or gaseous conditions, which would otherwise be suitable for germination (Bénech-Arnold *et al.*, 2000).

Until recently, most of the quinoa cultivars studied lack dormancy, and field observations confirm the existence of a high susceptibility to sprouting in the period just before harvest (Bertero and Bénech-Arnold, 2000; Bertero *et al.*, 2001). Germination behaviour was studied in seeds from two quinoa genotypes with dormancy ('2-Want' and 'Chadmo', originating from Bolivia and Chiloé, Chile, respectively), by combining cropping environments (sowing dates), storage and incubation. The objective was to determine the influence of the environment on the level of dormancy of quinoa seeds and the possible mechanisms involved (Ceccato *et al.*, 2011).

2.1 Environmental control of dormancy

As occurs in other species with a spring–summer cycle (Benech-Arnold *et al*., 2000; Benech-Arnold, 2004; Allen *et al*., 2007; Batlla and Benech-Arnold, 2007), dormancy release in quinoa seeds is manifested by a broader temperature range which allows germination, and after harvest the germination capacity at lower temperatures gradually increases.

Spring sowing, in which grains fill out in the summer, promotes dormancy in quinoa seeds, while sowing dates with autumn ripening reduce dormancy. The effect of sowing date could be due to differences in the photoperiod and/or the temperatures experienced during the development of the mother plant. Conditions of greater photoperiod and temperature at this stage are already associated significantly with higher levels of dormancy ($p < 0.05$; Ceccato *et al*., 2011). Similarly, for the variety 'Olav', germination at 6°C was higher with a delay at the time of harvest, associated with the mother plant's exposure to lower temperatures and shorter days (Jacobsen *et al*., 1999). These effects need to be assessed under controlled conditions so that the effect of each factor can be quantified independently.

In addition, seed storage at relatively high temperatures (25° vs 5°C) accelerates the process of dormancy release in both quinoa genotypes (Ceccato *et al*., 2011), as well as in *C. album* (37°-23°C, corresponding to ambient temperature vs 4°C; Karssen, 1970).

2.2 Structural aspects: importance of seed-coat

The seed-coat can largely explain the dormancy expressed in seeds. A perforation of the episperm and pericarp resulted in an increase of up to 80% in the germination capacity of seeds that developed in the summer for two accessions of different origin (Ceccato, 2011). Seeds of *C. polyspermum* responded to perforation in a similar way (Jacques, 1968) and embryos isolated from other genotypes of *C. quinoa* reached 100% germination at physiological maturity, while whole seeds (with pericarp) did not germinate (Bertero *et al.*, 2001).

This effect decreased with late sowing, and seeds that developed in winter did not respond to perforation. Nonetheless, they expressed a level of dormancy that could not be induced by the coat, revealing the presence of embryonic dormancy in quinoa seeds (Ceccato, 2011). This reduction in dormancy determined by the coat may result from the influence of environmental conditions on coat thickness and/or other coat properties.

With regard to the coat thickness, a significant reduction was observed with late sowing at the end of summer–autumn compared with spring sowing for the Bolivian accession ('2-Want'). The Chilean accession with a higher level of dormancy had a significantly thicker episperm for all sowing dates, even without variations between dates (Ceccato, 2011). The maternal environment's influence on the seed-coat's characteristics is associated with the level of dormancy in seeds from three other species of *Chenopodium*. For *C. polyspermum* and *C. album*, the thickness of the seed-coat and seed germination capacity are affected by the photoperiod experienced during their development (Jacques, 1968; Karssen, 1970; Pourrat and Jacques, 1975). For *C. bonus-henricus*, the altitude at which the plants develop increases the thickness and polyphenol content of the coats of harvested seeds and reduces their percentage germination rate. The average temperature during the 30 days before harvest had a positive correlation with germination (Dorne, 1981). In archaeological studies, it was found that *C. berlandieri* and *C. quinoa* had thinner seed-coats associated with domestication and it was suggested that this was linked to selection in favour of lower levels of dormancy (Gremillion, 1993a, b; Bruno, 2005, 2006). Nonetheless, a clear association between coat thickness and dormancy has not yet been verified for this species.

2.3 Hormonal control of dormancy

The hormonal control of dormancy is exerted through the balance between the two most important hormones that regulate it: abscisic acid (ABA), which increases dormancy, and gibberellic acid (GA), which reduces it. Their impact is caused by variations in the content, as well as the sensitivity of seeds to them (Karssen *et al.*, 1983; Bewley and Black, 1994; Hilhorst, 1995; Steinbach *et al.*, 1997; Koornneef *et al.*, 2002; Kermode, 2005; Feurtado and Kermode, 2007). The application of solutions that inhibit the synthesis of both hormones, sprayed directly on the quinoa panicles during seed development, revealed that quinoa seeds require GA to germinate (Ceccato, 2011).

Dormancy release in quinoa could be mediated by the reduction in its sensitivity to ABA. Its application in an incubation medium prevented seed germination. This effect decreased during the post-harvest period, and this occurred faster at 25°C than at 5°C, associated with an increased rate of dormancy release in seeds stored at this temperature. In a comparison of genotypes, the Chilean accession 'Chadmo' was more sensitive and persistent; this is coherent with its higher level of dormancy (Ceccato, 2011).

In addition, the coats could act as a constraint to the release of germination inhibitors outside the seeds, given that a higher quantity of ABA was released into the incubation medium of perforated seeds than in the case of whole seeds. On the basis of these observations, a possible hypothesis is that a variation in coat thickness in response to the maternal environment regulates the diffusion of ABA outside the seed during incubation, and that this mechanism helps modulate the level of dormancy (Ceccato, 2011). Figure 2 summarizes the principal factors of dormancy control in quinoa seeds. It shows the different factors (environmental, structural and hormonal) involved in determining and regulating dormancy, how they interconnect and hypotheses that arise.

3. Potential longevity and ageing

Seeds from the majority of species have the capacity to tolerate water loss at varying degrees and maintain viability during the anhydrous period, recovering vital functions rapidly when rehydrated. In terms of storage, seeds that dehydrate naturally to a water content that is in equilibrium with the environment are classified as orthodox. They tolerate subsequent artificial drying up to approximately 5% water content without losing viability (Ellis *et al.*, 1990). The stability of these orthodox seeds has been a crucial factor in agricultural development.

Three important fundamental factors are involved in the control of seed longevity: water, temperature and oxygen (Roberts and Ellis, 1989). The longevity of orthodox seeds is quantifiable and predictable: it increases with a reduction in water content and temperature, within a certain range (Ellis and Roberts, 1980). Predicting seed viability during storage is important, both for the management of germplasm collections and for the management of commercial seed production and storage. Although quinoa seeds demonstrate orthodox type behaviour, they can lose their viability in a short time, particularly in conditions of high temperature and humidity (Ellis *et al*., 1993).

In quinoa cultivars or accessions that originate from contrasting environments – Coastal ('Chadmo' and 'NL-6') or Altiplano ('Sajama' and '2-Want') – differences in behaviour have been observed during storage under different conditions, although it has not been possible to establish a link between the accessions' tolerance and origin (Castellión, 2008).

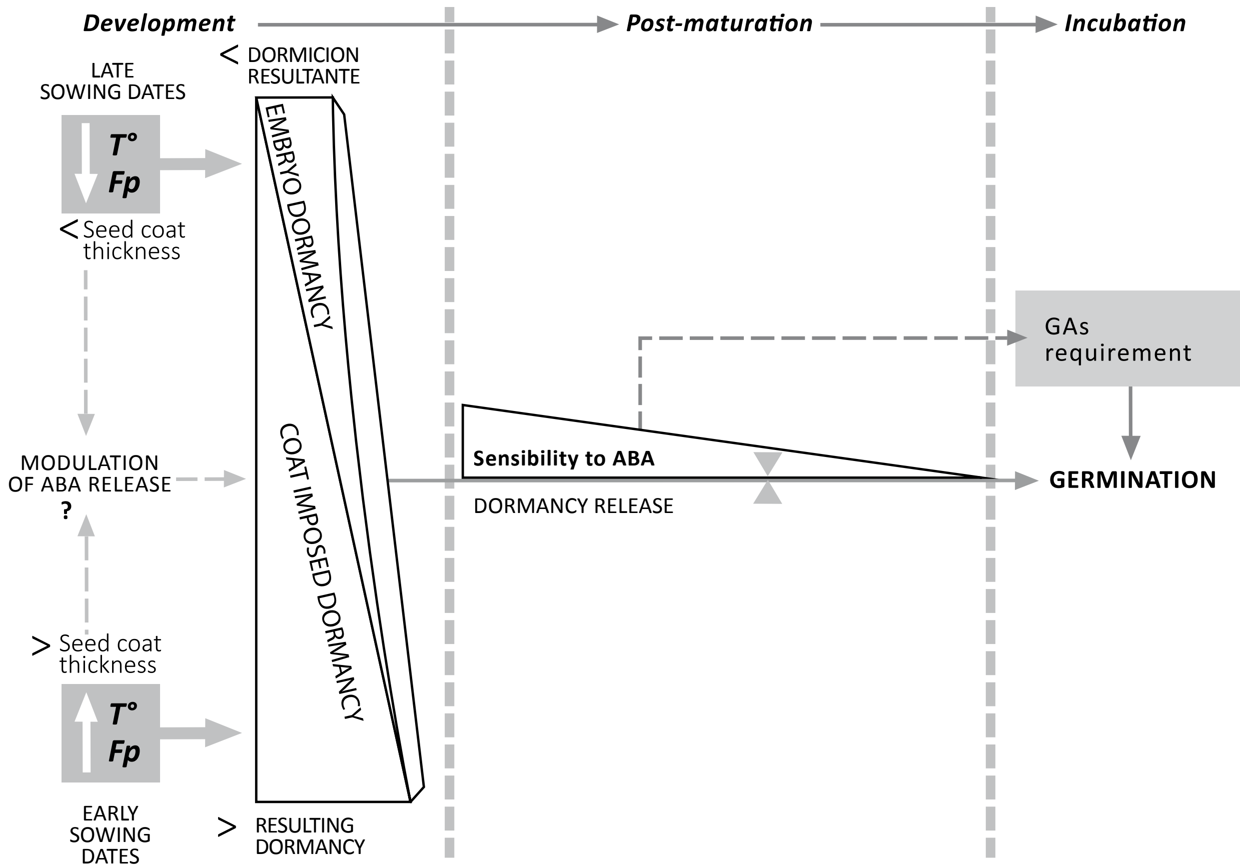

Figure 2. Conceptual schematic model of dormancy control in quinoa seeds, including the actual or hypothetical relationships between the different factors involved in its regulation. The dotted lines indicate regulation and the continuous lines indicate direct effects. During seed development, the environmental conditions determine a level of dormancy that will result from the levels of dormancy induced by coats and embryos. A mechanism proposed to induce dormancy via the coats involves regulating ABA release by varying coat thickness in response to the cropping environment. During post-ripening, dormancy release is regulated by sensitivity to ABA, which is gradually lost, and by storage temperature. Lastly, seed germination is determined by the presence of GA and the incubation temperature. Modified by Ceccato (2011).

Seeds from the four accessions studied, stored at 43% relative humidity (RH), maintained high values for normal germination and viability. However, only seeds from the accession 'Chadmo' maintained high levels of normal germination for 14 weeks, even in less favourable storage conditions (75% RH), demonstrating greater longevity than the other cultivars studied (Castellión, 2008).

Water content is an important factor in the kinetics of the reactions of deterioration that occur in seeds and in ageing (Justice and Bass, 1978; Priestley, 1986). In ripe orthodox seeds, enzymatic reactions do not have an important role in ageing due to the fact that enzymatic metabolism requires higher water content. Nonetheless, some spontaneous non-enzymatic reactions can occur even at lower water content (Priestley, 1986; Wettlaufer and Leopold, 1991; Sun and Leopold, 1995). These reactions can occur during non-enzymatic glycation with reduced sugars, like the Maillard and Amadori reactions, or with aldehydes produced from lipid peroxidation involving free radicals (Priestley and Leopold, 1983; Priestley, 1986; Wettlaufer and Leopold, 1991; Sun and Leopold, 1995; Murthy and Sun, 2000). With greater water activity, this is located in multilayers in the condensed phase, and the system's mobility increases, while the water remains available for enzymatic reactions involved in degradation. In equilibrium, at a constant temperature, the water activity (or water potential, a measure of the water that is available for different reactions) of the components in a mixture is equal, whereas the water content may not be.

When seeds are stored at a constant temperature in atmospheres of different relative humidity, their water content gradually reaches equilibrium with the environment. Thus, the final water content will be a function of relative humidity at which the seeds are stored. Relative humidity and water content can be represented by equilibrium curves or sorption isotherm. The sorption isotherms of orthodox seeds generally form a sigmoid shape, which indicates the three regions of water interaction (Vertucci and Roos, 1993; Walters, 1998) and may vary between different species due to the differences in seed composition (Vertucci and Leopold, 1987). The intensity and nature of the water's interaction with the seed's solid matrix affect the speed of the reactions of deterioration (Vertucci and Roos, 1990; Leopold and Vertucci, 1989). Therefore, the characteristics of sorption of the seeds can influence the variation in seed longevity between species (Eira *et al.*, 1999).

Sorption isotherms obtained in four quinoa accessions ('Chadmo', 'NL-6', '2-Want' and 'Sajama') were similar. However, their sensitivity to deterioration was different (Castellión *et al.*, 2010 b). In this way, the lack of a correlation between the longevity and the sorption properties in the different accessions indicates that the water content in multilayers is not a limiting factor *per se* in seed deterioration.

Water status and the metabolic changes have been studied in many biological systems using time domain proton nuclear magnetic resonance at a low resolution (TD-NMR). By using this technique for the accessions mentioned previously, 'Chadmo' showed lower values for transversal relaxation associated with water protons, indicating less molecular mobility in relation to the other cultivars studied (Castellión *et al.*, 2010 b).

Although the sorption isotherms of water were very similar, the degree of water mobility in the multilayer was correlated to the loss of viability and may be considered a determining factor in the kinetics of the reactions of deterioration involved in the loss of viability of these seeds. In this way, the information provided for the time of transversal relaxation of water will make it possible to predict the longevity of the different cultivars (Castellión *et al.*, 2010 b).

The Amadori and Maillard reactions refer to a complex series of reactions in which the proteins are aggregated, contributing to the ageing of seeds caused by the chemical alteration of functional proteins. This reduces the metabolic capacity and the metabolic system's capacity to limit the damage caused by free radicals and to repair the damage during germination (Murthy *et al.*, 2002).

In studies conducted with the quinoa cultivars 'Ollagüe' and 'Baer II', a significant increase in insoluble proteins was observed during storage, associated with the glycation of the Maillard reactions and correlated with longevity. Nonetheless, protein solubility was partially restored by priming in both cultivars, independently of their germination capacity (Castellión *et al.*, 2010a).

Traditionally, the analysis of protein fluorescence has been used to study the modification of proteins due to Maillard reactions during seed storage. The fluorescence spectrum of the Advanced Glycation End (AGE) products varies between species (Wettlaufer and Leopold, 1991; Murthy and Sun, 2000; Murthy *et al.*, 2002; Baker and Bradford, 1994; Murthy *et al.*, 2003). Correlating these trials with seed deterioration is often inefficient, as in the case of quinoa. This is attributed to interference with other fluorescent compounds in the seeds (Baker and Bradford, 1994; Castellión *et al.*, 2010a). Quantifying carboxymethyl-lisine is a novel alternative method for quantifying AGE products. This method detected high AGE levels in aged quinoa seeds with a low germination capacity. Seeds subjected to priming showed a slight reduction in AGEs, demonstrating a strong association between the latter and ageing in quinoa seeds (Castellión *et al.*, 2010a).

The composition of reserve lipids in the seed was determined by genetic (Knowles, 1988) and environmental conditions, such as light and temperature during development (Tremolières *et al.*, 1982). However, because of the ageing that seeds undergo during storage, the unsaturated fatty acids are susceptible to peroxidation: polyunsaturated fatty acids are more sensitive than mono-unsaturated fatty acids. Consequently, the variation in lipid composition during storage can be used as an indicator of ageing.

The proportions of fatty acids in the seeds of the quinoa accessions 'Chadmo' and 'Sajama', show that the main difference is the different proportion in the abundance of oleic (mono-unsaturated) and linoleic fatty acids (polyunsaturated). Surprisingly, 'Chadmo' was the accession that was richer in polyunsaturated fatty acids susceptible to oxidation, which demonstrated greater tolerance to storage (Castellión, 2008).

The damage caused to the fatty acids due to ageing, the effects of lipid peroxidation, may be evidence of a reduction in the relative composition of polyunsaturated fatty acids (linoleic and linolenic acid) and the formation of short chain fatty acids. In addition, in the accessions analysed, no indicators of the occurrence of lipid peroxidation were detected, such as the presence of short chain fatty acids or variations in the relative compositions of polyunsaturated fatty acids during storage. The analysis of the relative composition of polyunsaturated fatty acids, as well as the sum of the relative contents of linoleic and linolenic acids, did not show a correlation with germination and viability in the accessions studied (Castellión, 2008).

In the same accessions, the membranes were reported to have a high stability during storage. Their deterioration was associated to the auto-oxidation of fatty acids (Castellión, 2008). In addition, the lipids in the quinoa seeds were reported to have a high oxidative stability (Ng *et al.*, 2007). All this could be explained by the high tocopherol content previously reported in seeds of this species, given that they may prevent the spread of oxidation reaction, like anti-oxidants (Ruales and Nair, 1992).

Discussion

Quinoa's huge genetic variability across its geographical distribution means that genotypes can be found that are adapted to extreme climatic and soil conditions, even in such a vulnerable stage of the crop cycle as germination. This increases the possibility of finding accessions that are adapted or adaptable to very diverse conditions and encourages the expansion of this crop worldwide. Therefore, knowing the different accessions' limits of tolerance to adverse conditions and which qualities characterize them is useful in order to facilitate their selection and/or inclusion in breeding programmes based on the in-depth knowledge of the germination response to different stress factors.

While the optimum temperature for the germination of quinoa seeds lies between 15° and 23°C, the base temperature calculated for the variety 'Olav' demonstrates its capacity to germinate at temperatures around 0°C. Temporary exposures to temperatures below zero (freezing) affect germination in quinoa, although this depends on the germination stage when they occur. During stage III, the effects are less and depend on the accession and, consequently, in many cases they may achieve good germination percentages. Low osmotic potentials, the product of a water deficit, also affect germination, though to a lesser extent in accessions from saline and arid zones. This shows their adaptation and high tolerance to water stress. As far as salinity is concerned, Salare accessions are more tolerant – as can be expected. Nonetheless, by differentiating between the effects (osmotic and ionic), accessions from the coast of central Chile had a high tolerance specifically for the ionic factor (Delatorre and Pinto, 2009).

Dormancy is presented as an uncommon characteristic in quinoa seeds, considering the huge variability in the species' genotypes. Its regulation is complex, combining physical and hormonal factors, which in turn are influenced by the environment. It is important to identify molecular markers, which are simpler and more economic than physiological markers. They could be used in breeding programmes because this characteristic could improve the performance of quinoa crops in warm humid regions and reduce losses due to pre-harvest sprouting. In turn, the management of sowing dates is an option to ensure that the environmental impact is conducive to an adequate level of dormancy.

During storage, quinoa seeds have demonstrated ageing dynamics that vary significantly between accessions or cultivars. Therefore, the parameters calculated to estimate seed longevity in this species (consistent with the equation for viability) are not very accurate, particularly for predicting how long a batch of seeds will remain viable. Nonetheless, certain seed characteristics have demonstrated a correlation with the dynamics of ageing between accessions. Thus, water mobility in multilayers and protein insolubility can be measured and used as indicators to predict seed longevity in different quinoa accessions. In addition, the lipid composition is not a good indicator due to the high oxidative stability of the lipids that compose the seeds.

It should be noted that the accession 'Chadmo' was shown to have a high level of dormancy and tolerance to pre-harvest sprouting and, in turn, specific tolerance to adverse storage conditions, which ensures better longevity compared to the other accessions or cultivars. The existence of a causal relationship between both characteristics has not been demonstrated until now.

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CHAPTER 2.3.

Tolerance to saline conditions

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Abstract

Salinity is today one of the most widespread constraints in irrigated agriculture. Thus, salt tolerance is an agronomically important trait receiving increasing attention among scientists worldwide. Quinoa is tolerant to soil salinity and other adverse environmental factors, hence it attracts the attention of researchers as a possible crop in a changing world scenario in which scarcity of water resources and increasing soil and water salinization are the primary causes of crop loss. Quinoa's exceptional tolerance to salinity, frost, drought and other types of abiotic stress also makes it a model species for investigating cellular, physiological, biomolecular and morphological mechanisms at the basis of stress tolerance in halophytes and in plants as a whole. There are quinoa ecotypes adapted to valley, highland, salt desert, sea level and tropical environments, displaying broad genetic variability in salinity tolerance. For this reason, quinoa represents a valuable resource for selection of the most suitable material and for breeding new varieties adapted to different environmental and geographical conditions. In this chapter, scientific studies on salinity tolerance in quinoa conducted in the last decade by numerous research groups operating in at least nine different countries are described. We focus

on studies in which different quinoa genotypes are compared for their response to saline conditions, demonstrating that salt tolerance is a complex, multigenic trait involving a plethora of physiological and structural adaptations. Results available to date regarding the effect of salinity on the nutritional properties of quinoa are reported.

1. Introduction

Quinoa belongs to the chenopods (family Amaranthaceae), a group of plants comprising the highest number of halophytic (i.e. "salt-loving") genera. Quinoa is considered a facultative halophyte, and some varieties are able to cope with levels of salinity as high as those present in seawater, i.e. electrical conductivity (EC) of approximately 50 dS/m (corresponding to about 600 mM NaCl). Not surprisingly, therefore, quinoa grows on saline soils, from the Salare (salt flats) of the Bolivian Altiplano to the coastal zones of Chile. The halophytic nature of quinoa has been confirmed also under experimental (pot, hydroponic etc.) conditions. In a greenhouse experiment, Hariadi *et al.* (2011) tested six salinity levels for 70 days on cv. 'Titicaca' and observed a significant inhibitory effect on seed germination only for concentrations higher than 400 mM NaCl, while optimal plant growth was obtained between 100 and 200 mM NaCl. This is in accordance with previous results showing that yield of quinoa was highest under moderately saline conditions (10–20 dS/m) (Jacobsen *et al.*, 2003).

Quinoa is indeed tolerant to other types of adverse environmental factors (collectively known as "abiotic stress"), such as frost (Jacobsen *et al.*, 2005, 2007; Rosa *et al.*, 2009) and drought (Bosque-Sanchez *et al.*, 2003; Pulvento *et al.*, 2010; Jacobsen *et al.*, 2009, 2012; Fuentes and Bhargava, 2011; Razzaghi *et al.*, 2011a, b). For this reason, it is attracting the attention of researchers worldwide both as a possible alternative crop in the face of diminishing freshwater resources and increasing soil salinization, and as a model species to unravel the mechanisms at the basis of stress tolerance in plants. Today, research on quinoa is progressing beyond salt and drought tolerance, and includes studies on the effects of other abiotic (e.g. heavy metals, high and low temperatures, UV/FR radiations) and biotic (pathogens) stress-inducing factors.

2. Genotypic differences

Cultivating quinoa is a family heritage and the Andean farmers have proven to be a valuable instrument in preserving the genetic diversity of quinoa in their fields (Fuentes *et al.*, 2012). This biodiversity has been assessed by molecular methods (Christensen *et al.*, 2007; Fuentes *et al.*, 2009), and quinoa seeds of numerous accessions are being conserved in gene banks around the world (see Chapter 1.5 "State of Genetic Resources").

The existence of five categories (ecotypes) of quinoa, adapted to different conditions, suggests that the species must exhibit a broad genetic variability in tolerance to adverse climatic conditions. One approach towards evaluating and understanding salinity tolerance in quinoa has, therefore, been to compare different genotypes in terms of seed germination, growth and yield under saline conditions, and to investigate the morphological and physiological mechanisms responsible for these genotypic differences.

Many of the almost 2 500 quinoa accessions available to date have been shown to differ in their response to salinity during seed germination and later during the growth cycle. Jacobsen et al. (2003) observed that seeds of the Peruvian cultivar 'Kancolla' were able to germinate under conditions of salinity close to those of seawater (i.e. up to 57 dS/m). In a comparison between the Bolivian cultivars, 'Robura' and 'Sajama', the former was found to be more sensitive to salinity during germination with a tolerance limit of 100 mM NaCl (Schabes and Sigstad, 2005). Out of the 182 Peruvian accessions tested by Gómez-Pando *et al.* (2010) only the 15 most tolerant ones showed a high percentage of germination (60%) at a salinity level of 25 dS/m.

Ruiz-Carrasco *et al.* (2011) tested the *in vitro* germination, growth and short-term physiological responses to salt of four Chilean coastal genotypes originating from a latitudinal gradient going from central to southern Chile ('PRJ', 'PRP', 'UdeC9', 'BO78'). The aim was to link these modifications to the expression levels of two sodium transporter genes cloned in quinoa, *Salt Overly Sensitive 1 (CqSOS1)* and *CqNHX* (para. 3.3). They found a significant reduction in germination rate only at the highest salinity level (300 mM NaCl) and in the southernmost accession ('BO78'), where also root length was inhibited. The root/shoot fresh weight ratio was differentially affected by salt, with the lowest values in 'BO78'. Proline and polyamines, known to be associated with the response to salt stress (para. 3.2.3), as well as transcript levels of the two genes, were also modified in a genotype-specific manner upon exposure to 300 mM NaCl. Overall results indicated that, of the four accessions tested, 'BO78', originating from an area with relatively less harsh conditions in terms of precipitation, was the least salt-tolerant, suggesting a link between drought and salinity tolerance.

Delatorre-Herrera and Pinto (2009) tested four different Chilean genotypes and found that with 200 mM NaCl the most affected selection was 'Hueque' (50% decline in germinability), while the decrease in 'Amarilla' was only 6%. At 400 mM NaCl, the germination rate was lower for all genotypes, particularly those from non-saline areas, which germinated after 22 hours compared to 10 hours for those originating from a saline area, suggesting that salinity not only reduces germination percentage but also delays the process. Moreover, the relative contribution of the osmotic effect (i.e. drought generated by high soil salinity) and of ion toxicity (due to excessive accumulation of Na+ and Cl- in plant tissues) was also analysed, and indicated that salinity had a different effect on germination in different quinoa genotypes (Delatorrre-Herrera and Pinto, 2009). This may explain why data regarding the contribution of these effects on quinoa germination are contradictory. At the same time, this genotype-specific differential contribution of the two factors may provide a basis for breeding improved varieties adapted to particular field conditions. Thus, high osmotolerance during germination may be an advantage on drought-affected and slightly saline soils, while tolerance to ion toxicity would be advantageous under highly saline conditions.

Gómez-Pando *et al.* (2010) also studied the 15 most salt-tolerant Peruvian accessions at the mature stage and found that some genotypes exhibited a reduction in height under saline conditions, while others did not, or even showed an increase. The same was observed for leaf and root dry weight and yield. In particular, results indicated a dramatic influence of quinoa genotype on root dry mass per plant under saline conditions. While an 80% reduction in root dry mass relative to controls was observed in one accession reflecting its low salt tolerance, another accession surpassed the control in this characteristic, reflecting high salt tolerance. Overall, low plant height, short duration of life cycle, and maximum seed yield and harvest index are regarded as desirable agricultural traits.

In a pot experiment comparing 14 quinoa varieties in terms of biomass production, Adolf *et al.* (2012) reported that two varieties belonging to the 'Real' type ('Pandela rosada' and 'Utusaya'), adapted to the extremely harsh climatic conditions of the southern Altiplano of Bolivia, and a cultivar from the southern Andes of Peru ('Amarilla de Maranganí') were the least affected varieties in terms of relative biomass production and height at maturity (Figure 1).

In a comparative study between quinoa and the "model" halophyte *Thellungiella halophila*, Morales *et al.* (2011) used two genotypes of the Salare ecotype ('Chipaya' and 'Ollague') and two genotypes of the valley ecotype (the Peruvian 'CICA-17' and the Chilean 'KU-2'). Results indicated a greater reduction in fresh weight under saline conditions in *T. halophila* than in quinoa. In fact, at 300 mM NaCl, *T. halophila* averaged a tenfold decrease in fresh weight but Chipaya and Ollague only decreased twofold compared with their respective controls. Under strong salinity (450 mM NaCl), the quinoa genotypes belonging to the Salare ecotype maintained a relatively higher transpiration rate than the valley ecotype, 'CICA-17' (approx. 50% and

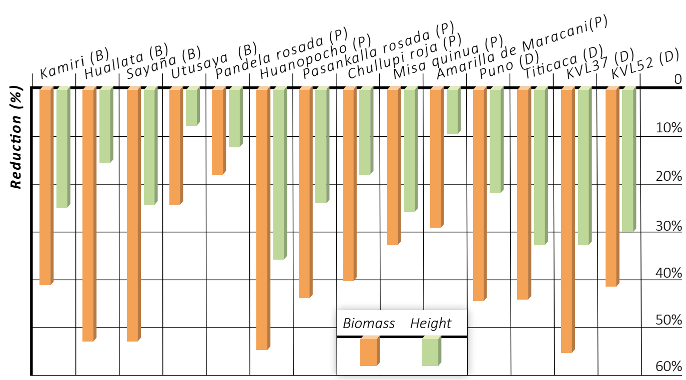

Figure 1: Name, origin (B = Bolivia, P = Peru, D = Denmark) and reduction in biomass and height under saline conditions relative to non-saline conditions of several quinoa genotypes. Adapted from Adolf *et al.* (2012).

40%, respectively, of control levels). No remarkable differences were observed, either between the quinoa cultivars in terms of ion accumulation and compatible solutes, or in leaf transcript levels of several genes with a putative role in salt tolerance; however, gene expression profiles in roots displayed some significant differences between Salare and valley ecotypes (para. 3.3).

A considerable amount of evidence has, therefore, accumulated, proving that there is wide genetic variability in salinity tolerance in quinoa. This represents an important resource for selection and breeding for even higher tolerance, and for cultivars adapted to different altitudes, latitudes, and a broad range of soil and climatic conditions (Christiansen *et al.*, 2010; Bendevis *et al.*, 2013).

3. Unravelling the basic mechanisms

It is believed that halophytes and glycophytes have a similar physiology and anatomy, but that salt-adapted plants may make more efficient use of the same salt-tolerance mechanisms (Shabala and Mackay, 2011). However, it cannot be excluded that halophytes display special salt-tolerance mechanisms that differ from those of glycophytes. Compared with glycophytes and even other halophytes, it must be asked whether quinoa possesses unique (as yet unknown) ways of adapting, and therefore growing and completing its life cycle, under high salinity. This is what today's researchers are investigating and it is the reason why, in the last decade, the number of scientific publications on this topic with regard to quinoa alone have soared, reaching 14 in 2010–12. The topic was recently reviewed by Jacobsen and co-workers (Adolf *et al*. 2013).

3.1. Morphological features

3.1.1. Seed structure

Several studies have shown that even halophytes can be sensitive to salt stress during the stages of seed germination and seedling emergence (Debez *et al*., 2004). Understanding the mechanisms which are responsible for the relative tolerance or sensitivity of the seed, such as if and where Na is accumulated, and if it affects seed viability, are important issues. Since salinity tolerance largely depends on the plant's ability to preserve ion homeostasis (Hasegawa *et al.*, 2000), concentration and distribution of other ions is also an important feature in both seeds and adult tissues.

Koyro and Eisa (2008) reported that in the Peruvian cultivar 'Hualhuas' the distribution of minerals in seeds harvested from plants grown under various salt treatments, including a very high concentration (500 mM NaCl), was altered, but ultimately highly regulated. These changes did not cause evident damage to the seed nor did they affect seed viability. The authors raised the question as to whether seed structure and compartmentation could have an influence on seed viability under high salinity, especially since quinoa seeds are of the campylotropous type, i.e. the embryo is peripheral around the perisperm (storage tissue) and therefore occupies a rather external position. Although seed weight decreased at high NaCl concentrations, dry matter reduction was compensated for by an increase in ash content. The salt-induced increase in ash content was due to increased Na concentration, but also to an increase in K, Mg and Ca concentrations. Although Na increase was very high, the K/Na ratio never fell below 1. Thus, there was a stable accumulation of K and other essential nutrients (such as P and S) even at high levels of salinity. Indeed, the seed-coat limited the passage of possibly toxic Na and Cl to the seed interior (> 90% Na and Cl was located in the pericarp). The study therefore demonstrated that in the seeds of salt-grown plants, an important tolerance mechanism was based on the integrity of the seed-coat and perisperm as protective barriers ensuring the exclusion of Na and Cl from, and the maintenance of a high K/Na ratio in, the seed's interior. Hariadi *et al.* (2011) likewise suggested that seed viability was dependent on its ability to exclude Na+ from the developing embryo in order to avoid ion toxicity.

3.1.2. Salt bladders

A typical feature of halophytes is the presence of specialized trichomes known as salt glands or salt bladders. Sequestration of absorbed salt into these structures appears to be an efficient strategy contributing to salinity resistance in some drought- and salt-tolerant species (Agarie *et al.*, 2007; Ben Hassine *et al.*, 2009). They are presumably involved in compartmentalizing potentially toxic ions, thereby excluding them from the other leaf tissues, in particular from the underlying photosynthetically ac-

Figure 2: Salt bladders on the leaf surface of quinoa.

tive mesophyll. Salt bladders may also be useful for reducing water loss and UV-induced damage to the photosynthetic apparatus. In chenopods, these salt glands are known as epidermal bladder cells (EBCs), and in quinoa they are present on the stem, and on both upper and lower leaf surfaces (Figure 2). In a Chilean genotype ('BO78'), no significant differences in EBC densities in untreated vs salt-treated plants and relatively modest ion excretion through salt bladders were reported (Orsini et al., 2011), suggesting that in this case EBCs may not play an important role in limiting ion accumulation. In the halophyte *Mesembryanthemum crystallinum*, EBCs were shown to accumulate water and metabolites, such as betalaine, malate, flavonoids, cysteine, pinitol, inositol and calcium oxalate crystals (Agarie *et al.*, 2007; Jou *et al.*, 2007). Thus, the protective role of EBCs may derive from the accumulation of organic compounds with ROS-scavenging or chaperone ability. Further studies are necessary to ascertain the composition, importance and function of EBCs in quinoa, also in relation to genotype-specific variations in salinity tolerance.

3.1.3. Stomata

Saline conditions generally decrease transpiration rate, but also CO_2 uptake, and hence photosynthesis (Iyengar and Reddy, 1996), through decreased stomatal conductance (see para. 3.2.1). The observed reduction in stomatal conductance in halophyte leaves is assumed to be important for better water use efficiency (WUE). This may originate from both physiological (e.g. control over stomatal aperture) and morphological (e.g. stomatal density and size) adaptive responses to salinity. In the former case, reversible and rapid regulation of the opening and closing of the stomatal pore is achieved via ion fluxes in and out of guard cells, a process that is under the control of the plant hormone abscisic acid (ABA). Early increases in ABA, and decreased leaf and soil water potential, are indicative of osmotic stress caused by salinity.

Gas exchange and transpiration have been shown to decrease in quinoa under salinity (Bosque Sánchez *et al.*, 2003). Quinoa exposed to different salinity levels and to the combined effect of salt and drought stress had an increased concentration in shoot and root ABA in accordance with its role as a signal to close stomata and regulate stomatal conductance (Razzaghi *et al.*, 2011a).

Recent studies have highlighted that a morphological mechanism for controlling transpiration and thus, WUE, under saline conditions in quinoa is through a reduction in stomatal size, density or both (Orsini *et al.*, 2011; Shabala *et al.*, 2012; Adolf *et al*., 2013). A reduction of up to 50% under very saline conditions accompanied by a reduced stomatal length was reported in the relatively salt-sensitive Chilean genotype 'BO78' (Orsini *et al.*, 2011). In a comparative study between 14 varieties of quinoa differing in salinity tolerance, Shabala *et al.* (2013) and Adolf *et al.* (2012) demonstrated that, while all had reduced stomatal density under saline conditions, this morphological parameter was affected in different ways, depending on the genotype.

3.2. Physiological and metabolic parameters

3.2.1. Gas exchange, stomatal conductance and photosynthetic rate

Razzaghi *et al*. found that when salinity increased, soil water potential decreased and, as a consequence, there was also a decrease in leaf water potential and stomatal conductance in quinoa (cv. 'Titicaca') plants that were either fully irrigated or subjected to progressive drought treatment. Similarly, 50–60% reductions in leaf gas exchange and conductance were reported by Orsini *et al*. (2011) for the Chilean accession 'BO78' already under moderate salinity (150–300 mM NaCl). Decreased stomatal conductance reduces water loss (transpiration rate) but also CO_2 entry. Stomatal conductance and photosynthetic CO_2 assimilation were analysed in two contrasting varieties of quinoa ('Utusaya' and 'Titicaca') under salinity. 'Utusaya', originating from the Salare region of Bolivia, was less affected, with only 25% reduction in net CO_2 assimilation compared to a 67% reduction in 'Titicaca' (Adolf *et al*., 2013). However, stomatal conductance, and therefore photosynthetic rate, were low in 'Utusaya' even under non-saline conditions – a typical trade-off between stress tolerance and productivity, and an aspect that should be taken into consideration when selecting varieties for cultivation under different conditions and for breeding. Irrespective of the effects of high salinity on CO_2 entry via stomata and hence its assimilation, several reports have indicated that in quinoa plants grown under salinity, the maximum photochemical efficiency of Photosystem II (PSII) was not affected, which suggests that PSII is not the main target of salinity stress (Hariadi *et al*. 2011, Adolf *et al*. 2013a).

3.2.2. Osmotic adjustment, K^+ retention and carbohydrate metabolism

LHigh salinity produces an osmotic (drought) effect, and can lead to ion toxicity due to the over-accumulation of Na^+ and Cl^- (Munns and Tester, 2008). In order to survive, plants must activate appropriate mechanisms to deal with these effects. Plants adjust to high external salt concentrations by accumulating a variety of organic molecules, the so-called organic osmolytes also known as "compatible solutes" (e.g. proline and glycine betaine), or inorganic ions, or both (Flowers, 2004; Shabala and Mackay, 2011). This accumulation of osmolytes is necessary for maintaining cell turgor and enabling cell expansion under conditions of increased external osmolality. While some tolerant glycophytes restrict ion movement to the shoots by limiting ion influx into the root, thereby avoiding the risk of ion toxicity, halophytes readily absorb, translocate and accumulate ions in the aerial parts (Flowers and Colmer, 2008). The accumulated ions (mainly Na^+, Cl^-, K^+) are supposedly used for osmotic adjustment, thus facilitating water uptake and transport, and presumably lowering the metabolic costs of production of organic osmolytes. Using cv. 'Titicaca' plants treated with NaCl at a concentration of 0–500 mM (approx. 0–50 dS/m), Hariadi *et al.* (2011) showed that 80–95% of osmotic adjustment in leaves was achieved by means of accumulation of inorganic ions (Na^+, K^+ and Cl^-). A similar situation was reported for the Chilean genotype 'BO78', where an increase in other cations (Ca^{2+}, Mg^{2+}) was also observed (Orsini *et al.*, 2011).

Wilson *et al.*(2002) investigated salt tolerance and ion accumulation in C. quinoa cv. 'Yecora Rojo' by treating plants with a salt mixture ($MgSO_4$, Na_2SO_4, NaCl and $CaCl_2$) similar to that which would occur in a typical soil in the San Joaquin Valley of California, where drainage waters are used for irrigation. No significant reduction was found in plant height, leaf area or fresh and dry weight in response to increasing salinity levels. The salinity response of quinoa was characteristic of a halophyte with a growth increase (leaf area and dry weight) even at moderate salinity levels. In both stems and leaves, increasing salinity reduced the K^+/Na^+ ratio. A similar situation was observed in wheat grown under the same conditions, but the decrease in the ratio was much more dramatic with wheat than with quinoa. In plants, high salinity induces K^+ efflux or impaired K^+ uptake, and the consequent reduction in cellular K^+ levels can be highly detrimental (Demidchik *et al.*, 2010). Thus, the regulation of K^+ homeostasis is an important aspect of salt tolerance, and the ability to retain an optimal K^+/Na^+ ratio is believed to be crucial for tolerance or adaptation to salt stress (Munns and Tester, 2008). Suhayda *et al.*(1992) found a strong relationship between tissue K^+/Na^+ ratio and salt tolerance in barley, and suggested this trait could be used as a selection criterion in the breeding of salt-tolerant cultivars. Moreover, an increase in the vacuolar Na^+ content must be accompanied by a concurrent increase in cytosolic osmolality. This is achieved not only by accumulating organic osmolytes in the cytosol, but also by increasing K^+. In salt-treated quinoa ('BO78') plants exposed to high salinity, a concentration of K^+ three times higher than in controls or plants exposed to lower NaCl concentrations was reported, whereas proline concentrations were not significantly affected, suggesting that the inorganic ion played a more important role in osmotic adjustment than the organic osmolyte (Orsini *et al.*, 2011).

Increases in organic osmolytes (soluble sugars, proline, glycine betaine) have nonetheless been reported in quinoa (Jacobsen *et al.*, 2007; Ruffino *et al.*, 2010). Morales et al. (2011) reported large quantities of betaine, trehalose and especially trigonelline in 'Chipaya' and 'Ollague' cultivars, and negligible quantities of sorbitol, pinitol and proline. In response to salt stress, trigonelline accumulated very high concentrations in both leaves and roots (800–7 000 µmol/g DW, depending on the tissue and treatment regime). This concentration far exceeds that reported in other crop species (soybean, corn, tomato).

Osmotic adjustment is particularly important during seed germination and seedling establishment, because if these fail, there will be no plant! The response of seedlings to salinity in terms of carbohydrate metabolism (enhanced production of soluble sugars) seems to be a major aspect allowing quinoa to adjust osmotically to a saline environment in its early stages of development, an important factor of plant salt tolerance. Prado *et al.* (2000) observed changes in glucose, fructose and sucrose content between salt-treated and non-treated seedlings. Rosa *et al.* (2009) analysed sucrose–starch partitioning and related enzymes in salt-stressed and salt-acclimated seedlings under low temperature. They reported higher activities of sucrose–phosphate synthase and soluble acid invertase in salt-stressed plants, and an increase in soluble sugars and proline, both of which are essential for the maintenance of osmotic balance under saline conditions.

A reduced matric potential in the seed interior may also counteract water loss under conditions of high external osmolality. Koyro and Eisa (2008) suggested that increased protein levels in seeds harvested from salt-treated quinoa plants may contribute to lowering this potential. They also argued that the acceleration of germination in these seeds could be the result of enhanced water uptake through the accumulation of Na^+ and Cl^- in the seed pericarp and of organic solutes in the seed interior.

3.2.3. Osmoprotective and other protective molecules

Dehydrins were first reported to accumulate in cotton seeds during the late stages of embryo development (Rorat, 2006). In addition, dehydrins have been found in nearly all vegetative tissues under stress conditions, such as drought, cold and high salinity (Battaglia *et al.*, 2008; Rorat, 2006). The ectopic expression of a wheat dehydrin has been shown to improve tolerance to high salinity and dehydration in the model plant, *Arabidopsis thaliana*. The mutation of a dehydrin gene in the moss, *Physcomitrella patens*, causes severe impairment of the plant's capacity to resume growth after salt and osmotic stress – further evidence of the role of dehydrins in stress tolerance mechanisms. Several dehydrin bands were detected in mature embryos of two quinoa cultivars adapted to two contrasting environments (high altitude vs sea level), with some bands showing quantitative differences in the two cultivars (Carjuzáa *et al.*, 2008). More recently, Burrieza *et al.* (2012) studied the effect of salt on the dehydrin composition of mature embryos of cv. 'Hualhuas', adapted to the arid and salty conditions typical of the Altiplano. Western blot analysis detected at least four dehydrins in seeds harvested from control and salt-stressed plants; no additional bands were detected under salinity conditions, and only one band (30-kDa dehydrin) increased under NaCl treatment (Figure 3).

As already mentioned, salinity stress causes a reduction in water availability (i.e. drought and osmotic stress), leading to stomatal closure and reduction in stomatal density, and accumulation of toxic Na^+ ions. Both stomatal closure and Na+ accumulation impair photosynthetic activity, which can result in the formation of reactive oxygen species (ROS). ROS are potentially capable of causing lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown and an impairment of enzymatic activity (Noctor and Foyer, 1998). Thus, oxidative stress is a third component of salt stress, and tolerance is strongly linked to a plant's ability to control ROS accumulation under stressful conditions. Although the accumulation of organic osmolytes is regarded as contributing to the plant's osmotic adjustment in a saline environment, it is now known that such compounds also play an important role in oxidative stress tolerance. Four major classes of organic osmolytes (amino acids, sugars, polyols and quaternary amines) are known; some may act as molecular chaperons protecting PSII against oxidative stress, while others directly scavenge ROS (Shabala *et al.*, 2012). All of these classes appear to be present in quinoa tissues (Aguilar *et al.*, 2003; Ruffino *et al.*, 2010; Orsini *et al.*, 2011; Ruiz-Carrasco *et al.*, 2011). In support of this hypothesis, exogenous application of glycine betaine was shown to substantially

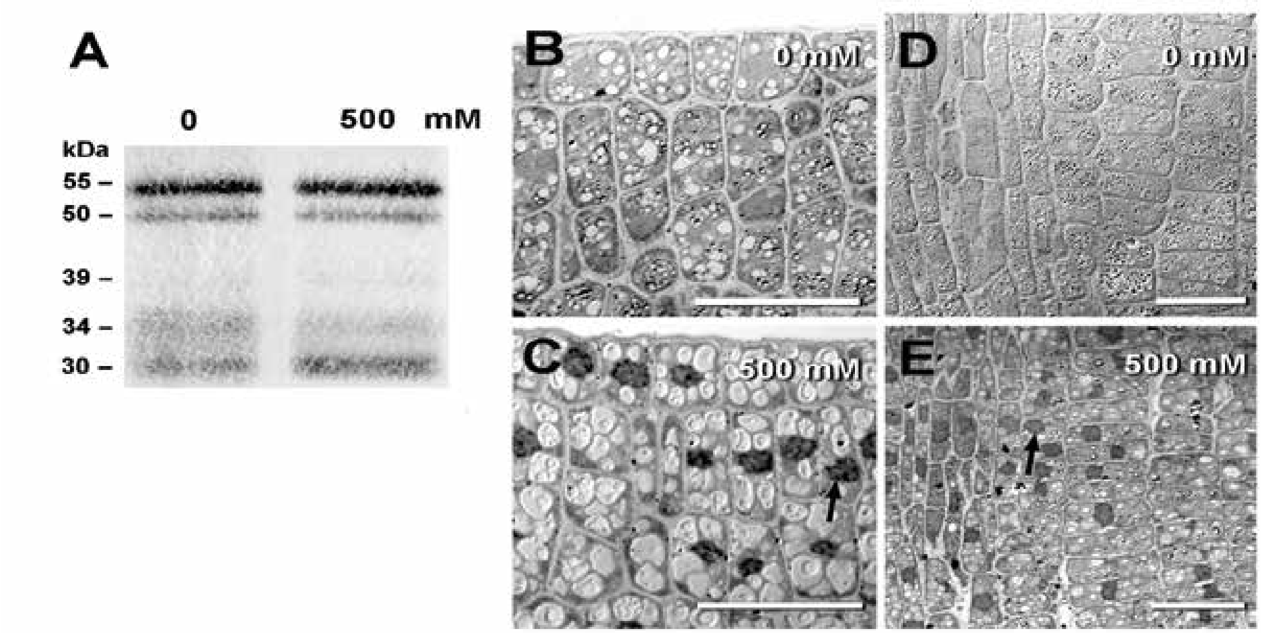

Figure 3: A. Western blot analysis of dehydrins in mature embryos of quinoa. Seeds were obtained from plants grown in 0 and 500 mM NaCl. **B-E**. Dehydrin in situ immunolocalization in the cotyledons (B, C) and embryo axis (D, E) observed under Differential Interference Contrast (DIC) microscopy. Labelling (arrows) was mainly observed in nuclei, specifically in chromatin. B, D : control plants; C, E : salt-stressed plants. Bar = 20 µm. After Burrieza *et al*., 2012. © Springer

mitigate the detrimental effects of UV-induced oxidative stress on photosynthetic efficiency (Shabala *et al.*, 2012).

Proline accumulation during salinity stress has been investigated thoroughly, and the role of this amino acid as osmoprotectant in protecting subcellular structures and macromolecules and as signal molecule has been established (Szabados and Savouré, 2010). In accession 'BO78', Orsini *et al.*(2011) reported that leaf and stem proline concentrations increased significantly under saline conditions: at the highest NaCl concentrations (600 and 750 mM), the increase was approximately ten times greater than in 0 mM NaCl. In another study, moderate salinity (300 mM NaCl) induced an accumulation of proline in 15-day old seedlings of four Chilean accessions ('BO78' and others); a distinction can be made between those that exhibited a moderate increase, and those that accumulated three to five times more proline than control levels (Ruiz-Carrasco *et al.*, 2011). In the same study, these authors analysed changes in polyamine (PA) levels in the different genotypes under salt treatment. PAs, of which putrescine (Put), spermidine (Spd) and spermine (Spm) are the most common in higher plants, are aliphatic polycations regarded as plant growth regulators also involved in stress responses (Alcazar *et al.*, 2010). There is evidence supporting the idea that PAs exert a protective function during stress (ROS scavenging, membrane stabilization, cell wall stiffening); they also seem to have a function as ion channel regulators (Kusano *et al.*, 2008). An inverse relationship between Put and Na^+ or K^+ levels in plant tissues is in accordance with the purported role of this PA in maintaining the cation/anion balance, while some reports point to the protective role of Spd and Spm in conferring salt tolerance. Results showed that the (Spd+Spm)/Put ratio was significantly lower in 'BO78' than in the other analysed genotypes, confirming the higher sensitivity of this southern genotype – this is in accordance with other parameters and with its provenance from the least stress-prone environment. Thus, while highest proline accumulation distinguished the most tolerant accession from the others, the PA response, on the other hand, distinguished the most sensitive (Ruiz-Carrasco *et al.*, 2011).

3.2.4. Sodium loading and translocation

In their experiment with a mixed-salt solution, Wilson *et al.* (2002) showed that in quinoa, Na+ levels increased only three- or fourfold in aerial tissues, while in a moderately tolerant wheat variety, the increase was over sixfold. Recently, Shabala *et al.* (2013) reported that genotypic differences in salinity tolerance were associated with differences in Na^+ uptake, with the most tolerant cultivars exhibiting lower xylem Na^+ content. The 14 genotypes tested could be separated into two groups, Na^+ includers and Na^+ excluders, with the most tolerant varieties falling into the latter group. It would therefore appear that also in quinoa, although rapid uptake and accumulation of Na^+ in the leaves is required for osmotic adjustment, ion toxicity is avoided in the most tolerant genotypes by limiting to some extent Na^+ loading into the xylem sap (exclusion mechanism). Indeed, Na^+ exclusion has always been considered a beneficial trait in glycophytes (Munns and Tester, 2008). In *Arabidopsis*, this exclusion is mediated by a Na^+/H^+ exchanger located at the plasma membrane of epidermal root cells (Blumwald *et al.*, 2000) encoded by the *Salt Overly Sensitive 1 (SOS1)* gene (Qiu *et al.*, 2002). SOS1 gene expression in quinoa under salinity has been investigated by several groups (Maughan *et al.*, 2009; Morales *et al.*, 2011; Ruiz-Carrasco *et al.*, 2011).

3.3. Gene expression studies

As described in previous paragraphs, capacity for ion uptake and translocation in quinoa under saline conditions has been investigated by measuring leaf sap Na, K and other ions. The topic has also been studied using molecular biology techniques, based on the fact that pivotal genes related to Na+ transport have been cloned in several species, and their role in salt tolerance assessed (Shi *et al.*, 2002). In *Arabidopsis thaliana, NHX1,* the gene encoding a tonoplast-localized vacuolar Na^+/H^+ antiporter, is regarded as being responsible for Na^+ compartmentation (and possibly K^+ homeostasis) in the vacuole. Compartmentation of Na^+ into vacuoles is a critical mechanism for avoiding the toxic effects of this ion in the cytosol, while providing additional osmoticum for water uptake and turgor maintenance. The plasma membrane SOS1 gene also controls ion homeostasis in the cytoplasm under saline stress conditions. Given quinoa's extraordinary salt tolerance, it is of interest to understand how genes associated with Na^+ antiporters are regulated in this species, as similar studies have been done in another salt-resistant species, the perennial grass Aeluropus lagopoides (Ahmed *et al.*, 2013). Maughan *et al.* (2009) cloned and characterized two SOS1 gene homologs in quinoa and found a high level of similarity between these gene sequences and SOS1 homologs in other species. Gene expression analyses of CqSOS1A and CqSOS1B in a cultivar originating from the Salare of the Bolivian Altiplano showed a stronger expression in roots than in leaves in the absence of salinity; however, saline treatment caused an up-regulation of both genes in leaves but not in roots – an observation which would suggest that Na+ exclusion at root level was not induced by this treatment (Maughan *et al.*, 2009). Gene expression analyses of CqSOS1 and CqNHX1 in four Chilean genotypes differing in salinity tolerance confirmed that the level of expression of these sodium antiporter genes was different in shoots and roots, and that these genes were differentially regulated in different genotypes (Ruiz-Carrasco *et al.*, 2011) (Figure 4). Transcriptional changes in CqSOS1 and CqNHX1 were also

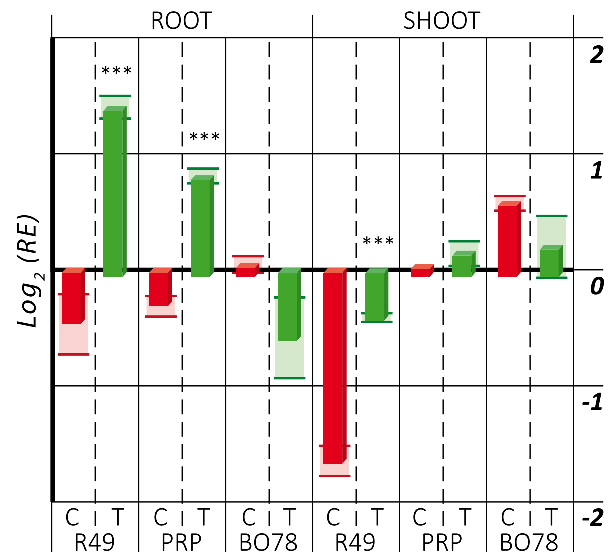

Figure 4. Expression of *CqNHX1* in roots (left) and shoots (right) of control (C) and 300 mM NaCl-treated (T) quinoa plants from northern (R49), central (PRP) and southern (BO78) Chile. The salt treatment was applied 60 days after germination in pots, and leaves were sampled 24 hours after the salt treatment. Results indicate a differential increase in *CqNHX1* expression in an organ- and genotype-dependent manner. Under salt stress, the northern and central genotypes (R49 and PRP, respectively) accumulate *CqNHX1* transcripts in the roots, while BO78 accumulates more transcripts in the shoots (K. Ruiz Carrasco, unpublished data).

measured under salinity (450 mM NaCl) and during recovery from saline treatment in two genotypes of the Salare ecotype and two of the valley ecotype (the Peruvian 'CICA-17' and the Chilean 'KU-2') by Morales et al. (2011). Differences in gene expression levels between accessions were reported for roots, but none were observed in leaves. SOS1 was more strongly up-regulated in salt-stressed roots of the Salare ecotypes, suggesting that cytoplasmic Na+ was moving out of the roots. Up-regulation of the gene encoding for an enzyme involved in the biosynthesis of the compatible solute glycine betaine, i.e. betaine aldehyde dehydrogenase (BADH), was observed in roots of both cultivars of the Salare ecotype ('Chipaya' and 'Ollague') and in the valley ecotype ('CICA-17'), without notable differences between genotypes.

3.4. Interaction of salinity with other environmental factors (temperature, drought)

Plants are able to display what is known as "cross-tolerance", which means that if a plant is tolerant to one type of stress it can also tolerate others (Hamed *et al.*, 2013). This is an important aspect to consider when selecting or breeding for a new variety since, in many regions of the world, particularly arid and semi-arid ones, heat, drought and salinity occur simultaneously.

González and Prado (1992) showed that at higher temperatures the detrimental effect of salinity in quinoa was generally less severe, and the ability of salt-treated seeds to recover after transfer to non-saline conditions was also temperature-dependent. This was confirmed by Chilo *et al.* (2009) who reported that lowering temperature and increasing salinity delayed and reduced seed germination and seedling growth. Rosa et al. (2009) also demonstrated that growth of quinoa seedlings was negatively affected by low temperature (5°C), and that salt-treated and low-temperature seedlings grown without added salt exhibited the same growth inhibition as unstressed controls. They also showed that low temperature induced different effects on sucrose–starch partitioning in cotyledons of salt-stressed seedlings. These preliminary results indicate that further investigations are needed to assess the combined effect of temperature and salinity both at early (germination, seedling establishment) and later stages of quinoa growth. Adolf *et al.* (2014) demonstrated in the Bolivian cultivar 'Achachino' that warm temperature conditions prolonged the flowering period, but shortened the time of seed filling compared with plants grown under cooler conditions. The result was more seeds of smaller size and weight in the warm climate. No differences in seed yield were revealed between salt-treated plants grown under the two temperature regimes (warm and cool).

Few studies have been performed with regard to the combined effects of drought and salt stress on quinoa under controlled field conditions. Razzaghi *et al.* (2011b) evaluated the effect of salinity and soil drying on radiation use efficiency (RUE[1]), yield and productivity in 'Titicaca'. Plants were exposed to five salinity levels (within the range 0–40 dS/m) from flower initiation onwards during the seed-filling phase; salinity treatments were divided into two irrigation levels – full irrigation (95% of field capacity) and non-irrigated progressive drought. Results showed that there was no significant interaction between drought and salinity on RUE, seed yield, harvest index and water productivity (i.e. seed or total dry matter per unit of water used). Another field trial was conducted in southern Italy using the same quinoa cultivar (Cocozza *et al.*, 2012). Results showed that, since seed yield was not compromised, 'Titicaca' can be cultivated in drought and salt stress conditions typical of Mediterranean-type agro-ecosystems (for further details see Chapter 5.15).

4. Does high salinity affect the nutritional properties of quinoa?

There is little information regarding yield and quality, in particular nutritional properties, of quinoa seeds under highly saline conditions. The Peruvian cultivar 'Hualhuas' (Koyro and Eisa, 2008) and 'Titicaca', the cultivar bred in Denmark (Hariadi *et al.*, 2011; Jacobsen *et al.*, 2010), could complete their life cycle and produce seeds even at 500 mM NaCl (approx. 50 dS/m). However, yield, number and size of seeds, as well as C/N ratio, were lower at high salinity levels (> 300 mM) than under control conditions. The lowered C/N ratio was mainly the result of an increase in protein content accompa-

[1] RUE (g DM MJ) was calculated for different fractions of yield obtained at final harvest, such as seed (RUEseed), straw (RUEstraw) and total dry matter (RUEDM) as RUE= Yield/IPAR.

nied by a decrease in total carbohydrates. In a field trial conducted in southern Italy (see Chapter 6.13), seed quality (protein, lipid, carbohydrate) was not significantly altered by irrigation with saline water, but fibre content was higher under saline conditions, probably due to a different relative amount of hull vs the rest of the seed (Pulvento*et al.*, 2012). Seeds of ten quinoa cultivars, nine from the Andean highlands (Patacamaya site in Bolivia/Argentina, 3 960 m asl) and one from northwest Argentina (Encalilla site, 2 780 m asl) were analysed for seed yield, protein content and amino acid composition when grown under drought conditions at the two different agro-ecological sites having different soil characteristics (EC of 2 and 7 dS/m in Encalilla and Patacamaya, respectively). The findings revealed that seed protein composition depended primarily on genotype, but also on environmental factors and their interactions, and that the essential amino acid profile was more affected than grain yield and total protein content (Gonzalez *et al.*, 2011). Mineral composition and protein content of seeds harvested from plants grown under neutral (L1) and saline-sodic (L2) soil conditions in central Greece were evaluated in eight quinoa varieties originating from Denmark, Chile, Brazil, the United Kingdom and the Netherlands by Karyotis *et al.* (2003). Protein concentration was significantly different between varieties at L1 but not at L2, and was on average 20% higher at L2 than at L1, indicating a negative correlation between grain protein and grain yield. At L2, seed phosphorus and iron content was not significantly different from that observed at L1, whereas the contents of most of the other minerals analysed (Ca, K, Mg, Zn and Mn) were, on average, significantly higher at L1, indicating that the marginal soil properties at L2 restricted the accumulation of these elements. The varieties from South America adapted well to soil conditions of both locations and were superior in accumulating mineral elements in seeds.

Vitamins and other molecules exerting antioxidant properties, such as phenolics, that can scavenge harmful radicals and reduce membrane lipid peroxidation, contribute to the nutritional and nutraceutical quality of quinoa. Gómez-Caravaca *et al.* (2012) examined the effects of irrigation and salinity on the seed phenolics content of a Danish cultivar. They found only limited changes in these compounds under reduced irrigation with or without salinity, suggesting that unfavourable soil conditions do not seem to affect the seed's content in these important bioactive compounds. With regard to another important category of antioxidant molecules, preliminary results have shown that the tocopherol (vitamin E) profile of seeds and leaves of four Chilean genotypes grown under saline (300 mM NaCl) conditions was altered, and in some cases enhanced, compared with controls grown without NaCl, and that the response was genotype-dependent (Antognoni and Biondi, unpublished data).

Saponins have a wide range of biological activities (antimicrobial, insecticidal, antifungal etc.) and can be used in industry as detergents and surfactants. They have a bitter taste – a negative characteristic in terms of attractiveness for human consumption. On the other hand, high saponin production may represent an asset in quinoa as an alternative and renewable source of saponins (Woldemichael and Wink, 2001; Carlson *et al.,* 2012). Under optimal irrigation, saponin content was 30% higher under salinity than in the absence of salinity (Gómez-Caravaca *et al.*, 2012). In a two-year field trial with 'Titicaca', Pulvento *et al.* (2012) reported a dose-dependent increase in seed saponin concentration with increasing salinity. This could be interpreted as a stress response, but further studies are needed to fully understand the mechanism connecting salinity with saponin production.

Although there is not yet sufficient information regarding genotypic differences and salinity on the nutritional and nutraceutical properties of quinoa to allow conclusions to be drawn, it would appear that these properties are, on the whole, not negatively affected or are even enhanced (e.g. protein and fibre content) under stressful conditions.

5. Conclusions

Given its halophytic nature, assessed and confirmed by a vast array of experiments conducted under conditions of moderate to high salinity, quinoa is certainly the ideal crop for the increasingly salinized agricultural soils worldwide. The information accumulated in recent years and summarized here, indicates that the broad genetic diversity of quinoa is associated with a wide range of tolerance to high salinity under multiple agro-ecological conditions (drought, cold etc). Thus, while quinoa genotypes possess a higher level of salt tolerance than all other crop species, some genotypes are more tolerant than others. This variation represents a precious resource, which can be usefully exploited to select and breed cultivars adapted to the most diverse soil and climatic conditions. Quinoa also represents a good model plant in which to unveil the mechanisms at the basis of salt tolerance: first, because it is the only halophyte seed crop and second, because its tolerance mechanisms may differ from those of other species in this small group of salt-adapted plants. Some of the information (morphological, physiological and molecular) available to date can already aid breeders in selecting for useful traits. Last but not least, there is a fair amount of evidence indicating that the nutritional properties of quinoa are not severely affected under high salinity and that, in some cases, they are even improved. This aspect corroborates the notion that quinoa is a crop which can offer communities living in harsh environments options to improve their livelihoods, generate income, achieve food security and enjoy better nutrition and health.

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CHAPTER 2.4.

QUINOA DROUGHT RESPONSES AND ADAPTATION

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Abstract

Quinoa thrives under a wide range of soil and climate conditions, from cold and arid areas to wet tropical regions. The adaptability of quinoa to various levels of drought is due to the differentiation of a diversity of ecotypes originating in contrasting agro-environments. Plants display various adaptive strategies to drought stress, from morphological to physiological adaptations that serve a range of responses to water deficit, from avoidance to resistance and tolerance. Plants cope with drought stress by changing and modifying key physiological processes, such as photosynthesis, respiration, water relations and antioxidant and hormone metabolism. Whole-plant responses to drought involve changes in leaf and root growth, in some cases with strong ontogenetic variation. These drought responses at both physiological and morphological levels show intraspecific variation related to ecotypic differentiation. This chapter explores the responses to this abiotic stress and reviews possible mechanisms concurring at both whole plant and tissue level, including recent determinations from architectural, morphological, physiological and molecular perspectives. Quinoa thus represents an invaluable opportunity, both as a potential crop in consideration of present and future climate change challenges, and as an important source of genes with biotechnological applications.

Keywords: *Chenopodium quinoa*, drought, ontogeny, physiological responses, morphological traits, plant architecture, molecular responses, intraspecific variation.

1. Introduction

The Andean seed crop, quinoa (*Chenopodium quinoa* Willd.), was domesticated and has been traditionally cultivated in the area for at least 7 000 years. Quinoa diversity is described with five major ecotypes linked to diversity subcentres: Altiplano (Peru and Bolivia), Inter-Andean valleys (Bolivia, Colombia, Ecuador and Peru), Salare (Bolivia, Chile and Argentina), Yunga (Peru, Bolivia and Argentina) and Coastal (Chile) (Risi and Galwey, 1989a, b; Bertero *et al.*, 2004). Its great diversity is characterized by exceptional adaptation to environmental conditions and edaphoclimatic conditions, including altitudes from sea level to 4 000 m asl, annual precipitation from 2 000 mm to extreme aridity (e.g. Las Quinas-Antofalla in Argentina, where it rarely rains and quinoa is totally dependent on irrigation – Bertero *et al.*, personal communication), significant variability in soil and nutrient availability, and climate conditions ranging from tropical to cold arid. Its adaptability to natural and cultivated ecosystems has made this species an outstanding model for the study of intra- and interspecific variation in growth and development patterns and in the response of shoot and root architecture to water deficit. The physiological adaptability that allows this species to grow under drought and other adverse conditions represents an invaluable opportunity and offers immense potential in the face of present and future climate change challenges.

2. Quinoa Responses to Water Deficit

LPlant responses and mechanisms for dealing with low water availability can be divided into two major categories: stress avoidance and stress tolerance (Claeys and Inze, 2013). The aim of stress avoidance mechanisms is to balance water uptake and water loss. Water uptake is enhanced by the accumulation of solutes which lower tissue water potential and by increased root growth. Water loss through evaporation is limited by closing the stomata, resulting in restricted shoot growth and accelerated leaf senescence. Stress tolerance mechanisms are aimed at protecting against cell damage when stress becomes more severe and stress avoidance mechanisms are no longer sufficient. Stress tolerance mechanisms include detoxification by reactive oxygen species (ROS) and the accumulation of protective proteins such as late embryogenesis abundant (LEA) proteins and solutes (e.g. proline, which has a dual role as both osmolyte and osmoprotectant) (Claeys and Inze, 2013). Both avoidance and tolerance responses are mainly orchestrated by abscisic acid (ABA), although ABA-independent mechanisms, such as those involving dehydration responsive element binding (DREB) proteins, also play a role (Nakashima *et al.*, 2009).

Quinoa possesses an exceptional innate ability to cope with water shortage based on its intrinsic low water requirement, and the aptitude to resume rapidly its former photosynthetic level and its specific leaf area after a period of drought (Galwey, 1989; Jensen *et al.*, 2000; Jacobsen *et al.*, 2003, 2009). This makes quinoa suitable for growing in arid and semiarid regions (e.g. India, sub-Saharan African countries), where there is no irrigation and farmers need to rely on seasonal rainfall (Bhargava *et al.*, 2006). Drought tolerance of quinoa has been attributed to its branched and deep root system that penetrates up to 1.5 m in sandy soils (Álvarez-Flores, 2012), and the presence of leaf vesicles containing calcium oxalate, which could reduce transpiration (Jensen *et al.*, 2000; Siener *et al.*, 2006). It has been demonstrated that high instantaneous photosynthetic efficiency (measured either as photochemical efficiency or as radiation use efficiency) is maintained in quinoa despite water deficit (Winkel *et al.*, 2002; Bosque Sanchez *et al.*, 2003). The plant also avoids drought thanks to: reduction of its leaf area by leaf shedding; small and thick-walled cells preserving turgor even after severe water losses; and stomatal regulation (Jensen *et al.*, 2000). In addition, quinoa can escape drought through precocity (i.e. early genotypes), which is important in areas where the risk of drought increases towards the end of the growing season (i.e. terminal drought), and also through low osmotic potential and the ability to maintain positive turgor even at low leaf water potential (Jacobsen and Mujica, 2001; Bhargava *et al.*, 2006). Drought escape manifests itself as a prolongation of the growth cycle in response to drought in the early vegetative stages and as early maturity in response to drought in the later growth stages (Jacobsen *et al.*, 2003; Geerts *et al.*, 2008). Given the vast genetic variability of the different quinoa ecotypes and genotypes for this characteristic, there is no agreement concerning the level of drought resistance of quinoa (Jacobsen and Mujica, 2001).

2.1. *Long-distance signals controlling leaf expansion and stomatal conductance*

The above-mentioned drought modifications and mechanisms – rapid stomatal closure, increased levels of ABA and increased content of osmoprotectants (i.e. betaine and proline) – are also common and shared with other plants (Jacobsen *et al.*, 2009). However, other mechanisms are still not completely understood, such as the accumulation of calcium oxalate, increased protein stability and thermostability of chlorophyll, which could be due to mechanisms which are genetically different from those already reported (Morales and Zurita, 2010; Shabala and Mackay, 2011).

The effects of drought on leaf water potential (ψl), stomatal conductance (gs), transpiration (Tr), photosynthesis rate (A_{max}) and crop yields were previously determined under the natural climatic conditions of the southern Bolivian Altiplano (Vacher, 1998). Drought caused large decreases in the parameters measured, and there was a major, rapid stomatal closure with an associated two-thirds reduction in Tr and Amax; and as drought continued, these parameters remained relatively stable, while the minimum potential reached values below -4 MPa. Interestingly, it has also been observed that stomata do not seem to respond to abscisic acid (ABA), except in conditions of extreme drought, and that quinoa plants can photosynthesize for a long period under very low irrigation, even for 3 days after stomata are closed (Jacobsen *et al.*, 2009). When stomata are closed, a phenomenon occurring in many plant species - but not yet demonstrated in quinoa - is that oxalic acid is reconverted to carbon dioxide for photosynthesis, allowing excellent water use efficiency (Sen *et al.*, 1971). In the study of how chemical and hydraulic signalling from the root system controlled gas exchange in plants growing in a drying soil, Jacobsen *et al.* (2009) determined that photosynthesis was maintained after stomata closure and, interestingly, only a slight increment of ABA in the xylem was detected. ABA was also documented when the crop encountered very mild stress, thus demonstrating that chemical signalling can also play an important role in maintaining stomatal conductance under these conditions (Hariadi *et al.*, 2011; Razzaghi *et al.*, 2011). Other mechanisms to maintain turgor under increasing drought could be osmotic adjustment, as suggested in other quinoa cultivars, and antitranspirant compounds other than ABA in the xylem sap (Jacobsen *et al.*, 2009; Hariadi *et al.*, 2011). The authors concluded that during soil drying, quinoa plants present a sensitive stomatal closure, by which the plants are able to maintain ψl and Amax, resulting in an increase in water use efficiency (WUE). The modest role of root-sourced ABA regulation means that quinoa must depend also on hydraulic regulation through a change in turgor or other chemical substances yet to be determined (Jacobsen *et al.*, 2009).

Natural candidates for regulatory roles include other hormones which have been shown to play an important role in adjusting growth to water availability. Indeed, transcript analysis of proliferating and expanding leaf tissue from *Arabidopsis* plants exposed to mild osmotic stress revealed a role of ethylene and gibberellic acids (GAs) in acclimation to both short- and long-term mild drought stress (Skirycz *et al.*, 2011; Claeys and Inze, 2013). This important role for GAs in growth regulation was corroborated by other studies that profiled leaf tissue at different developmental stages in *Brachypodium distachyon* and maize subjected to mild drought (Verelst *et al.*, 2013; Claeys and Inze, 2013). The role of the "stress hormone", ABA, is confusing in quinoa and other species, but current consensus suggests that ABA can both directly inhibit growth and indirectly stimulate growth by reducing ethylene biosynthesis, due to signals controlling growth that are organ- and tissue-specific, and finally in severe drought conditions ABA can activate aquaporin expression, thus controlling hydraulic conductance (Tardieu *et al*., 2010; Wilkinson and Davies, 2010; Claeys and Inze, 2013).

2.2. Turgor maintenance and osmotic adjustment

Recent evidence suggests that quinoa apparently uses a different system for adapting to water-deficient soil than that previously reported in maize, showing interactions between N, ABA and xylem pH to stomatal behaviour during soil drying (Jacobsen *et al.*, 2009). The mechanisms possibly used by quinoa to maintain turgor under increasing drought, in which ABA apparently plays a minor role, may include osmotic adjustment (Jensen *et al.*, 2000). Both high net photosynthesis rate and specific leaf area (SLA) values during early vegetative growth probably result in early vigour of quinoa, supporting early water uptake and thus tolerance to subsequent drought. The leaf water relations were characterized by low osmotic potential and low turgid weight/dry weight (TW/DW) ratio during later growth stages, sustaining a potential gradient for water uptake and turgor maintenance (Jensen *et al.*, 2000). The inherent low osmotic potential in quinoa probably causes drought tolerance, as in the case of lowering the osmotic potential by osmotic adjustment in other crop species such as wheat.

Another possible explanation for drought-induced stomatal closure is that quinoa produces antitranspirant compounds other than ABA in the xylem sap. Cytokinins, the classical antagonists of ABA, may play a role. When cytokinin transport is reduced in the xylem, for instance as a result of limited N supply, stomatal sensitivity to xylem ABA may be increased (Jacobsen *et al.*, 2009). These authors concluded that during soil drying, quinoa plants have sensitive stomatal closure, maintaining leaf water potential and photosynthesis and resulting in increased water use efficiency. The apparent lack of significant root-sourced ABA regulation means that quinoa must depend also on hydraulic regulation through a change in turgor or activity of other biological compounds yet to be determined.

A salt stress-induced increase in the total level of soluble sugars, proline and glycine betaine was reported in quinoa (Jacobsen *et al.*, 2007, 2009; Ruffino *et al.*, 2010). Glycine betaine and other betaine derivatives have long been recognized as major osmolytes in several species. These two compatible solutes may account for around 3% of the total osmolality values measured in experiments on quinoa's responses to salinity (Hariadi *et al.*, 2011), consistent with and suggesting an indirect role for compatible solutes in plant osmotic adjustment.

A very different and surprising form of interplay between tolerance and growth is mediated by proline, which accumulates in response to many abiotic stresses and acts as an osmolyte osmoprotectant regulator of redox balance and signalling molecule. Proline is also considered the only osmolyte able to scavenge free radicals, thereby ensuring membrane stabilization and preventing protein denaturation during severe osmotic stress (Szabados and Savouré, 2010; Shabala *et al.*, 2012). Recently, proline was shown to be transported to growing tissues to act as an energy source to support both root and shoot growth, as proline catabolism directly transfers electrons to the mitochondrial electron transport chain (Sharma *et al.*, 2011).

Since salinity and drought share common osmotic responses, the accumulation of sugars and proline allows plants to maintain the cellular turgor pressure necessary for cell expansion under stress conditions; they also act as osmoprotectants. Indeed, 300 mM NaCl induced an accumulation of proline in all quinoa genotypes evaluated; these could be divided into those that exhibited a moderate increase, and those that accumulated three to five times more of this osmolyte over control levels. Considering that this compatible solute acts as an osmoprotectant with a positive function in mitigating abiotic stress, the highest proline accumulation correlated with the most salt-tolerant quinoa genotype (Ruiz-Carrasco *et al.*, 2011).

2.3 Leaf growth, morphological and anatomical adaptive changes

Inhibition of leaf growth improves water balance and stress tolerance by limiting water loss, and thus ensures plant survival under water deficit. However, if this constraint is not only temporary, limiting growth too extensively (risk avoidance) can lead to a competitive disadvantage and unnecessary yield losses. Conversely, continued growth (taking risks) can threaten survival when water limitation turns out to be long and severe. Therefore, a balance between growth and survival, or in other words a choice between risks, is tightly regulated (Claeys and Inze, 2013).

Thus, growth regulation aimed at limiting shoot growth and thereby transpiration area is an integral part of the drought response of several plants. It has become evident that a very rapid and actively regulated response is not merely a consequence of altered hydraulics, as it cannot be abolished when xylem water potential is maintained, and it occurs in different species even when leaf water potential is not affected. Growth is also much more sensitive to water limitation than photosynthesis, and as a consequence carbohydrates as starch often accumulate in stressed plants, showing that growth reduction is not just the consequence of carbon starvation. There is a rapid and sharp decrease in leaf elongation rate in many species, termed "acute growth inhibition", followed by recovery of a new steady-state growth rate, referred to as "acclimation" (Skirycz and Inzé, 2010).

Indeed, the leaf expansion rate (LER) determined for well-watered quinoa grown in pots in a controlled environment greenhouse was rather high (up to 500 mm^2/day/plant), whereas it decreased to 0 and was significantly lower than the control from the onset of drought. Drought reduced LER on average to about 50% during the first 10 days compared with well-watered plants. Moreover, plant leaf area was determined by both the area of individual leaves and the number of leaves, and drought may affect both. Nevertheless, the authors observed that reduction in single leaf expansion and whole plant leaf area occurred at a similar soil-water status (Jacobsen *et al.*, 2009).

Other quinoa responses to drought were mentioned earlier in this chapter (Dizès, 1992; Vacher, 1998), for example, massive leaf senescence and the existence of many bladders or glands in the stems and leaves whose volume varies depending on water deficit. Although quinoa leaves wilt under severe drought, thus decreasing leaf transpiration by reducing the leaf surface exposed to direct solar radiation, quinoa has evolved a remarkable ability to resume leaf formation quickly after a major drought stress, and its wilting point is also lower than other Andean crops such as bitter potato (*Solanum juzepczukii*) and sweet potato (*Ipomoea batatas*) (Dizès, 1992). Expanded leaf surfaces are smooth, since trichomes are lost in mature leaves and leaves have a thick cuticular epidermis, whereas young leaves are covered by multiple bladders containing calcium oxalate and silicic anhydride that are hygroscopic in nature and reduce transpiration, as determined by scanning electron microscopy in young leaves and cortical parenchyma, which suggests an indirect role in water economy and turgor maintenance (Dizès, 1992; Shabala and Mackay, 2011). Another anatomical feature likely to confer drought tolerance in quinoa consists of stomata deeply sunken in the leaf epidermis (Dizès, 1992). Similarly, small thick-walled cells may be better adapted to large water losses without loss of turgor (Jensen *et al.*, 2000; Jacobsen *et al.*, 2003), suggesting a biophysical mechanism as well.

2.4 Importance of root morphology and architecture for drought tolerance

The root system is a complex plant organ with multiple critical functions: anchorage and support, soil exploration, water and nutrient acquisition and transport, secondary metabolite synthesis and exudation (Hodge, 2009). When soil water uptake by the roots or xylem water transport becomes insufficient to satisfy evapotranspiration, or water demand is not satisfied in time by root absorption and transport, as in transient water deficit in irrigated crops, plants enter into a water deficit, which may affect dry matter accumulation (growth) as well as plant phenology (Passioura and Angus, 2010). Growth and development are crucial for plant productivity and, more specifically, for the economic yield of grain crops.

The capacity of plants to explore the soil and exploit water resources depends firstly on the spatial configuration of the root system and its growth dynamics during the vegetative cycle (Malamy, 2005; Hodge, 2009). The general configuration of the root system, or architecture, is described on the basis of dichotomic (without any predominant root axis) or "herringbone" (with a main root axis supporting lateral roots) patterns. With regards to the growth dynamics of the root system, plants with an enhanced capacity for root expansion can reach soil layers with higher resource availability more rapidly than those with slow or spatially limited root growth.

In addition to root system architecture, water acquisition by plants also depends on root morphology and anatomy. Specific root length (SRL: root length/dry matter ratio), for example, is associated with capacity for root elongation (Eissenstat, 1992; Roumet *et al.*, 2006). Similarly, the diameter and order of appearance of the roots may modify the absorption and transport of water to upper plant tissues (Pregitzer *et al.*, 1997; Ito *et al.*, 2006). These traits of root architecture and morphology may vary because of many interacting factors: plant phenology, growth conditions, drought intensity and duration, soil properties (Fitter, 1991; Kranner *et al.*, 2010; Nicotra *et al.*, 2002).

In this context and considering the ephemeral character of soil water resources, root system capacity to adjust itself to these changes appears of fundamental importance (Reader *et al.*, 1993). Several root traits, such as SRL or the root/shoot ratio (R/S: ratio of root dry mass/aerial part dry mass), display some degree of variation, a feature known as root phenotypic plasticity (Fitter, 1991). These traits associated with other anatomical modifications would allow for a higher transport capacity or greater exploration capacity in dry soil layers (Nicotra *et al.*, 2002). However, these root system responses may also have a high carbon cost (Fitter, 1991) and slow down the development of other basal or adventitious roots (Walk *et al.*, 2006), or may even generate inter-root competition.

As for quinoa's root system, with the exception of short descriptions of some botanical traits (Mujica *et al.*, 2001), and of the chemical composition and R/S ratio (Schlick and Bubenheim, 1996; Bosque Sanchez *et al.*, 2003), interesting studies have recently tended to focus on root hormonal signalling (Jacobsen *et al.*, 2009; Razzaghi *et al.*, 2011), and on the impact of water and environmental factors on the R/S ratio and root length (González *et al.*, 2009a, b). In terms of morphological responses to water deficit, these studies have shown that biomass allocation between roots and shoots is unaffected by water deficit in quinoa (Bosque Sanchez *et al.*, 2003; González *et al.*, 2009a), which suggests the intervention of other adaptive mechanisms in response to drought.

3. Adaptations and traits

Enhanced shoot growth is seen as a contributing factor boosting plant performance under water-limiting conditions, as this reduces evapotranspiration and is also coordinated with enhanced root growth and better water uptake. However, factors controlling growth and tolerance mechanisms are important for continued growth in mild drought conditions, as this allows a plant to de-activate growth inhibition while maintaining a certain level of protection against damage (Claeys and Inzé, 2013). Focusing on particular traits may exacerbate the problems under severe drought, where lack of CO2 from stomatal closure, photosynthesis inhibition and reduced turgor will passively limit growth (Tardieu et al., 2010). In this case, different strategies need to be adopted to endure the stress as long as it occurs, while limiting plant transpiration and cell damage as much as possible and maximizing water use. Nevertheless, the idea that water use efficiency is synonymous with drought resistance and high yield under drought stress conditions is considered erroneous. Indeed, breeding for maximized soil moisture capture for transpiration is the most important target for yield improvement under drought stress, thus supporting the notion of effective use of water through physiological traits to minimize yield variations (Blum, 2009; González *et al.*, 2011).

3.1. Gas exchange, stomatal control and water use efficiency

Genotypic variations of leaf gas exchange and seed yield of ten quinoa genotypes adapted to high altitude in northern Argentina were analysed under drought conditions. The results showed that quinoa could produce interesting grain yields (i.e. promising varieties yielding up to 3 855 kg/ha) in arid regions other than the Bolivian Altiplano under reduced irrigation (González *et al.*, 2011). This study raised the possibility that leaf stomatal conductance is a heritable trait associated with heat stress prevention and increased yields. Since biomass production is closely related to the rate of transpiration, the most important breeding objective to optimize yields under drought conditions is to maximize the absorption of soil moisture for transpiration (González *et al.*, 2011). Previous studies revealed that quinoa evolved adaptive mechanisms to cope with drought through high water use efficiency and high root/shoot ratios. The maximum photochemical efficiency of photosystem II (chlorophyll fluorescence Fv/Fm ratio) and quenching analysis (qP and qN) showed that dehydrated quinoa plants were less protected from photo-inhibition than salt-stressed plants (Bosque Sanchez *et al.*, 2003). Similar chlorophyll fluorescence studies demonstrated a fast recovery of photosynthesis in young quinoa plants after a drought stress period, suggesting the maintenance of high photochemical efficiency despite water deficits (Winkel *et al.*, 2002).

Other physiological and biochemical traits have also provided useful information about plant adaptations to arid and semi-arid conditions using yield and chlorophyll concentration, since chlorophyll degradation under stress is an adjustment to reduce the electron flow between photosystem I (PSI) and PSII that could prevent photo-oxidative damage. Thus high carotenoid and chlorophyll content are desirable characteristics, as they indicate low levels of photoinhibition (González *et al.*, 2011). Moreover, stomatal conductance was relatively stable with low gas exchange, but steady under very dry conditions and low leaf water potential. Quinoa maintained high water use efficiency to compensate for the decreased leaf stomatal conductance and carbon gain by minimizing water loss (Vacher, 1998).

Drought effects on stomatal conductance, photosynthesis and leaf water relationships at different phenological stages have been determined (Jacobsen and Mujica, 2001; Razzaghi *et al.*, 2011), concluding that some quinoa varieties exhibit gas exchange parameters within the normal C3 plant range, and water relations are characterized by low osmotic potential that can be a major trait associated with drought tolerance. In general, measurements are expensive and difficult to implement, and also have generally limited spatial significance. A different approach to evaluate the effect of drought stress on quinoa development was assessed with three different indicators in field experiments: the number of days that the soil water content of the root zone was above a threshold, average relative transpiration and the standardized sum of daily actual transpiration, $\Sigma(T_a/ET_0)$ (Geerts *et al.*, 2008a). The best indicator to quantify the effect of pre-anthesis drought stress on phenological development was $\Sigma(T_a/ET_0)$ accumulated until 60 days after sowing (Geerts et al., 2008a). Recently, the use of stable carbon isotopes provided reliable measurements, which were positively correlated with grain yields and negatively with intrinsic water use efficiency (González *et al.*, 2011). This study indicated that genotypes with higher yield under stress had higher stomatal conductance and increased transpiration, consistent with reports for other crops (Blum, 2009).

A recent evaluation assessed grain yield and environment interaction of nine quinoa genotypes of different origins, which were exposed to two watering regimes (dry and irrigated) over two seasons in a Mediterranean environment in central Chile (Garrido *et al.*, 2013). Genotype yields were reduced to less than 50% when irrigated at 44% and 80% reference evapotranspiration. The authors determined significant interactions between genotype and environment for yield, harvest index and grains/m^2. Interestingly, a principal component analysis (PCA) showed a strong and significant association among yield, harvest index and grain number/m^2, low variability among genotypes when stressed, and much higher variability when the stress was not present (Garrido *et al.*, 2013). Low yields resulted from the effect of drought on the key stages of pre-flowering, flowering and pasty grain, which were previously determined as the most sensitive stages to water stress in quinoa, with a negative effect both on total grain yield and WUE (García, 1991; Geerts *et al.*, 2008a).

3.2. Root morphology and architecture of quinoa ecotypes

Recent studies open new perspectives on the morphology and architecture of the quinoa root system, its intraspecific diversity and plasticity in response to drought (Álvarez-Flores, 2012). For this reason, it is helpful to consider the contrasts existing among quinoa ecotypes, such as the Salare ecotype from the southern dry Altiplano of Bolivia and the Coastal ecotype from the humid coastal lowlands of Chile, two ecotypes that differ in their morphophysiological traits (Risi and Galwey, 1989a), as well as in the pedoclimatic conditions of their native habitats (Table 1). The southern Altiplano is characterized by altitudes near 3 700 m asl, sandy or rocky soils, a cold and arid climate with more than 250 days of frost per year in the most extreme areas, and mean annual precipitation of 150–300 mm (Aroni *et al.*, 2009). The low and infrequent precipitation, high evaporation rate and low soil water retention capacity are extremely adverse factors for crop growth and development (Garcia *et al.*, 2007). The crop environment is quite different 2 000 km further south in the high latitudes and rainy environments of Chile's Pacific coastal lowlands. The temperate and humid habitat creates much more favourable conditions for agriculture, with more than 1 200 mm of precipitation distributed throughout the year and soils with a high water retention capacity (Tosso, 1985).

Table 1. Origin of the two studied ecotypes of quinoa

Ecotype	Locality	Latitude	Longitude	Altitude
Salares	Jirira, Bolivia	19°51’S	67°34’W	3 700 m
Lowlands	Cunco, Chile	38°56’S	72°03’W	200 m

Rhizotron studies under controlled conditions allowed to compare the root growth of quinoa plants grown in sandy soil with non-limiting (12% volumetric humidity) or restricted (7% volumetric humidity) water availability, during 2 months beginning after seed germination (Álvarez-Flores, 2012). This period corresponded to the critical phase of crop establishment and plant vegetative growth, representing nearly half of the complete crop cycle. Under non-limiting water conditions, both quinoa ecotypes revealed a herringbone pattern in the root system architecture. In general, this topology reduces competition among roots of the same plant, as well as among roots of neighbouring plants, thus optimizing the exploitation of soil resources, even more so when these resources are limited (Fitter, 1991; León *et al.*, 2011). Furthermore, the presence of a strong main root axis allows to explore deep soil layers more rapidly and efficiently, a critical feature in early stages of plant development (Glimskär, 2000; Paula and Pausas, 2011).

In spite of their similarity in root topology, the quinoa ecotypes studied differed with regard to their growth dynamics and the features of their root system architectures. Under non-limiting conditions, primary root elongation was rapid during the first 6 weeks of crop growth, and slowed down thereafter. In the 6th week, when shoots of both ecotypes hardly reached 6–8 cm above the soil surface, the primary root of the Salare ecotype reached a depth of 1 m. The Coastal ecotype reached the same length a week later, a delay that reflects early vigour differences between the seedlings in relation to average seed size in the studied ecotypes (4.9 vs 2.1 mg per seed for the Salare and Coastal ecotypes, respectively – Álvarez-Flores, 2012). From the sixth week onwards, when primary root elongation began to slow down, growth of the rest of the root system began to accelerate due to ramification and elongation of the lateral roots. Consequently, total root system length reached up to 650 m/plant without significant differences between ecotypes at week 9 under non-limiting water conditions (Álvarez-Flores, 2012).

The differences in root architecture determined among ecotypes only appeared when two components of total root length were considered, namely number and length of root segments (i.e. root elements situated between two ramifications or between a ramification and a root meristem). During the first week, the Salare ecotype produced a primary root with longer segments than the Coastal ecotype (7.3 vs 2.5 cm average). This allowed the Salare ecotype to explore deep soil layers rapidly (Figure 1, 28 DAS [days after sowing]) and a major part of the lateral roots were formed at depth in the subsequent stage of root ramification (Figure 1, 42 DAS). These lateral roots displayed segments with 50% greater average length compared to those of the Coastal ecotype, which allowed to compensate for the equal or similar number of segments of the Salare ecotype. The final result was that the Salare ecotype did not produce a greater total root length than the Coastal ecotype, but it displayed a much faster colonization rate and dense in-depth root system (Álvarez-Flores, 2012).

3.3. Ecotype responses to water deficit in the quinoa root system

Root systems of Salare and Coastal ecotypes presented a more “herringbone” topology under water deficit, which implied greater reduction in lateral root growth than in primary roots. In fact, when drought occurs at early plant growth stages, the elongation of the primary root is considered beneficial for the acquisition of deeper, more reliable water resources, while a dense root ramification could result in rapid exhaustion of an unreliable water resource in the shallow soil layers (Padilla and Pugnaire, 2007). Indeed, differences between the ecotypes studied were that the root system of the Salare ecotype presented faster elongation and denser in-depth colonization. The architectural traits of the root system of the Salare ecotype may be the reason for a common practice in the driest areas of the Altiplano: cultivating quinoa fields every other year, so that water can accumulate in the deep soil layers during the crop-free year.

Water deficit also reduced the total length of root systems, although to a lesser extent in the Salare ecotype (-38% vs -57% in Coastal). These growth reductions were greater in the aerial plant parts than in the underground plant parts, since the root/shoot ratio of both ecotypes increased in water-stressed plants. In general, water deficit did not affect the **mean** length of the root segments. On the other hand, there was a significant reduction in the **total** length of the root system as the actual number of

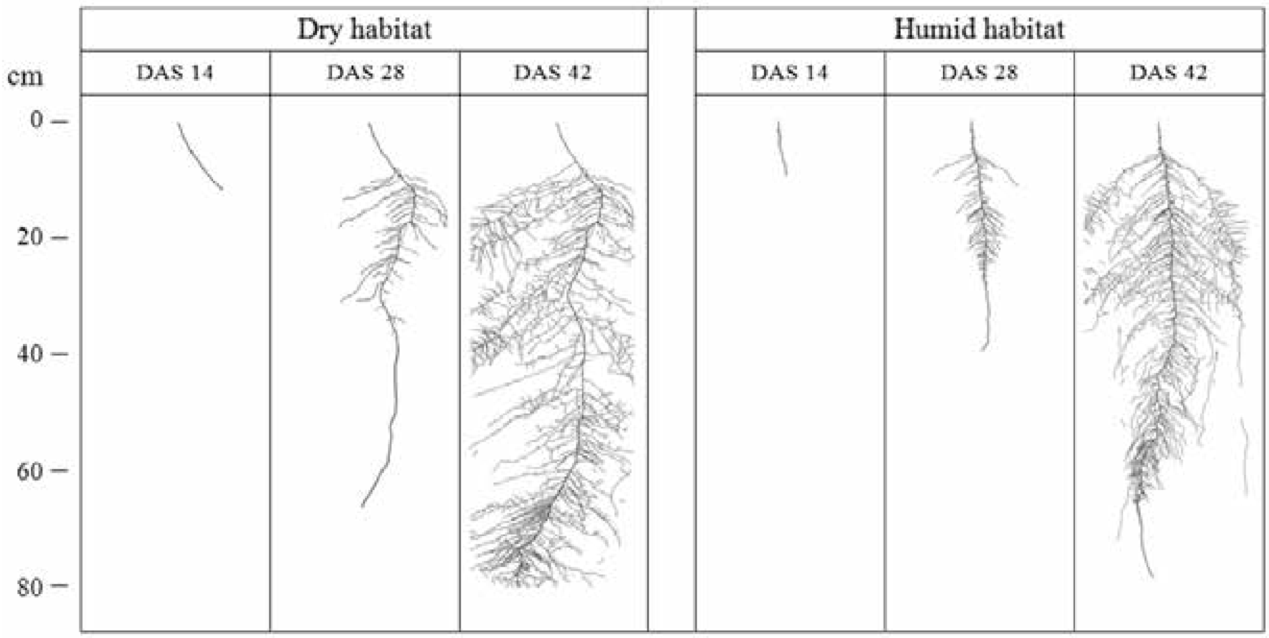

Figure 1: Time-course of root growth in two quinoa ecotypes from contrasted habitats at 14, 28 and 42 DAS (days after sowing).

root segments was reduced, with a difference between ecotypes (-8% in Salare vs -23% in Coastal). This could imply a significant ecotypic difference in root systems with regards to water absorption and sensitivity to water deficits (Álvarez-Flores, 2012).

It should be noted that water deficit – compared with non-limiting availability of water – stimulated primary root elongation in both ecotypes. In the Salare ecotype under non-limiting conditions, primary roots grew up to 50 cm during the first four weeks of the plant cycle, whereas they reached 75 cm in the same time interval under water deficit. In the Coastal ecotype, they grew to 35 and 40 cm, respectively (Figure 2). The rapid elongation of the primary root allowed the Salare ecotype to produce lateral roots distributed evenly throughout the entire soil profile, with a root density similar to that of plants growing with higher water availability. In contrast, the Coastal ecotype concentrated its lateral roots in soil layers between 5 and 50 cm, and exhibited very low root density in deeper soil layers (Álvarez-Flores, 2012).

4. Molecular Studies and Gene Discovery

Efforts to improve the crop have led to an increased focus on genetic research. The first study was published in 2005 by Maughan's group (Coles *et al.*, 2005): an EST database for quinoa using immature seed and floral tissue. These sequences were analysed for homology with known gene sequences and also for the identification of single nucleotide polymorphisms (SNPs) for quinoa. They compared 424 cDNA sequences of quinoa with sequences in the publicly available databases. Two-thirds (67%) of the quinoa proteins showed homology to Arabidopsis proteins with putative function, 18% had no significant matches, 9% had significant homology to *Arabidopsis* proteins with no known function and 6% shared significant homology with plant proteins of species other than *Arabidopsis*. Fragments of 34 ESTs were amplified and sequenced in five quinoa accessions and one related weedy species, *C. berlandieri*. Analysis of the quinoa EST sequences revealed a total of 51 SNPs in 20 EST sequences.

A recent paper from the same group (Maughan *et al.*, 2012) reported the identification of 14 178 putative SNPs; a diversity screen of 113 quinoa accessions was used for comparison with the five accessions used in the former study. The study also recovered the two major subgroups corresponding to Andean and Coastal quinoa ecotypes. Therefore the SNPs identified represent a valuable genomic tool that will be very useful for emerging plant breeding programmes looking for important agronomic

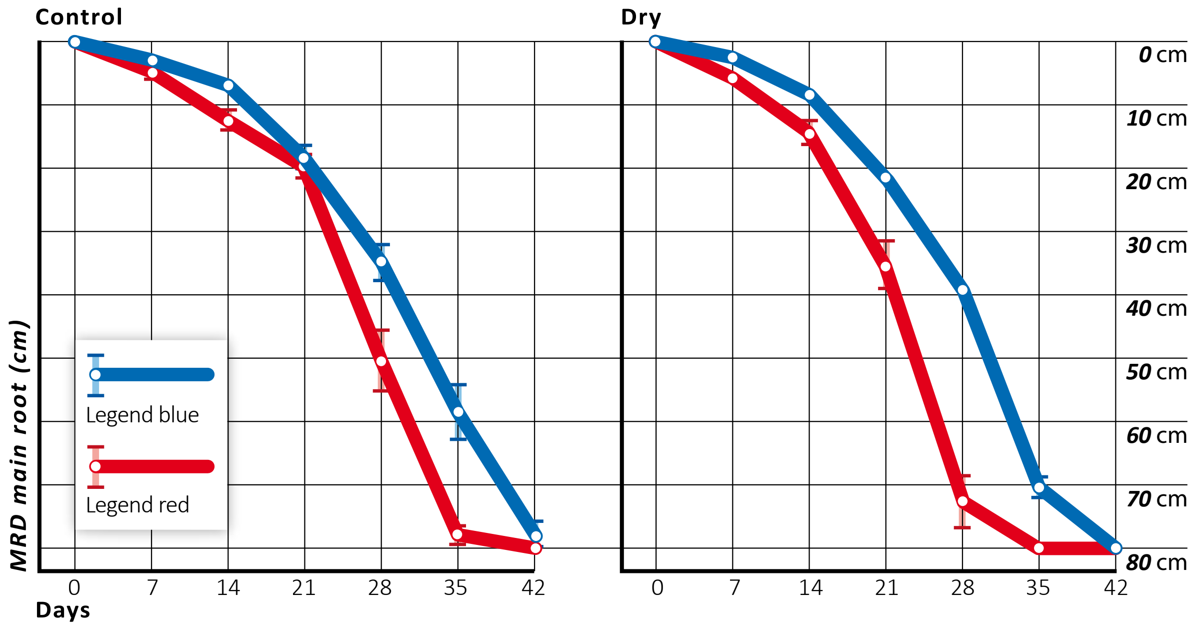

Figure 2: Dynamics of the maximal root depth (MRD) in two ecotypes of quinoa from Salares (Δ) and Lowlands (▲) (n = 5, the vertical bars show the standard errors, the gray areas show the water content in the soil: dark gray = 12% vol., light gray = 7% vol.).

traits in quinoa. Furthermore, a linkage mapping of the SNPs in two recombinant inbred line populations produced an integrated 29 linkage group map, spanning 1 404 cM with a marker density of 3.1 cM per SNP marker.

Unfortunately, quinoa EST generation based on Sanger sequencing is still very limited compared to other species. At present, only 424 ESTs can be found in the public domain (http://www.ncbi.nlm.nih.gov/nucest/?term=chenopodium+quinoa). Most of the work done on quinoa has been based on response to salt stress and some important genes have been characterized. Maughan *et al.* (2009) cloned and characterized two SOS1 gene homologs (*CqSOS1A* and *CqSOS1B*) of quinoa and found a high level of homology of these gene sequences to orthologous *SOS1* of other species. The expression of *CqSOS1* upon application of NaCl was investigated in a cultivar originating from the Salare region in the Bolivian Altiplano. Gene expression analyses showed greater expression in roots than in leaf tissue in the absence of salinity. However, the presence of 450 mM NaCl caused an up-regulation of both genes in leaf but not in root tissue (Maughan *et al.*, 2009). Ruiz-Carrasco *et al.* (2011) confirmed the different responses of sodium antiporters to NaCl in shoots and roots, and also cloned and analysed the expression of *CqNHX*. Interestingly, genes were differentially regulated in different genotypes. Different studies related to this abiotic stress have been used to study a salt tolerance mechanism in quinoa (Adolf *et al.*, 2012). However, more studies and discovery of new genes are needed, as reviewed by Jellen *et al.* (2013).

Studies have also been conducted on early drought stress effects (up to 9 days after sowing – Morales *et al.*, 2011a). These authors used an Altiplano Chilean quinoa genotype and performed a transcriptome sequencing analysis under dry and normal irrigation conditions. The transcriptome was sequenced by Illumina paired ends. The results were 53 million reads under control conditions and 50 million reads under drought conditions, which were assembled into 18 000 contigs measuring > 1 kb. In this study, a digital expression gene analysis was performed, resulting in 529 genes induced and 201 genes repressed under drought conditions (Morales *et al.*, 2011b; Zurita-Silva et al., 2013, unpublished data). This drought RNA-seq database is being used to discover/identify transcription factors in response to salt stress, given that these two stresses share similar molecular/physiological mechanisms for dealing with osmotic stress and ion toxicity (Ruiz and Silva, personal communication).

4.1 *The Future of Molecular Studies and Gene Discovery in Quinoa*

Most molecular studies in quinoa have been developed under salt stress conditions and gene identification has not kept the pace required to understand the genetic basis of differential physiological responses. The genome has still not been sequenced. An RNA-seq transcriptome analysis in different tissues of *Chenopodium quinoa* using four water treatments (from field capacity to drought) on an Inter-Andean valley ecotype ('Ingapirca') and a Salare ecotype ('Ollague') was recently released (http://www.ncbi.nlm.nih.gov/bioproject/195391). It is important to mention that a transcriptomic analysis of amaranth, a pseudocereal like quinoa, has been published and could serve as a reference for annotation and gene discovery (Délano-Frier *et al.*, 2011). Other strategies include the study of different genotypes of quinoa in search of genes induced by drought conditions. A full-length cDNA library was generated for transforming *Arabidopsis*, and transgenic lines obtained were assessed for their tolerance to drought conditions. Consequently, the genes that suggest tolerance in *Arabidopsis* were sequenced and identified, resulting in candidates corresponding both to orthologous and unknown genes, which may help to identify novel drought-tolerance genes (Zurita-Silva *et al.*, 2013, unpublished data).

5. Conclusions and Perspectives

Quinoa endures harsh climate conditions in various regions of its distribution area, particularly in the southern Altiplano of Bolivia, northern Chile and northwestern Argentina. In southern Bolivia, the world leader in quinoa production for export, the crop faces frequent drought events due to low and irregular precipitation and high evaporative demand (Vacher *et al.*, 1994; Geerts *et al.*, 2006; Jacobsen, 2011), and there is also high probability of frost (Jacobsen *et al.*, 2005, 2007; Winkel *et al.*, 2009; Pouteau *et al.*, 2011), as well as extreme solar radiation due to high altitude (Vacher *et al.*, 1994).

Although the causes of the variability in the physiological responses of quinoa to the environment remain largely unknown, it is often considered that the diversity of local quinoa varieties reflects selection and adaptation to the local soil and climate conditions of different habitats. However, a clear morphophysiological adaptation of these genotypes to local ecological conditions had not previously been demonstrated (Del Castillo *et al.*, 2007; Winkel *et al.*, 2009; Garcia *et al*., 2007). The diversity of the five major quinoa ecotypes and their tolerance features makes quinoa an interesting plant model (Fuentes and Zurita-Silva, 2013), mostly for studies of the functioning of shoot components related to photosynthesis (Bertero, 2001; Winkel *et al.*, 2002; Jacobsen *et al.*, 2005, 2007; Ruiz and Bertero, 2008), hormonal regulation (Jacobsen *et al.*, 2009; Gómez *et al.*, 2011), nutrient absorption (Razzaghi *et al.*, 2012a) and deficit irrigation responses (Geerts *et al.*, 2008a, b, c) – just some of the features included in other chapters in this volume.

As for other crop species, the responses and mechanisms of quinoa for coping with low water availability are included in two major strategies: stress avoidance and stress tolerance. However, this species has shown an outstanding ability to balance water uptake and water loss, and thus avoid water deficit. Quinoa enhances water uptake in various ways: by accumulating solutes (e.g. proline) which lower tissue water potential; by modulating root architecture; and through tight stomata control, which restricts shoot growth and accelerates leaf senescence, limiting water loss through evaporation. These mechanisms require fine regulation through, for example: hormonal signalling; balancing leaf growth and stomatal conductance; turgor maintenance; and dynamic osmotic adjustment. Indeed, Geerts *et al.* (2008a) demonstrated the high phenotypic plasticity of quinoa as a drought escape mechanism. Although they did not present a complete drought stress-thermal time interaction model, quinoa plasticity in response to pre-anthesis droughts was quantified for field conditions; the proposed model should be validated for other quinoa varieties and regions, and also improved by considering post-anthesis drought (Geerts *et al.*, 2008a). Reported values of seed yield per unit of water consumed ($WP_{Y/ET}$) are rather low (0.3–0.6 kg/m^3) as a result of the generally prevailing low fertility conditions (Geerts *et al.*, 2009). Quinoa plants have also evolved morphological and anatomical features that allow adaptive changes in response to drought, for example: leaf senescence control, vesicles containing calcium oxalate in the stems and leaves, thick cuticular epidermis and more sunken stomata than other Andean crops.

Variations in root architecture among quinoa ecotypes under water-limiting conditions, such as primary root elongation rate and root density in deep soil layers, point to a genotypic differentiation possibly associated with the selection of habitats with different resource availability, combined with selection by local growers. These root system traits could be of crucial significance in the dry conditions of the Altiplano, where the average annual precipitation does not satisfy water requirements for a complete crop cycle. They could also be useful for breeding new cultivars for agroecosystems with reduced input requirements (Lynch and Brown, 2012). Breeding for maximized soil moisture capture for transpiration is the most important target for yield improvement under drought stress (Blum, 2009).

With the incorporation of new technologies and approaches, such as the integration of genomic, transcriptomic and reverse genetic studies, the full potential of quinoa genetic variability could be exploited in order to generate new cultivars; this also represents a novel source for gene discovery that might serve in other crops of agronomic importance. These considerations are made in the face of current challenges, such as climate change and oscillations that constrain food production in the world, and quinoa is therefore an outstanding crop model for stress tolerance studies. Considering also its superior nutritional attributes (covered in Chapter 3.4), quinoa represents both a challenge and an opportunity to contribute to food security and sovereignty, not only in the Andes, but also in Africa, Asia and other parts of the world.

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CHAPTER: 2.5

Quinoa breeding and modern variety development

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Abstract

Quinoa can be genetically improved through various approaches. Conventional breeding methods, such as mass and individual selection, hybridization or interbreeding (intra- and intervarietal), and induced mutation, can all be used in conjunction with modern biotechnological tools. The principle objective is to create exceptional varieties that combine high yield potential, tolerance and resistance to biotic and abiotic factors, adaptability to diverse agroclimatic zones and suitable grain quality for food and industry. Due to quinoa's increasing popularity and the expansion of growing areas, other objectives have recently emerged, including morphological and physiological modifications that allow for mechanized harvesting, improve salinity tolerance and resistance, and reduce agricultural inputs. Breeding methods have made it possible to develop superior varieties in countries such as Bolivia, Peru, Ecuador, Chile, Denmark and the United States of America. Given the considerable genetic diversity and variation in centres of origin, continual genetic improvement and the introduction of the crop to other countries, quinoa could become humanity's new staple food crop.

This is especially true in countries with limited protein sources or poor food production capacity. The expansion of quinoa cultivation would increase the range of potential food sources (currently based on very small number of species, e.g. wheat, maize, rice, potato or soybean), while quinoa's high nutritional value could help combat malnutrition. It could also provide a solution to climate change issues.

Introduction

Quinoa breeding began thousands of years ago when people started selecting seeds and plants to alter the phenotypic and genotypic traits in areas around the Andean region. Evidence of wild and cultivated species of the *Chenopodium* genus can be found in Ayacucho in the Central Sierra, Peru, dating back to 5000 B.C.; in the Preceramic Chinchorro Complex in northern Chile (3000 B.C.); in indigenous tombs of the Tarapacá, Calchaquí-Diaguita, Tiltil and Quiligua peoples in Chile; and at the archaeological site of Punta de la Peña 4, layer 3, in the city of Antofagasta de la Sierra in Catamarca Province, Argentina (760–560 B.P.) (Uhle, 1919; Bollaert, 1860; Latchman, 1936; Nuñez, 1970; Tapia *et al.*, 1979; Rodríguez *et al.*, 2006; Lumbreras *et al.*, 2008). Trait improvement achieved through natural or human selection is visible in the seeds. The chenopods group features thinner pericarps and truncated margins that are the result of selection by farmers aiming to reduce seed dormancy and increase viability (Smith, 1992; Murray, 2005). Another important trait is the selection of white grains, evident in the decline in the proportion of black grains in the various samples collected through the years (Tapia *et al.*, 1979). Domesticated chenopods present more compact inflorescence, no seed dispersion system, uniform fruit maturity, reduced seed dormancy period and improved tolerance against shattering (Smith, 1984; Gremillion, 1993a; Bruno, 2001).

This process of natural and human selection occurred over several millennia alongside natural evolutionary processes, such as mutation and intra- and interspecific hybridization. As a result, complex populations with high genetic diversity developed in regions like the Andes, which has countless microclimates characterized by a wide range of moisture (very dry to very wet), temperature (very cold to very hot), altitude (sea level to 4 000 m asl), latitude (approximately 4°N to 40°S), soil types and numerous other factors to uses. Quinoa, maize and potatoes were probably the staple foods of people living in the Andean region until the Spanish conquerors arrived in 1534. The importance of quinoa and other Andean crops declined as the Spaniards replaced them with crops such as wheat, barley, oats, beans and green peas. For more than 500 years, quinoa was an underutilized crop, unknown to the world outside the Andean region. Farmers grew it for their own consumption, mainly in the Peruvian-Bolivian Altiplano. Traditional farming methods helped preserve quinoa's extensive variability, which would otherwise have been irretrievably lost (Cusak, 1984; National Research Council, 1989; Mujica, 1992; Jacobsen and Stolen, 1993).

Research on quinoa since the 1960s has highlighted the crop's exceptional nutritional quality and ability to thrive in marginal environments, resulting in worldwide interest and an increasing demand for quinoa that can only be met with greater production. There are various ways to increase production, and one of the most important is to develop new varieties adapted to current needs.

Quinoa breeding programmes began in the 1960s in Bolivia at the *Estación Experimental de Patacamaya*, financed by FAO–OXFAM (Oxford Committee for Famine Relief) and the Bolivian Government, and in Peru at the *Universidad del Altiplano*, Puno (Gandarillas, 1979; Tapia *et al.*, 1979). Today, there are quinoa breeding programmes in countries all around the world.

Breeding objectives

Quinoa breeding programmes must aim to balance the needs of farmers, industry and consumers, which vary over time and depend on the specific region or country where the crop is grown.

In the 1960s and 1970s, breeding efforts focused on yield, large grain size, eliminating saponins, single stems or no-branched stems with a well-defined panicle, disease resistance and excellent cooking quality (Gandarillas, 1979). In the 1980s and 1990s, other objectives were added, such as early maturity, black and red grains, and mildew resistance. Between 2000 and 2010, breeders began attempting to create varieties with hail and drought resistance and excellent industrial and nutritional quality, while being suitable for mechanized harvesting. This final objective was the natural result of new agricultural management approaches.

In Bolivia, particularly in the southern Altiplano ('Quinoa Real' commercial production export zone), there is a need for intermediate and very early-maturing varieties with large white, red and black seeds. In the central Altiplano, early-maturing varieties are required with medium mildew resistance and medium to large seeds (white or red) that are hail and frost resistant. Varieties in the north Altiplano must have high mildew and hail resistance, with small, medium and large seeds, depending on the final production destination. In valley areas, preferred varieties are semi-early to early maturing, mildew resistant and produce medium to large seeds. Interest has also been shown in producing quinoa in Santa Cruz as a winter crop, where varieties must be mildew resistant and heat tolerant, as well as suitable for mechanized harvesting.

In Peru, breeding objectives focus on high yields of large white and red seeds, although recently early maturity has also gained ground as the rainy season comes later in the year, resulting in later planting times. This makes growing varieties with long life cycles difficult. Breeders also aim for heat and salinity tolerance and a plant architecture adapted to mechanized harvesting and high-tech farming techniques, given the great potential for planting quinoa along the Peruvian coast.

In Ecuador, breeding objectives are centred on yield, large white seeds and mildew resistance. During selection, researchers also take into consideration agricultural management requirements, with the aim of facilitating mechanized harvesting and threshing.

Improving yield

Breeding efforts aim not only to improve morphological and physiological traits, but also to increase resistance to biotic and abiotic stresses, taking into account the many interactions between them.

High yield

To improve productivity or yield, it is important to determine the degree and type of genetic variation, genotype-by-environment interaction effects and heritability. Given the wide range of environments in regions where quinoa is grown, breeding programmes must consider evaluation strategies to measure the effects of a major genotype-by-environment interaction Bertero et al. (2004).

Mujica *et al.* (2001) reported yields of 2.28 and 3.96 tonnes/ha from genotypes selected for the *Prueba Americana y Europea de Quinua* trial, hosted by FAO in countries such as Italy and Greece. Bonifacio (2003) reviewed cultivars obtained at the *Estación Experimental de Patacamaya* in Bolivia that yielded nearly 1.2 tonnes/ha in the Altiplano, although greater yields (3 tonnes/ha) were achieved when cutting-edge technology was adopted and inputs increased. This is a considerable gain over the 700 kg/ha obtained by farmers growing traditional varieties. Trials along the Peruvian coast have shown that it is possible to reach yields of 6 tonnes/ha when a fertilization and irrigation system is adopted.

Life cycle

Early maturity is a trait that allows a variety to escape the adverse effects of frost and drought. Earliness is important because it allows quinoa plants to cope with climate change, namely delayed onset of the rainy season. Selection for early maturity is relatively easy since days to flowering have a high heritability at 0.82 (Mujica, 1988). However, early maturity does have its limits due to plant sensitivity to the short day length and cloudy conditions of the rainy season.

Resistance/tolerance to biotic stresses (disease and insects)

Improving quinoa's resistance and tolerance to biotic factors is of the utmost importance because small farmers sell their crops to organic markets. Improved resistance to biotic stresses must take into account the genetic components of both the organisms and the host crop. A number of complex interactions – anatomic, biochemical or physiological – take place between them.

Disease resistance to downy mildew (*Peronospora variabilis*) is particularly important, given: 1) the movement of varieties from dry zones to zones with higher relative humidity; 2) the significant rainfall in a short period of time together with high relative humidity conducive to mildew growth; 3) the high interest in quinoa in areas where it is not traditionally grown (Inter-Andean valleys, including the subtropical zone); and 4) mildew's transmission via seeds.

Furthermore, pest damage has reached considerably high levels, especially from insects that feed on the grains and inflorescence, as is the case with *kcona-kcona* (*Scrobipalpula* sp.) moth larvae. Resistance can be achieved through antixenosis (nonpreference for food or oviposition), antibiosis and tolerance. Another important issue is losses caused by birds; these can be reduced by increasing saponin content.

Tolerance to abiotic stresses

Hail and frost tolerance are increasingly important to compensate for climate change and allow expansion of quinoa cultivation to new locations. Delayed onset of the rainy season results in plants being subject to frost during their reproductive stage. Moreover, hail, which frequently occurs during the growing season, poses a major risk for plants (broken or detached leaves, stem lesions, panicle damage) and mature grains (shattering). For these reasons, breeders in Bolivia are attempting to improve these traits.

Drought resistance and salinity tolerance are much more complex traits in terms of both heritability and resistance mechanisms. Recent research has examined the functioning and expression of resistance genes. In Peru and Bolivia, as well as in laboratories specialized in molecular genetics (Brigham Young University), researchers are beginning to see encouraging results in evaluating and selecting for drought resistance (Jacobsen *et al.*, 2001, 2002, 2003, 2005, 2007; Gómez *et al.*, 2010; Ruiz-Carrasco, 2011; Verena *et al.*, 2013).

Morphology: plant architecture

As growing areas have spread and rural labour has become scarce, there is a certain urgency to replace or complement traditional harvesting methods with mechanized techniques. Trials in mechanized harvesting of quinoa have faced considerable difficulties under current crop management practices and the varieties used. In addition to taking advantage of increased inter- and intrapopulation variation, plant architecture must also be altered: standard branching patterns must be eliminated and new varieties developed with a suitable height and single stems with a single inflorescence.

Harvest index

This trait measures photosynthetic capacity and the rate of translocation of photosynthates to the seeds. It is influenced by both farming practices and the environment (Bertero and Ruiz, 2010).

Quinoa germplasm studies in Bolivia show harvest index variations of 0.06 to 0.87. The lower values are typical of Inter-Andean valleys ecotype quinoa varieties, characterized by their tall height and branching patterns (Rojas *et al.*, 2003).

Improving quality

Grain size

The market has traditionally preferred quinoa varieties with large grains that are white – or white once processed. They are sold as pearled (cleaned or washed) products and can be prepared like rice. Small grain quinoa is generally used to make flakes, flours and other products. One important aspect to improve is grain size uniformity in the panicle. Currently, a single panicle can contain three different grain sizes, depending on the genotype, on the basis of the proportion of hermaphrodite flowers (larger) and pistillate flowers (smaller) in the panicle (Rea, 1969; Bhargava *et al.*, 2007).

Bolivian 'Quinoa Real' has large grains and is much appreciated by the export market. It has been selected and grown for several millennia in the southern Bolivian Altiplano, while local varieties from the northern Altiplano have small grains. Native or ecotypical 'Quinoa Real' varieties have been crossbred to incorporate the trait for large grain size through hybridization and later selection to obtain large-grained cultivars that can be grown in the central and northern Bolivian Altiplano (IBTA–DNS, 1996; Bonifacio *et al.*, 2002; Bonifacio and Vargas, 2005).

Saponin Content

Another component of grain quality is saponin content. Saponin, found in the pericarp (outer shell), gives the grain a bitter taste and must be removed before the quinoa can be eaten. Processing technology has been developed and is currently being used to remove the bitter coating. While this makes it possible for the market to not reject products based on this trait, cleaning processes add to the cost and use considerable amounts of water, which could pose a problem in the future. However, saponin is a natural detergent and organic foaming agent that could have uses in industry as soap or other products. Quinoa cleaning processes include technologies to recover discarded saponin.

Quinoa germplasm features a range of saponin contents, from very sweet (no saponin) to very bitter. In some areas, there may be a need for very bitter quinoa varieties to provide a natural defence against certain bird species. However, this does not hold true for insect infestation: *qhuna-qhuna* (*Eurysacca melanocampta* Meyrick and *Eurysacca quinoae*) or *tikuna* (*Helicoverpa quinoa*) moth larvae attack both bitter and sweet varieties alike.

Obtaining sweet quinoa cultivars with large grains was achieved by crossing native varieties in the northern Altiplano with those in the south and using later selection. A series of sweet varieties with large grains and mildew resistance were released (Gandarillas, 1979a; IBTA–DNS, 1996; Bonifacio *et al.*, 2003; Bonifacio and Vargas, 2005).

Grain protein content

Breeding for traits such as increased protein or amino acid content is a significant improvement consideration. Quinoa protein content ranges from 7% to 24% (Koziol, 1992; Wright *et al.*, 2002; Repo-Carrasco *et al.*, 2003; Bhargava, 2007; Gómez and Eguiluz, 2011). High protein content is generally the result of low accumulation of carbohydrates, while increased yield is associated with low protein content. However, Bhargava (2007) reports a direct correlation and a significant decline between yield and grain protein content in several studies on quinoa varieties of various origins. These outcomes could help in the development of varieties with good yield potential and high protein content.

Other nutritive and nutraceutic principles

Recent studies have shown that quinoa's fibre content has a beneficial effect on human health and point to the potential for breeding research in this area. Grain fibre content can vary between 3.5% and 9.7% (Rojas *et al.*, 2010a).

Various companies in Bolivia have been processing quinoa-based products for over a decade. The diversity and marketing of processed products has risen considerably in recent years. However, such products are created using a mix of grains from different varieties and a homogenous quality is not consistently maintained. Breeding programmes have taken this into account and attention has been given to the selection of new traits, such as starch, amylose, amylopectin, starch granule diameter, reduced sugars and water in grain filling. The aim is for modern varieties to meet the specific demands of the food processing industry.

Trait heritability and selection index

To understanding the inheritance of traits selected as breeding programme objectives is important for determining and prioritizing the most appropriate breeding methods.

Espíndola (1980) indicates that yield is closely related to grain diameter, panicle length, plant height and stem diameter. Espíndola and Gandarillas (1985) studied the components of yield in 36 accessions from the germplasm bank and found a strong correlation between yield and plant height, panicle length and stem diameter, in addition to a high association between plant height, stem diameter and panicle length. Espíndola (1988) looked at heritability from a broader perspective based on 11 traits and calculated heritability percentages between 22.4% and 59.11%.

Mujica (1988) examined 32 quinoa variables and identified seven traits with a higher heritability value and a positive yield correlation. The variables were days to flowering, plant height, stem diameter, panicle diameter, central glomerulus diameter, dry weight of the central glomerulus and the number of seeds in the central glomerulus. He also reported that the number of days to flowering presented the highest heritability (0.82) among the traits studied and the lowest yield heritability, meaning that direct selection for higher yield is not very effective. However, the variables of plant height, stem diameter and panicle diameter have greater heritability, and selection for these traits is potentially much more effective. These observations helped identify a series of selection indices for quinoa.

These and other similar studies reveal more information about the heritability of qualitative and quantitative traits, such as plant colour, axillary pigmentation of the stem, inflorescence types, saponin content, seed colour and type, genetic and cytoplasmic male sterility, early maturity and plant height (Gandarillas, 1968, 1974, 1979a, b, 1986; Rea, 1969; Espíndola, 1980; Bonifacio, 1990, 1995; Saravia, 1990; Ward, 2000).

Trait stability

Quantitative traits, such as yield, plant height, panicle length and diameter, stem diameter, fresh and dry plant weight, harvest index, volumetric weight, grain size, weight per 1 000 seeds and protein content, are quantitative inheritance and are of major economic and nutritive importance. These traits are controlled by various genes that confer small additive effects and which are strongly influenced by the environment. Few studies on quinoa have dealt with the stability of these traits. However, breeding for these traits in any crop is more difficult.

Qualitative traits are by nature less influenced by the environment, and some are quite important, such as saponin content and grain colour. Breeding for these traits is easier.

Grain colour has shown an unquantified instability percentage, particularly in the case of white grains, which frequently change to dark colours and in some cases influence other traits. Bonifacio (1995, 1996) attributes the genetic instability of basic traits to spontaneous variation, the action of mobile genetic factors and paramutation. This type of segregation was initially attributed to crossing cultivated species with their wild relatives (ajara). However, recent careful observation has shown that it is due to a phenomenon of natural selection present in quinoa as an adaptation mechanism to stresses.

Gómez and Eguiluz (2013) mention the frequent change in colour of grains in quinoa research when using mutagenic agents such as gamma rays.

Biological and genetic principles in crop improvement

Reproductive biology

One of the first aspects to consider when determining the variety and breeding method to use is the understanding of the crop's reproductive system. Quinoa has a sexual reproduction process. Sexual reproduction is a biological process in which meiosis and pollination result in genetic variation, which is exploited in genetic crop breeding. Ruiz (2002) reported on *in vitro* vegetative propagation of quinoa.

Floral polymorphism

Quinoa is mainly considered a gynomonoecious species. The inflorescence has hermaphrodite flowers (perfect flower) and pistillate flowers, or only gynoecium (imperfect flowers) in varying proportions and sizes (Simmonds, 1965; Rea, 1969; Gandarillas, 1979). There are also plants with sterile androecious flowers or perfect flowers that produce non-functional pollen (Gandarillas, 1979; Saravia, 1990; Ward and Johnson, 1992). Lescano (1994) reported similar results on reproductive biology and possible protandry.

Type of pollination

Simmonds (1965) found that quinoa (*Chenopodium quinoa* Willd.), qañawa (*Chenopodium pallidicaule* Aellen) and Huauzontle (*Chenopodium nuttalliae* Safford) are self-pollinating species, and this was confirmed by Gandarillas (1979) and Wilson (1988).

Reported percentages of cross-pollination or outbreeding vary. Gandarillas (1976) found that cross-pollination ranged from 1.5% for plant-to-plant spacing of 20 m to 9.9% for plant-to-plant spacing of 1 m. Lescano (1994) reported 5.78% outbreeding and 94.22% self-pollination. According to the results for quinoa's centre of origin, the outbreeding percentage exceeds 10% of natural crossing. Quinoa has a 90% self-pollination rate, which is more or less the same as rice and sorghum (House, 1982; Jennings *et al.*, 1981). A study by Silvestri and Gil (2000) in Mendoza, Argentina, showed an outbreeding rate of 17.36%, confirmed by Gandarillas. However, the results were achieved under environmental conditions that were quite different to the species' natural adaptation conditions.

It is recommended that self-pollinating techniques be adopted for quinoa breeding.

Male sterility

Quinoa shows genetic and cytoplasmic-genetic male sterility, explained by inheritance (Saravia, 1991). Ward and Johnson (1993) reported hybrid vigour in hybrid progeny of quinoa using male sterility. This opens the door to numerous possibilities for developing commercial quinoa hybrids similar to rice and sorghum. Experiments in Bolivia showed a certain degree of heterosis in intervarietal crosses and even more in interspecific hybrids. However, use of interspecific hybrids was limited due to infertility problems. The use of male sterility in quinoa breeding offers interesting perspectives; it is important to identify lines with the greatest potential for species combinations.

Quinoa polyploidy

Polyploidy is an important factor to consider in plant breeding due to its influence on reproductive compatibility, fertility and the expression of phenotypic traits and the degree of variability.

The number of chromosomes in cultivated quinoa (*Chenopodium quinoa* Willd.) is $2n = 4\times = 36$ (Cárdenas and Hawkes, 1948; Gandarillas and Luizaga, 1967; Gandarillas, 1986). Gandarillas (1979) reported that quinoa has 36 somatic chromosomes with four sets of $x = 9$ chromosomes, the basic number for the genus Chenopodium, which means that quinoa is an allotetraploid. Studies by Simmonds (1971) and Gandarillas (1986) showed that two genomes from diploid species participate in the allotetraploidy of quinoa to create a sterile interspecific hybrid. This hybrid undergoes later duplication of the number of chromosomes, resulting in a self-pollinating allotetraploid.

With regards to genetic inheritance (chromosomal), quinoa displays disomic inheritance (Simmonds, 1971). This type of inheritance was noted, at least for qualitative traits, in various studies by Gandarillas (1968, 1971, 1979), Saravia (1990), Bonifacio (1990, 1991) and Silvestri and Gil (2000), who observed that the segregation of traits in F2 concordant with the classic proportions of 3:1 and 9:3:3:1 corresponded to one and two genes, respectively.

Genetic resources

Quinoa breeding first requires germplasm collections from which genetic material can be selected for use in the various breeding methods. Germplasm of quinoa and its wild relatives is mainly kept in ex situ banks in Ecuador, Peru, Bolivia, Chile, Argentina, Colombia, the United States of America and other countries around the world (Mujica, 1992; Bonifacio *et al.*, 2004; Fuentes *et al.*, 2006; Christensen *et al.*, 2007; Bravo and Catacora, 2010; Rojas *et al.*, 2010b; Gómez and Eguiluz, 2011).

In terms of accession quantities, Bolivia and Peru have quinoa germplasm collections that are the most representative of the Andean region and the world. The entire Bolivian quinoa collection has been characterized and evaluated for morphological and agronomic traits, 17% for nutritional value, 8% for agro-industrial traits and 86% for molecular attributes (Rojas et al., 2010b; Rojas and Pinto, 2013). In Peru, of the quinoa collection at the *Universidad Nacional Agraria La Molina* comprising 2 089 accessions, 100% have been characterized for morphological and agronomic traits and 43% for quality traits such as protein and saponin content and grain size (Gómez and Eguiluz, 2011).

With regards to i*n situ* conservation, there are many special niches and agro-ecological zones throughout the Andean region with specific traits that have made it possible to develop the greatest genetic diversity in quinoa, both in wild and cultivated species, which can still be found under natural conditions and in fields cultivated by Andean farmers. Altiplano, Salare, Inter-Andean valleys and Yunga ecotypes can be found in Bolivia, while Altiplano and Inter-Andean valleys ecotypes are grown in Peru. Quinoa is mainly conserved in these agro-ecological zones through traditional farming systems using continual planting and harvesting techniques in agro-ecosystems such as *aynokas, sayañas, huyus* and *jochiirana* (Ichuta and Artiaga, 1986; Rojas *et al*., 2010b).

Studies on quinoa collections in Ecuador and Argentina show less diversity, which indicates that Ecuadorian material was possibly introduced from Peru and Bolivia, while quinoa was originally introduced to Argentina from the Andean and Chilean coastal (southern) regions (Christensen *et al.*, 2007). Another reason for this lower diversity could be genetic drift and the cessation of cultivation or the isolation of communities.

Chilean quinoa is divided into two groups: Altiplano and Coastal ecotypes. Quinoa was mainly grown by the Aymara peoples in Chile's northern Altiplano. Its morphologic diversity was achieved through natural and human selection in addition to genetic drift after being introduced to Chile's central and southern areas. After evaluating quinoa lines in the subtropical zone of northern India, Bhargava (2007) reported that Chilean lines showed better adaptation in countries that have climates with cold winters and hot summers, such as in India.

This and other similar research has increased understanding about the genetic resources and degree of genetic variation for many traits that facilitate breeding. Some studies have even led to the creation of core collections in Peru that simplify and improve the management and use of quinoa genetic resources (Ortiz *et al.*, 1998). A core collection has also been created in Bolivia, and with 267 accessions (Rojas, 2010), it has undeniably helped breeding programmes in selecting materials with tolerance to abiotic factors.

Breeding methods

The > 100 commercial varieties in the Andean region have been developed using diverse breeding methods based on selection and hybridization. Significant progress has been achieved during this process with regards to varieties, ecotypes and germplasm.

Introduction

Breeding consists of introducing species, varieties or germplasm developed in one zone to another. Success depends on the degree of adaptation of the genetic material to its new environment and its acceptance by farmers and end users.

In the Andes, the introduction from the southern Bolivian Altiplano of 'Quinoa Real' varieties (Salare ecotype) to the conditions of the northern Altiplano were not very successful due to the varieties' susceptibility to mildew. Other Bolivian cultivars obtained in the *Estación Experimental de Patacamaya* (central Altiplano) and introduced to the Peruvian Altiplano by research institutions and individual farmers have shown positive results and are currently being grown commercially with good acceptance; they include 'Sajama', 'Kamiri', 'Chucapaca' and 'Huaranga' varieties. The 'Salcedo INIA' variety was derived from 'Huaranga'.

As quinoa's nutritional and agricultural value has been recognized, there has been a rise in germplasm movement around the world. Among the first introductions to other continents was that of Risi (1986), who introduced and studied the adaptation of approximately 300 quinoa accessions from Bolivia, Peru and Chile in the United Kingdom. Genetic material was later introduced on a broader scale to other countries in the Andean region and overseas through regional quinoa trials or the *Prueba Americana y Europea de Quinoa*, led by FAO (FAO/RLAC/UNA, 1998). Preliminary results of this trial showed that the accessions of the Peruvian and Ecuadorian Inter-Andean valleys ecotypes demonstrated good mildew tolerance, while the Bolivian material was highly susceptible to mildew. Coastal accessions matured extremely early but were very susceptible to hail under Sierra conditions (Mujica *et al.*, 2001). Bhargava (2007) reported the adaptation of several quinoa accessions to India's northern subtropical area, where 27 lines of *Chenopodium quinoa* and two lines of *C. berlandieri* subsp. *nuttalliae* were studied over a two-year period.

Currently, the introduction of genetic material from one region to another must comply with access standards and ensure equal distribution of the benefits produced by using the material. It must also ensure that breeders' rights to the varieties are respected according to current regulations. At the Andean Community of Nations (CAN), Decision 391 establishes a Common Regimen on Access to Genetic Resources, based on Decision 345 for a Common Regimen on the Protection of Plant Breeders' Rights. However, the regulation has not been applied as anticipated for various reasons.

Selection

Selection is one of the first breeding methods used for quinoa. It relies on the genetic variability developed over thousands of years throughout the Andean region through both natural processes, such as mutation and intra- and interspecific recombination, and natural and human selection. Selection involves the modification of allele frequency in plant and/or variety populations.

Mass selection

Mass selection is based on identifying exceptional phenotypes and selecting a mix of seeds at harvest. This method should be applied when breeding for traits with high heritability. The following mass selection approaches are used for quinoa.

Simple Mass Selection

Simple mass selection involves the selection of 100–200 superior plants (or more, depending on the breeders' objectives). The plants are harvested together and later planted, where they are compared to the original material from which they were selected in order to determine the degree of improvement. The process can be repeated over two or three selection cycles or in a period defined by the breeder. The result is a new improved and heterogeneous population made up of genotypes displaying a high degree of homozygosity. The population continues to maintain adaptability to the new growing area, with genetic variability to cope with various stresses such as climate and soil. Agromorphologic traits are homogenous in this population (plant height, life cycle and especially grain colour), which makes for easier management by the farmer.

Simple mass selection is an easy, low-cost and quick breeding method. It is currently used to create commercial varieties with more uniform agromorphologic traits. This type of improvement based on phenotypic selection requires excellent understanding of the crop and variety. Cultivars developed using this method cannot be used in certified production processes and are recommended for use in marginal areas. Seed production must be constantly monitored by breeders to ensure the appropriate proportion of phenotypes and a stable yield characteristic of the varieties.

Stratified Mass Selection

Stratified mass selection is a modified version of mass selection that involves improving the identification of superior plants and reducing the effect of environment variation on the selection process. There are generally variants or gradients in texture, fertility, moisture or soil depth in the selection location; these determine major variations in plant phenotype. Stratification involves sowing the same number of plants from the original population with equal plant-to-plant spacing and establishing blocks or grids in the research field according to the environmental factor variation. Selection is made in each grid by gathering an equal or proportional number of plants based on the estimated total.

Selection is made from 100–200 superior plants presenting the selection criteria determined by the breeding programme. Each plant is harvested separately, selected for a second criteria if relevant (e.g. grain size or saponin content) and conserved in individual labelled bags.

The seeds from the best plants are mixed together to widen the genetic base of the future variety. The lines must be standardized so that the mixes produce agronomic traits that facilitate farming labour and quality traits for final use. This method is much more effective but is also more costly in terms of labour and required materials. However, it does allow monitoring of genetic quality of the plants and the environment. Stratified mass selection is used in certified seed production for 'Quinoa Real' varieties in the southern Bolivian Altiplano.

The most important varieties obtained through mass selection include 'Royal' (Bolivia); 'Baer' (Chile); 'Dulce de Quitopamba' (Colombia); and 'Pasankalla', 'Chewecca', 'Blanca de Juli', 'Amarilla de Marangani', 'Blanca de Junín', 'Rosada de Junin' and 'Blanca de Hualhuas' (Peru).

Individual selection

Native quinoa varieties, which have a self-pollination rate of around 90%, are heterogeneous populations in which each genotype has a greater degree of homozygosity. In these original populations, plants are generally found to be vigorous, early maturing, resistant to disease, low temperatures, drought etc., and can be selected individually.

The seeds from these individual plants are planted in furrows or separate plots to maintain the purity of each progeny, and are thus considered pure lines. Evaluation of and selection for yield and other traits should be carried out in different locations over several years using appropriate statistical methods. Afterwards, seeds are multiplied from the exceptional line(s) and can be registered as new improved varieties.

A modification to individual selection, applied to quinoa by Gandarillas (1979), is called "panicle-furrow selection", in which approximately 200 plants are identified during the vegetative phase. The plants self-pollinate during the reproductive stage, and the self-pollinating plants are selected for a second criteria if relevant (saponin). They are threshed individually, assigned an identification code and the seed is conserved in an individual bag. The units are planted according to the assigned number in individual furrows, and selection is repeated between and within the furrows. The process can be repeated over two or three cycles until homogeneity of the material is achieved. This selection method enables mother plants and their progeny to be monitored and material can be recovered in the event of progeny losses from the remnant sample of the mother plant. This method is more costly in terms of equipment and labour, but is more effective in achieving the determined selection criteria.

For quinoa to self-pollinate, the following steps must be taken: 1) preparation, consisting in removing several leaves and axillary glomeruli at the base of the panicle; 2) isolation of the prepared panicle or glomeruli group by using suitable paper bags (15 × 25 cm or 10 × 15 cm); and 3) weekly monitoring of the plants for possible pest or disease infestation. For self-pollination of the entire panicle, it is recommended that the plant be supported with wiring to avoid flattening. For self-pollination of the glomeruli group, three leaves should be included to maintain humidity inside the bag. Commonly used materials used for self-pollination are 10 × 15-cm and 15 × 25-cm glassine paper bags, 4 × 6-cm and 6 × 10-cm labels, small fine-tipped scissors and 32-mm clips.

Individual selection produces a variety characterized by its genetic purity and acceptance by seed certification systems. Seed production should be carried out in isolation or maintaining adequate spacing to avoid contamination by pollen from other varieties. This method of individual selection is effective for obtaining new varieties from local or natural populations, as well as for maintaining the purity of the varietal identity from cultivars already developed. The 'Sajama Amarantiforme' variety, also known as 'Samaranti', is a cultivar obtained using this method.

Hybridization

Hybridization is another major breeding method that aims to quickly recombine favourable traits from several genotypes into a single genotype and produce novel genetic variability. This breeding method has been used for quinoa (Rea, 1948; Gandarillas, 1967, 1979; Lescano and Palomino, 1976; Bonifacio, 1990, 1995) and generally follows the steps described below.

Determining the breeding criteria

For hybridization, the priority criteria for the quinoa breeding programme must be determined from the start. According to Gandarillas (1979), hybridization offers strong possibilities to achieve objectives such as yield, grain size, disease resistance and other important traits related to improving nutritive quality.

Progenitor selection

Progenitors should be selected according to the breeding programme priorities, since hybridization is an oriented cross-pollination method. Various traits are generally improved simultaneously. Progenitors are selected from the available germplasm, which needs to have been previously characterized so that favourable gene resources can be easily and more accurately identified.

Progenitors can be selected between plants of different varieties, ecotypes, accessions and advanced lines from the *Chenopodium quinoa* species for intraspecific crosses. Plants from other species can also be selected for interspecific crosses – including intergeneric hybrids – depending on the aims and availability of gene sources in other categories of the quinoa gene pool. The use of *Chenopodium* and *Atriplex* genus species is reported by Bonifacio (1995).

Artificial crosses

Hybridization requires understanding of reproductive biology, applicable techniques and equipment and tools. The equipment used for crossing includes 10 × 15-cm glassine paper bags, 4 × 6-cm labels, small fine-tipped scissors, histological or dissection scalpels, tweezers, forehead magnifying glass, laboratory watch glass, cotton balls, #4 and #6 camel hair brushes, 70% alcohol, 32-mm clips, record and field notebooks and a plant-breeding box or container.

It is also necessary to have a nursery for selected progenitors, which will be sown according to the genotype life cycle to ensure synchronized reproductive stages to carry out cross-breeding. The cross-breeding technique consists of the following steps: 1) identification of the progenitors according to the breeding objectives; 2) preparation of the mother plant or female progenitor (removal of the panicle tip, leaves and flowers at anthesis; 3) emasculation for 10–14 consecutive days without causing lesions to the gynoecium or breakage of the pollen sacs; 4) gathering of pollen from the previously selected father plant or masculine progenitor; 5) pollination via various passes with the brush dipped in pollen over emasculated flowers with receptive stigmas from the mother plant; and 6) isolation via glassine-type paper bags. The process is very simple, but for traits such as inflorescence and smaller flower size, it can be very tedious and labour-intensive.

Crossing without emasculation is possible in mother plants with partial or complete male sterility or those displaying protogyny (Gandarillas, 1969, 1979; Saravia, 1991; Ward and Johnson, 1992).

When cross-bred plants reach physiological maturity, the plants are harvested individually and threshed separately to maintain their respective records.

Development of the F_1 population

F_1 ogeneration, or seeds obtained via the artificial crossing of different progenitors, is sown with greater plant-to-plant and furrow spacing than usual for better plant development and increased seed production for the next generation (F_2). For F_1, it is essential to remove non-hybrid plants resulting from self-pollination, which can be done using morphological or molecular markers. Hybrid plants demonstrate the markers or dominant traits of the male progenitor, while self-pollinators show the recessive traits of the female progenitor. Morphological markers are easily observable qualitative traits in plants and seeds with a high degree of heritability. Some, such as plant shape, colour or size, can be observed before flowering (Bonifacio, 1988). These markers will later become “descriptors”. Once the true hybrid plants are identified, they are labelled before anthesis. It is important to promote self-pollination, whether artificial or natural, by maintaining the corresponding isolation. At physiological maturity, seeds are harvested individually and kept in separate labelled bags. Seeds obtained from F_1 plants (carrying the F_2 embryo) will be used to produce the segregating F_2 population, to which various selection methods can be applied.

Managing the segregating population

The degree of genetic variation of the F_2 population will depend on the differences between the parents, the allelic series, linkage and other factors. The F_2 population, which is obtained from planted F_1 seeds, must be sufficiently large to promote the expression of all of the possible segregants and to form the genetic basis for pedigree selection. Gandarillas (1979) suggests an initial population of 2 000 plants per cross.

There are many ways in which to handle this material, and they depend on the breeder’s objectives and knowledge. Conventional methods used for quinoa are mass selection, pedigree selection (individual selection) and single seed descent.

Mass selection

This method, recommended for self-pollinating species (Jennings *et al.,* 1981) due to its simplicity and low cost, has proved successful in quinoa (Gandarillas, 1979).

Once hybridization has been completed and the F_1 obtained, the seeds from the F_2 generation (and up to F_5 or F_6, obtained via the above-described processes) are planted and harvested using natural (not artificial) mass selection. The populations should be planted from generation to generation in marginal areas where natural biotic and abiotic stresses are present year after year, in order to achieve genetic gain. During each reproduction cycle, the progeny self-pollinate in such a way that by the F6 generation, the plants develop a high degree of homozygosity and as a result display relatively stable trait expression. Progeny with limited adaptability, tolerance or resistance to stresses are eliminated or reduced due to the effect of the stresses or natural selection and because of intrapopulation competition. Artificial selection is started for desirable traits from F_5 or F_6. Selection pressure is established for breeding and yield evaluation in various locations over several years. Appropriate statistical models are used to identify the new improved line(s) that will constitute new varieties.

Mass selection, following artificial cross-breeding, is economical and requires little labour and few resources to carry out selection and observations. However, plant breeders face a risk of losing or failing to observe some desirable genotypes due to the underutilization of selection material and the loss of data from genotype-by-environment interactions.

This type of selection can be modified with a view to increasing its effectiveness. One possible modification is panicle-farrow selection, where evaluation of the progeny can be improved and superior lines can be more easily identified.

This method produced the sweet variety ‘Sajama’ (Gandarillas 1960), as well as ‘Huaranga’, ‘Chucapaca’ and ‘Kamiri’ (Saravia and Gandarillas, 1986).

Pedigree selection

Gandarillas (1979) and Lescano (1994) described the pedigree selection process used for quinoa.

The F_2 population is planted seed by seed with equal plant-to-plant spacing, and each plant is labelled to identify its origin or pedigree. Selection in the population is carried out based on individual evaluation and the plants are harvested separately. For the F_3 generation, seeds from each plant or its progeny are planted in separate furrows identified by family name. Because this generation already has a high rate of heterozygosity, selection should be made from individual plants within and between families, maintaining the identity or pedigree of each plant, which should be harvested individually. A similar process is followed and selection is carried out for F_4 and F_5 or F_6. Each plant selection should have sufficient data to enable identification of its progenitors and descendants. Given the high level of homozygosity in the F_6 generation, the progeny of the plants may be called lines. Selection at this level occurs between lines and not within lines, with mass harvesting of each line. Quality trials are carried out on this generation, particularly on those with industrial applications. The advanced lines, obtained using this method, are pure lines with all of the data belonging to its pedigree.

Preliminary assessments on yield and seed production increases for later trials are carried out starting from the F_7 generation. The F_8 to F_{11} or F_{12} generations are used to evaluate yield for the lines selected in various locations and years, so as to later select the line with the best behaviour and stability, which must then go through variety registration trails.

It is important to note that qualitative traits can be selected from generation F_2, while quantitative traits can be selected from F_4. Starting with F_6, material can be selected that is highly homozygote and on the basis of traits such as yield and seed size.

In each selection cycle, a control furrow should be included with a known variety or with progenitors at intervals of 15–20 units in order to compare the expression of various traits based on the selection criteria.

The pedigree selection method monitors individual plants very precisely and the lines require that the breeder have considerable experience with regards to the crop and selection protocol. Varieties obtained using this method display strong genetic homogeneity with a narrow genetic base. This disadvantage can be overcome by employing a mixed method with mass selection during the final stage. Finally, this technique is highly effective for protecting breeders' rights or registering cultivars and carrying out inheritance studies.

Single seed descent

Single seed descent emerged from the necessity to accelerate the development of new self-pollinating varieties. Following normal processes, between 12 and 15 life cycles or crop years are required to develop a new variety (pure line) of self-pollinating species. Of those 12–15 years, nearly half the period is simply spent waiting to achieve the degree of homozygosity required to evaluate economically important traits such as yield, which reflect hybrid vigour through heterozygosis (Poehlman and Sleper, 2003).

This method is based on managing the environment in such a way as to accelerate the plant life cycle. Segregating genetic material (F_2 to F_5 or F_6) is planted under controlled conditions with a high temperature, few nutrients, shallow soil, limited water and constant light. These conditions speed up plant life cycles so that they move from the vegetative to the reproductive stage very quickly, producing very little fruit or just enough to perpetuate their future survival ("principle of survival"). This method requires that just one of each plant be represented in the next generation for its progeny to be labelled "single seed descent". No selection is carried out during this process and more than three generations must be obtained in a year. Plant loss should be avoided; if it does occur, it should be at random.

Once the F_2 population is obtained, one seed from each F_2 plant is sown according to the original method so that if there are, for example, 8 000 plants in one F_2, there should be 8 000 seeds planted. In the next generation, the 8 000 seeds are planted. At maturity, a single seed is also taken from each plant according to the same process. This process continues through F_5 or F_6. Given that the material has already reached a sufficiently high degree of homozygosity required by the breeder (F_6 or F_7), all of the seeds are harvested and can be planted in the field to increase seed production.

Subsequent generations should be evaluated and selected for established criteria in a similar manner to how pure lines are managed (as described for other methods).

This method has been slightly modified for use with quinoa. Around five seeds are taken from each plant to replace units that are easily lost due to seed size and seedling vigour. Seeds are planted in small plastic containers (bags or disposable cups), with two to three seeds per container. After the initial danger period is over, they can be thinned to one plant per cup. In the field, planting is done in a similar way on a larger scale. Seeds are planted in successive cycles, alternating between the field environment and the greenhouse to reduce the time required to achieve reasonable homozygosis. This method is relatively easy and the only challenge lies in handling the very small seeds. From F_6 onwards, the lines obtained can be evaluated and selected in the fields of farmers applying participatory research techniques.

Backcross method

Backcrossing is a method designed to improve a commercial variety preferred by farmers and consumers but with one or two defects that limit its use. Often, these defects are susceptibility to a disease or a negative quality trait. This method requires a recurrent progenitor (commercial variety to be improved) and a donor progenitor with the desirable trait. To speed up the process and ensure success, the disease susceptibility or negative quality trait must be governed by recessive genes and the resistance or positive trait must be governed by dominant genes. If the desirable trait from the donor gene is recessive, the breeder must allow for an additional self-pollination cycle to identify the transferred recessive trait in homozygous state before commencing backcrossing. This doubles the time required to obtain a new variety.

The recurrent progenitor is crossed with the donor plant to obtain F_1 from which a backcrossing programme can be implemented with the recurrent progenitor from each generation. The plants used for the cross with the recurrent progenitor should have the resistance genes or the positive trait of the donor. For each backcross with the recurrent, half of the donor genome is lost. Between 6 and 10 backcrosses and final self-pollination are required for the genetic material to achieve the desired combination from the original variety plus the resistance or quality trait in homozygous state. This material is increased and can be used directly as a commercial variety.

Gandarillas (1979) and Lescano (1994) reported the use of backcrossing in quinoa. 'Quinoa Real' ('Pandela' variety) accession 1638 with large bitter grains was used as the recurrent progenitor, while the donor progenitor was the 'Patacamaya' variety, green in colour with sweet grains. The F_1 of this cross was a bitter, Pandela-type. F_1 plants were backcrossed with the 1638 Pandela-type accession. The plants from the first backcross (BC1) were self-pollinated to obtain Pandela-type plants with large, Royal-type sweet grains. These plants were backcrossed a second time with 'Pandela' and the progeny self-pollinated; sweet Pandela-types were selected and so on.

Interspecific and intergeneric hybridization

Interspecific hybridization refers to the crossing of individuals from two different species of the same genus. Gandarillas (1986) created interspecific crosses of the *Chenopodium* genus to study the origin of cultivated quinoa. Later, Bonifacio and Gandarillas (1992) selected a series of lines and obtained the 'Sayaña' variety using 'Sajama' (*C. quinoa*) crossed with 'Ajara' (*C. carnosolum*). Research on interspecific hybridization carried out by Gandarillas (1986) and Bonifacio (1995), as well as additional research discussed in papers by Wilson and Manhart (1993) and Ward and Johnson (1993), showed that interspecific crosses are viable, especially *C. quinoa* crossed with *C. nuttalliae*, *C. berlandierii, C. petiolare and C. carnosolum*.

Lescano (1994) suggested that interspecific hybridization between *C. quinoa* and *C. pallidicaule* could be used to obtain hybrids with a wider range of adaptation to higher altitudes and sweet grains. However, difficulties were encountered with regards to ploidy, small flower size and the high fragility of the pedicel, thus limiting this possibility.

Hybridization between species from the *Chenopodiun* and *Atriplex* genus has been attempted, producing intergeneric hybrids between C. quinoa and *Atriplex hortensis* (high protein content) and *A. joaquiniana* (Bonifacio, 1995). However, F_1 plants show complete male sterility in hybrids.

Intergeneric and interspecific crosses are methods that have not been thoroughly explored in quinoa breeding. However, the wild species of the *Chenopodium* genus and others from the Chenopodiaceae subfamily display considerable genetic resources that could be incorporated into cultivated quinoa (e.g. hardiness, frost and drought resistance).

Genetic improvement for induced mutation

Natural mutations: paramutation and transposition and selection by natural segregation

Natural segregation, fairly frequent in quinoa, is mainly associated with the change in plant and grain colour, causing green plants to turn purple, and white grains to turn black or brown and vice versa. This can be attributed to paramutation phenomena reported in several species, to genetic transposition or to both simultaneously (Bonifacio, 1995, 1996).

The varieties that show the most obvious natural segregation include 'Pandela', 'Sayaña', 'Jacha Grano' and Coastal quinoa ecotypes. It is assumed that this trait was more frequent during the species' evolution and was appreciated by ancient cultures during domestication and selection. This natural genetic variation has been identified in quinoa and exploited through panicle-furrow selection to obtain such varieties as 'Mañiqueña' and 'Blanquita', as well as the more recently released 'Qanchis Blanca'.

Induced mutation

Traditional quinoa varieties have desirable combinations resulting from natural and human selection over hundreds of years that have determined its degree of adaptation to various environments and its nutritional value. Currently, quinoa is being planted under high-tech conditions which require slight modifications to obtain the desirable gene combinations, particularly life cycle and plant height, which can be achieved through induced mutation.

Induced mutation is known for being quick and for retaining the existing desirable combination once the defectives traits are corrected in the material. In quinoa, it was used to improve the 'Pasankalla' variety, for which seeds were irradiated with doses of 150, 250 and 350 Gy gamma rays. In the M_1 generation, the germination process slowed as radiation doses rose. Similarly, seedling height, root length and leaf development were reduced more with higher doses; no plants receiving the 350 Gy dose survived.

In the M_2 generation, a broader spectrum of chlorophyll mutations was observed in the 150 Gy dose, with and maximum frequencies in the 250 Gy dose. The *chlorine* type was predominant, followed by the *xantha* type. The mutations recorded for the two doses concerned branching, pedicel length, reduced plant height and life cycle, stem and plant leaf colour, leaf shape and improved plant type. More than two types of mutation were seen in each plant, particularly with the 250 Gy dose. In the M_3 generation, the same spectrum of mutations was found, and the most valuable mutations were in plant height, life cycle and grain colour (Gómez and Eguiluz, 2013).

Modern biotechnology: techniques and tools applied to quinoa breeding

Besides the adoption of classic quinoa breeding techniques, major advances have been made in breeding improved varieties. However, in the last few years, the need to deal with more complex traits in less time has compounded the need to adopt new breeding techniques and use new biotechnological tools, with remarkable progress. Molecular techniques are now available to make conventional methods more effective, and some are already being applied in quinoa.

Doubled haploid breeding

This is an ideal technique to speed up the process of obtaining homozygous lines and new plant varieties. Homozygous genotypes obtained with doubled haploid techniques using F_1 generation plants can originate with single nucleus microspores derived from microsporogenesis or megasporogenesis in this generation. Doubled haploid lines reflect the randomness of the reproductive cells produced by the donor. Each microspore or megaspore is potentially able to regenerate an embryo. Each plant represents the variation that exists in the microspore population (Ferrie *et al.*, 1995, 2003). The production of doubled haploids from F_1 hybrids saves time for plant breeding programmes because it enables breeders to obtain completely homozygous lines in a shorter time frame. Preliminary studies have been carried out on quinoa to obtain doubled haploids from *in vitro* cultivation of anthers (microspores) from 'Roada de Huancayo' and 'Blaca de Hualhuas' varieties (Soplin, 2009).

Molecular marker-assisted selection (MAS)

The use of biochemical and molecular markers in quinoa selection began with the attempt to establish phylogenetic relationships between the *Chenopodium* genera. The use of Chenopodiaceae isozymes was reported by Wilson (1988) and Terrence and Walters (1988). RAPD markers were used to identify the hybrid state of progeny from interspecific and intergeneric crosses (Bonifacio, 1995) and the genetic relationship between wild and cultivated quinoa (Ruas *et al.*, 1999), and similar studies were also carried out by Maughan *et al.* (2006).

Molecular markers are essential tools in modern plant breeding because they are "linked" to genes of interest. Selection for these markers results in indirect selection of the gene of interest and is fundamental in marker-assisted selection (MAS). The closer the marker and the gene in question are, the more effective selection will be. It is also important with regards to germplasm conservation and characterization of core collections and their application in breeding and in MAS (Eathington *et al.*, 2007; Ganal *et al.*, 2009).

In quinoa, the first set of 208 polymorphic microsatellites, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs), were developed by Mason *et al.* (2005). Jarvis *et al.* (2008) later reported an additional set of 2 106 new SSR markers and a linkage map with 275 molecular markers, including 200 SSR markers. Joint efforts by PROINPA (*Promoción e Investigación de Productos Andinos*), PREDUZA (*Proyecto Resistencia Duradera en la Zona Andina*) and the McKnight Foundation led to the development of molecular markers for important traits, such as mildew resistance, saponin and protein content, and composition (Maughan *et al.*, 2004; Coles *et al.*, 2005; Mason et al., 2005; Stevens *et al.*, 2006; Jarvis *et al.*, 2008; Rodríguez and Isla, 2009).

Wilson (1988a) and Christensen *et al.* (2007) revealed a strong genetic similarity between Coastal ecotypes and the Andean ecotypes. Fuentes *et al.* (2008) reported that quinoa varieties from the Andean region and the Chilean coast have 21.3% common alleles, and that quinoa from the Andean region has a single allele frequency of 28.6% while Coastal ecotypes reach 50%. According to Rojas *et al.* (2010b), 86% of the Bolivian germplasm has been characterized using molecular markers.

Maughan *et al.* (2012) reported the identification of 14 178 single nucleotide polymorphisms (SNPs) and the development of an analytic laboratory process for 511 SNPs. Using 113 quinoa accessions, they showed that the minor allele frequency (MAF) for SNPs varied from 0.02 to 0.05, with an average MAF of 0.28. Structural analysis examined both Coastal and Andean quinoa groups. The SNP linkage map in a population derived from the recombination of two endogamous lines produced a linkage map with 29 linkage groups; 20 of these represented major linkage groups and were separated by 1 404 cM, with a marker density of 3.1 cM per SNP marker. The SNPs identified in this research are a necessary genomic tool for emerging breeding programmes for advanced agronomic trait analysis in quinoa.

Quinoa breeding programmes and activities

The first quinoa breeding programmes were established in various countries around the Andean region and were mainly implemented by governmental research institutions, public/private entities and public or private universities.

In Bolivia, quinoa breeding was implemented in the 1960s at the *Estación Experimental Patacamaya* with the participation of the SAI (*Servicio Agrícola Interamericano*) joint project, and later with the Instituto Boliviano de Tecnología Agropecuaria (IBTA) in 1977.

Under the IBTA until its closing in 1997, 15 improved quinoa varieties were released. In 1998, PROINPA took over the quinoa research, enabling the release of six varieties. Efforts are underway to obtain quinoa varieties that can adapt to the valley environments where high yields are achieved. The tropical zone (Santa Cruz) presents another promising area for quinoa, where it can be rotated as a winter crop between commercial crops such as soya.

In Peru, breeding research was begun in the 1960s by the *Instituto Nacional de Innovación Agraria* (INIA), mainly in Puno and Cusco, and the *Universidad Nacional del Altiplano* in Puno. Later, breeding activities were initiated at the *Universidad San Antonio Abad* in Cusco and the *Universidad Nacional Agraria* La Molina. Breeding research has led to the release of ten varieties in the Altiplano zone around Lake Titicaca and more than five varieties in the Inter-Andean valleys. Promising lines have also been developed for the Peruvian coast.

In Ecuador, characterization of genetic material intended for breeding began in the 1980s. Later, the *Instituto Nacional de Investigación Agropecuaria* (INIAP) implemented selection programmes and released at least three varieties. More recently, the INIAP seed programme has undertaken breeding via hybridization and selection. Preliminary results are very encouraging as they have been able to select progeny with large grains similar to 'Quinoa Real'.

In Chile, the private companies, Semillas Baer and AGROGEN, have established collections of local material, introduced genetic material and carried out oriented hybridization and selection to obtain the 'Regalona-B' quinoa variety adapted to coastal conditions (Von Baer *et al*., 2009).

In Planaltina, Brazil, EMPBRAPA has researched the adaptation and selection of quinoa varieties that show potential for developing tropical varieties from studied material (Spehar and Santos, 2005). It was found that higher yield was associated with plant height, a long productive cycle and panicle size, providing guidelines for selecting varieties for cultivation in tropical zones.

There have been many new initiatives to introduce quinoa to other continents, and some industrial countries have initiated breeding programmes, such as at the University of Colorado in the United States of America (Ward and Johnson, 1993; Murphy, 2010). Brigham Young University conducts basic research programmes in molecular biology oriented towards its application in quinoa breeding programmes, genomics, origins and phylogenetics (Maughan *et al.*, 2004; Coles, 2005; Mason, 2005; Stevens *et al.*, 2006; Jarvis *et al.*, 2008; Maughan *et al.*, 2012). Researchers at Washington State University have begun quinoa varietal selection with a focus on organic farming and diversified agriculture (css.wsu.edu/people/faculty/ kevin-murphy). In Europe, Risi (1986) evaluated the adaptation of more than 300 quinoa accessions in the United Kingdom and Denmark, while researchers from the University of Copenhagen run a quinoa research programme, which includes a component on genetic improvement (Jacobsen *et al.*, 2005; Jacobsen, 2007; Verena, 2013).

Modern quinoa varieties

The majority of improved varieties in Bolivia were released by the IBTA. Released varieties are 'Sajama', 'Sajamaranti', 'Chucapaca', 'Qamiri', 'Waranqa', 'Surumi', 'Jiskitu', 'Intinaira', 'Sayaña', 'Jumataki', 'Jilata', 'Patacamaya', 'Amilda' and 'Mañiqueña' (IBTA–DNS, 1996). Some of these varieties, such as 'Sajama', 'Chucapaca', 'Huaranga' and 'Sayaña', were well distributed, while others have not been as successful due to the closing of the IBTA in 1997. However, these varieties were conserved thanks to the participation of PROINPA. Varieties bred since the 1990s have had sweet, white grains; this demonstrates the influence of local consumption, as sweet quinoa is easier to process and clean.

Since the IBTA closed, PROINPA (a private non-profit organization) has taken over the breeding research, releasing improved varieties through hybridization and selection, with cultivars such as 'Jacha Grano', 'Kurmi', 'Blanquita', 'Qusuña', 'Aynoqa' and 'Horizontes' (Bonifacio *et al*, 2003; Bonifacio and Vargas, 2005). This group includes varieties that are both sweet and bitter, early maturing, with large grains and mildew resistance.

In Peru 17, high-yielding and mildew-resistant varieties with medium and large grains have been released. They include 'Blanca de Juli', 'Kankolla', 'Salcedo INIA', 'Illpa INIA', 'Cheweka', 'Yocará', 'Tahuaco 1', 'Pasanqalla', 'Chullpi Rojo', 'Qoitu Negro', 'Choklito', 'Altiplano', 'Amarilla Sacaca', 'Blanca de Hualhuas', 'Rosada de Huancayo', 'Blanca de Junín' and 'Amarilla Maranganí'.

Twelve high-yielding and mildew-resistant varieties with medium and large grains have been released in Ecuador, including 'Tunkahuan', 'Ingapirca', 'Cochasqui', 'Imbaya', 'Chaucha', 'Tanlahua', 'Piartal', 'Porotoc', 'Amarga del Chimborazo', 'Amarga de Imbabura' and 'Morada' (Mujica *et al.*, 2004; Tapia, 1990; Mujica, 1992). According to Peralta (2006), the 'Tunkahuan' variety is currently in use and is the most planted in the Ecuadorian sierra. The 'Pata de Venado' variety was released in 2004.

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CHAPTER 2.6.

Principle quinoa pests and diseases

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Abstract

From countries of Andean region, Bolivia and Peru report the greatest damage and losses incurred due to pest infestation and the rapid expansion of growing area. Elsewhere in the region, production areas are smaller, and pests are therefore less of a problem. The situation is similar in new quinoa-growing areas around the world. Pests that cause the greatest economic losses are larvae of noctuids (butterflies from the Noctuidae botanical family) , polyphagous insects that feed on various plant species. Noctuids attack quinoa in a number of agro-ecological zones; in South America a variety of species are implicated including *Helicoverpa quinoa*, *Copitarsia incommoda, Copitarsia decolora* and *Agrotis ipsilon*. The most significant pest found in the largest Altiplano growing areas is H. quinoa. The larvae cause considerable damage to the plants as they mine developing panicles, feed on the plant leaves, bore into the stems at the panicle base and eat the grains. A major infestation of these larvae can wipe out an entire crop. It is likely that noctuid larvae will pose a serious problem wherever quinoa is cultivated in the world. The main quinoa pest, endemic to the Andean region is a moth of which there are various species, such as *Eurysacca quinoae, E. melanocampta* and *E. media*; the most widespread is *E. quinoae*, whose larvae damage developing flowers and grains. The most serious disease in the region and on a global scale is quinoa downy mildew, which is caused by the fungus *Peronospora variabilis* Gaum (formerly called *Peronospora farinosa* Fr.). The fungus has two types of reproduction: asexual (direct germination) and sexual (oospores, a survival structure). Wet areas or periods of relative humidity around 90% favour the spread of the disease. Oospores form when the crop is in senescence or when conditions become favourable to the pathogen. At plant maturity, the oospores adhere to the outside of the grain. The rapid movement of quinoa crop throughout the world in recent years could facilitate the pathogen's spread between countries and continents, with major consequences including high losses in yield and grain quality. It is important to note that, in the Andean region and North America, there are sources of medium to high mildew resistance. Pest and disease control strategies depend on whether production is conventional or organic. Conventional quinoa farming employs strategies for control similar to other crops, while organic farming requires an integrated approach that relies on various practices and inputs that meet organic standards.

Principle quinoa pests and diseases

As a crop, quinoa is a newcomer to the world scenario, and there are fewer studies on specific pests and diseases than for other native Andean crops, such as potatoes. This chapter focuses on experiences in the Andean region, where the majority of data have been gathered, and deals with two of the most significant pest complexes, the "noctuid complex" and the "moth complex". Integrated management and control strategies are mentioned only briefly, because every situation is unique and requires different measures. With regards to diseases, this chapter discusses the major disease affecting quinoa on a global scale: downy mildew.

Quinoa pests

Quinoa is affected by a range of pests at the various stages of growth. A review of existing literature produced a list of 56 species of phytophagous insects associated with quinoa cultivation (Table 1), of which 24 belong to the Lepidoptera order, 15 to Coleoptera, 10 to Homoptera, 3 to Hemiptera, 2 to Thysanoptera, 1 to Diptera and 1 to Ortoptera. Depending on their mouthparts, these species may be chewers, leaf miners, pollen feeders or biting–chewing insects. Of these many species, those that feed on leaves (called defoliators) and grains (noctuid larvae and the quinoa moth) are the most common and most widespread. The other species (i.e. the majority) are only fortuitous visitors, or they co-exist with the crop and no major economic losses related to them have been reported.

Table 1. Phytophagous insects associated with quinoa cultivation (arranged in order of frequency).

ORDER	FAMILY	GENUS	SPECIES
Lepidoptera	Gelechiidae	*Eurysacca*	*E. melanocampta* (Meyrick)
			E. quinoae Povolný
	Geometridae	*Perizoma*	*P. sordescens* Dognin
	Noctuidae	*Agrotis*	*A. ipsilon* (Hufnagel)
		Copitarsia	*C. decolora* Guenée
			C. incommoda Walker
			C. turbata Herrich - Schaeffer
		Dargida	*D. graminivora* Walker
			D. acanthus Herrich - Schaeffer
		Feltia	*F. experta* Walker
		Helicoverpa	*H. quinoa*
			H. titicacae Hardwick
			H. atacamae
		Heliothis	*H. zea* (Boddie)
			H. titicaquensis
		Peridroma	*P. saucia* (Hübner)
		Pseudaletia	*P. unipunctata* Haworth
			P. interrupta Maassen
		Spodoptera	*S. eridania* (Cramer)
			S. frugiperda (J. E. Smith)
Lepidoptera	Pyralidae	*Herpetogramma*	*H. bipunctalis* (Fabricius)
		Spoladea	*S. recurvalis* (Fabricius)
		Pachyzancla	*Pachyzancla* sp.
		Hymenia	*Hymenia* sp.
Coleoptera	Bruchidae	*Acanthoscelides*	*A. diasanus* (Pic)

ORDER	FAMILY	GENUS	SPECIES
Coleoptera	Chrysomelidae	*Acalymma*	*A. demissa*
		Calligrapha	*C. curvilinear* Stal
		Diabrotica	*Diabrotica* spp.
			Diabrotica speciosa
		Epitrix	*E. subcrinita* LeConte,
			E. yanazara Bechyne
	Curculionidae	*Adioristus*	*Adioristu* ssp.
	Meloidae	*Epicauta*	*E. latitarsis* Haag
			E. marginata Fabricius
			E. willei Denier
		Meloe	*Meloe* sp.
	Melyridae	*Astylus*	*A. luteicauda* Champ
			A. laetus Erichson
	Tenebrionidae	*Pilobalia*	*Pilobalia* sp.
Homoptera	Aphididae	*Aphis*	*A. craccivora* Koch
			A. gossypii Glover
		Macrosiphum	*M. euphorbiae* (Thomas)
		Myzus	*M. persicae* (Sulzer)
	Cicadellidae	*Bergallia*	*Bergallia* sp.
		Borogonalia	*B. impressifrons* (Signoret)
		Empoasca	*Empoasca* spp.
		Paratanus	*Paratanus* spp.
			P. exitiousus (Uhler)
			P. yusti Young
Hemiptera	Lygaeidae	*Geocoris*	*Geocoris* sp.
	Miridae	*Rhinacloa*	*Rhinacloa* sp.
	Nabidae	*Nabis*	*Nabis* sp.
Thysanoptera	Thripidae	*Frankliniella*	*F. tuberosi* Moulton
			F. tabaco Lindeman
Diptera	Agromyzidae	*Liriomyza*	*L. huidobrensis* Blanchard
Ortoptera	Gryllidae	*Gryllus*	*G. assimilis* Fabricius

Source: Zanabria and Mujica, 1977; Mujica, 1993; Zanabria and Banegas, 1997; Mujica *et al*., 1998; Lamborot and Araya, 1999; Ortiz *et al.*, 2001; Rasmussen *et al.*, 2003; Saravia and Quispe, 2005; Valoy *et al.*, 2011; Rodríguez, 2013.

In general, the frequency and intensity of pest infestation in quinoa fields vary depending on geographical location, the presence of natural enemies and the environmental conditions. In the agro-ecological zones of Salare and Altiplano, where more than 80% of the world's quinoa is produced, the main pest issues are related to the quinoa moth and noctuid complexes. This chapter discusses both in more detail.

1. The noctuid complex

The noctuid complex refers to a group of insects belonging to the *Helicoverpa, Copitarsia* and *Agrotis* genera, whose larvae cause serious damage to quinoa crops, especially in crop areas of Bolivia and Peru, although they have also been reported in Chile, Argentina, Ecuador and Colombia.

The noctuid complex comprises the *Helicoverpa quinoa, Copitarsia incommoda* and *Helicoverpa titicacae* species in Bolivia, and the *Copitarsia turbata* and *Agrotis ipsilon* species in Peru. The adults of these species are nocturnal moths and their common name varies from region to region. For example, in Bolivia they are called *rafaelitos* or *alma kepis* and are considered to be a bad omen, while in Peru they are known as *palomillas*. The larvae also have different names: *ticonas*, *ticuchis* or earthworms in Bolivia, and earthworms also in Peru. The various noctuid species are described below.

Helicoverpa quinoa

Recent research based on mitochondrial DNA and genitalia dissection by Michael Pogue (currently being published) from the United States Department of Agriculture (USDA) and in cooperation with entomologists from the Fundación PROINPA, clarifies that the species *Helicoverpa gelotopoeon* corresponds to *Helicoverpa quinoa*. Pogue also explains that it is difficult to distinguish between the *H. quinoa, H. gelotopoeon* and *H. titicacae* species on the basis of morphological traits alone.

The most common and widespread quinoa pest in the Bolivian Altiplano is, therefore, *H. quinoa*, responsible for sizeable yield losses of up to 20%. It is also reasonable to assume that reports of quinoa infestations of *H. gelotopoeon* in other countries actually involve *H. quinoa*. The Bolivian Altiplano agro-ecosystem where this pest is found is extremely diverse. It includes dry areas near the Uyuni and Coipasa Salares as well as very wet zones around Lake Titicaca. Given the dynamic movement of the quinoa crop, there is an imminent risk of spreading this pest to similar Andean agro-ecosystems in other South American countries, including Peru, Ecuador, Chile and Argentina.

Additionally, it is not clear whether *H. gelotopoeon* – a species that infests many crops throughout the world – is also a quinoa pest. However, given its polyphagous nature, it could pose a significant problem in new growing areas.

Taxonomic classification

Helicoverpa quinoa is classified as follows:

Class:	Insect
Order:	Lepidoptera
Family:	Noctuidae
Genus:	*Helicoverpa*
Species:	*H. quinoa* Pogue & Harp

Life cycle of *Helicoverpa quinoa*

According to studies by the Fundación PROINPA, *Helicoverpa quinoa* has a very particular life cycle. Out of a total of 400 larvae observed, 50% had a duration of 223 ± 36 days from egg to adult (including the full adult life span), 5% remained in the pupal stage until the next crop year and 15% died before reaching adulthood. Figure 1 shows the duration of each development stage of *H. quinoa* reared in the laboratory at 25°C and 60% relative humidity.

As shown in Figure 1, the incubation period lasts 5 ± 1 days, the larval stage 26 ± 3 days, the prepupal stage 9 ± 1 days and the pupal stage 175 ± 29, while the adult lives 6–10 days.

Adult behaviour

In Bolivia, adult *H. quinoa* moths are generally crepuscular, but can often be observed feeding during the day on q*illu-qillu (Hymenoxys robusta), chachacoma (Senecio eriophyton)* and malva or qura (*Taras satenella*), flitting from flower to flower feeding on nectar (Figure 2).

Copitarsia incommoda

Copitarsia incommoda Walker is a noctuid insect. The polyphagous larva is found from Mexico to Chile (Angulo and Weigert, 1975) and causes considerable economic losses in many crops (Angulo

Figure 1. Life cycle of *Helicoverpa quinoa*. Source: Entomology laboratory at Fundación PROINPA, Quipaquipani, La Paz

Figure 2. *H. quinoa* feeding on *qillu-qillu* flowers.

and Weigert, 1975; Yabar and Baca, 1981; Serna, 1996; Vélez, 1997).

This species is reported in Bolivia and Peru as one of the principal quinoas pests, especially in the area around Lake Titicaca where this pest and the quinoa moth cause economic losses of around 30%. The polyphagous behaviour of this insect, i.e. that it feeds on various plant species, and the fact that it is present in many areas around the world, make it a potentially highly destructive pest anywhere quinoa cultivation is introduced and developed.

Taxonomic classification

Copitarsia incommoda is classified as follows:

Class:	Insect
Order:	Lepidoptera
Family:	Noctuidae
Genus:	*Copitarsia*
Species:	*C. incommoda* Walker

Life cycle of *Copitarsia incommoda*

As seen in Figure 3, egg incubation averages 5.5 days, the larval stage 26.13 days, the prepupal stage 3.09 days and the pupal stage 16.3, while the adults live 19.85 days, with the species completing its life cycle in 70.87 days.

Copitarsia decolora

Reports on the taxonomy for *Copitarsia* reveal some confusion in identifying *Copitarsia decolora* and *Copitarsia turbata*. The taxonomy of the *Copitarsia* genus was revised by Simmons and Pogue (2004),

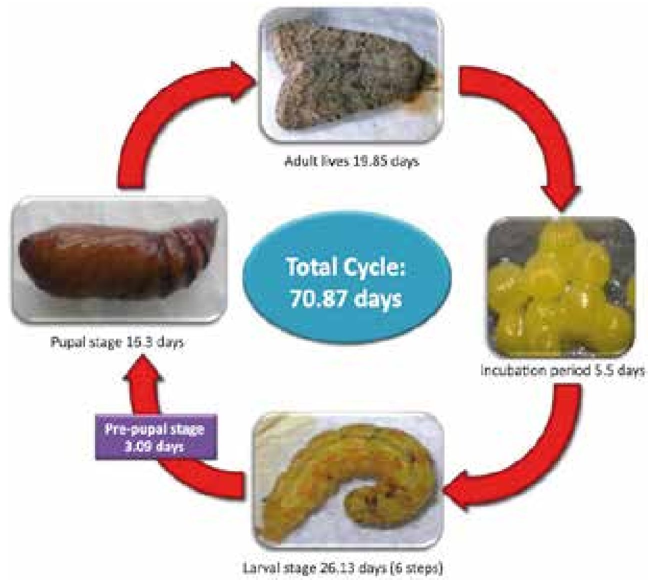

Figure 3. Life cycle of *Copitarsia incommode*.
Source: Choquehuanca 2011

who found that *Copitarsia incommoda* had been erroneously identified in the past as *Copitarsia turbata*. These authors designated *Copitarsia decolora* (Guenée) as the principal name of this pest, relegating *C. turbata* (Herrich-Schaeffer) to a synonym.

Angulo and Olivares (2009) later arrived at the same conclusion based on the morphology of the eggs and larvae. Thus, *C. decolora* (Guenée) is the correct taxonomy for the species commonly known as *C. turbata* (Herrich-Schaeffer). According to Angulo and Olivares (2003), *C. decolora* has been found in Venezuela, Uruguay, Peru, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Argentina and Chile. *C. decolora* larvae attack numerous crops (Castillo and Angulo, 1991; Angulo and Olivares, 2003).

Taxonomic classification

Copitarsia decolora is classified as follows:

Class:	Hexapoda
Order:	*Lepidoptera*
Family:	Noctuidae
Genus*:*	*Copitarsia*
Species*:*	*C. decolora* (Guenée 1852)

Synonyms attributed by Simmons *et al.,* 2004
Copitarsia turbata Hampson 1906.

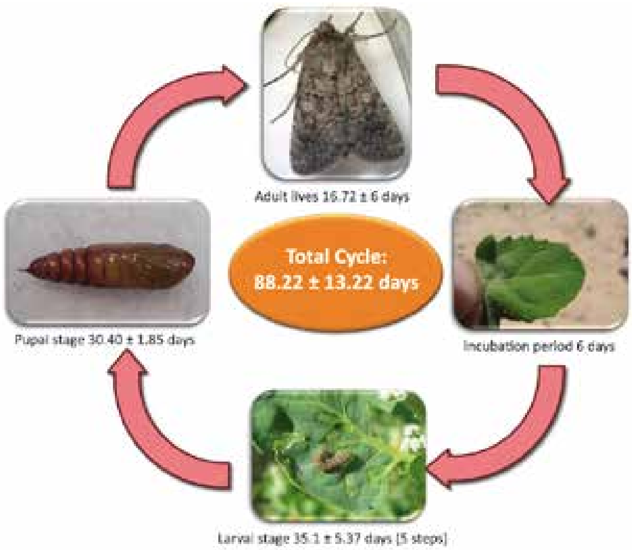

Figure 4. Life cycle of *Copitarsia decolora*

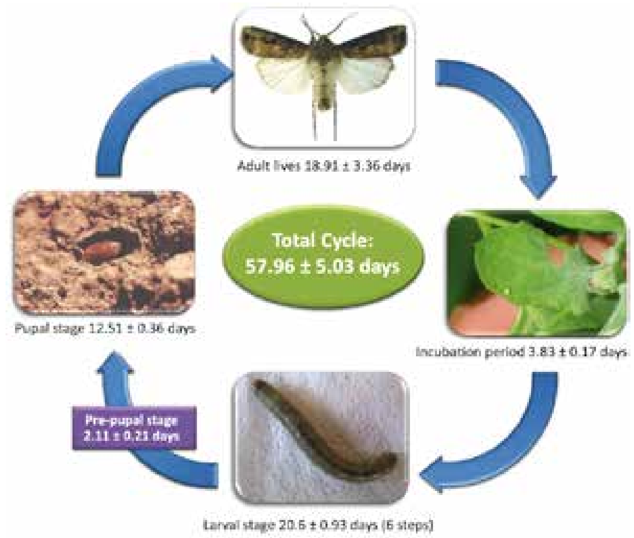

Figure 5. Life cycle of *Agrotis ipsilon*

Life cycle of *Copitarsia incommoda*

As seen in Figure 3, egg incubation averages 5.5 days, the larval stage 26.13 days, the prepupal stage 3.09 days and the pupal stage 16.3, while the adults live 19.85 days, with the species completing its life cycle in 70.87 days.

Life cycle of *Copitarsia decolora*

According to Moreno and Serna (2006), *C. decolora* has an average duration of 71.50 ± 7.22 days from egg to adult emergence when reared in a greenhouse at a temperature of 17.72°C and 65.26% relative humidity.

Males have a life span of 18.44 days and females 15 days. Each female lays around 1 000 eggs.

As Figure 4 shows, the full life cycle for *C. decolora* from egg to adult (including adult life span) is 88.22 ± 13.22 days.

Agrotis ipsilon

Agrotis ipsilon larvae, commonly known as earthworms or cutworms, are found throughout the world, particularly in the Andean region where they are considered pests for various crops (Artigas, 1994; Pastrana, 2004). Larvae live below the soil, where they build a protective cell. At dusk and night, they come out to feed on seedling stems, leaves and roots. The larvae in the first stages of development are mainly defoliators, becoming cutters in later stages. They can spend summer as larvae – a biological phenomenon known as estival diapause. Pupation occurs in the same underground cell. Adults can emerge nearly year round, but do so primarily in the autumn. The insect can overwinter as a larva or pupa (Artigas, 1994).

Taxonomic classification

Agrotis ipsilon is classified as follows:

Class:	Insect
Order:	Lepidoptera
Family:	Noctuidae
Genus:	*Agrotis*
Species:	*A. ipsilon* Hufnagel

Life cycle of *Agrotis ipsilon*

Figure 5 shows the development stage duration for *A. ipsilon* according to Blenk *et al.* (1985) reared at 27°C and 65–75% relative humidity with a photoperiod of 14:10. Figure 5 also shows that the eggs hatch in 3.83 ± 0.17 days, the larval stage lasts 20.6 ± 0.93 days, the prepupal stage 2.11 ± 0.21 days, the pupal stage 12.51 ± 0.36 days and the adults live 18.91 ± 3.36 days, for a complete life cycle of 57.96 ± 5.03 days.

Damage caused by noctuid complex larvae

Adult insects do not damage quinoa crops because they feed only on flower nectar and sweet secretions from plants such as tol (*Parastrephia lepidophylla, P. lucida*), *qillu-qillu (Hymenoxis robusta)* and q*ueñoa (Polylepis tarapacana)*.

The damage caused by the noctuid complex varies depending mainly on the noctuid species, the plant phenological stage and the larval stage. *Agrotis* larvae cut the seedling stem at the ground, but can also feed as defoliators.

In the Bolivian Altiplano, *Helicoverpa* and *Copitarsia* larvae are present year round throughout the crop's entire vegetative cycle, inflicting damage on multiple fronts. Recently hatched larvae mine the developing inflorescence, causing branching in the quinoa plant (Figure 6) on which smaller panicles form. During the plant development stage and when the larvae are bigger, they feed as defoliators (Figure 7).

During the crop's flowering and physiological maturity stage, larvae cause major damage as they bore into the panicle rachis (Figure 8), leading it to break off, which results, in defoliated plants. They also feed on developing grains. The most significant damage by these noctuid species occurs during the grain's milk stage or dough stage, when these pests behave as grain feeders (Figure 9) and have a direct impact on yield.

Methods of managing the noctuid complex

There are two global markets for quinoa: organic and conventional. This difference impacts farm management, with regards to both the crop and the use of organic inputs (see Appendix 1). The recommended methods for managing the noctuid complex are described below.

Monitoring for the presence of larvae

For any pest management strategy to be successful, it is extremely important to monitor and quantify the pests. It is then possible to make decisions early and determine the necessary control measures.

Two parameters used to evaluate infestations of pests are "incidence" and "severity". Incidence refers to the number of plants with individual pests divided by the total number of plants observed (percentage). Severity refers to the number of individuals found on each observed plant. This information helps the farmer assess the level of damage and determine whether control measures should be implemented to reduce pest severity. In the case of quinoa, it is recommended to spot-check ten plants per hectare. If the average number of larvae per plant is higher than one, a control method should be implemented. There is little research on

Figure 6. *Helicoverpa quinoa* larvae feeding on the developing panicle.

Figure 7. *Copitarsia incommoda* larvae

Figure 8. Damage to the panicle rachis caused by *Copitarsia incommoda* larvae

Figure 9. *Helicoverpa quinoa* larvae feeding on quinoa grains.

this subject, but it is essential to decide whether or not to apply a control method.

Crop rotation

Crop rotation is a practice that aims to avoid soil fertility exhaustion and break the pest life cycle. Because moths overwinter in the pupal stage, crop rotation requires that soil be ploughed before planting a new crop in order that the pupae be exposed to birds and other predators

Using light traps

Light traps are devices that attract adult moths to capture and kill them. The basic design is a bright light source and a capture mechanism containing water and a small amount of detergent to reduce the surface tension and prevent the insects from escaping (Figure 10).

One disadvantage of light traps is that they attract and capture a wide variety of moths, many of which are not pests. As such, for light traps to be helpful in decision-making, the species captured by the traps must be evaluated.

Using pheromone traps

In recent years, pest management strategies in Bolivia have included the use of pheromone traps (Figure 11).

Sex pheromones were produced in a joint effort by entomologists from the Fundación PROINPA and a Dutch company. To synthesize these pheromones, the insects were reared to pupal stage and then genital glands were sent to Pherobank.

The company used established methods to synthesize protopheromones, and the organizations then worked together to optimize the pheromones. There are currently pheromones for *Helicoverpa quinoa, Copitarsia incommoda* and *Agrotis andina*.

Sex pheromones are glandular secretions from the males that cause specific attraction reactions in males of the same species.

The pheromones can be used to monitor pests, to control adult insects or to disrupt mating.

One of the advantages of using pheromones is that they can target specific species: they attract and capture the insects at which they are aimed. They do not harm the environment, are accepted

Figure 10. Light trap used to capture noctuid complex adults.

Figure 11. A prototype of a barley trap with sex pheromones for *Helicoverpa quinoa*

in organic farming and are effective for at least 3 months.

Using bioinsecticides and ecofriendly pesticides

Bioinsecticides and ecofriendly pesticides are generally used in organic farming. They are biodegradable and do not harm the environment.

The organic insecticides and pesticides recommended for moth larvae control are shown in Table 2.

For organic quinoa production in Bolivia, the Fundación PROINPA developed an integrated pest management (IPM) strategy that has been implemented on thousands of hectares with considerable success. The noctuid or *ticona* complex management strategy focuses on monitoring of larvae and adults, preventive treatment, alternating treatments (active ingredients and modes of action), strategic application, the use of adjuvants and the safe use of organic inputs. The components of the IPM strategy are as follows:

- **Installation of pheromone traps.** Four traps per hectare are installed inside the plot (with minimum spacing of 25 m between traps) to identify the initial presence of adults and the moment that oviposition begins. In areas where the noctuid population is still low, the use of four traps per hectare makes it possible to maintain larvae populations at levels that do not cause significant damage (5-10% damage).
- **Field inspections.** Periodic inspections should be carried out during at least four of the crop's development stages (six green leaves, initial panicle formation, grain formation and milk stage). At least ten plants per hectare should be spot-checked at random during each inspection. If eggs and/or first-stage larvae are found on 20% of the plants checked, preventive measures should be taken. If larvae in later stages are found, or if there is a higher incidence, a control treatment should be applied.
- **Preventive treatment application.** An application of lime sulphur should be made as it acts on contact (affecting the insect's central nervous system), enabling good control of eggs and first-stage larvae. Moreover, this product has a repellent effect on adults, protecting the crop from new egg laying for at least 15 days.
- **Control treatment application.** A control treatment should be applied during the panicle development stage. Pest control is crucial at this phenological stage because insect damage at this time causes lateral branching and can make crop management difficult and reduce yield. When at least one larva per plant is observed, an application of Spinosad (active ingredient of the insecticide) is recommended. Another critical moment is the milk stage, the phenological stage at which larvae begin to feed on the developing grains, causing potentially considerable economic losses. It is important to do field inspections at this point, and if two or more third-stage larvae are found per plant, an application of Spinosad is recommended. This highly effective (> 93%), ecofriendly insecticide acts through contact and ingestion, allowing for effective larvae control with minimal effects on any beneficial insects that may be present.
- **Alternating treatments.** As part of an overall IPM approach and to avoid the development of resistant populations, treatments should be alternated. This means that the application of bioinsecticides should take into account the active ingredients and different modes of action, and more than two applications of the same product per crop year should be avoided.

Table 2. Organic insecticides and ecofriendly pesticides recommended for *ticona* moth larvae control.

Organic insecticides Active ingredient	Dosage/20 litres	% efficacy
Bacillus thuringiensis	90 gr	63
Spinosad (*)	3 g/litre	93.5
Lime sulphur	500 cc	35

*Product approved for organic farming

- **Using adjuvants**. Because quinoa crops display a large amount of oxalates on the surfaces of leaves, stems and panicles, reducing product adherence, it is very important to use an adjuvant which helps sticking. For example, a vegetable oil spray acts as a dispersal agent, improves coverage and prevents the formation of large drops. The application of the vegetable oil spray adjuvant enhances product efficacy.

Conventional farming

Synthetized chemical insecticides are not permitted in organic farming, but they are used in conventional quinoa production. These insecticides have the advantage of being quick-acting, effective and economical. However, it is widely acknowledged that they have a long-term negative effect on beneficial insects, the environment and farmers' health.

The methods and strategies described to control noctuid complex larvae for organic farming are also valid for conventional farming when ecofriendly insecticides are replaced with chemical insecticides.
The most frequently used products in Bolivia for conventional quinoa farming are classified as pyrethroids: Cypermethrin and Lambda-Cyhalothrin.

2. The quinoa moth complex

The quinoa moth belongs to the *Eurysacca* genus of the Gelechiidae family and Lepidoptera order. Currently, more than 20 *Eurysacca* species have been recognized, of which three – *Eurysacca melanocampta, E. quinoae* and *E. media* – are reported as the major quinoa crop pests (Povolný, 1997; Lamborot *et al*., 1999; Rasmussen *et al*, 2001a; Rasmussen *et al.*, 2003; Saravia and Quispe, 2003; PROINPA, 2008; Valoy *et al*, 2011).

These moth species are found throughout the Andean ecological region, characterized by its arid and semi-arid habitats. The presence of *E. melanocampta* was reported in Andean agro-ecological zones where quinoa is produced at altitudes of 1 900-4 350 m asl, from Argentina and Chile in the south to Colombia in the north (Povolný and Valencia, 1986; Povolný, 1990, 1997; Lamborot *et al*, 1999; Rasmussen *et al*, 2003; Valoy *et al*, 2011). However, *E. quinoae* appears to have a more limited dispersal. At the time of printing, it has only been reported in Bolivia and Peru (Povolný, 1997; Rasmussen *et al*, 2001b; PROINPA, 2008), while *E. media* has been reported as a quinoa pest in Chile and Argentina (Lamborot *et al*, 1999; Valoy *et al*, 2011).

This chapter describes the major characteristics of *E. melanocampta* and *E. quinoae*, the main species causing serious economic losses for farmers in the Altiplano and Salare agro-ecological zones where more than 80% of the world's quinoa is produced.

Eurysacca melanocampta

The *Eurysacca melanocampta* quinoa moth is a microlepidoptera described in 1917 as *Phthorimaea melanocampta* by English entomologist Edward Meyrick using samples from Peru. It was later identified as *Gnorinoschema melanocampta* and *Scrobipalpula melanocampta* (Ortíz and Zanabria, 1979) and is currently classified as *E. melanocampta* Meyrick (Ojeda and Raven, 1986), the technical name accepted for this species (Sánchez and Vergara, 1991; Avalos, 1996).

Eurysacca larvae are known by many names depending on the language. For example, in Spanish they are known as *polilla de la quinua* (quinoa moth) and *pegador de hojas* (leafminer), in Aymara as *kcona kcona* or *qh'una qh'una* and in Quechua as *kjako* and *kjaco curo*, meaning "grinder" or "borer" due to their tendency to bore into quinoa grains (Saravia and Quispe, 2003; PROINPA, 2008).

As previously mentioned, *E. melanocampta* larvae are one of the most widespread quinoa pests in the Andean region, particularly in the Salare, Altiplano and Inter-Andean valleys agro-ecological zones. This is not to say that the insect is not present in the Coastal and Yunga agro-ecosystems where crop production has expanded; most likely, this moth is also a major problem there as well.

In these areas, *E. melanocampta* has been reported feeding on various species of plants from the Chenopodiaceae family: quinoa *(Chenopodium quinoa)* and cañahua (*C. pallidicaule*), as well as various wild relatives. The pest has also been observed in *Vicia faba* (broad bean), *Lupinus mutabilis* (tarwi) and *Senecio* spp., which are alternate host plants to *E. melanocampta*. The insect has been found in potato crops in Colombia and Peru, but without serious economic consequences (Povolný, 1979; Povolný and Valencia, 1986).

Taxonomic classification

The quinoa moth is classified as follows:

Class: Insect
Order: Lepidoptera
Family: Gelechiidae
Genus: *Eurysacca*
Species: *Eurysacca melanocampta* (Meyrick, 1917)

Life cycle of *Eurysacca melanocampta*

The quinoa moth is a species with a complete metamorphosis. Its life cycle includes four stages: egg, larva, pupa and adult. The duration of each stage varies depending on rearing conditions. In field conditions on the Bolivian Altiplano, two to three generations have been reported during a single crop year (September–April).

Recently, a large percentage of second generation moths have been observed overwintering as adults in diapause, notably in tufts of grass. A smaller percentage overwinters as pupae. When climate conditions improve towards spring, adult moth diapause ends.

Moth infestations in quinoa fields occur when the adult moths emerge from pupae in the soil and the adults come out of diapause. The female moths lay their eggs on the underside of leaves or between the panicle glomeruli. Most of the eggs hatch after a week, and the larvae go through five stages

Literature on the subject indicates that the first generation of moths live from November to December, a period during which the larvae live between the quinoa plant's leaves and stem, where they feed on and roll the leaves into a protective structure similar to a case, called *k'epicha* in Quechua. The second and third generations live from March to April or May, with the larvae living between the glomeruli inside the panicle, feeding on the grains and staying out of harm's way (climate, insecticides, natural enemies etc.).

Under laboratory conditions (20 ± 3°C, 60 ± 5% RH and a 12-hour photoperiod), the life cycle is significantly reduced from the 132 days recorded in the field to 75 days (Figure 12; Quispe, 2002). Studies carried out by Flavio (1997) show that the life cycle of *E. melanocampta* is only 28 days if reared at 24°C and 56 days if reared at 22°C, which demonstrates that this species' life cycle varies with temperature.

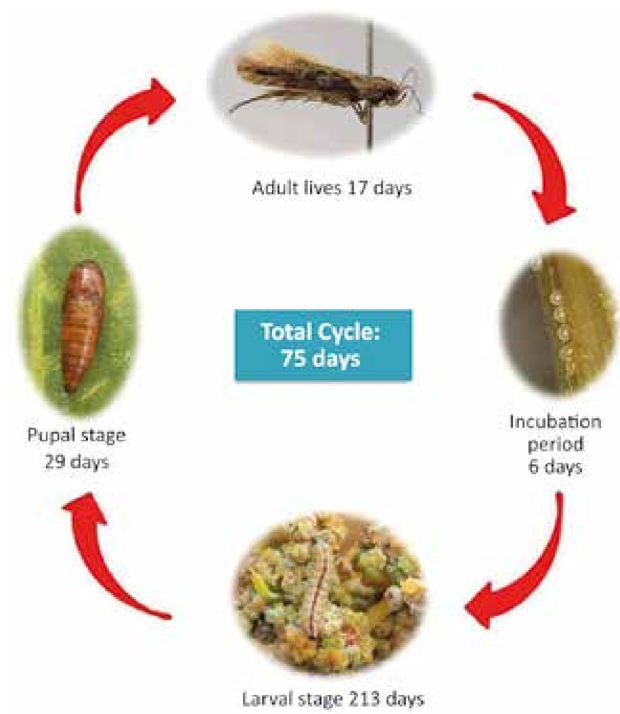

Figure 12. Life cycle of *Eurysacca melanocampta (*Quispe 2002)

Flavio also determined that the maximum number of eggs per female is 300.

Eurysacca quinoae

Eurysacca quinoae was described and reported as a quinoa crop pest by Povolný in 1997 based on specimens from La Paz, Bolivia. Over the last decade, the incidence of this pest has led to some confusion over the true identity of the moth species attacking quinoa crops in the Salare and Altiplano agro-ecological zones. The confusion arises from the difficulty of recognizing the local species in the fields at the egg, larval and pupal stages. These species can be differentiated at the adult stage.

Additionally, a particular characteristic of *E. quinoae* is its specialized feeding habit: at the time of printing, it has been reported in Peru and Bolivia as feeding only on quinoa.

Taxonomic classification

The *E. quinoae* moth is classified as follows:

Class: Insect
Order: Lepidoptera
Family: Gelechiidae
Genus: *Eurysacca*
Species: *Eurysacca quinoae* Povolný 1997

Life cycle of *Eurysacca quinoae.*

As with *E. melanocampta, E. quinoae* quinoae has four life stages: egg, larva, pupa and adult. The adult E. quinoae moth emerges from pupae in the soil, although they may also be found in developing panicles (inflorescence). The moths mate shortly after emerging, and the females lay their eggs mainly on the underside of leaves or in the inflorescence.

The eggs generally hatch after 5–7 days, and the larvae immediately begin feeding on the leaves (Rasmussen, 2006) before moving on to the developing quinoa grains. When the larvae arrive at their fifth development stage, they form pupae in the soil to later emerge as adult moths. According to PROINPA (2014), the *E. quinoae* life cycle can reach 73 ± 10.8 days (Figure 13) under laboratory conditions at 20 ± 3°C and 60 ± 5% RH with a 12-hour photoperiod.

Like *E. melanocampta, E. quinoae* displays two to three generations per crop per year in the Andean regions of Peru and Bolivia, depending on climate conditions (Zanabria and Banegas, 1997; Mujica *et al.*, 1998). Mujica *et al.* (1998) and Avalos (1996) reported that the first generation is found between November and December, while the second and third generations live from March to May/June in the Peruvian and Bolivian Altiplano. The distribution of both quinoa moth species is not uniform throughout the Andean region. According to Rasmussen *et al.* (2003) and Delgado (2005), while *E. quinoae* and *E. melanocampta* both exist in the Peruvian Altiplano, 98% of the larvae population collected during the second infestation period were *E. quinoae*.

Figure 13. Life cycle of *E. quinoae under laboratory conditions*

E. quinoae is also the most prominent species in Bolivia, with a 70–90% predominance in the Altiplano area; however, in the Salare area, no incidence of *E. melanocampta* was recorded, either in larvae collected in the first infestation period (November/December) or in the second (February/May).

The behaviour of *E. quinoae* larvae is similar to that of *E. melanocampta* during both infestation periods. Based on observations between November and December (first period), *E. quinoae* use leaves to create their protective case structures during the day and come out at night to feed on the quinoa leaves, causing indirect damage to the crop.

However, between February and May (second period), *E. quinoae* larvae are abundant in quinoa panicles, where they feed on the tender, mature quinoa grains, causing direct damage to the crop by eating the product destined for sale.

In the Bolivian Altiplano, *E. quinoae* and *E. melanocampta* have been observed overwintering in the adult stage (moth) in the native vegetation (grass and tola) that is abundant at this time of year.

Damage caused by moth larvae

E. quinoae and *E. melanocampta* larvae are initially found between the plant's apical leaves during the branching stage (Figure 15). The damage here occurs over the entire developing panicle. Nevertheless, the worst damage is observed between the stages of grain development and physiological maturity, when the larvae feed mainly on tender leaves if they are in their first stages or immature and mature grains in later stages (Figure 16; Mujica *et al.*, 1998; Rasmussen *et al.*, 2003).

The harm done by the quinoa moth occurs at two levels: indirect and direct larval damage to the plant. With regards to indirect damage, the photosynthetic area of the plant is reduced and first generation larvae feed on the parenchyma of the leaves, roll leaves and tender shoots and destroy developing inflorescences.

Second generation larvae destroy the developed inflorescences, milk and dough stage grains, and mature grains, thereby reducing the quality and quantity of the grain yield by 15–60% (Quispe, 1979; Ortíz *et al.*, 1979; PROINPA, 2008). This last generation reaches a growth rate of 30–35%, with more than 250 moth larvae per plant recorded. Measuring crop losses is challenging, and they are generally based on estimates by experts and calculations made using experimental methods.

Methods of managing the quinoa moth complex

The quinoa moth is the most common and frequently found species in quinoa crops in the Salare, Altiplano and Inter-Andean valleys agro-ecological zones, and their high numbers - due to the spread of quinoa-growing areas – have become serious, requiring control methods to prevent considerable economic losses.

The basic techniques and strategies described for managing the noctuid complex, both for organic and conventional farming, are also valid for controlling the quinoa moth complex, taking into account a few variations with regards to the time periods and number of applications. For example, in the Salare agro-ecological zone, pest management measures are implemented from the phenological stage of grain development, when large numbers of eggs are laid and first-stage larvae are found. They must be kept quickly in check to prevent their populations from multiplying. Preventive applications are recommended with products that are very effective for egg and first-stage larvae control, such as lime sulphur, which has shown an efficacy rate of over 80% in organic farming.

In this agro-ecological zone, the grain development stage begins between January and February, depending on time of sowing or resowing (common in this area due to seedling loss from strong winds). If the larva population exceeds the economic damage threshold (3–6 larvae per plant in a sample of 10 plants/ha), the use of Spinosad (an ecofriendly insecticide approved for use in organic farming) is recommended; its efficacy rate exceeds 90%.

In the Altiplano agro-ecological zone, there are generally two separate generations of insects: the first during the phenological stage of shoot emergence and the second during grain formation. In this area, control measures should be implemented during the critical plant growth stages, using ecofriendly insecticides or classic insecticides, depending on the type of production.

In the Salare and Altiplano agro-ecological zones, both pests are frequently found together (quinoa moth and noctuid complex larvae) during the grain development stage. The control strategies should be implemented conjointly.

2.1 Quinoa crop diseases

The majority of diseases affecting quinoa crops are due to fungi. Bacteria, nematodes and viruses are also a problem on a smaller scale. The incidence and severity vary according to the variety, phenological stage and environmental conditions. Overall, diseases have received little attention, with

Figure 14. Leaf damage caused by *E. melanocampta* and *E. quinoa*

Figure 15. Panicle damage caused by *E. melanocampta*

Figure 16. Quinoa plants severely affected by downy mildew

the first reports published in 1979 in the book *La Quinua y la Kañiwa* ("Quinoa and Kañiwa", Tapia *et al.*, 1979).

The most significant and well-known disease at a global level is downy mildew, although there are other minor diseases, such as damping off, green mould, leaf spot (caused by *Passalora dubia* [Riess] U. Braun, *Ascochyta hyalospora* and *A. chenopodii*), brown stem rot, eyespot, bacterial spot, nematodes and viruses. These diseases are not usually of great economic importance, but due to the rapid expansion of quinoa-growing areas in the Andean region, combined with the effects of climate change, they could become more serious. Furthermore, because quinoa is increasingly cultivated in other countries around the world with different agro-ecological and environmental characteristics, it is probable that new diseases will emerge. This chapter deals only with quinoa downy mildew.

2.1.1. Downy mildew

The primary quinoa disease on a global scale is downy mildew (Figure16), caused by the oomycete *Peronospora variabilis*[1].

[1] Formerly called *Peronospora farinosa* f. sp. *chenopodii* (Fr.) and later reclassified following research in 2008 and 2010 by Choi *et al.* using molecular techniques based on rDNA intergenic spacer regions.

Downy mildew was first reported in Peru in 1947, and has since been found in numerous countries across the globe, including Argentina, Bolivia, Chile, Colombia, Ecuador and Peru in South America; Mexico, Canada and the United States of America in North America; Portugal, France, the Netherlands, the United Kingdom, Sweden, Italy and Denmark in Europe; India in Asia; and Kenya in Africa (Danielsen *et al.*, 2000; Choi *et al.*, 2010; INIA, 2012; Mújica *et al.*, 2013). *P. variabilis* is an oomycete that is easily transmitted (via wind and rain). During crop growth, it is mainly disseminated through spores; however, at senescence or when crops are not present, it can be spread through oospores (sexual reproduction structures) which can adhere to the grain surface or inside the stubble that remains in the field. Essentially, the disease is spread over short distances through spores and over larger distances through oospores.

Because of the global interest in quinoa in recent years, there has been sustained seed movement between continents and countries which does not always comply with phytosanitary standards. The probability of transporting quinoa grains contaminated with oospores is very high (Danielsen *et al.*, 2004; Testen, 2012).

It is fairly certain that mildew can be found wherever quinoa is grown. The incidence and severity depend on the variety, crop management approach and environmental conditions. Crop losses due to mildew depend on the phenological stage of the plant when attacked and the variety's degree of resistance. When susceptible varieties are cultivated and environmental conditions are conducive to mildew growth – especially when relative humidity is high – the effects of mildew are severe.

If the attack occurs during the plant's initial development stages, the entire crop can be lost; in resistant varieties, losses range between 20% and 40% (Danielsen *et al.*, 2003; Figure 17).

The disease mainly reduces the plant's photosynthetic areas (appearance of chlorotic or necrotic spots on the leaves), causing partial or total leaf loss (as shown in Figure 18.), atrophied plant development, reduced panicle size and lower yield (small and/or defective grains).

Optimal conditions for mildew development are high relative humidity (> 80%) and temperatures between 18°C and 22°C, which promote spore formation and fungus growth. However, these processes may be interrupted during extended periods

Figure 17. Plants affected by mildew since seedling emergence (left) and plants affected by the disease during panicle stage (right).

Figure 18. Defoliation of quinoa plants: resistant variety (left) and susceptible variety (right).

of sun and drought. In some areas, plants may be covered by a thin layer of dew in the morning, which is enough to cause pathogen development. It has also been found that cloudy periods – even without rain – promote the appearance of the disease. The disease is less sensitive to temperature and can develop at temperatures between 0°C and 25°C.

Options for controlling this disease depend on the type of production (organic or conventional). For conventional farming, the newest generation of fungicides can provide satisfactory disease control if the products are applied early as a preventive measure. For organic farming, there are a number of considerations to make to ensure that acceptable levels of severity are maintained. They include the use of resistant varieties, early planting, low planting density and biofungicides approved for organic farming.

In the Inter-Andean valleys area, where average rainfall reaches 500 mm, it is important to implement control measures and sow resistant or tolerant varieties. Given the interest in planting quinoa in other areas of the world, mildew is a restrictive factor, in particular in areas with rainfall of > 500 mm where severe infestations would occur. The Salare agro-ecological zone, where average rainfall is 200–250 mm, is the main area of production of quinoa for export in Bolivia. In this extremely dry area, mildew is not a major problem, and certified organic farming and disease-free seed production are, therefore, facilitated.

Symptoms

The disease primarily affects foliage (leaves), although symptoms may also appear on the stems, branches, inflorescence and grains. Initial symptoms appear on the leaves as small, irregular spots that may be chlorotic, yellow, pink, red, orange or grey, depending on the plant colour (Figure 19).

Figure 19. *T*ypical spots caused by *Peronospora variabilis* that vary based on the colour of the quinoa plant

Figure 20. Abundant grey spore formation on the underside of the quinoa leaf.

Figure 21. Spore formation on the top and underside of a red variety.

As the disease progresses, these spots begin to merge (coalesce), the leaf becomes chlorotic and eventually falls (defoliation). When conditions are very conducive to disease development (high relative humidity, cloudiness and continual precipitation), all of the plant's leaves may become infected and fall off, halting plant growth.
The fungus forms spores on the underside of the leaves, and its spread depends on variety resistance or susceptibility.

In susceptible ecotypes, abundant spore formation is frequently observed as a greyish fungus (Figures 20 and 21); in resistant ecotypes, the fungus may or may not appear.

When the disease appears at the beginning of panicle development, panicle formation atrophies (slow growth) and grain filling and size are affected. If climate conditions are favourable during the dough stage, grains may turn black. In large grain ecotypes (Quinoa Real), reduced grain size and defective grains have been observed. In native and resistant varieties, however, mildew does not affect grain size.

It is at this stage when the oospores develop on the grain's pericarp, creating a major primary source of transmission if these seeds are used for planting. When the disease appears after flowering, it can be confused with the plant's natural senescence (generalized yellowing), but does not cause considerable losses.

Description of the pathogen

Peronospora variabilis (Choi *et al.*, 2008, 2010)) is an obligate biotrophic parasite from the Oomycetes group, Peronosporaceae family and Peronosporales order. *P. variabilis* has two types of reproduction: asexual and sexual. The asexual phase is characterized by the presentation of ovoid spores with direct germination. They have coenocytic hyphae and dichotomous mycelia (Figure 22).

Sexual reproduction occurs via the formation of ooSexual reproduction occurs via the formation of oospores (sexual survival structure) in the absence of a host plant. The pathogen is heterothallic, which means that for oospore formation to occur, two types of mating are required, P1 and P2 (genetically distinct but sexually compatible thalli), for the archegonia and antheridia to develop.

The archegonia grow via the antheridia, allowing fertilization before forming oospores (thick-walled structures). When conditions are favourable, oospores germinate and produce spores. Oospores can be observed through dye injected into the leaves and on the grain surface (Figure 23).

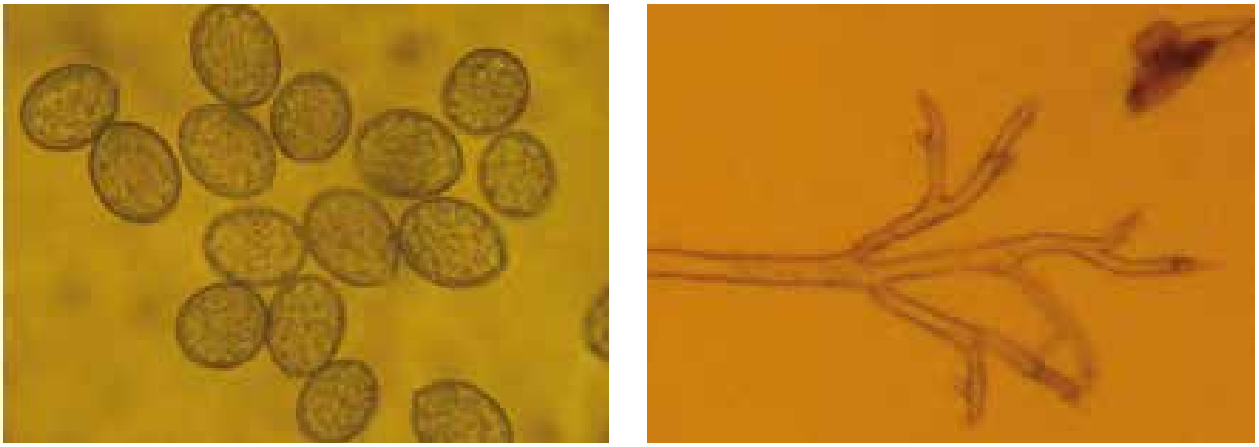

Figure 22. Spores (left) and dichotomous mycelium (right) of *Peronospora variabilis*

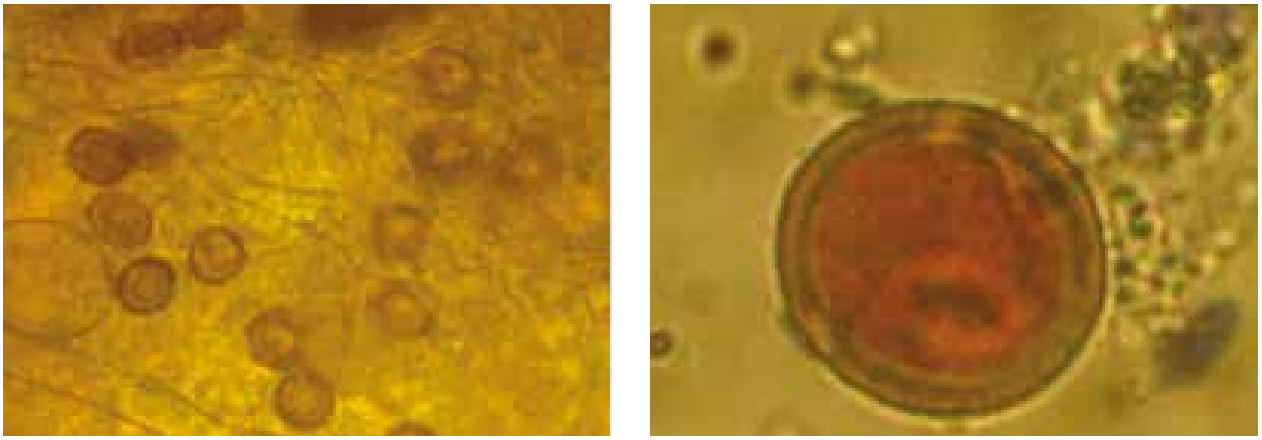

Figure 23. Oospores of *Peronospora variabilis* inside leaves (left) and on the grain surface (right).

Disease cycle

The source of the disease's initial spread are oospores found on the seeds or stubble from previous crops (Figure 24). The oospores become active under optimal environmental conditions (relative humidity > 80%), which stimulate germination and spore formation. When spores arrive at the leaf, they develop a germ tube, haustorium and appressorium, allowing their entry into the leaf. After 5 days, discoloration can be observed in the cell tissue followed by sporulation.

Mildew is considered a polycyclic pathogen: during the crop growth stage, the infection process is continuous, with various generations of the pathogen developing through asexual reproduction (only spores are produced).

When spots start to become necrotic, sexual reproduction occurs. The two mating structures appear and produce the oospore, the structure that conserves the pathogen for long periods of time without a host plant.

Epidemiology

There are three main aspects of the disease to consider with regards to epidemiology: pathogen (*P. variabilis*), host plant (quinoa) and favourable environmental conditions.

In the case of downy mildew, the most important factor is the environment, particularly relative humidity (> 80%) and cool temperatures.

These are the basic conditions for oospore and spore germination, multiplication and dissemina-

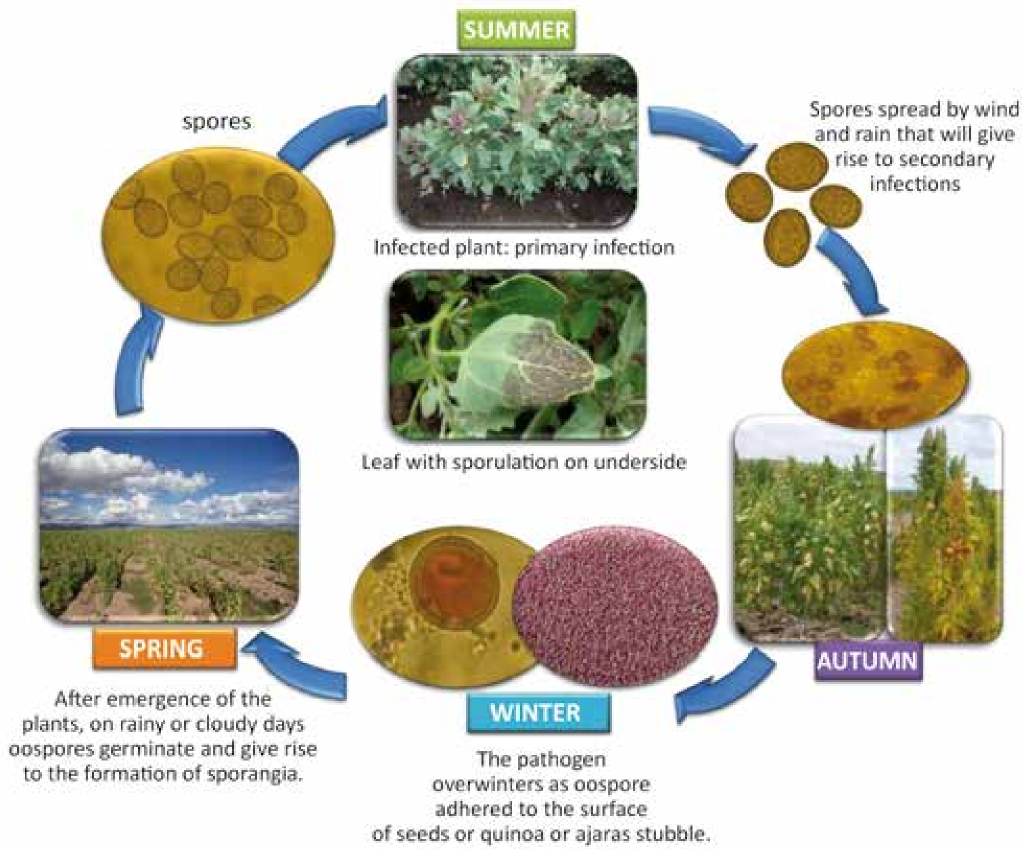

Figure 24. Quinoa downy mildew cycle

tion. If favourable environmental conditions continue over a prolonged period of time, polycyclic propagation can occur.

Spores are spread primarily by wind, as well as by rain that washes them to different parts of the same plant or spreads them through splattering. Morning dew also facilitates pathogen colonization on the underside of the leaves (Figure 25). However, if humidity drops, the spores dry out and sporulation ceases.

Oospores are the main source of infection. They stick to the quinoa seeds and remain in field stubble after harvest. Quinoa wild relatives – called *ajaras* in Bolivia, *ayara* in Peru, *quinua malla* in Ecuador and quingüilla in Chile – are somewhat susceptible to the disease and are a source of initial infection in the Andean region.

Because these wild species are found in nearly all agricultural areas around the globe, they could be an important source of infection in new quinoa-growing areas. The time of planting may also be

Figure 25. Leaf with dew in the early morning hours.

a determining factor in the appearance of the disease. In some areas, quinoa is planted after the first rains. The rain stimulates germination of the wild quinoa species at the same time as the cultivated quinoa, which promotes development of the disease in the very early stages of the crop.

Integrated management of quinoa downy mildew

Managing mildew depends on the growing areas and climate conditions, the varieties and their tolerance to disease, and whether organic or conventional farming practices are employed. There are various control methods which may be implemented according to each area. Furthermore, because genetic resistance is central to controlling mildew, this topic is discussed in more detail.

Genetic resistance

Genetic resistance is one of the most effective options in managing mildew. Farmers that use a disease-resistant variety can exploit it for several generations. A resistant variety requires fewer or even no fungicide applications, reduces production costs and is easier to integrate with other crop management methods.

In zones where the disease is endemic, such as the Inter-Andean valleys area, it is practically essential to use resistant or partially resistant varieties, otherwise the disease may decimate crops. For organic farming, synthetic fungicides are restricted, and ecofriendly fungicides or biofungicides with lower efficacy must be used to control mildew. Varieties with genetic resistance provide a good alternative.

In Bolivia, mildew is a restrictive factor for growing quinoa in Inter-Andean valleys and Altiplano agroecological zones. As such, a breeding programme has been developed based on the considerable existing genetic diversity in the country. There are currently numerous varieties with various productive cycles (late, semi-early and early-maturing), colours and sizes, and levels of saponin content and resistance (susceptible, partially resistant, hypersensitive and combined resistance).

Mildew resistance level can be governed by major genes (vertical resistance) or minor genes (horizontal resistance), as well as by a combination of major and minor genes, resulting in partial or durable resistance. These resistance genes are found in late-maturing quinoa varieties and other Chenopodiacea species: *Chenopodium hircinum, C. nuttalliae, C. petiolare, C. album* and *C. ambrosioides*.

At selection, it is important to consider several evaluation criteria, such as the phenological stage during which the first symptoms appear (spots, spot size, chlorosis etc.) and sporulation (a factor that determines whether the disease will spread) and defoliation occur (Bonifacio, 1997).

The most common type of resistance is horizontal resistance, also known as partial, minor gene, quantitative or durable resistance. The degree of resistance varies from highly susceptible to resistant, depending on the number of resistance genes the variety has. Moreover, resistance is related to the variety's life cycle. Varieties with a long cycle have better mildew resistance than early-maturing varieties; similarly, susceptible varieties have larger grains than resistant varieties.

The selection of varieties for traits of resistance, early maturity and large grains is feasible through breeding. Vertical resistance, also known as hypersensitive, major gene, non-durable or qualitative resistance, is characterized by the plant's swift reaction when infected: the affected section is isolated and necrotic spots in the leaves limit the disease's spread. This type of resistance can be lost over time, which is why it is known as non-durable.

Some Inter-Andean valleys ecotypes, germplasm accessions and improved lines show vertical resistance; however, varieties with this type of resistance are not yet being bred. It is theoretically possible to combine vertical and partial resistance, but so far no quinoa varieties with combined resistance exist.

Acquired resistance refers to plants that become resistant through interaction with the environment (it is not hereditary). For example, in Bolivia, acquired resistance is related to various aspects of crop management, such as early planting, soil fertility, daylight hours and plant vigour. The plant acquires resistance due to its exposure to longer daylight hours in the early stages of development and good plant nutrition. To generate and make use of this type of resistance, fields must be well prepared, plants fertilized, and moisture controlled or an irrigation system used for early planting.

With late sowing, plants are not exposed to longer daylight hours, but rather germinate under cloudy conditions or even rain, which prevents the plant from acquiring resistance and leads to disease susceptibility. For late planting under these conditions, preventive control measures should be taken in conjunction with fertilizer applications.

Molecular research has shown that populations of P. variabilis from Bolivia, Ecuador and the United States of America are identical; as a result, the sources of resistance identified in Bolivia should serve as a basis for breeding programmes in other countries.

In countries where quinoa is not a native species, resistant varieties from the Andean highlands should be planted; otherwise, hybrids should be developed, combining susceptible varieties adapted to the area with other local varieties.

Quality seeds

Since oospores remain on and spread via the seeds, it is important to collect seeds from disease-free plots. For conventional farming or when seeds are transferred between areas, it is recommended that they be disinfected.

Several fungicides are available for this treatment but they present a wide range of toxicity. It is important to note that the CTC mix presents acute hazard in normal use. It is classified by WHO as class U for products unlikely to present acute hazard. Others treatments exist, like Thiram (dimethyldithiocarbamate, WHO class II, Moderately hazardous), Fipronil (phenylpyrazole, WHO class II, Moderately hazardous) or Dividend (Difenoconazole, triazole: WHO class III, slightly hazardous).

All of these treatments require high attention in their use due to the concentration of individual product's active ingredients, which make a difference in terms of risk.

An organic alternative is to use biofungicides created with micro-organisms, such as *Trichoderma* spp. or *Bacillus subtilis*. These micro-organisms compete with the pathogens on the pericarp and promote improved root development.

Farming practices

It is well known that vigorous plants are better able to tolerate stress and disease infestation. To ensure good plant health, the soil should be well prepared with organic matter or other fertilizers.

Organic farming has achieved good results, with foliar biofertilizers formulated using humic acids, which activate plants' biochemical processes (respiration, photosynthesis and chlorophyll content) and supply essential nutrients and trace elements to improve plant vigour and disease resistance. Given that quinoa is the only host crop of *P. variabilis*, virtually any crop may be rotated with quinoa.

Early planting can be adopted as a means of avoiding disease: it is important to prevent periods of high rainfall coinciding with the most sensitive development stages (from the formation of two green leaves to initial panicle development).

Planting density can slow or prevent disease development, which in turn depends on the climate conditions of each area, the variety's degree of resistance and the soil fertility. In areas conducive to disease development (relative humidity ≥ 80%), the distance between furrows should be at least 0.5 m and plant-to-plant spacing at least 0.15 m.

Appropriate drainage should also be implemented, and the direction of the furrows with regards to wind and field slope should be considered, as should the planting method (furrows, broadcast sowing or pits).

Ecofriendly fungicides

Plants have been used to prevent human, animal or plant diseases since antiquity. Organic farming is well accepted for a wide range of reasons, for example: it does not contaminate the environment, is not toxic, does not create resistance to the active ingredient of pesticides used, is low-cost and breaks down quickly which avoids the permanence of the product in the soils.

As a result, many countries have turned to using plant extracts, exploiting their fungicide properties in disease control. Downy mildew has been treated with relative success using liquid extracts of horsetail (*Equisetum arvense* L.) and garlic (*Allium sativum*). Once the correct species is identified, ecofriendly fungicides can be developed and certified for use in organic farming.

As with synthetic fungicides, ecofriendly fungicides can be mixed. Trials have been carried out to alternate them with metabolites produced by fungi and beneficial bacterial.

It is essential that these products be applied preventively – or as soon as symptoms appear (5–10% infection) – and combined with an adjuvant (for organic farming, these can be prepared with cactaceae); the plant should be thoroughly sprayed with the product. The advantages of using medicinal or wild plants are that they leave no toxic residues on the crop, farmer or environment. They are also low cost, easy to find and can be prepared using traditional methods.

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Appendix 1

Organic and conventional farming

For quinoa crops, it is important to distinguish been between organic and conventional farming systems. This is because a large share of international demand is for organic quinoa; however, because of rising global demand for this crop, it is increasingly important to consider conventional farming in local and international markets.

According to the International Federation of Organic Agriculture Movements (IFOAM), "organic agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment, promote fair relationships and a good quality of life for all those involved." Organic agriculture considerably reduces the amount of external inputs required and does not use chemical fertilizers, pesticides or other synthetic products (IBNORCA, 2000). What sets organic agriculture apart is the fact that it is regulated through various standards and certification programmes. These principles, in addition to establishing general production standards, restrict and prohibit the use of most synthetic inputs, whether for fertilizing or controlling insects/pests, weeds or diseases. Standards also include principles for soil management with a view to maintaining or improving its fertility and structure, the cornerstone of agricultural production (AOPEB, 2002).

For quinoa, organic farming standards recommend the use of organic matter (guano, green manure etc.) to maintain or improve soil fertility, crop rotation, the use of light or heromone traps for preventive noctuid pest management, and the use of biofertilizers and biocide plant extracts for pest control. It should be noted that a main requirement of organic farming is that records be kept of all practices to ensure traceability. These records must be certified by accredited companies and the entire process approved to obtain the corresponding certification.

Conventional farming, which is defined as agriculture based on the intensive use of capital (tractors and highly efficient machinery) and external inputs (seeds with high yield potential, fertilizers and synthetic pesticides) to achieve maximum yield. Conventional quinoa production does not have to be certified. As such, improved seeds and synthetic fertilizers, insecticides and fungicides can be used and there is no obligation to practice crop rotation. However, conventional farming has changed in recent years due to shifts in market demands as consumers become more environmentally conscious and want products that are grown sustainably using good agricultural practices that respect the soil's productive capacity, use water efficiently etc.

Section 3
Nutritional and Technical Aspects

CHAPTER: 3.1.

Traditional processes and Technological Innovations in Quinoa Harvesting, Processing and Industrialization

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Abstract

The growth in global demand for quinoa has led to an increase in production in its areas of origin, as well as its introduction in other regions. Most of the increased production is of varieties and ecotypes rich in saponins; these need to be removed from the surface of the grain prior to consumption, because of their antinutritional properties and undesirable organoleptic qualities.

Industrial-scale innovations have, therefore, been introduced in the harvest and post-harvest phases (including reaping or cutting, placing in sheaves or arcs, threshing, winnowing and cleaning of grains, drying, selection, storing, processing, manufacturing of high value-added products, and direct use of products), to replace traditional practices that were generally conceived for small-scale production.

Successful production of high commercial quality grains depends to a large extent on what occurs at harvesting. The timely introduction of mechanized systems, such as mowers, blowers, winnowers, threshers, brushes, and combined threshing and sifting equipment, on medium and large-sized farms has various advantages over traditional manual practices. These technologies reduce impurities, as well as damage to and loss of grains; they also require less labour, which can be scarce in the farming areas. These systems have been introduced and improved to mitigate the intrinsic, negative environmental impacts.

In the processing stage, traditional saponin removal methods have been improved, with the development and use of industrial-scale equipment and technology. Combined methods are most commonly used; they guarantee the nutritional quality and morphological stability of the grain, and result in a final saponin content well below international standards. Such systems involve the removal of, saponins in two stages: hulling and washing, followed by centrifuging and drying of the grains. In optimized processes, up to 95% of saponins are eliminated in the hulling machine; the rest is washed away with water.

The volumes of water needed are still quite high, generally above 5 m3/tonne of quinoa processed, and the effluent generated is contaminated with saponins. Impurities, such as gravel, twigs, and unripe, broken or different coloured grains, are removed using sieves, sorters, spreaders, and magnetic or optical systems. These systems are almost always supplemented by manual work.

Market forces – combined with more stringent environmental standards, better prices and limited water resources in production areas – will continue to drive the development of increasingly efficient and innovative equipment and technology. There is a trend towards dry saponin removal methods; they do not require water, and also allow the collection of the saponins, which then fetch good prices on the market because they can be used in various areas of the industrial sector. Artisanal models for dry processing of quinoa are being researched, but further tests are needed before they can be proposed at industrial level.

Quinoa-based foods have been a part of the diet of Andean populations for centuries. Thanks to its nutritional qualities, quinoa is now used elsewhere in a wide variety of derived products (flour, flakes, popped seeds) or in blends with cereals, oleaginous seeds and other foods (mixed flour breads, noodles, extruded products and gluten-free pasta). It is hoped that the expansion of the quinoa market will lead to the development of other derived products, such as protein concentrates and isolates, oils, starches, and high value-added saponin derivatives.

1. Introduction

According to the human nutrition standards defined by the Food and Agricultural Organization of the United Nations (FAO), quinoa (*Chenopodium quinoa* Willd.) is the only plant food that provides all essential amino acids (Koziol 1992; González *et al*., 2012). Not only does quinoa have high nutritional value, it is also cheap to produce due to its broad genetic variability and its capacity to adapt to different climate and soil conditions (Fundación PROINPA, 2011).

These characteristics, combined with its multiple possible uses, have led to an increasing global demand for this strategic crop capable of contributing to food sovereignty in various regions. Countries in Europe, North America, Africa and Asia are aware of this and have begun to cultivate this Andean grain (Jacobsen, 2003).

For example, between 2005 and 2012, the demand for Bolivian quinoa in the United States of America increased by 1120%, in France by 207%, and in Germany by 361%. A total of 25 660 tonnes were exported, for a total value of USD78.9 million at a price of USD3 075/tonne (INE, 2013). Production of both conventional and organic quinoa has increased in recent years to meet this demand. Figure 1 shows that both the cultivated surface area and production increased considerably between 1990 and 2010. The area under quinoa quadrupled compared with 1970–1980, and had reached 69 970 ha by 2012. Total quinoa production also increased significantly from 23 240 tonnes in 2000 to 44 260 tonnes in 2012 (INE, 2013). Also, in Peru, according to the exporters' association (La República, 2013), in 2012, quinoa exports reached 10 402 tonnes and USD30.7 million, 23% more than in the previous year. Annual quinoa production was 39 398 tonnes in 2009 and increased to 44 207 tonnes in 2012 (MINAG, 2013). These two countries alone represent more than 90% of global production (Baudoin and Avitabile, 2013). Quinoa production in the Andean region of Ecuador (exports 941 tonnes, USD2 694/tonne), Chile and Argentina is still rather low (a few thousand tonnes per year).

To handle this increase in production, various industrial-scale innovations have been introduced in the harvest and post-harvest phases (including reaping or cutting, placing in sheaves or arcs, threshing, winnowing and cleaning of grains, drying, selection, storage, processing, manufacturing of high value-added products, and direct use of the product) to replace traditional practices initially conceived for small-scale production. The most significant innovations in quinoa processing are in the area of saponin removal.

This chapter seeks to describe the state of the art in current use of traditional practices, as well as the various technological innovations developed for the various harvest and post-harvest phases, with a particular emphasis on processing.

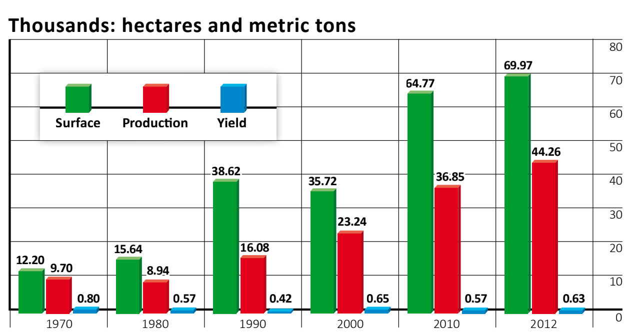

Figure 1: Cultivated surface area, quinoa production and yield per hectare in Bolivia between 1970 and 2012 (Source: IBCE/FAOSTAT, 2012)

2. Harvest

Quinoa is harvested when the plant reaches physiological maturity, a state that is easily recognizable as it changes colour, taking on a characteristic yellow, reddish, pink, purple or black tint, depending on the ecotype and/or variety. The state of maturity is confirmed by the hardness or resistance of the grain when pressed under a fingernail. The grains must be harvested within the recommended period in the reproductive cycle, to avoid losses from threshing or attacks by birds, and to avoid a deterioration in grain quality as a result of unexpected rain, hail or snow (Apaza *et al*., 2006).

Table 1 (Bonifacio *et al*., 2012) and Table 2 (Espíndola and Bonifacio, 1996) show the different phenotypic characteristics (e.g. tassel colour and grain colour) of various 'Quinoa Real' ecotypes grown in the southern Altiplano region of Bolivia, and of improved varieties, at the end of their respective vegetative cycles, when they have reached physiological maturity. Moisture content in the quinoa grain at maturity is 10–13% and in the plant, 16–20%. These characteristics can help identify the right time to harvest. Delaying the harvest by 2–3 weeks could lead to significant grain losses through wind-induced threshing (chafing between plants and tassels), in addition to threshing when the plants are cut and stacked in sheaves. Figure 2 shows 'White Quinoa Real' and 'Pink Quinoa Canchis' at physiological maturity.

Depending on the technology used, harvesting quinoa involves various stages. When harvesting is done manually with stationary threshers, the steps are: reaping or cutting, placing in sheaves or arcs, threshing, winnowing and cleaning of grains, drying, sorting, bagging and storage. When it is mechanized, using combine harvesters, reaping, threshing, and winnowing are done simultaneously, followed by sorting, bagging and storage.

2.1. Uprooting and reaping

Grains may be reaped manually in different ways. According to a survey carried out in the southern Altiplano in 2008, 57% of producers uprooted the plants, 42% used a sickle and 2% used a motorized mower (Aroni *et al.*, 2009).

Uprooting plants is an ancestral practice, especially used in areas with sandy soil. With this method, the lumps of soil that generally stick to the roots of the plant are partly removed by careful shaking or by lightly rubbing the roots together. The plants are then deposited on the ground in sheaves.

The mature plant may be reaped or mowed 10–15 cm above the surface of the land. Parts of the stem and the roots remain in the soil to protect it from erosion, and are subsequently converted into organic matter through a natural composting process (Aroni *et al*., 2009). Quinoa producers are gradually adopting the practice of using sickles, hoes or mechanical mowers for reaping. These slight innovations result in a significant reduction in contamination of the grain with sand, small stones and soil, which is extremely important for subsequent pro-

Figure 2: (a) "White Quinoa Real" at physiological maturity (Palaya, Potosí); (b) "Pink Quinoa Canchis" at physiological maturity (Chacala, Potosí) (Fundación PROINPA)

Table 1: Characteristics of improved varieties of quinoa at physiological maturity

Name of ecotype	Vegetative cycle [days]	Panicle (colour)	Whole grain (colour)	Pearl grain (colour)
Late maturing				
Achachino	180	Creamy red	Red	White
Chuku Puñete	172	Cream	Cream	White
Mok'o Rosado	172	Pink	Pink	White
Negra	172	Black	Black	Black
Pandela	175	Pink	Pink	White
Pisankalla	170	Reddish mocha	Reddish mocha	Cafe
K'ellu	172	Burnished gold	Burnished gold	White
K'ellu	176	Grey	Grey	Cafe
Real Blanca	171	Tobacco	Tobacco	White
Rosa Blanca	178	Pinkish grey	Pinkish tobacco	White
Timsa	180	Cream	Cream	White
Toledo	181	Orange red	Pinkish mocha	White
Tres Hermanos	176	Blend	Blend	White
Huallata	176	Blend	Blend	White
Semi-early ecotypes				
Chillpi Blanco	156	Cream	Cream	Crystalline
Kairoja	164	Pink	Pink	White
Lipeña	163	White	Tobacco	White
Manzano	167	Reddish mocha	Reddish mocha	White
Mok'o	161	Cream	Cream	White
Quinua Roja	164	Reddish mocha	Reddish mocha	White
Señora	161	Cream	Cream soft	White
Utusaya	165	Light cream	Cream	White
Wila Jipina	155	Cream pink	Soft cream pink	White
Early ecotypes				
Cariquimeña	144	Cream	Cream	White
Mañiqueña	143	Cream	Cream	White
Canchis Amarillo	144	Pale yellow	Light yellow	White
Canchis Rosado	147	Pink	Pink	White

(Source: Bonifacio *et al.*, 2012)

cessing of the grain. Figure 3 illustrates reaping with a sickle and with a manually operated mechanical mower.

Another task during harvest is sorting out atypical plants, in particular those with different seed colours, to avoid blends that reduce both quality and price. For example, in order to meet the Bolivian standard NB NA0038 of ≤ 1% of grains of another colour (IBNORCA, 2007), any plants with mocha or black grains must be removed if it is a white grain variety. Similarly, when it is a black or red-coloured

Table 2: Characteristics of some improved varieties at physiological maturity

Variety	Vegetative cycle [days]	Panicle (colour)	Whole grain (colour)	Pearl grain (colour)
Semi-late varieties				
Kurmi	170	Pink	White	White
Blanquita	176	Cream to white	White	White
Semi-early varieties				
Sajama	160	Yellowish cream	White	White
Chucapaca	160	Light pink	Grayish white	White
Surumi	165	Light pink	Light pink	White
Intinayra	165	Deep yellow	Yellow	White
Sayaña	165	Grainy	Soft yellow	White
Early varieties				
Jacha Grano	135	Light yellow	White	White
Aynoq'a	140	Cream	White	White
Horizontes	140	Cream	White	White
Patacamaya	147	Pink	White	White
Kosuña	150	Cream	White	White

(Source: Espíndola and Bonifacio, 1996)

Figure 3: (a) Reaping plants with a sickle (Palaya, Potosí); (b) Reaping plants with a mower (Palaya, Potosí) (Courtesy of: Fundación PROINPA)

variety, care must be taken to avoid the presence of white grains. Even when certified and/or selected seeds are used, there are almost always some atypical plants that could lead to undesirable blends. This phenomenon is the result of the natural genetic segregation that occurs in quinoa.

If plants are reaped a few weeks after their physiological maturity, there is a higher probability of grain loss during this exercise. In this case, it is recommended to harvest them during the morning hours when there is still dew on the plant, because the mature quinoa plant is highly hygroscopic and retains humidity.

Figure 4: (Left) Line-cut black quinoa sheaves (Chacala, Potosí); (Right) Sheaves crossed to facilitate drying (Rio Grande, Potosí) (Courtesy of: Fundación PROINPA)

2.2. Sheaving

Sheaving quinoa involves piling together the reaped plants in arcs or spikes to let the plants and panicles dry. It is thus possible to avoid wastage of the harvested plant due to adverse climatic events, such as unseasonal rains and hail that could leave marks on the grain (León, 2003; Apaza *et al*., 2006).

There is a wide range of forms and methods of sheaving. The most common is to make small heaps within the plot; another is to make linear sheaves with the panicles to one side, or circular sheaves with the panicles turned inwards. The most commonly used method in the southern Altiplano is to form an arc with the plants attached in the form of an "X" and the panicles pointing upwards. This form of sheaving allows for proper airing, and the drying process is much faster than with other methods. The sheaves must remain in the field no longer than necessary, to avoid further attacks by rodents and birds.

Linear, circular and arc sheaving all allow the harvest to be protected against late rains, as the upper part of the sheaves (panicles) are covered with polyethylene. If this is not done carefully, significant losses can occur as a result of seeds germinating within the panicle after being moistened by the rain. Figure 4 shows linear and cross sheaves.

2.3 Threshing

Threshing involves separating the grain from the panicle (*glomerulus*) (Calla and Cortez, 2011). Prior to threshing, it is important to check that the moisture content of the grain is ≤ 15%. (Apaza *et al.*, 2006). The method adopted for threshing depends on the available machinery and the local topography. Traditional threshing can still be observed in places where quinoa is produced on slopes (Figure 5a) – the "huajtana", a stout baton, is used to beat the panicles and remove the grains. In the plains, threshing is done with successive runs by a tractor (Figure 5b) or other vehicle, or using stationary threshers. Tractors and other vehicles are used for threshing on tarpaulins spread out on a raised threshing floor (platform). The tarpaulin must cover the entire surface to avoid the tyres of the vehicle coming into constant contact with the soil and/or sand, which would result in contamination of the grain.

For threshing on a raised platform, the dry plants are laid out in two parallel lines, generally with the heads turned inwards (Figure 6a). The gap between the rows is the same width as the gauge or the distance between the tyres of the vehicle. As the vehicle moves back and forth over the rows of panicles, the grain is separated from the heads. The chaff is gradually removed using rakes and is deposited outside the platform. This operation is repeated several times until a partially cleaned grain is obtained, although it may still be mixed with debris from the plant.

2.3.1. Threshing machines

Various types of stationary threshers have been tested in recent years, including the Vencedora (Figure 6b) and the Alban Blach. They have not been

Figure 5: (a) Traditional quinoa threshing on slopes (Miraflores, Potosí); (b) Threshing using a tractor (Palaya, Potosí) (Courtesy of: Fundación PROINPA)

Figure 6: (a) Threshing quinoa with a truck (Chacala, Potosí); (b) The Vencedor thresher (Villa Esperanza, Potosí) (Courtesy of: Fundación PROINPA)

widely adopted, however, because they are quite costly and tend to result in a high level of grain breakage (Aroni *et al*., 2009).Other types of machines are currently being promoted, including:

The TR-C thresher

The TR-C thresher (Figure 7) was developed by the FAUTAPO Foundation and the Mechanized Agriculture Research, Training and Extension Centre (CIFEMA) (Aroni *et al*., 2009). The machine comprises a huller and a system of sieves that separate the thick parts of the plant from the grain. Because this machine is smaller than other, similar machines, it can be carried on a light vehicle (small truck, cultivator etc.). The machine is easy to use, also for women. It has an easy–to-manoeuvre, low-consumption (5.5 hp and 1 litre/h) gasoline engine; it includes two exchangeable sieves, and its yield is 276–368 kg/h (CIFEMA, 2006).

MASEMA FAUTAPO I Thresher

This machine was financed by the FAUTAPO foundation of Bolivia and the PRONORTE foundation of Salta, Argentina. It was constructed by students at the *Universidad Tecnológica Nacional Regional* Córdoba, and tested in Uyuni (Figure 8, Turismo Rural Comunitario, 2013). The thresher uses a conventional transverse rotational cylinder equipped with plastic and rubber millstones, where the stems are separated and the fruit or grain of the plant is separated from its flowers. The grains and chaff are separated using two moving sifters; the first of these,

Figure 7: The TR-C Thresher (Source: CIFEMA 2006)

Figure 8: The MASEMA FAUTAPO thresher/winnower (Source: *Turismo Rural Comunitario* 2013)

commonly known as a "sacapaja" (straw remover), removes the larger pieces and only the grains or pieces with a diameter of < 3 mm pass through the second sifter until the last stage of separation. The grains are then winnowed and sorted by size, and small bits of flowers and straw are removed. This is done using a fan and a wind tunnel, where grains are selected according to size and weight and the lighter bits of grain are blown out of the machine. The prototype includes triphase electrical engines for each function, making it possible to adjust the specific capacity at each stage of the sorting process. Each stage has a speed control, and the energy source is an Otto cycle conventional electricity generator. Field tests showed no deterioration in grains. There is however a need to make some adjustments to the winnowing phase.

Modified Vencedora thresher

The Vencedora thresher is a Brazilian machine that yields 320 kg/h. In the Altiplano, it needs to be pulled by a tractor transported on a truck. It is not particularly suited to the conditions of small-scale producers with dispersed plots. It was, therefore, adapted locally in 2007 to reduce its size, while maintaining the threshing and fanning functions (Figure 9). The machine was tested in the northern and central Altiplano regions in Bolivia. It yielded 180–210 kg/h, with an efficiency of 85% grain and 15% chaff (leaves and crushed pedicels) (Aroni *et al*., 2009).

Figure 9: The modified Vencedora thresher (Source: Fundación PROINPA 2008)

Tubular thresher

The tubular thresher (Figure 10) developed by the Foundation for Promotion and Research on Andean Products (PROINPA) is a very light machine with an independent power take-off; it can be transported on a pick-up truck. Its components comprise: loading platform, thresher body, grain outlet sorter, chaff outlet, engine base, 5 hp gasoline engine, and collector for the grain after threshing. Its service life is > 10 years.

The tubular thresher has an average yield of 95 kg/h in processing quinoa grains, with 15% husks separated from the grain by the winnowing fan. The outlet sieve gives almost clean grain, minimizing the need for the successive sifting required with

Figure 10: Tubular thresher (Source: Fundación PROINPA 2008)

other threshers. Table 3 shows the yields obtained with mechanized threshing of three quinoa varieties (Fundación PROINPA, 2008).

2.4 Sifting or sieving

Sifting or sieving consists of separating the grain from the chaff, which includes bits of leaf, small stones, pedicels, inflorescences and small twigs (Apaza *et al*., 2006). The sieves used for this manual task generally measure 0.80 × 1.50 m and are made of mesh or of wood drilled with 3.5–4 mm holes. Operators shake them back and forth to separate the grain and the husks from the chaff. Sifting is a very tedious and dusty task (Figure 11). The wind can be a help or a hindrance depending on how hard it blows.

2.5. Winnowing

Winnowing involves the removal of small, light impurities. In traditional practices, wind energy is used, while mechanized winnowers use a blower or fan.

Traditional winnowing is done manually, using a tray or other recipient to collect a portion of sifted quinoa, which is then poured out in a stream, transverse to the direction of the wind. Since this method depends on the wind – variability of direction and intensity – it is not very efficient, the grain obtained is heterogeneous and not all impurities are removed.

Improved winnowing methods use mechanical winnowers, operated either manually or by engine power. These winnowers generate a regular air current with rotating blades, and are equipped with a receiving hopper where a constant, regulated quantity of grain is poured (Figure 12). These machines are relatively cheap. Their most important characteristic, however, is that they are not dependent on the wind and can be used for winnowing at any time of the year. They yield about 500–800 kg/h. By 2008, roughly 77% of the southern Altiplano farmers in Oruro, and 14% in Potosí were using mechanical winnowers (Aroni *et al*., 2009).

Figure 13 shows the motorized winnower at work on the harvest (grain + husks + chaff). This improved yield machine (1 600 kg/h) was built by *Consultora y Taller Mecánico Aroni* in Uyuni, Bolivia. The winnower includes a mechanism that separates the chaff, in addition to winnowing.

V-M winnower

The receiving hopper of the V-M winnower includes a rotary cylinder that ensures that the quinoa grains are fed in continually and also that the smaller quinoa grains are recovered during the winnowing process.

Table 3: Tubular thresher yield with three varieties of quinoa

Quinoa cultivars	Dry plant weight [kg]	Threshed grain [kg]	Chaff [kg]	Threshing time [min]	Threshing yield [kg/h]
Línea Purpura	50	16	34	10	96
Jacha Grano	56	19	37	12	95
Surumi	33	11	22	7	94
Average	**46**	**15**	**31**	**10**	**95**

(Source: Fundación PROINPA 2008)

Figure 11: (a) Sifting (Chacala, Potosí); (b) Sifting on a slope (Palaya, Potosí) (Courtesy of: Fundación PROINPA)

Figure 12: Winnowing quinoa (Salinas de Garci Mendoza, Oruro) (Courtesy of: Fundación PROINPA)

This machine, which is ideal for the working conditions in Bolivia, has a 5 hp, 1 litre/h diesel engine and is capable of processing 600–650 kg of grain and fragments per hour at the ideal blade rotation speed of 550–600 rpm (CIFEMA, 2007).

2.6 Combine harvesters

During the 2012/13 crop year, two types of engine-driven combine harvester were tested in Challapata and El Choro, in the Oruro district in Bolivia. The CLAAS and DIMA harvesters (Figure 15) are smaller models, designed for working on medium- and small-sized plots. This type of harvester does the shearing, threshing, sifting, and cleaning simultaneously and avoids contamination with impurities. Following the trials, it was seen that there was scope for improvement both in crop management and in the equipment itself.

In terms of crop management, improvements can be made in several areas: soil preparation (in particular in levelling or matching); appropriate sowing density; and use of varieties with a simple growth habit, homogeneous crop maturity, producing plants with a single panicle. The shearing system used by the machines also needs to be adjusted to reduce the high percentage of losses resulting from shattering and shorn panicles that remain on the ground.

2.7 Transport

Quinoa is transported from production zones to storage areas using various types of vehicle: pick-ups, trucks, tractors etc. (Figure 16).

Secondary roads provide access for vehicles to the growing areas in the plains and highlands, facilitating the transfer of the bags of grain to storage depots in the quinoa-producing communities.

2.8. Storage in harvest areas

Storage involves ensuring that the grain remains clean for a given period of time, and preserving grain quality (Calla and Cortez, 2011). Every year, there are more storage facilities on farmers' own premises to meet the requirements of organic production and food safety. Storehouses must be constructed according to set specifications regarding the materials. The construction must have the right environmental conditions (temperature and hu-

Figure 13: Quinoa cleaning and winnowing with a stalk shredder (Palaya, Potosí) (Courtesy of: Fundación PROINPA)

Figure 14: The V-M Winnower (Source: CIFEMA 2007)

Figure 15: (a) CLAAS combine harvester (Challapata, Oruro) (Bretel, 2013); (b) DIMA combine harvester (El Choro, Oruro) (Courtesy of: Fundación PROINPA)

midity), facilitate cleaning and provide protection from rodents and other animals that could cause contamination. Figure 17 shows a storehouse constructed with brick walls and the inside of another depot with gypsum wall coatings and a cement floor, both of which are appropriate for ensuring cleanliness.

It is worth noting here, in this section on harvest, that the CPTS has developed a range of technologies based on cleaner production principles for quinoa cultivation in the arid lands of the Bolivian Altiplano. Technologies include seed drills, fumigators–liquid fertilizer dispensers–sprinklers, harvesters, solar-powered dryers, and threshers–winnowers–seed sorters. The machines have reached the final prototype stage and are currently being tested in conjunction with appropriate agricultural methods, prior to moving on to commercial production.

3. Processing

Grains are not uniform in size after harvesting and winnowing. On average, grain size varies between 1.4 and 2 mm in diameter, and the grain contains impurities (especially chaff residue, twigs, leaves, and small stones, as well as broken, damaged, coloured, germinated, covered, and unripe seeds). Quinoa is processed to obtain grains that meet quality standards in terms of size, impurities or extraneous material and satisfy bromatological and microbiological requirements (IBNORCA, 2007). The grains therefore have to undergo a series of processes including: preliminary sorting and re-

Figure 16: (a) Quinoa being transported (Palaya, Potosí); (b) Quinoa transported by tractor (Palaya, Potosí) (Courtesy of: Fundación PROINPA)

Figure 17: (a) Construction of a quinoa depot, PAR project (Bella Vista, Potosí); (b) Farmers' organization quinoa warehouse (Courtesy of: Fundación PROINPA)

moval of impurities; saponin removal, which is normally carried out using both hulling (dry method) and washing (wet method); drying; sorting by size; separating of different coloured grains; and removal of residual impurities.

3.1 Preliminary sorting and removal of impurities

Before being transported to the processing plant, generally in 100-kg bags made of polypropylene or other materials, the initial product is sorted using simple sieves made of a plate perforated with 3 mm diameter openings and a woven mesh with a spacing of 1.2 mm between the threads (Quiroga *et al.*, 2010). The processing speed is 100 kg every 2–3 minutes. The machine runs on a 1.5 hp motor. The sorting process generates five products:

- Particulate matter (mainly dust and saponins)
- Light, coarse impurities (twigs, leaves)
- First grade grain (grain with a diameter of > 2.2 mm) (90–95%)
- Second grade grain (grain with a diameter of < 2.2 mm)
- Heavy impurities (stones)

Particulate matter is discharged into the atmosphere, impurities are discarded, and second grade quinoa is either returned to the farmer or purchased at a lower price at the same time as the first grade. Both products are weighed on a scale.

Some processing companies are equipped with the CIFEMA grain sorter (CIFEMA, 2013) or with similar prototypes that sort the grain by size using two sets of different-sized, interchangeable sieves, which can also be used to sort different varieties of quinoa. The sorter (Figure 18) runs on a 5 hp diesel engine and has a processing capacity of 700–1 000 kg/h. The sieves measure 60 × 100 cm, and are equipped with a mesh with 2 and 1 mm openings.

Once the quinoa has been purchased, it is stored in 100-kg bags of plastic or other materials in facilities that are capable of processing hundreds of tonnes of grain each month. Some large processing plants use metal silos (Figure 19), to avoid rodents and moths.

Figure 18: Grain sorter (Source: CIFEMA, 2013)

3.2 Saponin removal

The process of removing saponins is one of the most important stages in grain processing and in recent years, various appropriate technologies have been developed for removing saponins to levels within the acceptable limits, without affecting the grain's nutritional properties.

This section aims to: demonstrate the progress made in saponin removal; describe the main technologies currently adopted by quinoa processing companies; and outline the chemical and functional characteristics of saponins, and their concentration and localization in the grain structure.

3.2.1 Saponins

At least 20 different types of saponin have been identified in quinoa (Kuljanabhagavad *et al*., 2008). These chemical compounds include various monosaccharide units that are attached via a glycosidic bond to a triterpene skeleton, known as an aglycone or sapogenin. Depending on the number of saccharide chains in the structure, they may be classified as mono-, di- or tridemosidic. Monodesmosidic saponins contain a single saccharide chain, generally located in C-3. Bidesmosidic saponins contain two saccharide chains, one of them generally attached by an ether bond to C-3, and the other attached to C-18 or C-26 by an ester bond. The most common monosaccharides are D-glucose, D-galactose, D-glucuronic acid, D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose and D-fructose. Four aglycones

Figure 19: Storage silos at the *Complejo Industrial y Tecnológico Yanapasiñani* S.R.L. (CITY) Company. (Courtesy of: UPB).

have been identified in quinoa saponins: oleonolic acid, phytolaccagenic acid, hederagenin (Ridout *et al*., 1991; Ng *et al*., 1994; Ahamed *et al*., 1998). Some authors count serjanic acid as the fourth aglycone (Madl *et al*., 2006), while others consider it to be spergulagenic acid (Kuljanabhagavad and Wink, 2009).

Saponins in the quinoa seed are located in the first external coat of the episperm, which is itself made up of four layers (Villacorta and Talavera, 1976; Prado *et al.*, 1996; Jiménez *et al.*, 2010). This external coat is rough, brittle and dry, and can be partly removed using abrasive methods or by washing with cold water. Removal improves considerably when warm water or alkaline or acid solutions are used. Figure 20 shows the various parts of the quinoa seed and the layers of the episperm.

The physicochemical and biological properties of saponins have been used in many commercial applications in the food, cosmetics, agricultural and pharmaceutical sectors (Ahamed *et al*., 1998). Despite being considered as antinutritional substance (like tannins, phytic acid and protease inhibitors, Ruales, 1992), and although it has a negative effect on red blood cell levels in blood types A and O (González *et al*., 1989), there is scientific proof of their beneficial health effects due to their anticarcinogenic properties (Güçlü-Üstündağ and Mazza, 2007) and cholesterol lowering effect (Taka *et al*., 2005). Some studies have also demonstrated their antifungal properties (Woldemichael and Wink, 2001; Stuardo and San Martín, 2008).

In current characterizations, various quinoa varieties and ecotypes are designated as "bitter", "semi-sweet" and "sweet". This classification is based on saponin content, which is generally 0–3% in dry grains. Saponin content in "bitter" grains is 1–3%, in "sweet" grains 0.0–0.1%, and in "semi-sweet" grains 0.1–1% (Güçlü-Üstündağ and Mazza, 2007). Other authors believe that a variety or ecotype may be considered "sweet" if the saponin content is 20–40 mg/100 g dry weight, and "bitter" if the saponin content is > 470 mg/100 g dry weight (Mastebroek *et al*., 2000).

The only real proxy for determining if a type of quinoa may be classified as "sweet" is its organoleptic acceptability for human consumption, which varies between 0.06 and 0.12%. This is in line with the results obtained at the *Universidad de Ambato* (Ecuador), which indicated that the maximum acceptable limit of saponin content in the cooked grain is 0.1% (Nieto and Soria, 1991).

3.2.2 Bitter and sweet quinoa genotypes

Attempts have been made to obtain low saponin content varieties, for example, through conventional genetic selection. The 'Sajama' variety, which is considered "sweet", was obtained through selection, as were 'Kurmi', 'Aynoq'a', 'K'osuña' and 'Blanquita' in Bolivia (grain size around 2 mm), 'Blanca de Junin' in Peru and 'Tunkahuán' in Ecuador.

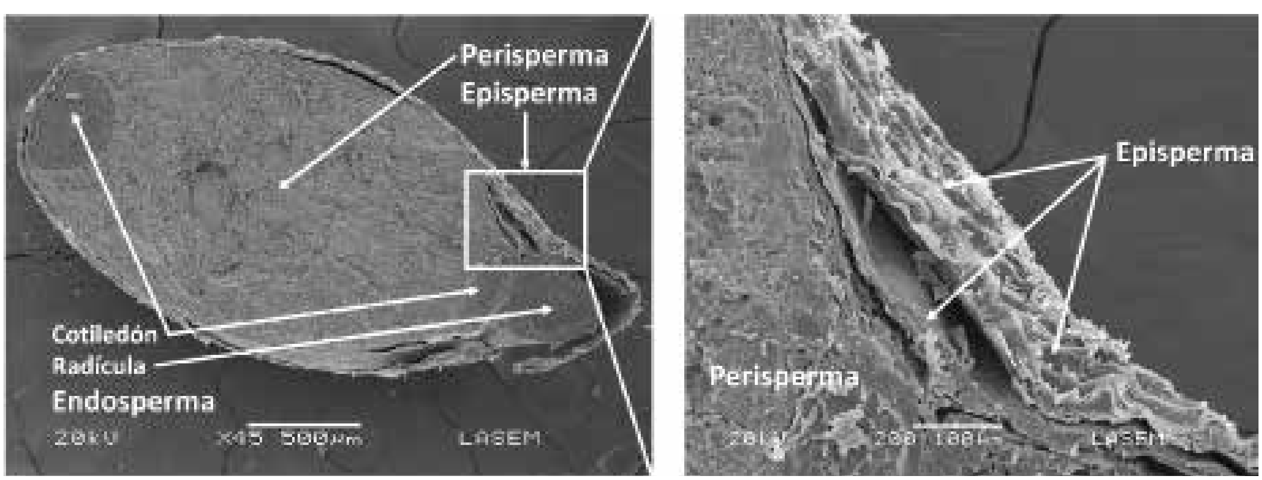

Figure 20: SEM micrograph, principle parts of 'White Quinoa Real' ecotype seed (Source: Quiroga *et al*., 2011)

In conventional improvement, two selected progenitors are artificially crossed and the first generations are then selected individually, followed by combined mass and individual selection in later generations (Fundación PROINPA, 2005). Although it is a predominantly autogamous species, cross-breeding may still occur. This means that in cropping, even low saponin content varieties and ecotypes could once again display a high saponin content. Nevertheless, with proper crop management techniques, saponin levels could be guaranteed over time, for example, by avoiding cross-breeding with "bitter" quinoa varieties and/or ecotypes.

Gandarillas (1979) suggested that the presence or absence of saponins in quinoa might be controlled by a locus (or loci). Using hybridization and pedigree selection, Ward (2000) attempted to reduce saponin content by taking into account the fact that F_6 progeny could be highly homozygous. However, it was found that after three pedigree selection cycles, the saponin content in plants with < 1 mg/g of saponins had increased by 3.57% in S1 and 11% in S4. These results led to the conclusion that, since this is an allotetraploid species with occasional recombination between homologous chromosomes, it is difficult to reduce the saponin content. Just the fact that there are over 20 types of saponin in existence (Kuljanabhagavad *et al*., 2008), suggests that a considerable number of loci may be involved in producing the various saponin levels detected. To a certain extent, this indicates that achieving homozygosis is not feasible, or at least would require greater knowledge about the genetics of the species. This conclusion was somewhat foreshadowed in the works of Risi and Galwey (1989) and Jacobsen *et al*. (1996), who reported that since saponin content was a continuous distribution variable, it might be subject to polygenetic control. It should be mentioned, however, that these studies did not specify the type of material used and whether it was a population that included "sweet" and "bitter" quinoa varieties and/or ecotypes in varying proportions, as expected in normal distribution.

The link between the presence or absence of saponins and enhanced resistance to certain pests has led some researchers to investigate the role of saponins in the plant. Evidence of its protective capacity has to date come from observations in the field, in particular in the northern, central and southern Altiplano regions of Bolivia, where – depending on the degree of humidity and the varieties and ecotypes of quinoa cultivated – it is possible to study the presence or absence of saponins and how this relates to known pests.

Figure 21: "Black Quinoa Real" ecotype at physiological maturity, "bitter" quinoa (Courtesy of: Fundación PROINPA)

Table 4 shows some of the quinoa varieties and ecotypes cultivated in the Andean region and their saponin levels (Miranda, 2010; Ward, 2000). They include the 'Quinoa Real' ecotypes found in the Bolivian southern Altiplano region, which are in high demand and obtain good prices on the international market because of their grain size (Bonifacio *et al.*, 2012). Figure 21 shows the crop on the farm. The list also includes some varieties currently being grown in Europe (Pulvento *et al*., 2010).

All of the above considerations have led to the development of agro-industrial processing for saponin removal (Bacigalupo and Tapia, 2000).

Table 4: Examples of some quinoa varieties and ecotypes, classified as "sweet", "semi-sweet" and "bitter" (Source: Miranda, 2010; Ward, 2000; Bonifacio *et al*., 2012; Pulvento *et al*., 2010) [a] Principal production is 'White Real' white, 'Toledo', 'Phisanqalla' (red- or mocha-coloured grain) and 'Ch'iara' (black grain)

"Sweet"	"Semi-sweet"	"Bitter"
Aynoq´a (Altiplano Central de Bolivia)	Chukapaca (Bolivia)	Horizontes (Bolivia)
Blanquita (northern Altiplano, Bolivia and the transitional zone between northern Altiplano and Central)	Kamiri (Bolivia)	Real (southern Altiplano, Bolivia)[a]
Huaranga (Bolivia)	Boliviana Jujuy	Amarilla de Marangani (Peru)
Kancolla (Bolivia)	Regalona Baer (Chile)	CICA (Peru y Argentina)
K'osuña (southern and central Altiplano, Bolivia)		KVLQ520Y (Denmark)
Kurmi (northern and central Altiplano, Bolivia)		Cochasqui
Ratuqui (Bolivia)		Huatzontle
Robura (Bolivia)		Imbaya
Sajama (Bolivia)		Witulla
Samaranti (Bolivia)		
Sayaña (Bolivia)		
Ingapirca (Ecuador)		
Tunkahuán (Ecuador)		
Blanca de Juli (Puno, Peru)		
Blanca de Junin (Junin, Peru)		
Chewenca		
Illpa INIA		
Nariño		
Pasankalla		
Witulla		

In the Andean region, most of the traditional varieties and ecotypes of quinoa are bitter and need to be hulled, washed and/or roasted, according to the end use, namely, for production of flour, soups, drinks, popped quinoa etc. (Alcocer, 2010). Table 5 describes the different stages and processing times for quinoa, according to its end use.

In some communities in the salt marsh areas of Uyuni and Coipasa in Bolivia, dry methods are used to remove saponins. In other communities (Chacala, Potosí), however, both dry and wet methods are used and the work is generally done by women. Quinoa grains are roasted in a metal container (*bateas*) for approximately 30–40 minutes, until they are golden brown. Removing moisture from the grain makes the episperm more fragile and facilitates its removal. While the roasted quinoa is still warm, it is mixed with an abrasive clay material extracted in the Llica region and known as "pojkera" and then trodden for 30–60 minutes on a rough stone surface known as a "saruna" or "tarquinaso". A large percentage of saponins are removed during this stage.

Subsequently, the rest of the episperm and the abrasives are winnowed away from the grain for 20–40 minutes. In the final stage of saponin re-

Table 5: Saponin removal on quinoa grain according to end use. [a]The data relate to processing of approximately 11 kg of quinoa. [b]Roasted and ground quinoa [c]Lightly roasted and ground quinoa. [d]Quinoa cooked in a light broth with meat or dried beef, tubers and vegetables. [e]Steam-cooked rolls made with quinoa flour, similar to *tamales* or *humitas*, with some dressing in the centre.

Steps	**Processing time[a] [min]**			
	Pito[b]	**Phisara[c]**	**Soup[d]**	**Mukuna[e]**
Roasting	29	36	33	36
Treading	24	60	40	60
Winnowing	20	40	40	40
Washing	25	35	30	35
Drying		180	180	180
Winnowing		10	10	10
Roasting-Milling	90	0	0	0
Total time	**188**	**361**	**333**	**361**

moval, and of removal of impurities such as small stones and seeds collected during harvest, the quinoa grains are washed in various stages over 25–35 minutes. The effluent is inspected visually to check for foam formation, the quality control parameter. When the effluent is clear of foam, this indicates that the saponins have been removed. Finally, the quinoa is dried for 2–4 hours, until the final moisture content is about 18%. Depending on what it is to be used for, the grain may sometimes be winnowed and roasted again (Figure 22).

The saponin removal process used in Argentina in the region close to the Chilean border (Santa Catalina, Jujuy) is very similar to the one described above. In the northeast, however, saponin is traditionally removed simply by washing. A certain quantity of grain (5–10 kg) is placed in 50-kg bags made of cloth or synthetic materials. The bag is then submerged in the water of a river and/or a brook and, held at both ends, it is moved up and down so that the grains rub together. The water helps the saponins dissolve and they are washed downstream. The movement is repeated until there is no longer any foam in the water. The grains are then dried on zinc sheets laid outside.

In Peru and Ecuador, saponins are traditionally removed from quinoa mainly using the wet method, i.e. manual washing with a large amount of water on an abrasive (stone) surface until the outer layers of the grain are removed (Nieto and Valdivia, 2001).

Traditional saponin removal takes time and effort. For example, in the quinoa-producing zones of the Bolivian Altiplano, it takes 3–6 hours to clean approximately 11 kg of quinoa. Such techniques are appropriate for small quantities of quinoa, for example, for family consumption.

(b) Modern saponin removal systems

For many years, quinoa processing companies used or adapted machines, equipment and technology initially developed for processing rice, wheat, soybean and sorghum. The low volumes of production, compared with these other crops, and the existence of only a small number of milling companies globally, provided little incentive for developing specific machines, equipment and technology for this sector.

In the last 10 years, however, quinoa has experienced a quiet boom: once a product consumed solely by the farmers growing it in the Altiplano and Inter-Andean valleys, it has become a global, high commercial value crop cultivated in extensive areas, not only in the countries where it originated, but in others where it has been introduced. This phenomenon is mainly due to: the increased demand for gluten-free cereals from the 0.4% of the world population that suffer from coeliac disease; the increased demand for high quality, affordable organic products; and the implementation of efficient food programmes in various countries by organizations such as FAO (Birbuet and Machicado, 2009).

There has also been a rising demand for appropriate machines, equipment and technologies to meet the particular requirements and characteristics of quinoa. Machines need to increase efficiency and processing capacity while being economically accessible for processing companies. Various teams of researchers and technicians have begun work on new, innovative options.

Bacigalupo and Tapia (2000) carried out an excellent review of the mechanized processes used in removing quinoa saponins in the Andean region (Peru, Bolivia and Ecuador), with a description of the various processes and configurations developed since 1950, both as pilot projects and on an industrial scale. They compared the advantages and disadvantages of the wet, dry and combined methods with respect to the effects on nutritional quality of the processed grain, effective saponin removal, water and energy consumption and the cost of these processes.

Among the dry methods, two studies in particular stand out: i) the 1980 Torres and Minaya huller, with 95% efficiency and a grain saponin content of 0.04–0.25%, depending on the quinoa variety or ecotype processed; and ii) the dry method continuous flow prototype developed in Ecuador by Valdivieso and

Figure 22: Traditional, artisanal saponin removal process (Bolivian Altiplano): roasting, treading, winnowing, washing and drying (Courtesy of: Fundación PROINPA)

Rivadeneira in 1992, where the grain saponin content in 75 kg/h bitter quinoa batches was reduced to 0.026% and broken grain was reduced to 1.5%.

Among the wet methods, the Huarina project stands out. In 1983, Reggiardo and Rodríguez developed a pilot washing system with three stages: soaking, centrifuging and rinsing, followed by drying in a tunnel of warm air; this produced a good quality grain that was well accepted on the Bolivian market.

Finally, among the combined methods (hulling, washing and drying), the process developed by Derpic in 1988 stands out. This method is characterized by its efficiency in removing the hulled layer (65%), the low amount of moisture absorbed by the grain during washing (17–30%), which makes it easier to dry, and the low saponin concentration in the effluent, which mitigates the possible environmental effects of the combined method (although, since saponins are soluble in water, they are not removed from the effluents). The work done by Zavaleta (1982) contributed greatly to understanding how saponins are extracted using this method. The authors recommend hulling for sweet varieties and the combined method for varieties with a high saponin content, because this method uses less water, ensures good protein quality in the processed grain, uses a minimum amount of energy and costs little.

In industry, most processing companies currently prefer the combined method, because it efficiently removes saponins and maintains grain quality, thus satisfying international requirements, in particular for organic ‘Quinoa Real’. The Bolivian National Association of Quinoa Producers has had a vital role in promoting the industry, resulting in the processing of larger volumes.

This section describes and analyses recent innovations based on previous experience and developed mainly since 2000. They apply Cleaner Production criteria in the design and operation of the hulling, washing and drying phases. Other innovations in dry processing at laboratory and semi-industrial level are also described, as well as small-scale developments in combined systems.

Medium-scale systems

In the 1980s, a small-scale Tangential Abrasive Dehulling Device was developed in Canada to simulate the abrasive action of industrial hullers (Reichhert *et al.*, 1986). The authors reported 85–95% saponin removal achieved for quinoa. The equipment (designed for hulling also other seeds) comprises a horizontally rotating abrasive wheel, with a stationary plate holding eight stainless steel bottomless cups, mounted vertically on the rotating wheel. A rubber fitted lid is used to cover the cups when the machine is operated. Wedges are used to adjust the space between the rotating disc and the cups where the grains are fed, so that hulls, broken grains and fine particles are blown by a fan into a container attached to the huller. The hulled grains are collected by means of a vacuum aspirating device (Opoku *et al.*, 2003).

In Argentina, industrial blenders/mixers adapted for grain washing are used to process greater volumes of seeds. Operating at low rotational speeds, they have a processing capacity of 10–20 kg for each 30-minute wash. The seeds are subsequently dried in tunnels used for drying pepper – aerial hothouses with a polyethylene floor and ceiling to create a differential heating effect. The two ends of the tunnel are open so that the air can enter and exit easily.

As part of a project in Bolivia aimed at facilitating quinoa processing and consumption and improving the nutritional status of rural quinoa-producing communities in the southern Altiplano, a small-scale saponin removal machine was developed, with the capacity to process 12 kg in 7 minutes using the traditional method of roasting, hulling, winnowing, washing and drying – processes which could take women up to 12 hours to complete (Astudillo, 2007). The operation of the machine was demonstrated in various areas and it was quite well accepted by rural women.

To promote quinoa consumption among producing families in the southern Altiplano in Bolivia, following a drastic reduction in consumption as a result of changing dietary habits, poor artisanal saponin removal methods and high prices on the international market, in 2008, the Rowland company built a small capable of processing 45 kg of quinoa per hour. PROINPA promoted the use of this equipment among producers in Chacala, Chita and other areas. The equipment weighs 30 kg and measures 70 cm (length) × 30 cm (width) × 80 cm (height). It runs on an electric engine (or gasoline, for those areas where there is no electricity). The smallest gasoline engine on the market is a 5.5 hp engine, but this machine only uses 0.5 hp, which corresponds to gasoline consumption of 0.25 litres/h. The quinoa grains are fed in through a 30° inclined receiving hopper before passing through a cylindrical huller (15 cm long and 60 cm wide) with a 2 × 6 cm inlet and outlet.

In the huller, the grains rub against each other and against the walls of the cylinder while they are transported by a constantly moving worm wheel through a meshed cylinder where the saponins are ejected by the air current generated by the movement of the blades mounted on the worm wheel. The feed rate can be controlled mechanically through an access hatch and the force exerted by an engine-operated pulley. *Pojkera* can be fed in with the quinoa. Figure 23 shows the small commercial.

In 2010, a group of researchers at the *Universidad Privada Boliviana* (UPB) developed a laboratory model of a novel application of the spouted bed that is commonly used to dry cereal grains, applying it to dry saponin removal from bitter quinoa. In a spouted bed, air is introduced upwards through nozzles, forming a central channel where grains are pushed to the top of the container, from where they fall in a ring-shaped solid downwards flow until they reach the base where they are once again pushed upwards at high linear speed. The momentum and energy generated by the process as the grains rub against each other cause the abrasion of the episperm. Figure 24 shows the pilot prototype.

Working on three commercial ecotypes of 'Quinoa Real' and their blends, in less than 30 minutes, the dry process reduced the saponin concentration in the grains to < 0.01%, in line with commercial export standards and well below the 0.12% required by the Bolivian NB 063 standard. The powdered saponins were also completely recovered (Escalera *et al*., 2010; Quiroga *et al*., 2011). Losses in mass were limited to < 5% (commonly accepted value in conventional processes using the combined method), and specific energy consumption was also reduced to 0.23 kWh/kg (Obando *et al*., 2011). Furthermore, saponin concentration in the recovered dust increased to approximately 6%, which is above the average of 3.9%, obtained during the hulling stage in the conventional combined method (Subieta *et al*., 2011).

The increase in protein and lipid content induced by the loss of the episperm mass also demonstrates that the grain does not lose its nutritional quality (Quiroga and Escalera, 2010). The processed quinoa grains show no visible signs of surface damage, including in the embryo. In the dry processing method suggested here, removal of the outer episperm

Figure 23: Small processing 45 kg/h of quinoa (Source: Astudillo, 2007)

Figure 24: Spouted bed reactor for dry saponin removal (Courtesy of UPB)

layers is more homogeneous and controlled than in the combined processing method, where grains are hulled, washed, dried and winnowed. The final appearance and thickness of the remaining episperm on the finished product is very similar to that of quinoa processed using the technology available on the market (Figures 25 and 26) (Quiroga *et al.*, 2010).

These results demonstrate the potential of this innovation to overcome the technical and environmental issues raised by existing technologies used for processing quinoa. The process now needs to be studied on a semi-industrial scale.

(c) Industrial methods

Quinoa processing companies mostly use the combined method to remove saponins and comply with the established market quality standards. Nevertheless, the process has always presented major difficulties with regard to removal of saponins and impurities and concerning grain moisture content. There are currently 62 processing plants in Bolivia (Table 6), comprising 16% artisanal processors, 27% semi-industrial and 57% industrial companies. Of the industrial processing plants, 40% are found in Oruro, 25% in La Paz and 35% in Potosí, Cochabamba and Chuquisaca. The technologies used range from artisanal technologies to very complex and sophisticated processes (IBCE, 2012).

One of the most significant industrial contributions has been the technology developed by the Sustainable Technologies Promotion Centre (CPTS), which uses the physical properties of the seed episperm. The grain undergoes a cleaning process to remove impurities in a preliminary sorter (Figure 27), followed by saponin removal in a huller (Figure 28) with dual compartments: i) the hulling system, and ii) the particle extraction and collection system.

Figure 25: Spouted bed reactor for dry saponin removal (Courtesy of: UPB)

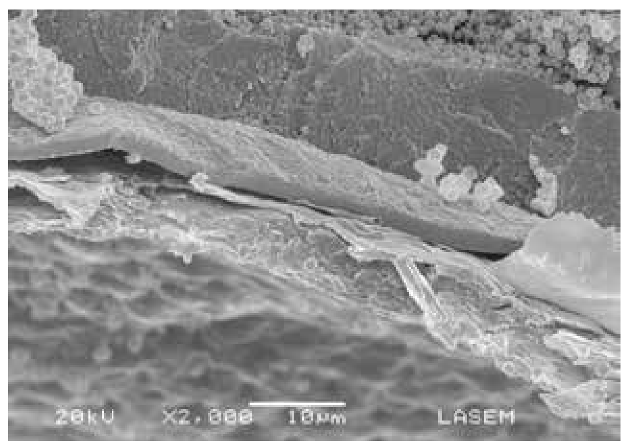

Figure 26: SEM micrograph of 'Quino Real' finished product from the Cereales Andina company, using technology from the *Centro de Promoción de Tecnologías Sostenibles* (Source: Quiroga and Escalera, 2010)

Table 6: Quinoa processing plants by department (Bolivia) (Source: IBCE 2012)

Department	Artisanal	Semi-industrial	Industrial
Chuquisaca	-	-	3
Cochabambaa	-	5	4
La Paz	3	8	9
Oruro	6	2	14
Potosí	1	2	5

Figure 27: Preliminary sorter (Courtesy of: CITY and UPB)

Inside the cylindrical drum of the huller is a revolving rotor equipped with "ribs" installed so as to push the quinoa grains, pressing them against each other. This design produces intense friction between the grains, resulting in a more uniform wearing down of the episperm. The lower part of the cylindrical drum is equipped with a perforated metal plate that does not allow the quinoa grain through, but allows the saponin powder (also known as "mojuelo", bran) to fall through for evacuation by the particle extraction and collection system. The episperm is extracted using the abrasive properties of the grain surface itself, thereby reducing the damage to the germ that occurs when grains are "brushed" or rubbed against an abrasive surface. The huller removes 90–95% of saponins.

The size of the outer diameter and the length of the cylinder, in conjunction with other design parameters, determine the processing capacity of the huller. The rotation speed of the rotor may vary between 1 200 and 1 600 rpm, and the pressure and ejector ribs are 8–12 mm wide.

The particle extraction and collection system comprises a trapezoidal collector, an air turbine for extraction and a system to collect the saponin dust.

Figure 28: Huller (Courtesy of: CITY and UPB)

The trapezoidal collector is built out of 1 mm thick common iron. At the end of the collector is a cylindrical outlet connected to the turbine air inlet by an elbow-shaped rubber, to reduce the pressure and facilitate maintenance of the turbine. The particle extraction section comprises an air turbine with a 25 cm diameter rotor. Finally, the saponin dust collector is made of two cubic jute containers, one inside the other. The total surface area of the inner container is just over 5 m², and the flow of air and dust expelled by the extractor passes through a tube inserted in the external container, terminating in the inner container.

Saponin removal is completed through a wet cleaning process where the grains are first picked to remove stones and then soaked. This is followed by a wash, a second picking and a pre-rinse, rinse (Figure 29) and finally centrifuging (Figure 30). The system includes pumps for the water supply and to recirculate the rinse water that runs out of the centrifuge.

Figure 29: Wet cleaning system (Courtesy of: CITY and UPB)

Figure 30: Centrifuge (Courtesy of: CITY and UPB)

The washer simulates a laminar trajectory of the grain through the turbulent water flow, which ensures that the first grains in are the first out. Grains remain in the water for approximately 5 minutes; due to the high efficiency level, this stage of hulling requires only 5–7 m3 of water per tonne of processed quinoa. The process eliminates 100% of high density and 60% of low density small stones, and reduces the saponin content in the washed grain to 0.01%.

The grain is subsequently dried in a drier comprising an LPG or natural gas-operated warm air generator (Figure 31), 4 drying tables (Figure 32) and a 38 m^3/min flow of air expelled by a high efficiency 2 hp turbine, for a processing capacity of 600 kg/h of dry grain (CPTS 2006).

The dry grain is then re-sorted to obtain the most homogeneous grain in a granulometric sorter. It is cleaned in a specific gravity cleaner (Figure 33) and straw is removed in an electric engine powered winnower. Different colour grains are separated through an optical-pneumatic sorter in two or three runs (Figure 34). Finally, the grain is picked manually, to eliminate 100% of any remaining impurities in the quinoa grain before being bagged as an end product for export.

It is currently estimated that about 75–80% of all organic ‘Quinoa Real’ exported from Bolivia is processed using this technology, which has made it possible to increase eightfold the continuous processing capacity. Implementation of Cleaner Production principles in designing and building equipment has resulted in: mitigation of the impact on the environment, especially with regard to water and energy consumption; and enhanced residue (saponin dust) reduction and recovery. Both the hulling system and the washing system have reduced material losses while maintaining the nutritional qualities of the grain.

Table 7 shows the results of the use of prototypes based on the technology developed by the CPTS in the Andean Valley company. These prototypes were installed in 2006 and are still functioning in the company.

This equipment is currently available from the builders, *Complejo Industrial y Tecnológico Yanapasiñani* S.R.L. (CITY) in El Alto, La Paz.

4. Quinoa processing (Agro-industry)

Processing, both small-scale and industrial, of quinoa produces pearled quinoa, granules, flakes, flour, expanded products, dyes, pasta and extruded

Figure 31: Warm air generator (Courtesy of: CPTS)

Figure 32: Drying tables (Courtesy of: CITY and UPB)

products etc. (Mujica *et al.*, 2006). This section describes the basic processes used to obtain some of these products, and presents the results of research into their effects on the nutritional quality of the by-products, and into the development of potential products (e.g. oil, concentrates and protein isolates).

4.1. Quinoa flakes

To obtain quinoa flakes, grain saponins are first removed using the process for pearled quinoa. The grains are then dried until the moisture content reaches approximately 15–16%. Quinoa flakes are obtained by pressing the grains between two converging rollers, a process very similar to that used

Figure 33: Specific gravity sorter (Courtesy of: CITY and UPB)

Figure 34: Optical-pneumatic sorter (Courtesy of: CITY and UPB)

Table 7: Situation at Andean Valley S.A. before and after implementation of the technology developed by the *Centro de Promoción de Tecnologías Sostenibles*.

Parameter	Situation		Difference
	Previous	Current	
Quinoa grain processing capacity [tonnes/h]	0.09	0.66	0.57 (800%)
Percentage raw material lost [%]	3.5	1.0	2.5
Percentage of saponin dust recovered [%]	0.0	85.0	85.0
Installed electric power of the replaced technology [kVA]	31.5	15.3	16.2 (51%)
Specific electricity consumption [kWh/tonne of quinoa]	101.6	23.2	77 (80%)
Specific water consumption [m^3/tonne of quinoa]	14	9	5 (36%)
Specific LPG consumption [kg/tonne of quinoa]	33	12	21 (64%)

for oat flakes. The size of the flakes depends on the variety and the end use of the product. It is possible, for example, to achieve a thickness of 0.1–0.5 mm (Mujica *et al.*, 2006). How well the flakes hold depends on the variety and, above all, the plasticity of the grain starch (perisperm) and the degree of adherence of the embryo to the perisperm. Sweet varieties better preserve the integrity of the leaflets, while bitter varieties tend to disintegrate resulting in a greater proportion of fine grit or embryo particles (protein).

Quinoa flakes have a wide range of potential uses: in juices combining quinoa with fruits (apple, pineapple and mango); in soups; and in pies, tarts and cakes. For soups and juices, quinoa flakes require less cooking time than the grain, making them easier to use and consume.

4.2. Expanded quinoa products or *pisankalla*

Expanded quinoa is made from the pearled grain. The processed grain, with a moisture content of 14–15%, is pressure cooked (145–165 psi) at high temperature and high pressure, then forcibly expelled. This causes a sudden change in temperature and a sharp drop in pressure, which makes the grains pop as they expand immediately, releasing their internal moisture in the form of vapour. The result is a good volume, light product that can be flavoured or sweetened (Mujica, 2013).

Reynaga *et al.* (2013b), studied ecotypes of 'Quinoa Real' in the grain-popping process, and found that the 'Pisankalla' and 'Mok'o' ecotypes have high expansion indices (1.95 for both ecotypes). The 'Pisankalla' ecotype or variety is known to expand more in the traditional roasting process;this is confirmed in the cited reports. Popped quinoa can be used in many ways, including as instant cereals and as a base for energy bars. In Peru and other areas, popped quinoa is known as quinoa manna (Mujica *et al.*, 2006).

The nutritional quality of quinoa may however deteriorate during this process. Talavera (2003, cited by Mujica *et al.*, 2006), found a wide range of protein levels in popped products of different varieties: 12.6% for 'Salcedo INIA', 10.4% for 'Sajama', 9.4% for 'Blanca de Juli' and 6.9% for 'Kancolla'. It appears that the percentage of protein diminishes considerably in popped products. According to Villacres *et al.* (2013), the process of popping also causes a drop in palmitic, oleic and linoleic acid levels.

In local lore, *pisankalla* is the popped form of quinoa processed using artisanal methods; it has been part of the local diet for several millennia. The specific varieties of quinoa used to make *pisankalla* may have red or black grains, depending on the colour of the episperm. These varieties are known as 'Pisankalla' and 'Quytu'. Grains are popped by putting a handful of conditioned grains (appropriate moisture level) into a clay pot (jiwki) and heating it over a fire fuelled by cow or llama dung. The grain is constantly stirred as it roasts. The roasted grain can be consumed directly or ground into an instant product.

4.3. Flour

Quinoa flour is obtained by grinding quinoa from which the saponins have been removed, using pressure and friction, and later airing it to obtain a light powder. Quinoa flour can be used in almost all products manufactured by the flour industry, and up to 40% quinoa flour may be used in making bread, 40% in pasta, 60% in sponge cakes and 70% in biscuits (Mujica *et al.*, 2006). Reynaga *et al.* (2013b) report that for bread-making, the suggested ratio is 19% quinoa flour and 81% wheat flour.

Quinoa flour is traditionally obtained through a process known as aku jupa, using appropriate varieties with small-sized grains. Once the saponins are removed, the grains are ground on a traditional grinding stone (qhuna). The flour obtained is used in various traditional dishes and pastries. Farmer experience has shown that flour processed on the qhuna keeps longer without spoiling. Reynaga *et al.* (2013b) suggest that flour obtained using the grinding stone has better particle size characteristics than flour obtained from a hammer mill.

Bonifacio *et al.* (2013) suggest that some varieties can be used in baby formula, due to the shorter time required for gelatinization of their starch. Furthermore, starch from white quinoa and 'Pisankalla' can be used as a thickener in creams and soups (Pumacahua *et al.*, 2013).

4.4. Noodles

Noodles or pastas are food products derived from kneading and moulding unfermented blends of wheat flours with potable water (Mujica *et al*., 2006). Quinoa flour provides an alternative for the noodle and pasta industry, although it is not yet known which of the different existing varieties are best suited to the needs of the pasta industry. Reynaga *et al*. (2013a) studied industrial quality Bolivian 'Quinoa Real' and found that the best ratio for noodles is 21% rice flour (type 45) and 79% quinoa flour (type 45).

Reynaga *et al*. (2013b) tried quinoa flour in the preparation of gluten-free pasta. They obtained good results with the local 'Pisankalla' variety and also with a blend of 50% rice flour and 50% quinoa. They experimented further by reducing the rice flour to 25% and increasing the quinoa flour to 75%, with satisfactory results.

4.5. Extruded products

Food extrusion is a cooking system that involves high temperatures, high pressure and tangential stress (shearing) in a short period. It is used as a means of restructuring starch and protein content food material, thereby producing different types of textured foods.

According to Mujica *et al*. (2006), the process includes the following events: a) starch gelatinization and dextrinization, protein texturing and partial denaturation of the vitamins present; b) melting and plasticising of the food; and c) expansion by flash evaporation of moisture.

In the case of extruded quinoa alone and/or combined, pearled quinoa is hydrated to 15% moisture every 25 minutes; it is fed into the extruder and goes through the mechanical thermal transition area, where the raw material is mixed, compressed and kneaded, transforming it from a granular structure to a semi-solid plastic dough. This process is carried out at 150°–160°C and 1.2 atm of pressure for 5–12 s. The dough is extruded through the openings at the mouth of the machine and sheared at the outlet with a rotatory cutter to obtain the desired shape for the final product. This system does not affect the nutritional and organoleptic quality: the chemical content and protein rating remain almost stable compared with non-extruded granular material. Indeed, the end product obtained is an aseptic food product is acceptable to the consumer (Mujica *et al.*, 2006).

4.6. Potential products

Oils

The oil content in quinoa is quite high and varied from 2% to 11% in the 555 Bolivian strains studied, with an average of 6.39%. The quality of oil is good, due to the high percentage of unsaturated fatty acids (approximately 89%), and includes 50–56% linoleic acid (omega 6), 21–26% oleic acid (omega 6) and 4.8–8.1% linolenic acid (omega 3) (PROINPA Foundation, 2011). On account of these characteristics, quinoa helps to reduce bad cholesterol (LDL) and increase good cholesterol (HDL), thus making it a potential source for the production of oil as a by-product.

Protein concentrates and isolates

Due to its high protein content (12–18.9% in the 555 Bolivian strains studied by PROINPA), and because it provides all the essential amino acids, quinoa is of particular interest for the production of protein concentrates and isolates (> 80%), for use as the main ingredients in high value-added food formulas.

To obtain protein concentrates or isolates from quinoa in a typical laboratory process (Mujica *et al.*, 2006), the fat-free germ or embryo of quinoa must first be isolated. To do this, the quinoa grain is first cleaned to remove all impurities, soil and small harvest residues before being washed to completely eliminate the saponins. The grain is left to soak until it germinates, at which point it is ground roughly to separate the embryo from the starch. Subsequently, the germ is dried and ground and the fat extracted. The quinoa germ from which the fat has been removed goes through a process of high temperature alkaline extraction (pH 11.5 at 50°C), centrifuging, washing with water, followed by another round of centrifuging. The result is a solid residue, which is subjected to isoelectric precipitation at a pH value of 4.8 to centrifuge it again to remove the liquid. The solid matter is subsequently washed with water, centrifuged and finally put through a vacuum drying process (30°C) toobtain quinoa protein isolates and concentrates with adequate functional characteristics.

Using the defatted germ of the 'Kancolla' variety, Guerrero (1989, cited by Mujica *et al.*, 2006) obtained a dry isolate in granulated form and a cream-coloured colourless, tasteless powder. The proximal chemical composition in the dry base was: 87.8% protein, 0.22% fat, 1.3% fibre, 1.4% ashes and 9.28% carbohydrates. Similarly, it contained an adequate balance of amino acids except for sulphur compounds, with a net protein utilization of 48.5.

Mufari *et al.* (2013) compared conventional isoelectric precipitation and the enzymatic method of obtaining quinoa protein concentrates. The enzymatic method used four enzymes: α-amylase, glucoamylase, pullulanase and cellulase, in the presence of a pH 5 sodium acetate buffer, to convert starch and cellulose into soluble glucose, producing a protein-enriched residue. The protein concentrations obtained were lower (38%) than the conventional method (53%). The enzymatic method allows for a higher recovery of initial proteins: 43% against 15% recovery using the traditional method, and it has the added advantage of producing a glucose-rich supernatant by-product. The authors suggest optimizing the conditions to obtain higher protein concentrations.

Starches

Quinoa is also a major source of carbohydrates. The starch content in the dry matter is 54%, the granule is polygonal in shape, with a size of 0.6–2.0 µm, and is located in the perisperm as individual entities or compound aggregates of spherical or oval shape and measuring 16–34 µm (Ruales and Nair, 1994a). Other authors (González *et al*., 1989) reported values of 32.6% for the 'Sajama' variety. The amylose content is 7.1–11.2% and the molecular structure of the amylopectin is very similar to waxy starch, with approximately 35% grade crystallinity (Tang *et al.*, 2002; Qian and Kuhn, 1999).

Starch digestibility does not vary significantly when grains are processed; unprocessed grain has a digestive utilization ratio of 72%, while grain that has been parboiled at 60°C for 20 minutes has a 77% digestive utilization ratio. A higher degree of starch dextrinization improves binding and savoury qualities, i.e. the taste and texture, of the final product (Ruales and Nair, 1994b).

Compared to wheat and barley starch, quinoa starch is more viscous and has better water retention and expansion capacities. Gelatinization also occurs at a slightly higher temperature. These results translate into better performance as a thickening agent for fillings, but are not so good for preparing quinoa starch-based breads and cakes (Lorenz, 1990). Compared with maize starch, however, quinoa starch is less soluble and less viscous (Ahamed *et al.*, 1996).

Due to its physicochemical properties, quinoa starch has been used in the preparation of baby foods. It has good stability when subjected to freezing and thawing – a phenomenon known as freeze-thaw stability – and is thus suitable for use in manufacturing preprepared frozen foods. Other authors also point to the opacity of the gelatinized starch, which makes it ideal for use in emulsified food products, such as salad dressings. (Ahamed *et al*., 1996).

5. Discussion

In response to the increase in quinoa production in recent years, there have been ongoing efforts to develop industrial-scale technological innovations for the harvest and post-harvest stages, to replace the traditional manual cropping practices used in producing the Andean grain. Initially, agricultural machinery designed for other types of grain was used. These technologies were later gradually adapted to suit the requirements of quinoa and finally, efforts were made to promote the development and construction of purpose-built machines for this crop.

The current mechanization of quinoa production has advantages and disadvantages. Despite increased recovery of the grain produced and a reduction of impurities (resulting in improvement of the final quality of harvested and processed grain), the environmental impact could still be negative due to loss of plant cover, soil degradation and erosion in production areas. It is, therefore, important to incorporate environmentally friendly and conservation-conscious principles when developing new technologies. The rising demand for organic quinoa makes a positive contribution in this direction.

Despite the fact that conventional breeding methods have produced low saponin content quinoa varieties and generated more knowledge about the genetic structure of this species, the most commonly cultivated varieties today are the bitter, high saponin content varieties and ecotypes with grains requiring saponin removal prior to consumption. It is believed that the saponins themselves are a defence mechanism protecting the plant against pests and diseases (i.e. invasion by insects, birds and rodents). Furthermore, some of the bitter ecotypes and varieties are more genetically stable and are endowed with special characteristics, as is the case of the 'Quinoa Real' ecotype – in high demand on the international market for its grain size of about 2.5 mm.

Although current saponin removal methods still apply the basic principles of traditional processes, it is worth noting that with enhanced scientific knowledge about the characteristics of the episperm and the properties of saponins, major progress has been made in the development of equipment and appropriate technology.

Saponins are easily removed because they are located in the outer layers of the grain. Dry saponin removal methods make use of the inherent abrasive qualities of the episperm resulting from the structure of the plant tissue. Removal is a lot more effective and uniform when grains rub against each other, since the frictional force is similar to or less than that when the grains are rubbed against a rough surface. It is, therefore, possible to better control the hulling process and obtain higher and more uniform episperm removal (and hence saponin-removal percentages). Despite the ovoid shape of the seed, the fragility of its embryo and exposure to the environment, the nutritional quality of the seed is not affected by the frictional force between the grains.

Heating, an element used in both traditional dry and wet methods, has not yet been incorporated into the design of new saponin removal processes. The traditional grain roasting technique is not seriously considered, because it colours the grain as a result of the reaction between proteins and reducing sugars present in the grain, and there is a possible breakdown of the saponins. Nevertheless, increasing the temperature of the water used to wash the grain may improve the process of extracting the saponins, as it softens the episperm tissue and makes it more soluble, which facilitates and accelerates leaching. In order to avoid modifying the physical and chemical properties of the grain, the temperature must under no circumstances exceed the protein denaturation temperature or the starch gelatinization temperature.

A greater understanding of water absorption mechanisms and the distribution of saponins in the grain has made it possible to identify the best periods for washing and to achieve more appropriate designs, so that the water penetrates only as far as the layers where saponins are found. Consequently, the other layers of the episperm are not hydrated, the amount of water used is significantly reduced and drying time is much shorter. Drying is also a critical stage that needs to be adequately controlled to prevent microbial growth. The final moisture content of the grain should be < 13.5 %.

Although combined methods have been improved, the amounts of water used in the washing phase are still significantly high at 5–15 m^3/tonne of processed quinoa, especially in regions where water is scarce. In the Bolivian Altiplano, for example, annual rainfall is only 150–200 mm (Fundación PIEB, 2010). These processes also generate residual waters contaminated with saponins that, in many cases, are discharged untreated into natural bodies, with the risk that they may upset the balance of the ecosystems. Furthermore, environmental regulations on water and soil pollution are becoming increasingly stringent with regard to accepted limits of discharge. This could lead to an overhaul or even the elimination of the wet or combined saponin removal methods.

Enhanced recovery of residues (episperm and saponins)s is another aspect to be considered when designing saponin removal equipment and technology. Saponins have multiple uses in the industrial sector (Kuljanabhagavad and Wink, 2009), and residue from hulling is no longer considered "waste" residue with no commercial value; on the contrary, it is seen as a by-product with a good market price. There is a need to develop methods that make it possible, not only to recover a greater quantity of saponins in dry removal, but to isolate the portions with the highest concentration of saponins. This comparative advantage could be used to promote the cultivation of other varieties and ecotypes of quinoa. Varieties and ecotypes with smaller grains and perhaps lower nutritional quality, but high saponin content, could be cultivated in regions outside the traditional producing areas; this is the case for quinoa cultivated in the Inter-Andean valleys.

When developing equipment and technology for saponin removal, it is important to consider, not only good processing capacity the ability to provide an end product of international quality standards, but also environmental protection and conservation factors: i) reduction of water and energy consumption; and ii) reduction of contaminated solid and liquid residues. To this end, many of the prototypes constructed have good potential for adaptation to industrial level, responding both to the technical requirements of efficiency and grain quality and to environmental and economic requirements.

There are currently many quinoa products on the market (e.g. expanded products, flour, noodles, flakes, extruded products, cereal and energy bars) made from saponin-free grains. In addition, research continues on the development of new combined products that could generate more interest in quinoa consumption. However, little has been done to date to develop products requiring more complex technologies for separating active ingredients and nutritional components, such as oil, protein concentrates and isolates, starch, quinoa milk, saponin derivatives, dyes from leaves and seeds. These high value-added products, which are still being researched, are considered to represent the economic potential of quinoa: they make use of not only its nutritional properties, but also its physicochemical characteristics. In the light of the vast genetic variety that exists in the Andean regions, quinoa could transcend the food industry to provide products for the chemical, pharmaceutical and cosmetic industries. In order to develop this potential, local production capacity needs to be boosted through appropriate planning, including research on process and product development and subsequent technology transfer.

6. Conclusion

Projections in the sector indicate that demand for this ancestral grain – especially organic quinoa – will continue to rise,. This will inspire the improvement of agricultural machinery currently available on the market, with the optimization of processes and technological innovation, not only in the harvest and post-harvest stages, but throughout the production chain. The objectives will be to increase yield, improve grain quality, reduce water and energy consumption and generation of waste, and mitigate the intrinsic negative environmental impacts.

Industrial saponin removal uses the combined method, to meet the quality standards for commercialization of quinoa grain, especially with regard to: i) grain integrity, ii) nutritional value, and iii) final saponin content. Current combined processes enable saponin removal to reach levels of 0.01–0.06% (as required on the international market), which is far below the values detected by the palate. The most effective systems use the dry method to remove up to 95% of saponins in the huller, with a grain mass loss of approximately 5–7%. The rest of the saponin is removed during washing, when the grain remains in contact with the water for barely 2 minutes – or even just seconds.

With the equipment and technology currently available, it is not yet possible to process large volumes of quinoa using the dry method, without compromising the nutritional quality and changing the grain shape. There are some artisanal dry-method prototypes with high efficiency in terms of saponin removal and recovery of the saponins, but these are yet to be developed on an industrial scale.

The latest technologies recognize the value of the saponin-rich episperm residues, which have multiple uses in the industrial sector and therefore seek to recover as much of these chemical components as possible during the removal the process. The presence of saponin should be considered yet another opportunity presented by quinoa.

Because of its physicochemical, rheological, nutritional properties and its agronomic versatility, quinoa is increasingly incorporated in the preparation of a range of foods; nevertheless, only a small portion of its potential has to date been explored, especially in terms of higher value-added products.

Today, the "golden grain" is considered a strategic crop poised to contribute to global food security.

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CHAPTER: 3.2.

Fodder and Animal Feed

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Abstract

Quinoa has been used to feed animals since pre-Hispanic times. Thanks to its nutritional properties and the by-products generated during harvesting and milling, the crop is used for feeding both ruminants and non-ruminating animals. Various studies shown that quinoa grain, administered whole or ground in varying proportions in the feed ration, can supply the needs of monogastric animals, especially poultry and pigs. Since saponins confer a characteristic bitter taste that inhibits consumption, they must be removed through washing before the grain is used in feed. Another option is to use sweet cultivars: they have produced interesting results and it is not necessary to remove the saponins. By-products from grain harvesting, threshing and milling (e.g. seed bran or husks) are another potential source of feed. However, if they come from bitter quinoa, the saponin content will be high and hinder consumption. This effect can be mitigated by mixing in other ingredients. For ruminants, studies have been carried out on forage and silage production and the addition of harvest residue (stalks and leaves) to animal diets. While dry matter yields are acceptable, they are mainly of interest because of their digestibility and high protein content, making quinoa a high quality forage. In non-monogastric animals, saponin does not have adverse effects; on the contrary, it has the advantage of controlling certain internal parasites. Quinoa is a multipurpose crop and may be used to feed animals in its major cultivation areas, given the quantity of residue produced when the grain is milled; it can also be used as fodder in areas where water is in short supply, and at high altitudes where other species cannot flourish. However, in the current situation of high grain prices, using quinoa as animal feed may not be commercially feasible.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) has been cultivated in the American Andean region since pre-Hispanic times (Canahua Murillo and Mujica Sánchez, 2013; Galwey, 1992), especially in those regions comprising modern-day Peru and Bolivia. It is found in areas ranging from sea level to altitudes of 4 000 m asl. During the Tiwanaku and Inca civilizations, quinoa had an important place in people's diets and was also used to exchange for products from outside the Altiplano region (Bonifacio, 2006). It is a highly nutritional food for both human beings and animals (Ahamed *et al.*, 1998; Bhargava *et al.*, 2006; Mujica*et al.*, 2001). The presence of saponins can affect quinoa consumption, so these must be removed from the grain before it can be fed to pigs and poultry. Fresh quinoa leaves and harvest chaff are quite attractive for sheep, bovines, camelidae, goats and fish (Francis *et al.*, 2002) and quinoa leaves may be used for silage (Montoya Restrepo *et al.*, 2005). Residue from milling has a high nutritional content adapted to animal feeds: low quality broken grains are used for poultry feed; stalks, bits of leaf, remains of the panicle, inflorescences, flowers and pedicels are used to feed sheep, bovines and pigs (León Hancco, 2003). The value of these by-products lies in the sheer volume produced, which makes them viable for animal feeding. From the last century to the present, studies have been carried on the contribution of quinoa to animal feeding. While it is undoubtedly a multipurpose crop, often only the grain is considered a major food source, and little is known about the other parts of the plant – or they are not appreciated (Bonifacio, 2006; Galwey, 1992). In recognition of its many attributes, the Food and Agriculture Organization of the United Nations (FAO) designated quinoa as a crop that could contribute to food security.

2. Quinoa use in animal feeding

The principal product is the grain, which is also the main source used in animal feed tests, as it can be used as a supplementary protein, to improve the balance of amino acids in animal diets (Jacobsen, 2003). Nevertheless, populations living on the Altiplano plateau used both the whole plant and its by-products from harvesting, threshing and milling to feed domestic animals, especially camelidae; with the arrival of the conquistadors, they were also fed to bovines, sheep, birds and pigs (Hernández Bermejo and León, 1994). Considering the scarcity of forage material in the high, dry, cold regions of the Altiplano and other latitudes, quinoa by-products represent an important supplement for use in livestock production and are a source of high quality, locally produced forage (Bonifacio, 2006; Jasso Cantú *et al.*, 2002).

The presence in the grain of saponin, which gives a characteristic bitter taste (Ahamed *et al.*, 1998; Bonifacio, 2006; Cuadrado *et al.*, 1995), has been studied in great detail. Grain with a sapogenin content of 4.7–11.3 g/kg of dry matter is classified as "bitter" quinoa, 0.2–0.4 g/kg as "sweet", and grain with a value between these two ranges is "intermediate" (Mastebroek *et al.*, 2000). Saponin is concentrated in the outer part of the grain, in the pericarp, and needs to be removed before the grain can be consumed by animals.

Saponins can modify the micro-organisms in the gastrointestinal tract, particularly in ruminants (Gee *et al.*, 1993) and reduce the protozoan populations in the rumen by bonding with the cholesterol in the cell membrane of the protozoan and causing cellular breakdown and death (Makkar and Becker, 1998). Nevertheless, Abreu *et al.* (2004) observed an increase in the number of protozoans in sheep fed with high saponin-content Sapindus saponaria fruit. Saponins may in certain cases have a negative effect on the feeding behaviour of mammals, and there are clear reports of the negative effects of its consumption, digestibility and productivity, diminishing its viability as forage (Rogosic *et al.*, 2008).

2.1 Grains

To derive the maximum benefit from quinoa grain, the saponins must be removed using either the "wet" method, where grains are soaked in pots and then rubbed together to eliminate the saponins and pedicels, or the "dry" method, where grains are hulled through heating, then rubbed together to eliminate the saponins in powder form (Borges *et al.*, 2010).

2.1.1 Feeding monogastric animals with quinoa grain

Research on the use of quinoa grain in animal rearing has focused mainly on poultry and pigs, comparing feed rations of grains containing saponins with rations of grains containing few or no saponins. The addition of varying percentages of grain to the diet has also been investigated, and it has also been compared with other feed sources. Gandarillas (1948), cited by Cardozo and Tapia (1979), studied the physiological effects of saponin on Leghorn hens, comparing feed rations that included washed, unwashed, raw and cooked quinoa with a maize-based feed containing 40% grain. There was no statistically significant difference in weight gain in hens fed with either quinoa or maize, for the 30-day duration of the experiment. The biggest weight gain was in hens that consumed washed and cooked quinoa, followed by unwashed, cooked quinoa – demonstration of the positive effect of cooking quinoa (Table 1). This finding was corroborated by Cardozo (1959), cited by Cardozo and Tapia (1979), when hens were fed with cooked, washed and raw quinoa supplemented with porcine brain extract and compared to a control group fed with milk (Table 2). Weight gain in the chickens fed with cooked quinoa was higher, similar to the control group, while consumption was higher when the quinoa was washed. Feed-use efficiency was however higher with unprocessed quinoa.

In a study to find protein substitutes for feeding chickens, Gandarillas et al. (1968) carried out tests using quinoa grains with very little or no quinoa

Table 1. Weight of Leghorn hens, with four quinoa-based rations and one maize-based control

	Average weight of birds (g)		
Feed ration	Initial weight	At 15 days	At 30 days
Washed and cooked quinoa	325	547	835
Washed, uncooked quinoa	304	500	757
Unwashed, uncooked quinoa	287	450	731
Unwashed, cooked quinoa	332	504	823
Control (yellow corn)	319	564	832

Source: Gandarillas (1948), cited by Cardozo and Tapia (1979)

Table 2. Increase in live weight and feed consumption in hens on a feed ration of quinoa and milk

Feed ration	Increase g/hen	Consumption g/hen
Cooked quinoa	550	4100
Washed quinoa	382	4250
Quinoa + cholesterol extract	450	3360
Unprocessed quinoa	406	2800
Control (milk)	545	3430

Source: Cardozo (1959), cited by Cardozo and Tapia (1979)

content. The first test compared sweet quinoa with bitter quinoa and a feed ration of milk and wheat bran (Table 3). The second experiment studied the effect of washing sweet quinoa (Table 4) and the results showed no difference between washed and unwashed quinoa, although feed-use efficiency was higher with the smaller amount of quinoa.

They also observed that up to 30% quinoa, there was no reduction in growth. Cardozo and Tapia (1979) reported that with higher levels of quinoa, the effect of saponins would manifest itself in the form of vitamin A deficiency, but this effect could be controlled with high doses of vitamin A and D supplements – inferring that quinoa may be lacking in vitamins A and D, or that quinoa saponins have a depressant effect on one or both of these vitamins. This confirms the interference of saponins in vitamin A and E absorption in hens reported by Jenkins and Atwal (1994).

Quinoa was also mixed with other Andean grains and other ingredients commonly used to prepare feed. Negron *et al.* (1976) cited by Cardozo and Tapia (1979) compared a quinoa- and kaniwa- (*Chenopodium pallidicaule*) based feed ration with industrial feed (Table 5). They found no significant differences in daily live weight increase, but feed-conversion efficiency was higher with quinoa and kaniwa grains and mortality was also lower (Table 6). With the price of these products at the time, the cost per kg of live weight was 10–50% lower than for industrial feed. The authors report that these grains somehow alleviate altitude sickness and make it possible to raise broiler chickens quite economically in environmental conditions > 3 500 m asl.

Two experiments were carried out to assess the effects of including quinoa grain hulled to eliminate saponins in wheat, rapeseed, pea and soybean flour feeds for broilers (Jacobsen et al., 1997). In the first experiment, the chickens were given a blend of feeds from 6 days old to 36 days old, with diets containing 100, 200 and 400 g/kg of unprocessed and unhulled quinoa. Linear growth reduced as the amount of quinoa increased (from 1.8 to 0.8% for every 10 g/kg of quinoa added), while hulling showed a non-appreciable beneficial effect, but only in the first week of the experiment. In the sec-

Table 3. Increase in weight and mortality of New Hampshire hens on a feed ration of sweet and bitter quinoa

	Control Milk	Sweet quinoa 60%	Sweet quinoa 30%	Bitter quinoa 30%
Feed protein content	248	20.1	19.1	19.7
N° of days of experiment	60	60	60	60
Weight increase, g	375c	427.5b	621.0 a	489.3a
Mortality, hens	---	3	---	5

The figures with a different letter show a 1% statistical variance from probability
Source: Gandarillas *et al.* (1968), cited by Cardozo and Tapia (1979)

Table 4. Feeding chickens with washed and unwashed sweet quinoa

Response	Washed 30%	Washed 60%	Sweet quinoa 30%	Sweet quinoa 60%
First stage: 27 days				
Daily weight increase, g	16.2	11.4	11.5	9.9
Average daily consumption, g	38,4	30.5	34.6	34.1
Feed-use efficiency	2.37	2.67	3.01	3.44
Second stage: 37 days				
Average daily weight, g	21.1a	20.4a	27.1a	25.0a
Feed-use efficiency	2.93	3.20	3.53	3.54

Source: Gandarillas *et al.* (1968), cited by Cardozo and Tapia (1979)

Table 5. Ingredients, percentages and nutritional value of a balanced quinoa- and kaniwa-based feed ration

Ingredients	Starter ration	Growing ration	Finishing ration
Quinoa, %	50,0	44,0	44,0
Kaniwa, %	34,4	35,4	34,4
Starch paste, %	6,0	7,0	7,0
Fishmeal, %	9,0	13,0	10,0
Common salt, %	0,5	0,5	0,5
Vitamin supplement, %	0,1	0,1	0,1
Nutritional value			
Protein, %	19,4	21,2	19,6
Fat, %	4,2	4,4	4,1
Fibre, %	8,0	8,0	7,9
Ash, %	6,1	7,0	8,0
Moisture, %	8,1	7,9	7,7

Source: Negron *et al.* (1976), cited by Cardozo and Tapia (1979)

Table 6. Chicken feeding with three industrial rations and a blend of quinoa and kaniwa

Response	Ration 1	Ration 2	Ration 3	Quinoa + Kaniwa
Daily live weight increase, g	23.0a	24.5a	23.0a	24.2a
Feed-use efficiency	2.66	2.15	3.31	1.99
Dead animals	11	8	11	6
Economic value ×100	-3.05	22.97	-18.27	32.27

Source: Negron *et al.*, (1976) cited by Cardozo and Tapia (1979)

Table 7. Effect of quinoa flour supplementation on weight gain, consumption and feed conversion in broiler chickens in the last 21 days of fattening

Ingredients	Quinoa flour			
	0%	45%	30%	15%
Quinoa flour	0	45	30	15
Fishmeal	7	8	8	8
Soybean cake	20	14.3	15	16.5
Plantain flour	16	6	13.5	13.8
Ground corn	24	9.7	16	20
Corn husks	24	8.5	8	18
Molasses	4.5	4	5	4.2
Premixed vitamins + minerals	0.5	0.5	0.5	0.5
Mineral salt	1.5	1.5	1.5	1.5
Eggshell powder	1.5	1.5	1.5	1.5
Bone meal	1	1	1	1
TOTAL	100	100	100	100
Weight gain (g)	143.13	108.94	108.57	106.07
Feed consumption (g)	97.2	100.62	97.2	97.2
Feed conversion	1.08	1.20	1.22	1.11

Source: Muñoz Tunubala *et al*. (2007)

ond experiment, the chickens were fed from 0 to 39 days with pellets containing 150 g/kg of unprocessed quinoa, 150 g/kg of hulled quinoa and 50 g/kg of quinoa sprouts. The hulled quinoa showed no effect, and with 150 g/kg of quinoa, live weight fell at 20 and 39 days - from 627 to 601 g and from 1 760 to 1 709 g, respectively - while feed conversion increased at 20 days - from 1437 to 1486 g of feed/kg of live weight. The yield for broilers that were fed quinoa sprouts was as good as those in the control group, which led the authors to conclude that while quinoa could potentially be used as feed for broiler chickens, the quantities included in the diet should not exceed 150 g/kg. Studies continue to define the effects of various quinoa processing methods in broiler chickens. Unprocessed, hulled and washed quinoa were assessed with four different levels of protein in the diet (13.2, 18.0, 13.3 and 23.0% CP), compared with a diet based on wheat, sorghum and corn (Improta and Kellems, 2001). Growth and the survival rate for chickens fed with unprocessed quinoa were lower than in chickens fed with washed or hulled quinoa; but the results of those fed with washed quinoa were similar to those of chickens with a diet based on corn and soybean cake, and better than those fed with hulled quinoa. It would appear that washing is more efficient than hulling in terms of reducing the factors affecting quality

Table 8. Live weight gain in young turkeys fed with different quantities of quinoa and fishmeal

Feed ration	Growth		Fattening		Finishing	
	g/animal	Feed use efficiency	g/animal	Feed use efficiency	g/animal	Feed use efficiency
Quinoa 15%	1284	2.27	6360	4.31	2392	5.36
Quinoa 10%	1283	2.39	5808	4.25	2214	5.84
Quinoa 5%	1240	2.19	5362	4.48	2125	5.90
Fishmeal	1224	2.18	4368	5.29	1594	6.80

Source: Mogollón and Rentería (1975) cited by Cardozo and Tapia (1979)

and lowering the productive performance of chickens. Raising the protein content of the diet – from 13.2% to 18.0% and 23.0% – led to a marked improvement in growth and survival of the quinoa-fed groups. Washing and hulling the quinoa grain, and increasing the protein content of the diet or slightly reducing the amount of quinoa by adding soybean cake, therefore improves growth and survival rates in broiler chickens.

A study to find alternative sources of protein and energy, included an experiment to assess the effects of introducing 15%, 30% and 45% of quinoa flour into feed rations used to fatten broiler chickens. With each chicken receiving 148.47 g/day (Muñoz Tunubala *et al.*, 2007), the control group gained more weight and demonstrated better feed conversion, while the group given a ration including 15% quinoa flour demonstrated the best feed conversion, despite gaining the least weight (Table 7). The authors concluded that, given that quinoa flour in the feed produced only slight differencesi, it was a viable alternative to other sources of energy such as corn.

Mosquera *et al.* (2009) needed to substitute part of a poultry diet with alternative feeds and evaluated the addition of 0%, 5%, 15% and 25% sweet quinoa with saponins in two phases: a starter phase (1–4 weeks) and a finishing phase (4–6 weeks). Feed consumption in the starter phase was lower in the control group (1 265.4 g), while there were no differences between the test groups receiving quinoa (1 553.8, 1 543.4 and 1 547.93 g, respectively). In the finishing phase, there were no differences between the groups (1 993.99, 2 098.19, 2 080.97 and 2 005.83 g, respectively). In terms of weight gain, no differences were found, either in the initial phase (816.63, 839.84, 840.50 and 881.64 g, respectively) or in the finishing phase (1 075.4, 1 127.19, 1 171.56 and 1 066.16 g, respectively). Feed conversion in the initial phase revealed differences between the control group (1.47) and the quinoa diets (1.75, 1.74 and 1.67, respectively), but in the finishing phase there were no differences (1.86, 1.86, 1.79 and 1.90, respectively). In terms of feed efficiency, there were differences in the initial phase, with better performance in the control group (68.35), while the test groups receiving quinoa were statistically similar (57.36, 57.59 and 59.99, respectively). In the finishing phase, however, there were no differences (54.02, 53.74, 56.28 and 53.07, respectively). The control group had the best yield (74.075%) and the quinoa diets provided the lowest yields. Finally, mortality was higher in the control diet (10.94%), while in the quinoa groups it was 1.56%, 0% and 1.56%, respectively. The authors concluded that it was possible to use quinoa as an unconventional ingredient in preparing feed concentrates for broiler chickens and that sweet quinoa (no saponin removal) could adequately replace other protein sources, such as soybean cake, by up to 25%, without affecting the normal growth of the animal. The group that received 5% quinoa demonstrated the best economic profitability, without affecting productive parameters, such as feed conversion and efficiency in the finishing phase, and weight gain in both phases. Birds that were fed rations containing different quantities of quinoa demonstrated similar behaviour in feed consumption, weight gain, feed conversion, feed efficiency and dressed yield, in both phases evaluated. Furthermore, due to the low saponin content in the sweet quinoa varieties used in the feed rations for broiler chickens, there was no impact on consumption. There is therefore no need for saponin removal in these varieties.

Experiments were also carried out by Mogollón and Rentería (1975), cited by Cardozo and Tapia (1979, who added 5%, 10% and 15% quinoa to feed rations for young turkeys, compared with a feed ration based on fishmeal (Table 8). With the exception of the growth ration, feed-use efficiency was higher with the higher quinoa content in the diet.

In quail (*Coturnix japonica*) feeding, Nossa and Garzón (1976), carried out a 35-day study during the growth period, looking at the effect of adding four isoprotein rations (20%) with two different energy levels (2 800 and 3 100 kcal/kg). The difference between the two levels was the proportion of quinoa included in the feed ration (0%, 5%, 10% and 15%). Another study on egg-laying used an isoprotein feed ration (20%) with 2 800 kcal/kg of energy and the same portions of quinoa, also over 35 days. In growth, there were no observed differences with the control group, although the latter showed better performance using a low level of energy, with a daily weight gain of 2.04 g and a total weight gain of 858g. A clear trend appears, with a greater proportion of quinoa leading to improved results; for example, with a low level of energy and 15% quinoa, the daily weight gain was 2.13 g and total weight gain was 898 g. The best daily and total weight gains (2.18 and 915 g, respectively) were obtained with a low level of energy and 5% quinoa. Differences were observed in egg laying, with the best performance in the control group (67 eggs), followed by the group whose feed contained 15% quinoa (64 eggs). The lowest production was in the group with 5% quinoa (40 eggs), with no differences regarding consumption.

Quinoa grain was also used in pig feed Cardozo (1959), where it was found that the development of pigs fed with unwashed quinoa was adversely affected by the saponin content of the grain. This effect is however modified in monogastric animals by their response to vitamin supplementation, as observed by Cardozo and Tapia (1979), when they added vitamins A and D to chicken feed, due to the low levels of these vitamins in quinoa grain. Gandarillas *et al.* (1968) tested two levels of quinoa (30% and 50%) (Table 9), and found no statistically significant differences in weight gain, although the highest increases were obtained with the feed that included broad bean flour, as well as a mix of powdered milk and bran. A comparison between the two levels of quinoa showed that weight gain and feed-use efficiency were higher in the diets with the lower percentage of quinoa (30%). The findings of Cardozo and Tapia (1979) are confirmed by observations in the saltmarsh regions of the Bolivian Altiplano Sur, where bitter ‘Quinoa Real’ is cultivated. Feeding pigs with washed, cooked quinoa mixed with barley and alfalfa flour appeared to produce positive results.

Table 9. Feeding piglets with industrial feed and different proportions of quinoa

Ingredients	Control 0%	Quinoa 30%	Quinoa 50%	Altiplano 30%
Corn	25.0	5.5	-	-
Barley	20.0	20,0	7.3	20.7
Fishmeal	17.2	19.5	17.7	14.3
Milk powder	12.8	-	-	-
Bran	20.0	20.0	20.0	-
Levabol yeast	5.0	5.0	5.0	5.0
Washed quinoa	-	30.0	50.0	30.0
Broad bean flour	-	-	-	30.0
	100.0	100.0	100.0	100.0
Total increase, kg	29.2 a	27.1a	25.8a	32.1a
Daily increase, g	423	393	371	464
Feed-use efficiency	3.6	3.2	3.3	2.9

Source: Gandarillas *et al.* (1968) cited by Cardozo and Tapia (1979)

Table 10. Calculation of total digestible nutrients (TDN) in quinoa grain

Nutrients	Content (%)	Digestibility (%)		Digestible nutrients
Proteins	14.29	81		11.57
Fat	4.94	68 x 2.25		7.56
Fibre	4.01	67		2.69
NFE	58.61	85		49.82
			TDN =	71.64

Source: Ugarte (1956), cited by Cardozo and Tapia (1979)

Table 11. Weight gain in calves according to the different types of supplementation

Supplement	Live weight increase (kg/day)	No. of days to reach 100 kg	Achievable live weight in 1 year
2 kg Barley	0.679	147	250
1.5 kg Barley + 0.5 kg Wheat	0.776	130	280
1.8 kg Barley + 0.2 kg Broad beans	0.800	125	292
1.8 kg Barley + 0.2 kg Ground quinoa	1.133	88	414

Source: Martínez Claure (1946), cited by Cardozo and Tapia (1979)

Diaz *et al.* (1995)worked with a group of 8-week old piglets weighing 10.1 kg, that were fed a basic concentrate that served as the control. The cereals were then partially replaced by quinoa flour (5% and 10%). After 5 weeks, there were no observed differences in final weight (20.4, 20.0 and 18.9 kg) or in daily weight gain (294, 285 and 248 g). There were however differences in the feed-conversion ratio: 3.6 for the group that received the control diet, 4.6 for the group whose diet included 10% quinoa flour. The authors concluded that 5% of the cereals could be replaced with quinoa flour.

2.1.2 Feeding ruminants with quinoa grain

Although ruminants are not usually fed with quinoa grain, there are some examples of quinoa added to ovine feed to determine the total digestible nutrient value of quinoa grains (Table 10), based on in vivo digestibility values found by Ugarte (1956), cited by Cardozo and Tapia (1979).

The results indicate that the energy content of quinoa grains is 2.97 kcal/kg of dry matter (i.e. not very high).

Also, after adding quinoa to feed rations for calves, Martínez Claure (1946), cited by Cardozo and Tapia (1979), reported a positive effect (Table 11). Mixing 200 g of ground quinoa with 1.8 kg of barley produced 1.133 kg/day increase in live weight, while it took 88 days to reach 100 kg of live weight. With such figures, it would be possible to achieve 414 kg of live weight in 1 year, thus increasing not only weight gain, but also the speed at which calves can reach their commercial weight.

According to Cardozo and Tapia (1979), by-products of the quinoa harvest, such as the residue from threshing (10.7% protein) and chaff, are commonly used to fatten bovines in the regions around Lake Titicaca in Peru and Bolivia.

2.1.3. *Feeding other animal species with quinoa grain*

To assess the feed value of sweet and bitter quinoa, Pate et al. (2006b) prepared feed rations based on sweet cultivars (‘Surumi’, ‘Patacamaya’, ‘Sayana’ and ‘Chucapaca’) and bitter cultivars (‘Real’), and compared them to feed rations based on corn, barley and oats for Andean guinea pigs (*Cavia porcel-*

Table 12. Proximal composition of the reference diet, experimental diets, apparent digestibility coefficients of fishmeal, poultry offal meal and quinoa flour defined for *Oreochromis niloticus*.

Diet	DM (%)	% Dry matter			GE (Kcal/Kg)	Digestibility coefficient		
		PB	EE	Ash		Dry matter	Protein	Energy
Reference diet	89.5	29.9	2.2	11.3	4118.6			
Fishmeal 10%	88.3	33.8	3.8	11.8	4398.8	75.9 ± 1.7 a	88.1 ± 2.1 a	63.9 ± 8.0 b B
Fishmeal 20%	92.5	40.4	4.2	11.7	4385.2	74.3 ± 2.7 a	89.4 ± 0.6 a	53.0 ± 1.3 b B
Fishmeal 30%	90.3	45.9	5.0	12.2	4517.1	71.3 ± 6.2 a	90.9 ± 2.4 a	88.5 ± 0.7 a A
Poultry offal meal 10%	91.2	33.1	4.6	10.1	4427.9	72.3 ± 4.1a	96.8 ± 0.6 a	53.8 ± 3.1 b
Poultry offal meal 20%	88.3	34.6	8.1	10.4	4564.1	78.7 ± 2.8 a	94.9 ± 1.2 a	52.6 ± 4.4 b
Poultry offal meal 30%	90.9	39.7	9.8	10.6	4695.9	74.1 ± 6.4 a	94.1 ± 2.1 a	59.7 ± 1.0 b
Quinoa flour 10%	84.8	27.1	4.3	9.4	4346.4	58.8 ± 5.5 b	67.7 ± 7.6 b	29.0 ± 7.4 c B
Quinoa flour 20%	87.9	26.4	3.7	8.7	4227.6	64.4 ± 4.2 a	73.5 ± 5.1 b	52.0 ± 3.2 b A
Quinoa flour 30%	89.7	24.0	3.0	8.1	4227.0	59.9 ± 4.1 b	77.5 ± 2.9 b	66.1 ± 5.6 b A

Different lower case letters in the same column indicate statistical significance ($p < 0.05$) in the comparison of all ingredients; different upper case letters in the same column indicate differences in inclusion levels for each raw material ($p < 0.05$).

Source: Gutiérrez-Espinosa *et al.* (2011)

lus). The bitter quinoa was added in both unwashed (containing saponins) and washed (saponin-free) forms. The guinea pigs fed with sweet quinoa gained more weight (353.5–414.4 g) than those fed with bitter quinoa in both unwashed (307.4 g) and washed (308.0 g) form. Weight gain in guinea pigs fed with corn (337.7 g) was statistically similar to the results with sweet quinoa, but lower than with 'Surumi' (414.4 g), while the lowest weight gain was registered in guinea pigs fed with barley (245.8 g). Feed conversion was more efficient in the groups receiving sweet quinoa (4.15–4.37) than in those receiving corn (5.71) and barley (5.71). Feed consumption was lower with bitter quinoa (1 278 g) and higher with corn (1 896 g). These results indicate that sweet quinoa is a more promising feed source for guinea pigs than bitter quinoa (with or without saponins), it exceeds barley and is similar to maize and oats. The exception is the 'Surumi' cultivar, which surpassed all the others (quinoa, corn and barley). The same authors (Pate *et al.*, 2006a) suggested that sweet quinoa varieties were more palatable than bitter quinoa and had the same palatability as washed (saponin-free) quinoa, corn, oats and barley. To ascertain this, they carried out experiments using these cultivars, first comparing bitter quinoa with sweet quinoa, and then comparing sweet and bitter quinoa with corn, oats and barley. The results showed a preference for sweet varieties (11.5–24.2% consumption), while consumption of washed and unwashed bitter quinoa was 21.9% and 1.1%, respectively. As for the other cereals, there was a preference for oats (45.6%), followed by corn (17.1%). Consumption of sweet quinoa was lower than corn: 1.6% ('Sayana') and 10.6% ('Surumi'). Consumption of bitter quinoa was almost nil (0.3%), but washing produced an increase in consumption (7.4%). Barley consumption was similar to sweet quinoa consumption. Although saponin removal through washing significantly improves the palatability of bitter quinoa, when a choice of oats, corn and barley is available, the preference is for oats and corn over quinoa (both sweet and bitter); this goes against the initial assumption.

In terms of weight gain, eliminating the saponins from bitter quinoa confers no advantages, since the sweet cultivars are superior to bitter cultivars. On the other hand, when feeding is independent, sweet quinoa cultivars are equal and sometimes superior to corn or oats. Traditionally, quinoa is only edible once saponins are removed (through cleaning or by some other means); this limits its direct use by humans or animals, compared with other cereal grains. It can thus be inferred that sweet quinoa cultivars are as palatable as washed quinoa; dissemination of sweet, rather than bitter, cultivars would eliminate the need for saponin removal (an extremely time-consuming process).

New Zealand rabbits (*Oryctolagus cuniculus*) were fed with a diet of 30% sweet quinoa in various forms (washed, sprouts and parboiled) and 70% commercial concentrate. The highest levels of consumption were recorded in the group that received quinoa sprouts (4 692 g), with a weight gain of 1 231 g after 6 weeks; the lowest levels of consumption were with parboiled quinoa (4 345 g), with a weight gain of 1 106 g (Vargas Ramírez and Carreño Salamanca, 2007).

In a study with Nile tilapia (*Oreochromis niloticus*), the apparent digestibility of dry matter, protein and energy in fishmeal, poultry offal meal and quinoa were compared, at three levels of inclusion (Table 12). While there were no differences in digestibility for dry matter and protein in fishmeal and poultry offal meal, there were differences in quinoa flour, which produced lower values. In terms of energy, fishmeal recorded the best digestibility coefficient with 30% inclusion, while quinoa flour recorded the lowest with 10% inclusion. These results show that the digestibility coefficients of dry matter and protein were not affected by the level of inclusion. The apparent digestibility coefficients of quinoa flour protein were also similar to those observed in wheat, rice and maize, and exceeded 67%, which indicates that it can be included in diets for Nile tilapia. The authors concluded that quinoa flour could be used as a substitute for other cereals, with the additional advantage that it has a higher protein content (Gutiérrez-Espinosa *et al.* 2011).

2.2. Quinoa by-products

Harvesting, threshing and subsequent milling of quinoa generate a variety of by-products with a range of traditional uses: bran, stalks and dry leaves can be used for animal feed.

2.2.1. *Feeding animals with quinoa grain bran*

Quinoa hulling produces bran that comprises the remains of the pericarp and the hull of the grain. Quinoa farmers, in particular those in the Altiplano region, use these by-products in various ways: some use them as feed for sheep, llamas and guinea pigs, others use them for compost. The nutritional protein contribution of quinoa bran varies between 11.14% and 14.94%, depending on how the grain is processed. These protein levels show that quinoa bran has high potential for use in preparing animal feed (Aduviri, 2007).

The effect of adding 30% and 60% quinoa bran (obtained using the dry and wet methods) to the rations of guinea pig (*Cavia porcellus*) was studied, with a control group that received wheat bran. The results showed that the most satisfactory average live weight gain values were obtained with the guinea pigs that were fed rations containing 30% quinoa bran from dry hulling (7.80 g/day), followed by bran produced using the wet method (7.62 g/day) and the control group (7.35 g/day). At 60% inclusion of quinoa bran, consumption fell, with low values of 16.50 g/day for wet method bran, compared with 21.34 g/day for the group receiving 30% wet method bran and 21.25 g/day for the control group. This low consumption was attributed to the excessive amount of by-product included in the ration, which made it less palatable. However, 30% quinoa bran obtained by the dry or wet method can be substituted for wheat bran. Furthermore, it is worth underscoring the antiparasitic effects of quinoa in the digestive tract of the guinea pigs (Aduviri, 2007).

Tuquinga Tuquinga (2011) studied guinea pigs and evaluated diets with different levels of quinoa residue in the growth and fattening phase, comparing with a control group (without quinoa residue). The study used 96 weaned, female guinea pigs at 28 days. The best results were obtained with the inclusion of 40% quinoa bran: in the growth phase, the final weight obtained was 813.23 g, with a weight gain of 366.25 g at 64 days and a daily weight gain of 10.17 g. The feed conversion rate was 4.5. In the fattening phase, the best responses in all areas were again obtained with the inclusion of 40% quinoa bran in the diet. Final weight was 1 107.50 g, with a weight gain of 294.17 g at 100 days, a daily weight gain of 8.17 g and a feed conversion ratio of 8.33.

Table 13. Effect of quinoa bran supplementation on weight gain, consumption and feed use in piglets in the first 28 days of weaning

	Origin of the quinoa bran						
	South America				Denmark		
Dose (mg/kg)	0	100	300	500	300	SEM	p-value
Initial weight (kg)	8.2	8.2	8.2	8.2	8.2	0,2	0.93
Final weight (kg)	16.4	16.6	16.8	16.0	16.5	0.5	0,41
Consumption (g/day)	412	415	426	387	412	18	0.17
Weight gain (g/day)	294	301	307	280	296	16	0.41
Gain: Consumption (kg/kg)	0.71	0.72	0.72	0.71	0.72	0.06	0.99

Values are least square means

Source: Carlson *et al.* (2012)

Carlson *et al.* (2012) assessed the addition of South American quinoa bran in the feed rations of piglets in doses of 100, 300 and 500 mg/kg, as well as bran from Denmark with 300 mg/kg (Table 13). Quinoa bran supplementation did not influence growth rate, feed consumption or feed use by the piglets, despite a large difference in saponin content of the bran from South America and Denmark, accounting for 28.7% and 2.0% of the weight, respectively. The origin of the feed source therefore had no effect on the behaviour of the piglets, although consumption and weight gain were numerically lower in piglets fed with 500 mg/kg of bran; this could indicate that the concentration was not high enough to have a negative effect on production and, to a certain extent, that the lower consumption might be related to the bitter taste of the saponins in the bran.

2.2.2 Feeding animals with quinoa stalk

Due to the shortage of forage in the Altiplano regions of Peru and Bolivia, the possibility of using quinoa stalks as fodder is being studied, especially in ground form, although the nutritive value may not be very high. Cardozo *et al.* (1968) compared quinoa stalks with other forms of forage in the Bolivian central Altiplano. In an initial trial with sheep, they were able to achieve live weight increases comparable to those obtained with green or dry barley-based feeds. Subsequent experiments assessed feeds where quinoa stalks replaced oat and barley hay and found no significant differences among the groups. In these experiments, the main feed source was from native grasslands, to which 200 g of supplemental rations were added each day. With the proportion of quinoa stalk in the feed ration varying between 35% and 65%, no difference in weight was observed. Nevertheless, the purpose of these experiments was to demonstrate that residue from quinoa farming could be used to feed animals.

Similarly, Rizo Patrón and Soikes (1968) used barley straw and quinoa stalks to feed sheep, with the addition of two antibiotics. Higher weight gains were observed with barley straw, but the economic analysis led them to prefer the use of quinoa stalks. Table 14 shows the analysis of quinoa stalks used in the ovine fattening experiment. The authors deduced that with the addition of the antibiotic chlortetracycline to the barley straw, there is no increase in live weight in relation to the diet including quinoa stalks. Furthermore, the link between the forage consumed and weight gain was much less in the quinoa stalk diet, and as such the difference in weight increase was mainly due to the differences in forage consumption, with an advantage for barley since quinoa straw contains a higher amount of fibre and saponin and is thus less palatable for the animal.

Table 14. Analysis of quinoa stalk

Component	Percentage
Moisture	12.60
Crude protein	5.52
Fat	0.77
Fibre	26.12
NFE	46.56
Ash	9.43

Source: Rizo Patrón and Soikes (1968), cited by Cardozo and Tapia (1979)

2.3. Feeding animals with quinoa forage

Quinoa is used as fodder for ruminants, in particular in areas where other species cannot thrive because of the prevailing soil and climate conditions (e.g. in the vicinity of the saltmarsh regions). Capelo (1980) indicated that quinoa harvested as forage at 135 days included 55% leaves and panicles and 45% stalks, with 66.6% moisture and a 10.2 tonnes/ha yield of dry matter. Montoya and Roa (1985) reported 2.322–4.242 tonnes/ha yields of dry matter containing an average of 15.42% protein from material coming from Peru and Bolivia in the 1970s and 1980s project entitled "Potential for readapting quinoa in Colombia". Nevertheless, these results were lower than those reported in Mexico by Bañuelos Taváres *et al.* (1995), who assessed the yield, chemical composition and digestibility of 18 quinoa varieties with different vegetative cycles (6 early, 6 intermediate and 6 late varieties) *in situ*. The plants were cut at the flowering phase and dry matter yields varied between 7.733 and 11.400 tonnes/ha, with protein values of 17.81–18.98% CP (Table 15). The authors concluded that in the light of its yields and high protein content, quinoa could be used as forage for ruminating animals. To determine its potential as fresh forage, von Rütte (1988) reported yields from week 9 to 17, with increases in green matter of 18–74 tonnes/ha, with 12.6–18.6% of dry matter, and 26–17% protein content (weeks 9 to 16), with peaks in week 10 (29.5%) and week 14 (28.5%).

In exploring alpaca (*Lama pacos*) rearing as a profitable alternative in areas outside the Altiplano with difficult conditions, a number of potential forage species were evaluated (López *et al.*, 1996). Digestibility and consumption of hay from the white goosefoot (*Chenopodium album*) weed were compared with three different types of alfalfa (*Chenopodium album*) hay (López *et al.*, 1996). White goosefoot protein digestibility was 73.8%, compared with 71.3–76.1% for alfalfa; cell wall digestibility (NDF) was also better for white goosefoot (60.3%) than for alfalfa (44.3–54.6%). The trend was similar for hemicellulose, with 17.2% digestibility for white

Table 15. Comparison between quinoa vegetative cycles on days for cutting, yield and composition

	Type			
	Early	Intermediate	Late	EE
Days at cutting	93.5a	98.40b	106.3c	0.85
Yield:				
Forage, kg/ha	59994a	67069b	74750c	831
DM, kg/ha	7733a	9243b	11440c	190
DDM, kg/ha	5171a	5892b	6688c	116
Composition:				
DM, %	13.60a	14.60b	16.00c	0.29
NDIF, %	59.13	60.53	57,53	0.65
CP, %	17.96	17.81	18.98	0.32
SP, %	50.14	50.34	51.05	1.12
ISDMD, %	66.22a	63.91a	58.81b	0.67

DM dry matter; DDM digestible dry matter; NDIF neutral detergent insoluble fibre; CP crude protein; SP soluble protein; ISDMD *in situ* dry matter digestibility.

Source: Bañuelos Taváres *et al.* (1995)

Table 16. Average values of productive parameters in forage production

Crop	DM (tonnes/ha)	Leaves (%)	Leaves (kg/ha)	Protein Leaves (kg/ha)	Protein planta (kg/ha)
Sunflower	7.11ª	48.35e	3437.68d	649.72a	1188.79a
Amaranth	2.25d	64.02a	1440.45c	319.78d	445.50d
Dolichos bean	1.76e	61.13b	1075.88d	231.13e	366.08e
Kenaf	4.47c	49.94cd	2232.31b	428.60c	826.95b
Soybean	2.71d	50.30c	1363.13c	298.53d	574.52c
Maize	6.22b	49.31cde	3067.08a	503.00b	833.48b
Quinoa	2.56d	48.36de	1238.60d	302.08d	560.64c

Averages with different letters in the same column show a significant difference of $P > 0.05$, according to Duncan's test.
Source: Ramos and Cruz (2002)

goosefoot and 6.6–9.4% for alfalfa, but voluntary consumption of dry matter did not exceed 2% of live weight in either case. White goosefoot consumption was 1.34 kg/100 kg, and alfalfa varied between 1.75 and 1.95 kg/100 kg. Expressed as a unit of metabolic size, this translates into 37.6 g/kg0,75/d for white goosefoot and 48.6–54.6 g/ kg0,75/d for alfalfa. The lower consumption of white goosefoot may be explained by its bitter taste. Since alpacas are rustic and capable of consuming lower quality forage, white goosefoot could be an alternative source of forage.

Ramos and Cruz (2002) compared quinoa plant yield for forage production to sunflower (*Helianthus annus*), red amaranth (*Amaranthus cruentus*), dolichos bean (*Lablab purpureus*), kenaf (*Hibiscus cannabinus*), soybean (*Glycine max*) and maize (*Zea mays*). Total plant height in quinoa was 82.52 cm and the percentages of dry matter, leaves and stalks on the whole plant were respectively 18.88%, 18.52% and 22.26%. The yield was 2 560 kg/ha, with 48.36% leaves. Protein content in leaves and the total plant was 302.08 and 560.64 kg/ha, respectively (Table 16). Quinoa produced the highest percentage of Ca (3.34%), with a protein content of 0.3% and 26.32% CF. The authors recommend sunflower and corn growing for greater forage production, while amaranth, soybean, quinoa and dolichos bean are recommended to produce high quality forage.

In order to identify alternative sources of forage able to withstand drought and freezing and adapt to poor soils and high altitudes, saponin concentration and composition in two quinoa cultivars, 'Sajama' and 'Chucara' were evaluated for their use as forage (Jasso Cantú *et al.*, 2002), with three levels of soil moisture deficit: low, medium and high. Differences in saponin content were observed: plants with a low moisture deficit had the highest saponin content of 0.456%, while plants with a high deficit had a saponin content of 0.386%. Saponin content was lowest at the branch development stage (0.309%) and highest at the flowering stage (0.608%). For all cultivars, biomass yield increased as the level of moisture deficit was reduced. With the high moisture deficit, the biomass value was 5.94 and 6.54 tonnes/ha, respectively, for 'Sajama' and 'Chucara' and 10.81 and 10.61 tonnes/ha, respectively, at the low deficit. Quinoa is thus an alternative forage for high altitude dry areas.

In a study to find alternative winter forage, alfalfa hay was substituted with quinoa in a fattening diet for rabbits, replacing 100%, 50% and 75% of alfalfa with quinoa (Primero Rubio and Rojas Lemus, 2007). The results showed that the productive parameters were not affected by the inclusion of quinoa in the alfalfa diet. Average consumption of the three diets was 83.92, 85.50 and 81.67 g, respectively, against 82.72 g for the 100% alfalfa diet. Over 6 weeks of fattening, weight gain was 1932.2, 2058.3 and 2023.3 g, respectively, against 1 945 g for the 100% alfalfa diet. Quinoa is thus a good alternative to alfalfa hay in periods of shortage.

Similarly, quinoa was evaluated as an alternative feed for goats, using the hay of ecotypes from the Altiplano region ('Mix') and from southern Chile ('BO25') and comparing them with alfalfa hay (Ortiz Munizaga, 2009). The results showed no significant differences in components from the proximal analysis and there were no observed differences in daily dry matter consumption. Average consumption was around 536 g/d or 3.6% of live weight, although there tended to be a stronger preference for alfalfa, while quinoa from the south was more often rejected. As for the apparent digestibility coefficient, there were significant differences in the values obtained for CP (83.5a%, 78.3b% and 67.3b%), NDF (49.3a%, 62.3b% and 57.3a,b%), and Hemicellulose (39.6a%, 73.8b% and 71.3b%) for alfalfa, 'Mix' and 'BO25', respectively. While no differences were found in live weight (average 14 kg) or body condition (average value 2.6), there was a clear trend of overall weight loss, both daily (107 g/d) and during the whole period (1.5 kg), in animals that were fed 'BO25'. This indicates that quinoa from the Altiplano is more suitable for forage and can be considered a good alternative forage for goats, especially in arid areas where forage is scarce.

2.4 Feeding animals with quinoa silage

In an effort to find alternatives to compensate for the shortage of feed in winter in the Altiplano region, experiments were carried out using silage from the quinoa plant. In the Chinolí experimental station of the *Instituto Boliviano de Tecnología Agropecuaria*, an experiment was carried out, feeding native lambs with quinoa and barley silage in various proportions: 100% quinoa; 75% quinoa + 25% barley; and 100% barley. The control group was fed by grazing, with the addition of harvest residue (basically corn stover) (Table 17). Consumption of the silage containing quinoa was higher, with an average 2.5 kg per day, which produced a daily weight gain of 140 g (Table 18). It therefore appears that including quinoa as silage in the diet of lambs represents a viable feeding alternative for sheep farmers, replacing corn stover. Furthermore, better responses could be obtained in sheep crossed with breeds specialized in meat production (Bilbao la Vieja Gutiérrez, 1995).

2.5 Cost of including quinoa grain and by-products in animal feed

Few publications have analysed the cost of adding the various forms of quinoa to animal feed. These costs were calculated at a time when the demand for quinoa was limited to the local market and it could be used as an alternative to other foods in animal diets. In silage production, the 100% quinoa plant ration cost USD0.0255/kg, 75% quinoa cost USD0.0276/kg and 100% barley silage was the most expensive at USD0.0340/kg (Bilbao la Vieja Gutiérrez, 1995).

For rations including quinoa bran (Aduviri, 2007): 60% bran was the most economical (USD0.0009/kg), followed by 30% (USD0.0014/kg), while the control ration was the most expensive at USD0.0015/kg (due to the high price of wheat bran, which accounted for 40% of the feed ration). Muñoz Tunubala *et al.* (2007) calculated the cost of rations containing 15%, 30% and 45% quinoa flour as USD0.5145, 0.4733 and 0.3630/kg, respectively, while the control cost USD0.4990/kg. At the time, the average price of processed quinoa grain was USD1.27/kg.

Mosquera et al. (2009) calculated the cost of rations containing 5%, 15% and 25% quinoa grain used during the initiation phase as USD0.3329, 0.3435 and 0.3540/kg, respectively, compared with a control with no quinoa that cost USD0.4285/kg, while in the finishing phase the cost was USD0.3432, 0.3537 and 0.3642/kg, respectively and USD0.3877/kg for the control ration. During this period, the average price of processed quinoa was USD2.60/kg.

The current international demand for quinoa grain leads to ever-increasing prices, with no indication that the trend will be reversed. According to Jacobsen (2011), the price of unprocessed quinoa increased from USD0.312/kg in 2000 to USD2.187/kg in 2008. Quinoa currently costs between USD2 500 and 3 000/tonne on the international market. Under the present circumstances, therefore, including quinoa grain in animal diets would probably cost more than the control feed rations, thus limiting its viability for use in conventional animal production. On the other hand, by-products and residue from the harvest are a more realistic alternative, although there may be competition for products containing saponin from the cosmetics and pharmaceutical industries, among others, as they also require this resource (Mujica *et al.*, 2001). The question arises therefore as to whether all the information required is available to be able to decide definitely about the use of quinoa grain and its derivatives as a substitute in animal diets. It would be worthwhile carrying out economic studies.

Table 17. Bromatological composition of quinoa and barley silage

	Treatments		
	Quinoa (100%)	Quinoa+Barley (75%+25%)	Barley (100%)
Crude protein %	13.07	9.61	4.32
Dry matter %	89.39	89.00	89.39
Ash %	14.38	11.90	7.56
Ether extract %	2.16	1.58	1.66
Crude fibre %	14.47	17.65	24.48
NFE %	51.72	48.26	45.31
TDN (%)	62.83	63.14	65.60
DE (kcal/kg MS)	2.76	2.77	2.88
ME (kcal/kg MS)	2.26	2.27	2.36
Calcium	1.54	0.83	0.20
Phosphorus	0.22	0.26	0.12

Source: Bilbao la Vieja Gutiérrez (1995)

Table 18. Weight gain and consumption of native sheep with quinoa and barley silage

Details	Treatments			
	Quinoa (100%)	Quinoa+Barley (75%+25%)	Barley (100%)	Control
Number of animals	4	4	4	4
Days of feeding	28	28	28	28
Initial weight, kg	19.37	18.75	17.75	19.25
Final weight, kg	23.37	22.62	19.75	19.87
Total weight increase, kg	4.00a	3.87a	2.00b	0.62c
Daily live weight increase, kg	0.142	0.138	0.071	0.022
Silage consumption, kg	295.70	277.55	207.10	
Daily consumption, kg	10.56a	9.91a	7.39b	
Individual consumption, Kg	2.64a	2.47a	1.84b	

Source: Bilbao la Vieja Gutiérrez (1995)

3. Concluding observations

Quinoa grain has a high nutritional quality, with high protein levels, and it represents a good feed alternative, in particular for the production of non-ruminating animals, mainly poultry and pigs. Residues from quinoa harvesting, threshing and milling also demonstrate the same quality.

Saponins limit and reduce consumption and thus affect live weight gain. They therefore need to be removed prior to inclusion of quinoa in feed rations. There is also the option of using grains from sweet cultivars with a low or zero saponin content not requiring saponin removal.

When quinoa grains or milling by-products are used as a substitute in feed rations for non-ruminating animals, the proportion must not exceed 30% of the diet in order to avoid consumption being affected by the presence of saponins. In the case of bitter quinoa, saponins must first be removed using the wet method.

For ruminants, quinoa is basically used in the form of forage, silage or by incorporating residues from the harvest (stalks and leaves), and the presence of saponins does not cause any difficulties; on the contrary, it appears to exert a certain control over internal parasites.

Quinoa is a multipurpose crop and a viable option for feeding animals in its major cultivation areas, given the quantity of residue produced in farming and milling the crop.

At present, the necessary economic information is not available to be able to state with certainty that quinoa grain, by-products and harvest residue can be used in commercial animal production.

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CHAPTER: 3.3

Saponins

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Abstract

The term saponin comes from the Latin word sapo, meaning "soap", reflecting a readiness to form stable soap-like foams in aqueous solutions. The biological role of saponins is not completely understood, but they are generally considered to be part of a plant's defence system against pathogens and herbivores, particularly because of their bitter flavour. Saponins comprise aglycones and sugar, each representing about 50% of the total weight of the molecule. In quinoa, saponins are a complex mixture of triterpene glycosides that derive from seven aglycones: oleanolic acid, hederagenin, phytolaccagenic acid, serjanic acid, 3β-hydroxy-23-oxo-olean-12-en-28-oic acid, 3β-hydroxy-27-oxo-olean-12-en-28-oic acid and 3β,23α,30β-trihydroxy-olean-12-en-28-oic acid, while the most common sugars are arabinose, glucose and galactose. Saponins are traditionally considered very antinutritional because of their haemolytic activity, and there is therefore a long-standing controversy about their functions in food. It is believed that saponins can form complexes with membrane sterols of the erythrocyte, causing an increase in permeability and a subsequent loss of haemoglobin. However, recent extensive studies of the biological activity of saponins *in vitro* and *in vivo* have identified associations with several health benefits, including anti-inflammatory, anticarcinogenic, antibacterial, antifungal and antiviral effects. Saponins are also of interest as valuable adjuvants and the first saponin-based vaccines have been introduced commercially. Traditionally, quinoa seeds are either abraded mechanically to remove the bran – which is where the saponins are predominantly located – or washed with water to remove bitterness prior to use. During washing, valuable nutrients are lost and the chemical composition and amino acid profiles of quinoa seeds can be altered. Following treatment, the level of saponin content in to-be-consumed quinoa seeds remains a major concern in terms of bitterness and possible negative biological effects. A mathematical model based on Fick's second law has been created to optimize the leaching process of saponins from quinoa seeds during washing with water.

Many studies have focused on the effects of agronomic variables (e.g. irrigation and salinity) on the saponin profiles of quinoa. It has been observed that saponins decrease in samples that have been exposed to drought and saline regimes – suggesting that irrigation and salinity may regulate the saponin content in quinoa and affect its nutritional and industrial values.

Studies are underway to evaluate and compare the saponin content in seven varieties of quinoa grown in Italy and six varieties grown in Chile under rainfed or low irrigation conditions. Seeds from the more arid or stressing Chilean localities have a higher saponin content.

1. Introduction

1.1 Saponin chemistry

Saponins are compounds found in many plants (Sparg *et al.*, 2004) and they have the distinctive feature of forming foam. The name probably comes from the plant *Saponaria* whose roots were historically used to make soap (Latin *sapo* = soap) (Augustin *et al.*, 2011). Chemically, they are glycosides with a polycyclic aglycone (glycoside-free portion), which may occur in the form of a steroid or a triterpenoid choline bound via the C3 carbon by means of an ethereal bond to a side sugar chain. The aglycone is commonly referred to as sapogenin, while the subset of steroidal saponins is commonly referred to as sarapogenin. Saponins are amphipathic because of their fat-soluble aglycone function and their water-soluble saccharide chain. This characteristic is the basis of the ability to form foam. Saponins are perceived as bitter, and this reduces the organoleptic characteristics and the palatability of any products rich in them. Only a few (usually those with a triterpenoic aglycone) have a nice flavour, reminiscent of liquorice root.

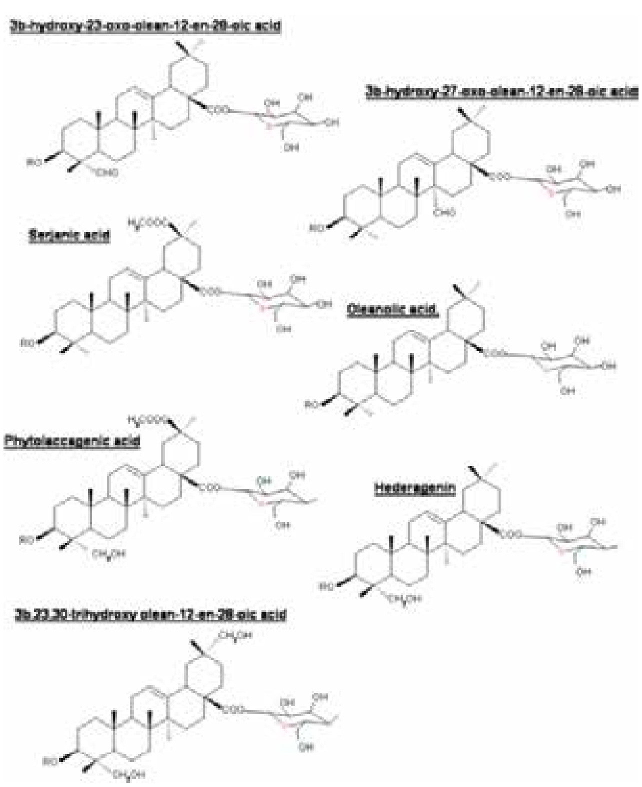

Figure 1: Summarizes the seven aglycones identified so far in the different parts of quinoa (flowers, fruits, seedcoats and seeds) (Kuljanabhagavad *et al*., 2008). These structures have been obtained by means of extensive characterizations in NMR (nuclear magnetic resonance) and mass spectrometry. Most of the variability is generated by the saccharide side chains – indeed, the seven aglycones give birth to more than 20 saponins (Table 1).

1.2 Saponin Biosynthesis

Evidence that the overexpression of squalene synthase may induce an up-regulation of saponins and phytosterols (Lee *et al.*, 2004) suggests that this enzyme is involved in the branching of biosynthetic pathways leading to the synthesis of phytosterols and saponins. This observation led to the theory (now consolidated) that saponins derive from the same anabolic process that leads to the formation of phytosterols. All terpenoids derive from condensation of 5-carbon building blocks designated IPP (3-isopentenyl pyrophosphate) and DMAPP (dimethylallyl pyrophosphate). In plants, IPP and DMAPP drift from condensation of acetyl-CoA in the mevalonate pathway or from pyruvate and phosphoglyceraldehyde. Terpenoid biosynthesis in plants is extensively compartmentalized: steroids, triterpenes and saponins are mainly synthesized in the cytosol utilizing IPP from the mevalonate pathway.

Flores-Sanchez *et al.* (2002) conducted experiments in which the activity of HMG-CoA reductase – a key enzyme in mevalonate and squalene synthesis – was inhibited, and this led to a reduction of phytosterols and of ursolic/oleanolic acid biosynthesis, confirming the hypothesis that the biosynthetic pathway of saponins is linked to that of plant sterols by means of squalene synthesis.

IPP and DMAPP undergo condensation to the 10-carbon intermediate GPP (geranyl pyrophosphate), and the addition of a second IPP unit leads to FPP (farnesyl pyrophosphate, C15), the common precursor of the vast array of sesquiterpenes produced by plants. Linkage of two FPP units leads to formation of squalene (C30). This is then epoxygenated to 2,3-oxidosqualene (C30), considered the last common precursor of triterpenoid saponins, phytosterols and steroidal saponins. The steps at which steroidal saponin and phytosterol biosynthesis diverge have not been elucidated, although Kalinoswska *et al.* (2005) suggest that cholesterol is a precursor of steroidal saponins.

Table 1: Saponins derived from the 7 aglycones found in quinoa.

Compound	Sugar side chain	Aglycone
1	β-D-Glc(1→3)-α-L-Ara	I
2		II
3		III
4		IV
5		V
6		VI
7		VII
8	α-L-Ara	III
9		V
10		VI
11	β-D-GlcA	III
12		IV
13		VI
14	β-D-Glc(1→2)-β-D-Glc(1→3)-α-L-Ara	III
15		IV
16		V
17	β-D-Xyl(1→3)-β-D-GlcA	IV
18	β-D-Glc(1→3)-β-D-Gal	V
19		VI
20	β-D-Glc(1→4)-β-D-Glc(1→4)-β-D-Glc	V

The first committed step in the biosynthesis of triterpenoid saponins and phytosterols is the cyclization of 2,3-oxidosqualene. During this process, internal bonds are introduced into the oxidosqualene backbone, resulting in the formation of predominantly polycyclic molecules containing varying numbers of 5- and 6-membered rings. The high number of possibilities for establishing different internal linkages during cyclization gives rise to a vast array of diverse structures, and over 100 different triterpene skeletons have been found in nature. However, from this vast range, only a limited number of possible cyclization products appear to be utilized in saponin biosynthesis.

Following the formation of basal sapogenin backbone structures, these common precursors usually undergo various modifications prior to glycosylation. The most common sapogenin modifications are small functional groups, such as hydroxyl-, keto, aldehyde - and carboxyl-moieties at various positions of the backbone.

Glycosylation patterns of saponins are often considered crucial for their biological activities. Typical triterpenoid saponin glycosylation patterns consist of oligomeric sugar chains of 2–5 monosaccharide units, most often linked at positions C3 and/or C28. Less often, 1–2 monosaccharide units have been reported to occur at positions C4, C16, C20, C21, C22 and/or C23. Glucose, galactose, glucoronic acid, rhamnose, xylose and arabinose are the most abundant hexoses and pentoses in the saccharide chains. Saponin glycosylation presumably involves sequential activity of different enzymes belonging to the multigene family of uridin diphosphate glycosyltransferases (UGTs).

1.3 Biological role

Saponins have different biochemical activities. Francis *et al.* (2002) reported, among others, strong haemolytic, antimicrobial, fungicidal, allelopathic, insecticidal and molluscicidal activity, while Vega-Gálvez *et al.* (2010) reported their effects as a vaccine coadjuvant. Therefore, although the true biological significance of saponins in quinoa still needs to be fully determined, the current line of thought is that they are part of the plant's apparatus to defend off predators.

1.3.1 Haemolytic activity

One of the systems used to probe the presence of saponins in a plant extract or in a drug is based on incubation of the extract with blood red cells and verification of the degree of haemolysis of the sample. The ability of saponins to break the membrane of the erythrocytes is linked to their ability to bind membrane sterols (Khalil *et al.*, 1994). When the membrane bursts, there is an increase in permeability and a loss of haemoglobin. Baumann *et al.* (2000) have investigated the effect of saponins on the membrane structure through haemolysis of human erythrocytes. The findings show that saponin-lysed erythrocytes do not reseal, indicating that saponin-induced damage to the lipid bilayer is irreversible. The level of haemolytic activity has been attributed to the type of aglycone and to the presence of the sugar side chains (Wang *et al.*, 2007).

1.3.2 Anti-inflammatory activity

In the carrageenan-induced oedema assay, many saponins isolated from plant sources produce an inhibition of inflammation. Kim *et al.* (1999) suggested that the anti-inflammatory activity of these saponins is related to anticomplementary action through the classical inflammation pathway. Oleanolic acid and ginsenoside Ro show the highest anticomplementary activity.

1.3.3 Antifungal/antiyeast activity

Triterpenoid saponins from the seeds of *Chenopodium quinoa* Willd. (Chenopodiaceae) have been reported to have antifungal activity (Woldemichael and Wink, 2001). A study by Bader *et al.* (2000) revealed that the antifungal activity of saponins against different Candida albicans strains can be influenced by variation of the etherglycosidically bonded carbohydrate units and the acylglycosidically bonded oligosaccharide at C-28 of the aglycone. However, only crude saponin mixture inhibits the growth of *Candida albicans*. Pure compounds show little or no activity, which suggests a possible synergistic effect between these saponins.

1.3.4 Antibacterial/antimicrobial activity

Saponins have also been reported to have antimicrobial activity (Killeen *et al.*, 1998). Alcohol soluble saponins have antimicrobial activity towards both prokaryotic and eukaryotic organisms, but only at low cell densities, and they do not inhibit microbial growth of dense populations.

1.3.5 Cytotoxicity and antitumour activity

Numerous reports highlight the highly cytotoxic properties of many saponins (Musende *et al.*, 2009; Man et al., 2010). In particular, oleananes show an antitumour effect in various pathways, including anticancer, antimetastasis, immunostimulation and chemoprevention. The detailed mechanisms are complex but involve dephosphorylate Stat3 in a variety of human tumour cell lines and lead to a decrease in the transcriptional activity of Stat3, which regulates proteins such as c-myc, cyclin D1, Bcl2, survivin and VEGF. Moreover, several immunostimulating activities, such as induced growth of human T lymphocytes, promoting apoptosis and triggering autophagic cell death have been reported. They decrease respiratory activity and induced ATP efflux after inhibition of the voltage-dependent anion channel in the outer mitochondrial membrane.

2. Saponin removal

Saponins are generally bitter, so before consumption they must to be eliminated from quinoa. Traditionally, quinoa seeds are either mechanically abraded to remove the bran, where the saponins are predominantly located, or washed with water to remove bitterness prior to use. Wright *et al.* (2002) report that during this washing process, valuable nutrients are also lost and the chemical composition and amino acid profiles in quinoa seeds may be altered. The final level of saponin content in to-be-consumed quinoa seeds remains a major concern in terms of its bitterness and possible negative biological effects.

2.1 Kinetic

The removal of saponins from quinoa seeds during washing can be described according to the rules governing solid–liquid extraction and by applying mathematical models generally used to evaluate process kinetics.

The total saponin concentration inside quinoa seeds rapidly tends towards an asymptotic value following an initial leaching. Fuentes *et al.* (2013) show that this asymptomatic value decreases as the washing temperature increases.

Saponin ratio (SR) – defined according to equation 1 – is the most commonly used parameter for modelling the saponin leaching kinetics of quinoa seeds. SR represents a dimensionless concentration used to study the leaching kinetics, supposing a mechanism of diffusion inside the solid and negligible external mass transfer under conditions of intensive stirring.

$$SR = \frac{X_{st}-X_{se}}{X_{s0}-X_{se}} \quad \text{Eq.1}$$

where X_{st} is the saponin content in real time ($g/100_{gdm}$), and X_{s0} and X_{se} are the initial and residual saponin contents.

Table 2 represents the most important model adopted for modelling SR in saponin removal.

2.2 Uses of Saponins

Saponins are used in industry as additives in foods and cosmetics. They can also be used in other industrial applications (Yang *et al.*, 2010; Chen *et al.*, 2010; Price *et al.*, 1987; Hostettmann and Marston, 1995) as, for example, preservatives, flavour modifiers, detergents (due to their chemical properties and abilities as foaming agents) and agents for cholesterol removal from dairy products.

Notably, saponins can also activate the mammalian immune system, arousing significant interest in their potential as vaccine adjuvants (Sun *et al.*, 2009). Their unique capacity to stimulate both the Th1 immune response and the production of cytotoxic T-lymphocytes (CTLs) against exogenous antigens makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens, as well as in therapeutic cancer vaccines.

3. Quinoa saponin content

3.1 Analytical methods

Several analytical methods have been developed for the determination of saponins from various matrices, including quinoa seeds. The simplest methods are used to detect typical saponin features, such as their ability to form foam or their haemolytic ability. The most commonly used methods, however, are chromatographic. Both liquid chromatography (with detection by mass spectrometry, DAD and

Table 2: Mathematical models selected to describe saponin leaching kinetics

Model	Equation	Reference
Midilli–Kuçuk	$SE = n_{11}\exp(-k_{12}t^{n_{12}}) + Ct$	Vega-Gàlvez *et al.* (2011)
Weibull	$SR = \exp(-(\frac{t}{\beta})^{\alpha})$	Corzo *et al.* (2008)
Logarithmic	$SR = C + n_5\exp(-k_6 t)$	Akpinar (2006)
Henderson–Pabis (modified)	$SR = n_8\exp(-k_9 t) + n_9\exp(-k_{10}t) + n_{10}\exp(k_{11}t)$	Sacilik & Elicin (2006)
Two terms	$SR = n_6\exp(-k_7 t) + n_7 + n_7\exp(k_8 t)$	Lahsasni *et al.* (2004)
Page (modified)	$SR = \exp(-k_3 t^{n_2})$	Togˇ rul& Pehlivan (2003)

ELSD), and gas chromatography (with detection by mass spectrometry and FID) have been employed. Gas chromatography has been widely used, although providing for a longer extraction protocol and a delicate silanization reaction. The first studies to include determination by gas chromatography were those by Ridout *et al.* (1991) and Price *et al.* (1986). In gas chromatography, saponins are generally extracted after acid hydrolysis of the degrased sample with a polar solvent; the extract after silanization is analysed with non-polar or slightly polar columns and eluted at high temperatures. The analysis in HPLC, on the other hand, entails a simpler preparation consisting of extraction with alcohols and purification with a C18 SPE. Separation is usually achieved with C18 stationary phases and elutions in water-acetonitrile gradient, both for photometric detection (DAD, ELSD) and in mass spectrometry.

3.2 Saponin evaluation in Chilean quinoa ecotypes

3.2.1. Ecotypes present in Chilean quinoa agro-ecological regions

Five quinoa ecotypes are described for the Andean region. They come from the Inter-Andean valleys of Colombia, Ecuador and Peru, the Altiplano of Peru and Bolivia, Yunga in the Bolivian subtropical forest, Salare (salt flats) in Bolivia, Chile and Argentina, and the Coastal (lowlands) or sea level areas of Chile. Their origins and possible expansion routes have been reviewed by Fuentes *et al.* (2012). In Chile, just two of the five ecotypes have been found (Salare and Coastal). However, within these two ecotypes many landraces or local farmers' varieties exist in the country. In the Altiplano (highlands) at 4 000 m asl (19°S), farmers hold at least 12 of these landraces (Alfonso, 2008; Alfonso and Bazile, 2009), known by the local Aymara people as, for example, 'Pandela' (red seeds), 'Jankú' (white seeds), 'Churi' (yellow seeds), 'Chullpe' (brown seeds), 'Khánchi' (dark pink seeds) and 'Chále' (mixed colours). In central (34°S) and southern (39°S) Chile, the landraces appear less abundant because there is less diversity of seed colour, as most are whitish, yellowish, beige and grey, the latter being more abundant at southern latitudes (39°S), as is also observed in seed bank collections used for testing comparative yields (Martínez *et al.*, 2007).

Of these three regions, the climatic conditions are more stressful in the high Andes of northern Chile where annual rainfall is 100–200 mm (Lanino, 2006), while in central and southern Chile, it is over 400 mm (Miranda *et al. 2013).*

3.2.2 Saponin content

The total saponin content evaluated in whole seeds of Chilean landraces and in one hybrid variety ('Regalona') is over 1%. They are, therefore, all bitter (i.e. saponins > 0.11%) but with significant variation among them. Unexpectedly, high Andes Salare landraces do not always contain higher values of saponins (2%). Those from central Chile have the highest values, reaching as much as 4% (Miranda *et al.*, 2012). When seeds are sown in a different locality, particularly cultivated under the drier conditions of arid Chile (at 30°S with no rainfall between October and May), harvested seeds increased their saponin content, at least for the 'Regalona' hybrid, from 2.2% to 3.2%. This phenomenon, however, is not observed for another landrace from Villarrica in southern Chile. The latter maintains a saponin content of 2.11–2.38% when cultivated in arid northern Chile (Miranda *et al.*, 2013). The higher saponin content in landraces from central Chile might be due to the particular stressing conditions of high salinity in some coastal soils. These soils are sometimes naturally irrigated in the winter with brackish waters from the neighbouring rivers influenced by the high tides of the Pacific Ocean (Orsini *et al.,* 2011).

3.2.3 Conclusions

1. Saponin content has to date been studied in seeds from Chilean landraces of quinoa belonging to the Salare and Coastal Andean ecotypes. Their saponin content is high (> 2%), compared with some sweet quinoas of the Altiplano (< 0.11%).
2. Unexpectedly, saponin content is higher in coastal landraces from central Chile
3. The saponin content of some quinoa seeds changes when grown under different conditions, normally increasing in a more stressing climate (drought).

3.3 Italian research activity

From 2006, different field trials have been performed at ISAFoM-CNR to test quinoa. The strategic objectives of these studies have been: to evaluate

Table 3: Saponin content (mg/100 g_{dm}) in the two accessions

Accession	Total saponin	Oleanolic ac.	Hederagenin	Phytolaccagenin
	mg 100 g^{-1} of DW	*% of total saponin*		
KV_{april}	238.9 ± 10.87	78.2	16.7	5.1
KV_{may}	213.8 ± 7.52	76.3	18.9	4.8
RB	329.0 ± 6.78	85.3	10	4.7

the quantitative and qualitative responses of quinoa accessions under combined abiotic stresses (salt and drought stress) and their adaptability in the Mediterranean environment of southern Italy (see Chapter 6.3); to improve food production by introducing quinoa as a possible alternative crop for this area (potentially high value food cash crops); and to verify the opportunities for use of quinoa seeds, flours and derivatives in product lines for children and for people with coeliac disease, with potentially interesting growth prospects in specialized sectors.

At the experimental station of the National Research Council (CNR), Institute for Agricultural and Forest Mediterranean Systems (ISAFoM) in Vitulazio (CE) (14°50'E, 40°07'N, 25 m asl), a 2-year (2006–07) field trial was carried out to compare two quinoa genotypes: 'Titicaca' ('KVLQ52') and 'Regalona Baer' ('RB') under rainfed conditions (Pulvento *et al.*, 2010). Comparison was also made between two sowing dates (April and May) for 'KVLQ52' ('KV'_{april} and 'KV'_{may}). In this period, quinoa was studied within the project "CO.Al.Ta. II" (Alternative Crops to Tobacco), set up by the European Community (CE), to explore the possibilities of diversification of Italy's traditional tobacco-growing areas and to evaluate seed quality, and in particular saponin content, in collaboration with the Department of Food Technology (DISTAAM) of the University of Molise.

Results show that April is the best sowing time for quinoa in the Mediterranean region (Table 2). Of the two genotypes, 'RB' records better growth and productivity, apparently being more tolerant to abiotic stress (high temperatures associated with water stress).

The study includes quantitative/qualitative assessment of saponins. Gas chromatography analysis shows that the two varieties of quinoa are in an intermediate position between "sweet" and "bitter" genotypes. In particular, the total saponin content of 238.9 and 213.8 mg/100 gdm for genotype 'KV'_{april} (sown in April) and 'KV'_{may} (sown in May), respectively, was obtained. For genotype 'RB', the saponin content is 328 mg/100 gdm. From a qualitative point of view, confirmed by bibliographic data (Ridout *et al.*, 1991), oleanolic acid is the main saponin component (76–85%), followed by hederagenin (10–18%) and phytolaccagenin (4–5%). Since saponins are mainly located in the outer layers of the seed, these components were removed through the process of pearling. The process was performed using a laboratory perlator model (TM-05-Takayama, testing Mill) with an abrasive roller (40P). A 50% reduction in total saponins % compared with the initial value for the product with a pearling degree of 20% was observed by gas chromatographic analysis. However, the final product still had a saponin content which could be detected at sensory level. Application of pearling at 30% reduced the saponin content by about 80%. In fact, saponin values dropped from 238.9 mg/100 g_{dm} to 33.47 mg/100 g_{dm} in the pearled product (Table 3). Ash, protein and lipid content in 'Titicaca' is higher after abrasion of the pericarp. In particular, the linoleic omega fatty acid is very high in 'Titicaca' seed and flour.

Seed abrasion tends also to increase oleic, linoleic and palmitic fatty acid in 'Titicaca'.

From 2008 to 2013, ISAFoM-CNR participated as a partner in the UE project "Sustainable water use securing food production in dry areas of the Mediterranean region" (SWUP-MED).

Quinoa genotype 'Q52' ('Titicaca') was grown in an open field trial in 2009 and 2010 to investigate the effects of salt and water stress on quantitative and qualitative aspects of the yield. Treatments irrigated with well water ('Q100', 'Q50' and 'Q25') and corresponding treatments irrigated with saline water ('Q100S', 'Q50S' and 'Q25S') with an electrical conductivity (ECw) of 22 dS/m were compared.

Saline and water stress in both years do not cause significant yield reduction, and quinoa may be defined as tolerant to salinity and drought (Pulvento *et al.*, 2012).

Chemical composition of quinoa seeds confirms a higher protein and fibre content compared with common cereals, while the highest level of saline water determines higher mean seed weight and, as a consequence, higher fibre and total saponin content in quinoa seeds. It has been observed that irrigation with 25% full water restitution, with and without the addition of salt, is associated with an increase in free phenolic compounds of 23.16% and 26.27%, respectively. In contrast, bound phenolic compounds are not affected by environmental stresses.

The effects of the different agronomic variables, such as irrigation and salinity, on the saponin profiles of quinoa were analysed.

Saponins were evaluated in terms of sapogenins (Gomez-Caravaca *et al.*, 2012; Lavini *et al.*, 2011) (Figure 2).

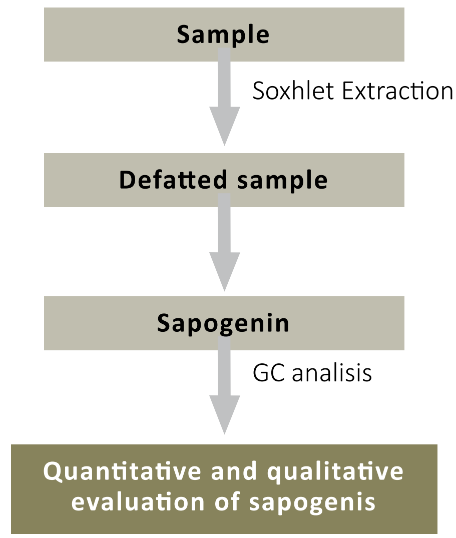

Figure 2: Schematic diagram for the extraction of saponins

A gas chromatographic procedure was applied for the evaluation of saponin aglycones (sapogenins) derived from the acid hydrolysis of samples (Ridout *et al.*, 1991; Woldemichael and Wink, 2001). Three major quinoa saponin aglycones were identified: oleanolic acid (36–50% total), hederagenin (27–28%) and phytolaccagenic acid (21–36%) (Figure 3).

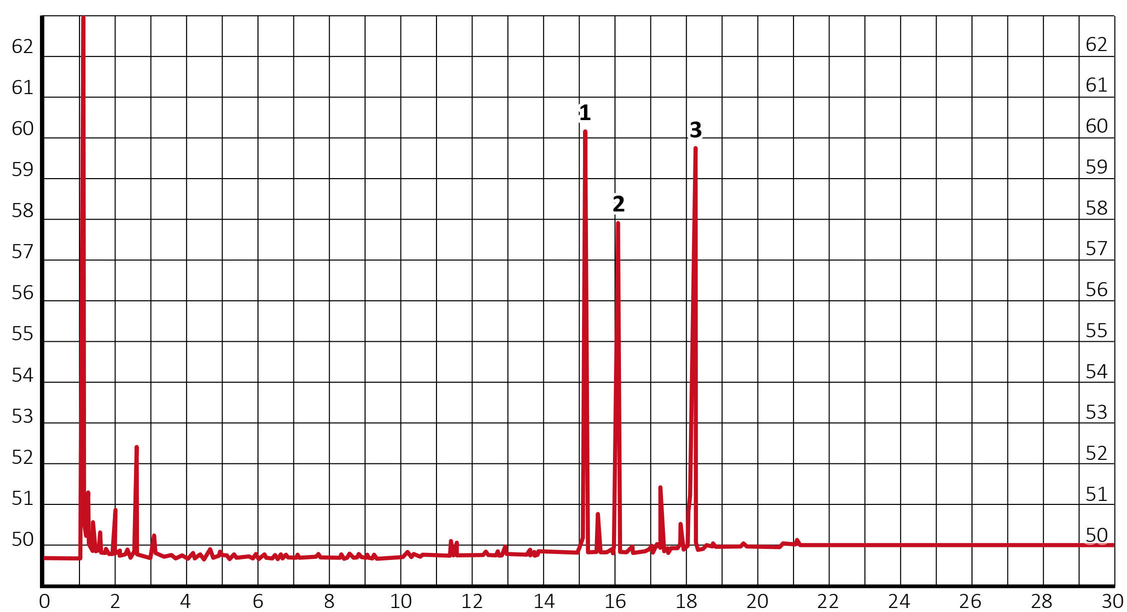

Figure 3: GC chromatogram of Titicaca saponin (1 Oleanolic acid, 2 Hederagenin, 3 Phytolaccagenic acid)

When considering the total amount of saponins (Table 5) it was observed that 'Titicaca' is a bitter variety. In fact, quinoa seeds with a saponin concentration > 0.11% are usually considered to be bitter genotypes (Vega-Gálvez *et al.*, 2010).

The highest saponin values were observed in samples obtained without deficit irrigation treatments (1 633.3 mg/100 g for 'Q100S' and 1 140.1 mg/100 gdm for 'Q100', respectively). The samples treated with a water deficit ('Q25' and 'Q50') showed a decrease in saponin content compared with 'Q100'. The 'Q50' samples, compared with 'Q100', showed a decrease in saponins of 32%; while the samples grown with a higher irrigation deficit ('Q25') showed a 45% decrease in saponins. These results are in agreement with the study of Soliz-Guerrero *et al.* (2002), who reported that saponin content is affected by a soil-water deficit, to the extent that high water deficits promote low saponin contents. Samples treated with saline water also show significant differences at different irrigation levels ('Q100S', 'Q50S' and 'Q25S'); the decrease in saponin content in the 'Q50S' and 'Q25S' samples is very high compared with 'Q100S' (40% and 42% for 'Q25S' and 'Q50S', respectively).

From 2011 to 2013, field trials were performed in Vitulazio on quinoa, and others are ongoing at ISAFoM within the "CISIA" project, funded by the National Research Council, and the "Quinoa Felix" project – Introduction of quinoa (*Chenopodium quinoa* Willd.) – in the Campania region for high nutritional and functional value food production, in collaboration with the University of Molise and CNR-Institute of Food Science (ISA) of Avellino. The aim of these activities is to evaluate yield and seed quality of *Chenopodium quinoa* varieties grown under rainfed conditions in southern Italy, and to assess milling performance and protein, ash, lipid and saponin content of the seed.

All analyses are performed on whole seeds and on "pearling" grain, after removal of the pericarp, to define the potential nutritional characteristics of each quinoa variety. Since there is no genetic resource of quinoa as a domesticated variety in Italy, the studies are conducted using seeds received from foreign institutions and of different origins. Testing is being done on the Danish quinoa cultivars 'Puno' and 'Titicaca' selected from material originating in southern Chile and provided by the University of Copenhagen; four Bolivian cultivars 'Kurmi', 'Janca grano' 'Blanquita' and 'Real'; the Peruvian 'Amarilla de Marangani'; and 'Jujuy rosada' originating in Argentina. The Danish cultivars 'Titicaca' and 'Puno' give the higher yield, while 'Janca grano', 'Real' and 'Kurmi' give the lowest yields; 'Blanquita' does not produce under Mediterranean conditions.

All seven aglycones have been assayed. The variety 'Jujuy Rosada' is richest in saponins (4.99%), while 'Real' is the poorest (0.1%). Although the concentration profiles of the seven aglycones vary greatly among the varieties – in particular, in 'Jujuy rosada', 72.5% of saponins contain 3β-hydroxy-23-oxo-olean-12-en-28-oic acid as aglycone, while in 'Real', oleanolic acid is the most represented aglycone (despite only 24.80%) – there is a more homogeneous distribution of all seven aglycones. However, 3β,23,30-trihydroxy olean-12-en-28-oic acid is the least represented aglycone in all the varieties studied.

4. Conclusion and perspective

Saponins present both an obstacle and an opportunity. The deployment as food of many pseudocereals, especially quinoa, is hindered by the presence of these antinutritional elements, both because of reduced palatability due to their bitter taste, and because of the serious effects they can have on human health. On the other hand, these molecules are proving to be extremely interesting in several fields: from pharmaceutical (as the basis for the development of new cancer drugs, new antifungals or adjuvants in vaccines), to chemical, but especially in the field of agronomy, where they are proving to be excellent and versatile insecticides. Saponin insecticidal activity is based on three different mechanisms (Chaieb, 2010): interference with feeding, entomotoxicity (various forms of chronic toxicity, such as female fertility reduction and decreased rate of blossoming eggs, are observed in many insect species) and growth regulation (research shows that saponins are able to regulate the growth of many insect species). The effects of saponins are generally associated with disturbance of the developmental stages and moulting failure. Nevertheless, there is still massive scope for understanding and improving this use of saponins, regarding in particular: stability (because the bulk of insecticide activity is due to the sugar side chains and these are very susceptible to pH values and enzymatic activity), application, action of residual saponins and their antinutritional properties, and, finally, their difficult synthesis. The latter could be solved by means of extraction protocols from varieties that produce large amounts of saponins or are grown under conditions that generate larger quantities (good water supply and high salinity of the soil), while knowledge of the pedoclimatic effects on saponin content may allow the development of varieties requiring sustainable agronomic treatments to eliminate these dangerous antinutritional agents.

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CHAPTER: 3.4

Quinoa: Nutritional Aspects of the Rice of the Incas

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Abstract

"Rice of the Incas" is one of the names given to quinoa. Some kind of basic grain for human consumption has originated in every major region of the world. The least known and the least disseminated is quinoa, in contrast with rice, the most widespread and, while of Asian origin, is grown almost everywhere in the world. In practice, quinoa and rice grains are treated in very similar ways for human consumption and share many culinary uses. Both grains can be dehusked before consumption, using machines. Neither possess gluten, but quinoa has other advantages which have not been fully exploited to achieve greater expansion of its consumption and cultivation. First, when rice is peeled, a large proportion of the proteins and other elements associated with the chaff are lost. On the other hand, quinoa, which has the structure of amaranthaceous seeds, loses almost none of its nutritional qualities when it is peeled or washed. The water requirement of quinoa is much less than that of cereals, which means that higher yields are possible, particularly in terms of protein production in relation to water consumption. Quinoa's adaptability to arid zones is one of the reasons for which FAO promotes greater use of the crop. Further studies are needed to determine more precisely the effective assimilation of its significant quantities of proteins, minerals and vitamins, as well as its high quality oils and flavanoids. Furthermore, the peel of quinoa produces saponins, a waste product with potentially valuable uses, including the control of snail pests that attack rice paddies. The fight against hunger and malnutrition – two major scourges of opposing worlds (developed and developing countries) – has an invaluable ally in quinoa.

1. Introduction

"Rice of the Incas" is one of the names given to quinoa, and it has been chosen in this chapter for several reasons. Ancient knowledge recognizes a basic food type for each major region of the world: for example, rice in the countries of the great Asian continent, maize in Central America, wheat in ancient Mesopotamia, sorghum and millet in Africa. In each large zone of the planet, therefore, one type of grain dominates (Table 1), and today, some species have spread to become acquired agricultural crops in different continents (Bazile, 2012). For example, maize reached Europe and wheat reached America during intercontinental journeys and colonization processes. Quinoa was not considered an important grain by the colonizers. On the contrary, it was rejected and only survived colonization in locations rendered remote by altitude or geographically isolated for other reasons (Fuentes *et al.*, 2012). Its nutritional value has recently been rediscovered through scientific studies revealing its numerous hidden nutritional merits, and today FAO affirms that quinoa could make a major contribution to world nutrition and agriculture.

Table 1. Zones of the planet and their respective originating grains

Continental zones	Originating consumption grains
North America	Sunflower
Central America	Maize, beans
South America	Quinoa
Europe	Colza
North Africa	Oats
Central Africa	Coffee
Southern Africa	Millet, sorghum
East Africa	Millet, sorghum
West Africa	Sorghum
Central Asia	Wheat
West Asia	Wheat, lentils, barley
South and Southeast Asia	Rice
East Asia	Soybean

Source: Bazile, 2012

The nutritional value of quinoa has been reviewed in the literature since the 1990s, in part by FAO itself – Tapia (1990, 1992, 2000) and Ayala *et al.* (2004) – but also in other independent studies and reviews, such as Galwey (1993), Schlick and Bubeheim (1996), and more recently Jancurova *et al.* (2009), Vega-Gálvez *et al.* (2010) and Rojas *et al.* (2010). FAO (2011) is the document laying the foundation for the declaration of 2013 as the International Year of Quinoa. It documents trial crops and commercial productions of quinoa in various regions of the world outside Central or South America, including North America, Europe, Asia and Africa, and it states that cultivation can be extended to other regions with climates and photoperiods that are very different from those of its origin. Evidence of this is provided by the experimental crops in Mali, a sub-Sahelian region with a very hot and arid climate and where rainfall is concentrated in the summer months (Coulibaly *et al.*, 2013).

Unlike the exhaustive studies on its nutritional properties (listed above), this chapter describes the nutritional value of quinoa in terms of its name "Rice of the Incas", suggesting uses similar to those for rice by the various populations of the world. This approach is a direct consequence of the emergency situation faced by the world today given the evidence of child hunger. In 2012, there were over 1.1 million children under 5 suffering from serious acute malnutrition in the belt of countries to the south of the Sahara alone (UNICEF, 2013). Rice is one of the dried-grain-based food products that is easiest to transport, store and consume without major processing. It can therefore be rapidly deployed as a palliative food in emergency situations where immediate intervention is necessary before local varieties can be improved (and then only if the physical and political climates of the affected countries allow so). Crop adaptation and enhancement are processes lasting years. In comparison with other grains such as wheat, oats and maize, rice has many practical advantages when it comes to cooking, and in this respect it is similar to sorghum and millet. This chapter compares quinoa with rice, as both crops are easy to use (cooking), store and transport. Quinoa, therefore, is in a stronger position than the other crops mentioned to help combat the scourge of hunger and malnutrition among children and their mothers. At the other extreme, excess weight and obesity have reached epidemic proportions during the last 25 years in all age groups and social strata. These two conditions affect 7% of under-5s, 25–30% of school-age children and ≥ 50% of the adult population (70% in the United States of America, Chile and Mexico) (Jacoby *et al.*, 2014). Quinoa and its nutritional qualities could also contribute to resolving this problem.

2. Practical advantages of quinoa over rice

Quinoa has more proteins than the other grains mentioned (Table 2), and its grains contain all the essential amino acids (tryptophan has the lowest concentration). The presence of essential amino acids has also been confirmed in the less well-known varieties of quinoa, such as those pertaining to Coastal ecotypes from central and southern Chile (Miranda *et al.*, 2012a). As mentioned, quinoa is similar to rice in terms of culinary uses, transportation and storage, but once processed quinoa grain offers superior nutritional quality. In most countries, rice is consumed mainly as “white rice”. White rice differs from the wholemeal rice because it has been dehusked, a process which results in a significant reduction in protein content (Table 3). On the other hand, in the case of quinoa, while peeling and/or washing is often necessary to remove the saponins from the epicarp (few varieties are sweet or lacking in saponins), far fewer proteins are lost than in the case of rice, where 16–17% of high quality proteins are lost during the peeling process. In contrast, with quinoa, removal of the outer seed layer , which is rich in saponins, fibre and flavonoids, but protein-poor, results in the consumable grain gaining roughly 6% in relative protein mass (Table 3). This is explained by the fact that in cereals such as rice, the protein is in the outer layer of the grain, which is mostly eliminated during dehusking, while in Amaranthaceae and Chenopodiaceae (i.e. quinoa), the proteins are in the embryo itself, which is barely touched during peeling or washing (the desaponification process). Although rice and quinoa are processed using similar systems (dehusking) and both maintain their culinary, transportation and storage qualities, peeled quinoa distinguishes itself by retaining superior nutritional properties compared with white rice. This is an important factor when countries consider use of quinoa as a rapid palliative to tackle conditions of hunger or chronic malnutrition. Likewise, quinoa can have an important role in the diets of pregnant women, as prenatal nutrition conditions have been clearly demonstrated to affect the survival, growth and health of children, in both the short and the long term (Pinho Franco and Nigro, 2003; Nauta et al., 2013). Given the exceptional characteristics of quinoa in terms of its comparative contents of calcium, iron, vitamins and high quality oils (Table 4), it is very important that quinoa be used in emergency situations to at least partially replace white rice, particularly among mothers and children at risk of malnutrition. It is even more important that its use be extended to all mothers whose poor nutritional habits during pregnancy could have a negative effect on the health of their children, also in the long term. This is well documented in quantitative studies of the frequency of chronic diseases in different groups for which prenatal nutritional conditions are known, and some long-term studies in developed countries correlate the conditions of children at birth and their health status 20 years later (Barker et al., 1989; Vieau, 2011).

Another advantage of quinoa is that it consumes less energy during cooking, particularly compared with wholemeal rice. Quinoa only takes 15 minutes to cook, and once the water is boiling, the heat is turned off and the pan is covered and set aside. The accumulated heat is sufficient to complete the cooking process and energy is saved. Only white rice – with its inferior nutritional quality – allows this level of energy saving.

3. Nutritional efficiency in relation to water use

Another reason for which FAO has chosen quinoa as a model crop is its physiology of adaptation to stress, particularly its highly efficient use of water (Martínez *et al.*, 2009). The quantity of grain obtained per

Table 2. Proteins (% dry) before and after the peeling process, in quinoa and rice

Stage of analysis	Rice+	Quinoa*
Before peeling (wholemeal grain)	8.4	12.8
After peeling (white grain)	6.7	13.7
Protein change (%)	-16.6	+6.4

+ Based on the information shown on the labelling of commercial rice of the same brand in organic trade in Aix-en-Provence, France (sampling by the author).

*Average of three samples taken from the locality of Cahuil, central Chile (data provided by the author, CORFO project) litre of water is therefore another useful criterion for comparing quinoa with rice and promoting the crop as a genuine, at least partial, replacement for rice. Table 3 shows the water-use footprint – yield per volume of water – of various grains. It can be seen that quinoa is higher-yielding than the other grains, in terms of water-use efficiency. Efficiency is even greater if one considers not only kg/ha of grain, but also quantity of protein/kg. Indeed, quinoa's water-use efficiency is also reflected in the crop's high nutritional efficiency, which is 10 times more water-efficient in terms of protein production than white rice.

4. Anti-oxidant effects and functional properties of quinoa

The earliest studies of the nutritional aspects of quinoa identified a number of functional attributes such as the high quality of its starch (Lindeboom, 2005; Ahamed *et al.*, 1998; Ogunbengle, 2003) and its low glucose and fructose contents, which help maintain a low glycaemic index (Oshodi *et al.*, 1999). This functional quality does not preclude another functional quality that is very important in today's world, namely the capacity to provide greater post-consumption satiety (Berti *et al.*, 2005). This is very important, because it contributes towards healthier nutritional habits, particularly given the increased risk of cardiovascular diseases and other non-communicable diseases among children who are on or beyond the thresholds for excess weight and obesity. Nowadays, this affects not only developed countries but also emerging ones, such as Chile (Mardones, 2009; Jacoby *et al.*, 2014). Recent studies of the functional properties of quinoa identify its anti-oxidant capacity, attributed to the presence of flavonoids (Zhu *et al.*, 2001; Repo-Carrasco-Valencia *et al*., 2010). These compounds are identified

Table 3. Water-use footprint (litres of water per kg of grain) and water efficiency of protein production (g-proteins in 100 g of grain × 1 000/water footprint) for a selection of other most widely used food grains worldwide, compared to quinoa

Type of grain†	Water footprint (litres/kg)	Water efficiency of protein production per 1000 kg of grain (% proteins × 1 000 litres/water)
Rice	2497	2.7
Maize	1222	7.7
Wheat	1227	10.3
Quinoa	500*	27.8

*Estimated from an irrigation deficit study in the arid region of Chile (Martínez et al., 2009), assuming a low average yield of 1 tonne/ha (1 000 kg/ha), using only worm humus as fertilizer. For the other crops, the water footprint values were obtained from Novo et al. (2008) and from the website: www.waterfootprint.org.

† The crop protein values are shown in Table 4 (white rice is used and 13.9% for quinoa).

Table 4. Comparison of selected nutritional qualities in quinoa and other grains, including proteins, vitamin B1 and two important minerals

Type of grain	Proteins g in 100 g (=%)	Vit B1 (mg/100 g)	Fe (ppm)	Ca (ppm)
(mg/100 g)	Fe	0.08	4.6	40
(ppm)	Ca	1.9	6.3	38
(ppm)	9.4	0.3	25	100
Mijo	11.0	0.3	30	201
Poroto Soja	36.5	0.9	157	2770
Sorgo	11.3	0.34	45	260
Trigo	12.6	0.3	40	360
Quinua*	9-16	0.39	133	1200

*A range is shown for proteins, which averages 13.9% between the studies of Gonzalez *et al.* (2013) and the review made by Vega-Gálvez *et al.* (2010). For the other elements and crops, average values were obtained from Schlick and Bubenheim (1996), Novo *et al.* (2008), Martínez *et al.* (2006) and Latham (2002) not only as anti-oxidants, but also as enhancers of memory and cognitive processes (Spencer, 2010). Many of these properties are lost in processes that involve high temperatures, such as extrusion (Brady *et al.*, 2007). They are not, therefore, recommended, although positive health effects have been recorded from cereal bars containing quinoa extrusions (Dogan and Karwe, 2009; Farinazzi-Machado *et al.*, 2012). Anti-oxidant capacity may be supplied by various constituents (Table 4), including flavonoids, vitamin E, and oleic, linoleic and linolenic oils, reviewed by Vega-Gálvez *et al.* (2010). Vitamin E is well recognized as an anti-oxidant and membrane protector, and it is exceptionally resilient to the high temperatures normally applied during processing (Miranda *et al.*, 2010).

Finally, quinoa's anti-oxidant capacities and other qualities yet to be discovered, may lead to new uses of the crop or its by-products, facilitating its introduction as food. One example is the recent discovery that some seeds of Chilean landrace varieties, in addition to anti-oxidant properties, exhibit notable antibacterial capacity against pathogenic bacteria such as Escherichia coli and Staphylococcus aureus (Miranda *et al.*, 2013a). Also the leaves have anti-oxidant properties and extracts containing anti-prostate cancer properties have been obtained (Gawlik-Dziki *et al.*, 2013).

5. Genotype and environmental effects on the nutritional quality of quinoa

There are studies that show that the same varieties of a crop (e.g. tomato) vary in their nutritional properties, and even in their bio-assimilation, if they are cultivated in contrasting environments. For example, tomato varieties grown in Spain, in an arid environment, have a high carotenoid content and good capacity of assimilation (Aheren *et al.*, 2010). In the case of quinoa, recent studies of crops of the same varieties (Bolivian and Argentinian) grown in different environments also reveal differences in total protein content – but without substantial losses and broadly maintaining the balance between the different amino acids (González *et al.*, 2012). For two Chilean varieties, a registered hybrid cultivar ('Regalona') and a landrace variety ('Villarica') from the humid south of Chile (39°S), when grown in a hyper-arid zone (30°S) showed no significant changes in total protein content, but did show significant changes in certain minerals. For example, more iron was found in seeds grown in an arid zone, despite the poor iron content of the soil (Miranda et al., 2013). Moreover, the seeds of the 'Regalona' and 'Villarica' varieties grown in the arid zone (low levels of irrigation) increased significantly ($P < 0.05$) in terms of yield (4.2 and 5.1 tonnes/ha, respectively), soluble dietary fibre (16.8 ± 0.4 and 28.9 ± -2.1 g/kg of dry matter [DM], respectively), vitamin B3 (2.44 ± 0.005 and 2.26 ± 0.04 mg/100 g DM, respectively), saponins (3.22 ± 0.38 mg/100 g DM for 'Regalona'), phenolic compounds (19.2 ± 5.8 and 31.92 ± 1.14 mg equivalent of gallic acid per 100 g DM, respectively), and in their proximate component analysis (except for proteins). In contrast, in their original environments (cold and rainy damp climate), the seeds were found to be larger (2.22 ± 0.17 mm for 'Villarica'), with greater 1 000-seed weight (3.08 ± 0.08 and 3.29 ± 0.08 g, respectively) and higher insoluble dietary fibre content (112.3 ± 23.8 g/kg DM for 'Regalona'). Moreover, vitamin C was greater in arid environments (31.22 ± 4.2 mg/100 g DM), but 'Villarica' had a higher content in its original climate (49.3 ± 5.36 mg/100 g DM). These results suggest that many of quinoa's properties are maintained with geographic changes involving different soils and climates. Nonetheless, the concentrations of some elements and molecules change, which may or may not be favourable and must be evaluated on a case-by-case basis: depending on the desirable value for a specific human nutritional need, an appropriate genotype and environment combination can be considered.

6. Prospects

Given the protein content of quinoa leaves (sometimes > 20%, as shown in this volume's chapter on forage properties, Blanco-Callisaya, 2013) the plant can be used for human consumption, not only of the grains, but also of the sproutings and leaves. While nutritional properties of the plant structure may be susceptible to change, there are many other advantages, both culinary and functional, and quinoa has good adaptations to new climates, soils and other cultures. Further studies are needed on bio-assimilation in both in vitro and in vivo models, as well as in humans. For example, it has been shown that the bio-assimilation of the iron contained in quinoa is greater in the case of germinated sproutings than in ungerminated seeds (Valencia *et al.*,1999). It is also possible that the recent detection of isoflavins in quinoa seeds (Lutz *et al.*, 2013) might mean that this food product could enhance the quality and quantity of milk for infant breast-feeding, with significant benefits for children under 5, considering also the presence of the insulin-type growth factor (IGF-1) (Ruales *et al.*, 2002).

Saponins also represent an opportunity for more widespread development of quinoa. Saponins are a waste product that to date have rarely been a major focus of applied research, but to valorize the saponin content of quinoa would help the crop gain recognition and even be considered as a partial replacement for rice. Studies on other saponins have shown that their flavonoids are potential anti-cancerogenous agents (Man *et al.*, 2010). They also act against pests and agricultural diseases (Stuardo and San Martin, 2008; San Martin *et al.*, 2008). While eliminating saponins may entail major costs, it has already been noted that this process does not reduce its nutritional quality (unlike in of rice), and the waste product generated could have a high value. Preliminary studies, for example, show a positive effect on the flower opening of ornamental plants, when the water used in the simple washing of quinoa before consumption is then used to irrigate the plants (Figure 1). All of these possibilities are opportunities requiring quality research to enable quinoa to recover the role it used to have in ancestral times in South America. Moreover, this role could be expanded to other regions of the world, and the Rice of the Incas could represent an opportunity for the entire planet, particularly for people whose nutrition is qualitatively and/or quantitatively deficient. In this case, the problems of adapting quinoa as a food solution could pose a problem, owing to the potential negative effects of loss of local agrobiodiversity resulting from the introduction of a new crop. Nonetheless, this type of introduction has occurred in the past, for example with potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum* L.) without major losses of local agrobiodiversity. It is important to study the local reality, case by case, prior to any massive introduction programme, particularly if the urgency of hunger or malnutrition means that mistakes must be avoided at all cost. For example, in Mali (12°N, West Africa), attempts have been made for 3 years to test the performance of previously unknown varieties of quinoa, with good results reported in the dry season, but many pests and diseases in the wet season (Coulibaly *et al.*, 2013). In this case, potential is observed, but at the same time every care must be taken to complete all research stages before launching into a massive introduction process.

7. Conclusions

The nutritional value of quinoa recognized in every literature review over the last 20 years can only be confirmed and enriched further by more research. The high quality proteins contained in its seeds are little affected by the cultivation conditions, particularly in water-deficit situations. This makes the plant very resilient, a useful quality where agriculture faces problems of aridity, degraded or salinized soils, and even excess greenhouse gas emissions. The 20 amino acids often maintain their proportions in different cultivation conditions, with little impact on the quality of their proteins. Their minerals (P, K, Ca, Mg, Mn, Zn), oils, vitamins (B1, B2, B3, C, E) and flavonoids seem to combine with each other synergetically, to give this plant highly nutritional and anti-oxidant qualities, maintained even under processing at high temperatures. Its advantages over rice, its similar culinary uses and post-harvest processes, and its low water demand, suggest that quinoa grains could be at least a partial replacement for rice, with excellent benefits for human health, in both deficit populations and in populations where nutrition problems arise from excess.

Figure 1. Ornamental flowers (*Gazania* sp.) Irrigated with water (controls) and with a solution of the same water after being used to wash quinoa grains (unpublished data provided by the author).

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CHAPTER: 3.5.

Nutraceutical Perspectives of Quinoa: Biological Properties and Functional Applications

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is an amaranthaceous plant that has been recognized for centuries as an important food crop in the South American Andes. Its grains are highly nutritious; with a high protein and bioactive compound content that surpasses traditional cereal grains in terms of biological value. Quinoa is a nutritionally well-balanced food product with multiple functions associated with the reduction of chronic disease risk, thanks to its anti-oxidant, anti-inflammatory, immunomodulatory, anticarcinogenic and other properties. This chapter provides an up-to-date overview of the nutraceutical perspectives of quinoa, based on various scientific studies of its biological properties and functional applications beneficial to human health.

1. Introduction

Quinoa is an ancestral Andean grain crop that has become the subject of worldwide attention in recent years because of its nutritional and functional value, its potential for pharmaceutical applications (Bhargava *et al.*, 2006; Hirose *et al.*, 2010; Vega-Gálvez *et al.*, 2010) and its capacity to prosper in adverse conditions (e.g. soil salinity, extreme pH, drought and frosts) (Jacobsen *et al.*, 2003; Fuentes and Bhargava, 2011). Due to these characteristics, quinoa cultivation has been introduced in new zones outside the Andean area, particularly in North America, Europe and subtropical regions of Africa and Asia; the good production results confirm its potential as a grain for human consumption (Mujica *et al.*, 2001; Casini, 2002; Jacobsen, 2003; Bhargava *et al.*, 2007; Pulvento *et al.*, 2010). In this context, the nutritional value of quinoa is now recognized for its high-quality protein (particularly rich in essential amino acids) and for its carbohydrate content (with a low glycemic index and generally higher nutritional and functional qualities than cereal grains such as maize, oats, wheat and rice) (Ruales and Nair, 1993a, 1994; Repo-Carrasco *et al.*, 2003; Repo-Carrasco-Valencia *et al.,* 2010). Accordingly, the Food and Agriculture Organization of the United Nations (FAO) declared 2013 the International Year of Quinoa, in recognition of its role in attaining food and nutritional security, and its potential for eradicating poverty (United Nations, 2011).

2. Nutritional and phytochemical composition of quinoa

Various studies have reported the nutritional composition of quinoa, highlighting in particular the biological value of its grains: high protein concentration (Bhargava *et al.*, 2007; Miranda *et al.*, 2012, 2013); starch and dietary fibre content – around 60% and 13%, respectively (Ruales and Nair, 1994; Tapia *et al.*, 1979; Repo-Carrasco-Valencia and Serna *et al.*, 2011); and oil content of 4.5–8.7% (Ruales and Nair, 1993a; Repo-Carrasco *et al.*, 2003) in the following proportions: 24% oleic, 54% linoleic and 4% α-linoleic (Wood *et al.*, 1993; Fleming and Galwey, 1995). Quinoa is also considered a good source of riboflavin, thiamine, folic acid and both α and γ-tocopherols. In comparison with other grains, it has high concentrations of calcium, phosphorus, magnesium, iron, zinc, potassium and copper (Torrez *et al.*, 2002; Jancurová *et al.*, 2009; USDA, 2013a; Table 1). In addition, significant quantities of bioactive components, such as phytosterols, betaines, squalene, ecdysteroids, fagopyritols, carot-

Table 1. Nutrient content of *Chenopodium quinoa* (USDA, 2013a).

Nutriente	Unit	Value per 100 g
Proximal		
Water	g	13.28
Energy	kcal	368
Energy	kJ	1539
Protein	g	14.12
Total lipids (fat)	g	6.07
Ashes	g	2.38
Carbohydrates, by difference	g	64.16
Fibre, total dietary	g	7.0
Starch	g	52.22
Minerals		
Calcium, Ca	mg	47
Iron, Fe	mg	4.57
Magnesium, Mg	mg	197
Phosphorus, P	mg	457
Potassium, K	mg	563
Sodium, Na	mg	5
Zinc, Zn	mg	3.10
Copper, Cu	mg	0.590
Manganese, Mn	mg	2033
Selenium, Se	µg	8.5
Vitamins		
Thiamine	mg	0.360
Riboflavin	mg	0.318
Niacin	mg	1520
Pantothenic acid	mg	0.772
Vitamin B6	mg	0.487
Vitamin C., total ascorbic acid*	mg	22.39
Pholate, total	µg	184
Betaine	mg	630.4
Luteine + zeaxantine	µg	163
Vitamin E (alpha-tocopherol)	mg	2.44
Tocopherol, beta	mg	0.08
Tocopherol, gamma	mg	4.55
Tocopherol, delta	mg	0.35
Lipids		
Fatty acids, total saturated	g	0.706
Fatty acids, total mono unsaturated	g	1613
Fatty acids, total polyunsaturated	g	3292

enoids, vitamin C and polyphenols (e.g. kaempferol and quercetin; Tables 2 and 3), have been identified in its grains (De Simone *et al.*, 1990; Berghofer and Schoenlechner, 2002; Taylor and Parker, 2002; Dini *et al.*, 2004, 2005; Wijngaard and Arendt, 2006; Álvarez-Jubete *et al.*, 2010), which have been widely reported as having beneficial health effects (Dini *et al.*, 2010). Moreover, the leaves of quinoa contain a considerable quantity of ash (3.3%), fibre (1.9%), nitrates (0.4%), vitamin E (2.9 mg α TE/100 g), sodium (289 mg/100 g), vitamin C (1.2–2.3 g/kg) and proteins (27–30 g/kg) (Bhargava *et al.*, 2006). Quinoa leaves, like its grains, also contain a large quantity of bioactive compounds, such as ferulic, sinapinic and gallic acid; kaempferol, isorhamnetin and rutine (Gawlik-Dziki *et al.*, 2013). Nevertheless, a number of so-called “anti-nutritional” elements have also been reported in its grains, including tannins, protease inhibitors, phytic acid and saponins (Chauhan *et al.*, 1992; Ruales and Nair, 1993b). The main shortcoming of quinoa is, therefore, the bitter taste of its grains, resulting from the saponins which are present in the external seed layers and have been widely described as an anti-nutrient due to their strong binding affinity to minerals (Brady *et al.*, 2007). However, there is increasing evidence that saponins can have beneficial health effects (e.g. anticarcinogenic and hypocholesterolemic effects) (Álvarez- Jubete *et al.*, 2010; Kuljanabhagavad *et al.*, 2008).

2.1 Polyphenols

Polyphenols are bioactive secondary metabolites of plants that are widely present in foodstuffs of plant origin. The three main types of polyphenol are flavonoids, phenolic acids and tannins, and they act as potent anti-oxidants (Repo-Carrasco-Valencia *et al.*, 2010). Polyphenols can also contribute to the bitterness, astringency, colour, taste and oxidative stability of food products (Han *et al.*, 2007; Scalbert *et al.*, 2005; Shahidi and Naczk, 1995).

Phenolic compounds are currently of major interest for their dietary effect resulting from their multiple properties, including: anti-oxidant (cardiovascular prevention), anti-allergic, anti-inflammatory, antiviral and anticarcinogenic (Nishibe *et al.*, 1996; Dini *et al.*, 2004; Aalinkeel *et al.*, 2008; Pasko *et al.*, 2008; Khan *et al.*, 2010). The anti-oxidant activity and presence of phenolic compounds in quinoa grain have been investigated using various methodologies (Zhu *et al.*, 2001; Nsimba *et al.*, 2008; Pasko *et al.* 2008, Repo-Carrasco-Valencia *et al.*, 2010; Miranda *et al.*, 2013). The total phenol content and free-radical ab-

Table 2. Total soluble phenolic acid content of seeds[1] and leaves[2] of *Chenopodium quinoa* (mg/100 g)

		Pasko *et al.* (2008)[*, 1]	Repo-Carrasco-Valencia *et al.* (2010)[†, 1]	Gawlik-Dziki *et al.* (2013)[£, 2]
Compound	**Unit**	**Value por 100 g**	**Value por 100 g**	**Value por 100 g**
Caffeic acid	mg	4.0	0.7 ± 0.4	s.d.
Ferulic acid	mg	s.d.	15.0 ± 3.0	76.2 ± 4.2
o-Coumaric acid	mg	s.d.	s.d.	0.23 ± 0.02
p-Coumaric acid	mg	s.d.	8.0 ± 7.0	3.3 ± 0.3
p-OH Benzoic acid	mg	7,7	2.9 ± 0.6	1.0 ± 0.1
Vanillic acid	mg	4.3	11,0 ± 2,0	2.3 ± 0.2
Gallic acid	mg	32.0	s.d.	16.3 ± 1.2
Cinnamic acid	mg	1.0	s.d.	s.d.
Chlorogenic acid	mg	s.d.	s.d.	3.7 ± 0.2
Syringic acid	mg	s.d.	s.d.	1.9 ± 0.01
Sinapinic acid	mg	s.d.	s.d.	19.3 ± 1.13
Benzoic acid	mg	s.d.	s.d.	0.15 ± 0.02
TOTAL	**mg**	**49.0**	**37.0 ± 9.0**	**124.4 ± 7.4**

† Average content of ten different genotypes of quinoa.
* Content of only one quinoa genotype from Bolivia.
£ Content of only one genotype (‘Faro’ cultivar, Chile), grown in Poland, obtained from three independent experiments.

Table 3. Flavonoid content in seeds[1] and leaves[2] of *Chenopodium quinoa* (mg/100 g)

		Pasko *et al.* (2008)[*, 1]	Repo-Carrasco-Valencia *et al.* (2010)[†, 1]	Gawlik-Dziki *et al.* (2013)[£, 2]
Compound	**Unit**	**Amount per 100 g**	**Amount per 100 g**	**Amount per 100 g**
Myricetin	mg	n.a.	0.5 ± 0.5	n.a.
Quercetin	mg	n.a.	36.0 ± 13.0	0.68 ± 0.06
Kaempferol	mg	n.a.	20.0 ± 20.0	4.6 ± 0.5
Isorhamnetin	mg	n.a.	0.4 ± 0.7	0.31 ± 0.02
Rutine	mg	36.0	n.a.	6.2 ± 0.6
Orientin	mg	107.6	n.a.	n.a.
Vitexin	mg	70.9	n.a.	n.a.
Morin	mg	8.9	n.a.	n.a.
Hesperidin	mg	0.2	n.a.	n.a.
Neohesperidin	mg	0.2	n.a.	n.a.
TOTAL	**mg**	**223.8**	**58.0 ± 13.0**	**11.8 ± 1.2**

† Average content of ten different genotypes of quinoa.
* Content of only one quinoa genotype from Bolivia.
£ Content of only one genotype ('Faro', Chile), grown in Poland, obtained from three independent experiments.

sorbance capacity (DPPH – diphenyl-P-picrylhydrazyl) in quinoa grains have displayed, respectively, average values of 1.11 and 42.3 mg of gallic acid equivalent/g. These concentrations are well above those found in the seeds of traditional cereals, such as barley (0.16 mg of gallic acid equivalent/g), wheat (0.36 mg of gallic acid equivalent/g), rice (2.5 mg of gallic acid equivalent/g) and millet (17.7 mg of gallic acid equivalent/g), suggesting that quinoa has great potential as a cereal substitute (Asao and Watanabe, 2010; Djordjevic *et al*., 2010).

Recently, the phenolic acid content of quinoa has been reported to comprise mainly caffeic, ferulic, p-coumaric, p-OH-benzoic, vanillic, gallic and cinnamic acids (Repo-Carrasco-Valencia *et al.*, 2010; Pasko *et al.*, 2008; Table 2). In addition, the flavonoid content consists predominantly of quercetin and kaempferol, while some varieties have abundant orientin, vitexin and rutine (Pasko *et al.*, 2008; Álvarez-Jubete *et al.*, 2010; Hirose *et al.*, 2010; Repo-Carrasco- Valencia *et al.*, 2010; Table 3).

As the presence of flavonoids in edible plants improves their nutraceutical value in terms of health-promoting effects, the flavonoid content of several plant species has recently been published online, based on a number of different studies (USDA, 2013). However, this valuable online dataset does not yet include the flavonoid content of quinoa, despite the fact that quinoa is a more effective functional food, in terms of a source of bioactive flavonoids, than conventional cereal and pseudocereal grains, with a flavonoid content even exceeding that of berries such as lingonberry (*Vaccinium vitis-idaea*) and American cranberry (*Vaccinium macrocarpon*).

The concentration in quinoa seeds of the glycosides, daidzin (4',7-dihydroxyisoflavone) and genistin (4',5.7-trihydroxyisoflavone), together with their respective aglycones, daidzein and genistein, was recently reported for the first time (Lutz *et al.*, 2013). These isoflavones are considered phytoestrogens due to their ability to bind with estradiol receptors (ER) (Ye *et al.*, 2009). Several analyses of these isoflavones in food products have determined a wide range of concentrations, found mainly in leguminous grains in the form of glycosides of daidzein, genistein and glycitein. Nonetheless, in cereal flour (oats, wheat and maize), trace or unquantifiable amounts of these compounds have been reported (Adlercreutz and Mazur, 1997; Horn-Ross *et al.*, 2000; Liggins *et al.*, 2002; USDA, 2008). Thus, the content of daidzein and genistein in quinoa seeds, based on local Chilean ecotypes, was reported as 0.7–1.15 and 0.05–0.25 mg/100 g, respectively; whereas in commercial quinoa seeds, the daidzein and genistein content was 0.78–2.05 and 0.04–0.41 mg/100 g, respectively. The concentration of these isoflavones in plants generally depends on factors such as genetic diversity, environmental influence, harvesting and processing conditions (Tsukamoto *et al.*, 1995). Interestingly, the darker coloured quinoa seeds obtained from high Andean (Altiplano) zones displayed a higher isoflavone content – demonstration of their potential as a source of health-promoting bioactive compounds (Lutz *et al.*, 2013).

2.2 Triterperpenoids

A group of triterpenoid compounds widely documented in quinoa are saponins. Located mainly in the outer layers of the grain (the pericarp), they are characterized by a bitter taste and the formation of foam (saponins soluble in water), making the grain basically unpalatable (Brady *et al.*, 2007). The saponin content in seeds varies between sweet and bitter genotypes: 0.2–0.4 and 4.7–11.3 g/kg dry matter (DM), respectively (Mastebroek *et al.*, 2000). Therefore, most quinoa seeds on the market have been treated to remove their cover, then washed with water or scarification – but not the sweet varieties, which have no saponins (or only in concentrations of < 1.1 g/kg DM) (Abugoch, 2009). To date, 20 types of triterpenoid of saponins have been described in quinoa, isolated from various parts of the plant (flowers, fruit, seed pericarp and the seeds themselves) (Mizui *et al.*, 1988, 1990; Cuadrado *et al.*, 1995; Mastebroek *et al.*, 2000; Kuljanabhagavad *et al.*, 2008). The saponins' structure has been illustrated through chemical analysis and 1D and 2D nuclear magnetic resonance spectroscopy (NMR) (Wink, 2004; Kuljanabhagavad *et al.*, 2008). It has thus been possible to distinguish monodesmosidic saponins (one carbohydrate chain) and bidesmosidic saponins (two carbohydrate chains), comprising units of arabinose, glucose, galactose, glucoronic acid, xylose and rhamnose (Kuljanabhagavad and Wink, 2009). It has been established that the saponins are molecules derived from oleanolic and hederagenin acids, phytolaccagenic acid, serjanic acid and 3β,23,30-trihydroxy olean-12-en-28-oic acid, which have C-3 and C28 hydroxyl and carboxyl groups, and are modulated by β-amyrin enzyme activity (Kuljanabhagavad *et al.*, 2008).

Studies of saponins isolated from the quinoa seed pericarp have revealed antimicrobial activity, toxicity in Artemia, antiviral activity, the capacity to reduce cholesterol levels, and increased drug absorption through mucous membranes, modifying intestinal permeability (Meyer *et al.*, 1990; Bomford *et al.*, 1992; Mahato and Kundu, 1994; Estrada *et al.*, 1999; Woldemichael and Wink, 2001; Stuardo and San-Martín, 2008; Kuljanabhagavad and Wink, 2009). Saponins may even act as an immunological adjuvant to boost the response of gene-specific antibodies (Estrada *et al.*, 1998; Verza *et al.*, 2012), and may induce cytotoxicity and apoptosis in cancer cell lines (Kuljanabhagavad *et al.*, 2008) – demonstration of quinoa's great potential in various therapeutic applications.

In addition to saponins, quinoa possesses a special class of triterpene molecules, known as phytoecdysteroids (Kumpun *et al.*, 2011), which present a wide range of pharmacological effects in mammals (Lafont and Dinan, 2003; Báthori *et al.*, 2008). Ecdysteroids are steroid hormones which control moulting and reproduction in arthropods. The main phytoecdysteroid found in quinoa plants is 20-hydroxyecdysone (20E), with an average concentration of 365 ± 51 mg/kg (Báthori *et al.*, 2005; Kumpun *et al.*, 2011). Several beneficial effects on the functioning of various organs have now been recognized (Dinan and Lafont, 2006) – for example, the anabolic property of 20E as a precursor of protein synthesis in muscle cells in humans and mice (Gorelick-Feldman *et al.*, 2008; Báthori *et al.*, 2008). Other studies have shown that 20E controls the regulation of blood glucose levels and activity against associated obesity (Chen *et al.*, 2006; Foucault *et al.*, 2011). The antidiabetic effect of this molecule has been observed in mice, through a reduction in adiposity when fat-enriched diets are used, supplemented with this phytoecdysteroid (Kizelsztein *et al.*, 2009). Furthermore, these compounds can be considered potent chemical agents capable of preventing or retarding damage to the skin associated with the activity of the enzyme collagenase and the effects of oxidative stress (Nsimba *et al.*, 2008).

3. Biological properties and functional applications

3.1 Anti-oxidant effect

Natural anti-oxidants have an important role in inhibiting free radicals and oxidative chain reactions at the tissue and membrane levels (Nsimba *et al.*, 2008). Most of the phenolic compounds in quinoa possess anti-oxidant activity (Gorinstein *et al.*, 2007; Repo de Carrasco and Encina Zelada, 2008; Pasko *et al.*, 2009). Nsimba *et al.* (2008) evaluated the anti-oxidant activity of several quinoa extracts (cultivated in Japan and Bolivia) and reported a high level of anti-oxidant activity in the grains, exceeding even that of amaranth. Different methodologies were adopted: ferric oxide/reduction potential (FRAP), 2.2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2.2-diphenyl- 2-picryl-hydrazyl (DPPH), with values of 4.97 mmol Fe^{2+}/kg DM, 27.19 mmol trolox/kg DM and 38.84 mmol trolox/kg DM, respectively.

The anti-oxidant activity of bitter and sweet quinoa seeds, both before and after cooking, was evaluated by Dini *et al.* (2010), with the aim of establishing which seed types have the best anti-oxidant property, and how it may be affected by traditional cooking methods. The results, obtained through the DPPH and FRAP methods, revealed a higher level anti-oxidant activity in the bitter seeds than in the sweet ones. This high level of activity depended mainly on the presence of phenols and flavonoids; on the other hand, in sweet seeds, the anti-oxidant activity was the result of the presence of compounds such as phenols, flavonoids and carotenoids. Cooking caused a significant drop in anti-oxidant capacity in both types of seed: 50.4% in sweet seeds and 45.4% in bitter ones (Dini *et al.*, 2010).

The bioactivity of the isoflavones has also been reported to have an anti-oxidant effect (Gopalakrishnan *et al.*, 2006; Lo *et al.*, 2007; Jian *et al.*, 2010). Given the beneficial effect of the isoflavones, they have been used in the formulation of several functional foods, following *in vitro*, *in vivo* and clinical studies, with the aim of reducing risk factors for chronic conditions such as cardiovascular diseases, neurodegenerative diseases, osteoporosis and cancer (Song *et al.*, 2007; Jian *et al.*, 2010; Lutz, 2011); they have also been used in food formulations to improve maternal milk production during lactation (Zhang *et al.*, 1995; Liu*et al.*, 1999; Groot, 2004). Indeed, the traditional use of quinoa by the Aymara population of the Altiplano (highlands) in northern Chile suggests that eating quinoa promotes lactation (Lutz *et al.*, 2013). Considered as a whole, these data support quinoa's potential as a food supplement capable of enriching a normal diet by providing sources of natural phenolic compounds with anti-oxidant properties.

3.2 Hypocholesterolemic and antihypertensive effects

Cholesterol, produced in the liver and absorbed through the diet, is necessary for normal metabolic processes. Nonetheless, high total cholesterol levels and high levels of low-density lipoproteins (LDLs) are associated with a high risk of developing coronary diseases (Quillez *et al.*, 2003). In this context, several studies have shown that the presence of sterols in plants inhibits the body's absorption of cholesterol (Moreau *et al.*, 2002). The evaluation of phytosterol content in quinoa seeds indicates the presence of β-sitosterol (63.7 mg/100 g), campesterol (15.6 mg/100 g) and stigmasterol (3.2 mg/100 g), and that the content of these components is greater than that reported for seeds of squash, barley and maize, but less than for lentil, pea and sesame seeds (Ryan *et al.*, 2007).

The use of protein isolates obtained from quinoa seeds (> 10% grain) significantly reduced plasma and liver total cholesterol levels in mice fed with fat enriched diets (Takao *et al.*, 2005). In addition, the use of these quinoa protein isolates displayed bile-acid binding activity *in vitro* and modulation of the expression in the liver of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) – an enzyme crucial for cholesterol biosynthesis. These results suggest that the prevention of increased plasma and liver cholesterol in mice fed with a diet containing quinoa protein isolates can be attributed to the inhibition of bile-acid reabsorption in the small intestine and the control of cholesterol synthesis and its catabolism. Other studies using quinoa flour, or hydrolyzed quinoa protein, have also shown that the bioactive properties of quinoa can significantly lower blood pressure in mice and rats (Aluko and Monu, 2003; Ogawa *et al.*, 2001). *In vivo* studies using a 3% concentration of quinoa grain pericarp as a dietary supplement also displayed a significant reduction in plasma and liver cholesterol levels in mice (Konishi *et al.*, 2000), possibly due to the content of water-soluble dietary fibre, as reported with oats, rice bran and other fibres (Anderson *et al.*, 1994; Truswell, 1995).

There is considerable evidence that the administration of fructose-enriched food products in rats induces an adverse alteration in metabolism and oxidative status, leading to hypertriglyceridemia, an increase in blood pressure, obesity, and impaired glucose tolerance and insulin resistance (Ackerman *et al.*, 2005; Tappy *et al.*, 2010; Pasko *et al.*, 2010a). Studies performed on rats to evaluate the effects of a diet supplemented with quinoa seeds on the biochemical parameters in plasma and tissues when fed with a high-fructose diet, showed that the addition of quinoa seeds to the diet affected oxidative status by reducing plasma malondialdehyde (MDA) and anti-oxidant enzyme activity, such as superoxide-dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), in plasma, heart, kidney, testicles, lungs and pancreas. Moreover, the incorporation of quinoa in the diet reduced total cholesterol, LDL, triglyceride and glucose levels, and lowered the total plasma protein level, without a reduction in high density lipoproteins (HDLs). These results suggest that quinoa seeds in the diet can act as a moderate protective agent against potential changes induced by fructose consumption by reducing lipid peroxidation and improving anti-oxidant capacity in the blood (plasma). They may also reduce most of the adverse effects caused by fructose in the lipid profile and glucose levels (Pasko *et al.*, 2010a, b). These studies point to quinoa's potential as a coadjuvant agent in the treatment of cardiovascular diseases.

3.3 Anti-inflammatory and immunomodulatory activities

Inflammation is clinically defined as a physiopathological process characterized by redness, oedema, fever, pain and loss of functional activity. Recent studies suggest that excessive inflammation and oxidative damage contribute to several acute and chronic conditions, including autoimmune, neurological and cardiovascular diseases and cancer (Grivennikov *et al.*, 2010; Reuter *et al.*, 2010). Although the current steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat chronic inflammatory diseases, prolonged use may produce unwanted side-effects (Roubille *et al.*, 2013). Thus, natural compounds traditionally adopted in the prevention and treatment of a range of pathologies have recently received much attention for the anti-oxidant and anti-inflammatory nature of their components, either separately or combined (Basnet and Skalko-Basnet, 2011).

Quinoa grain has traditionally been used by the Andean people as a natural remedy in the anti-inflammatory treatment of muscle sprains, twists and muscular strains, placing poultices made from quinoa grains (especially the "black" type) mixed with alcohol on the affected zones (FAO, 2011). The literature suggests that the quinoa saponins are responsible for anti-inflammatory activity (Mujica, 1994). One type of monodesmosidic saponin, called 3-O-β-D-glucopyranosyl oleanolic acid, isolated from the seeds of *Randia dumetornm* Lam., has been described as having strong anti-inflammatory activity in doses of 25 and 100 mg/kg (with LD50 values of 3 600 mg/kg in mice and 1 500 mg/kg in rats) (Ghosh *et al.*, 1983).

Interestingly, this type of monodesmosidic saponin has also been reported in quinoa seeds (Ma *et al.*, 1989), suggesting that the anti-inflammatory activity of quinoa could be linked to the monodesmosidic compounds present in its grain (Kuljanabhagavad and Wink, 2009). The analysis of monodesmosidic saponins based on different parts of the quinoa plant (seeds, seed-coat and flowers obtained from the commercial seed supplier *Compañía de Semillas Avelup* in Temuco, Chile), using liquid chromatography-mass spectrometry (LCMS), identified the component 3-O-β-D-glucopyranosyl hederagenin. This monodesmosidic saponin isolated from *Hedera colchica* fruits has also been described as having a high level of anti-oxidant activity (Gülçin *et al.*, 2006), and it could be related to the monodesmosidic 3-O-β-D-glucopyranosyl hederagenin found in quinoa seeds (Kuljanabhagavad and Wink, 2009).

Oleanane-type pentacyclic triterpene saponins are natural components of several plants (Mahato and Nandy, 1991; Vincken *et al.*, 2007), and many of them have been used as an anti-inflammatory remedy in traditional medicine (Sosa *et al.*, 2007; Wiart, 2007; Liu and Henkel, 2002; Kim *et al.*, 2002). Quinoa has oleanolic acid as its main aglycon in seeds and hederagenin in leaves (Mastebroek *et al.*, 2000; Cuadrado *et al.*, 1995), and they have been implicated in various anti-inflammatory molecular mechanisms (Hwang *et al.*, 2010; Wang *et al.*, 2013). Given the pharmaceutical potential of triterpene saponins, future research should focus on the characterization and use of quinoa saponins for their application as anti-inflammatory agents and coadjuvants in the absorption of certain drugs, given their capacity to induce changes in intestinal permeability (Gee *et al.*, 1989; Johnson *et al.*, 1986; Oakenfull and Sidhu, 1990). The low toxicity level of these natural compounds means that the triterpenes present in quinoa could serve as a natural source of new elements in the development of drugs (Kuljanabhagavad and Wink, 2009).

One of the classic illnesses with an important inflammatory component is coeliac disease – a chronic autoimmune enteropathy, triggered by the dietary gluten found in wheat, barley and rye (Abugoch, 2009). The proteins of these cereals can be classified, according to their solubility in alcohol and acids, as prolamins and glutenins, which are partially resistant to human proteases. When ingested by coeliac-disease patients, several immunogenic gluten peptides are generated, activating the immune system through multiple cellular channels and resulting in premature ageing of the mucus enterocytes that cover the intestinal tract (Schuppan *et al.*, 2009; Di Sabatino and Corazza, 2009; Tjon *et al.*, 2010).

The present-day treatment of this disease basically consists of adhering to a strict gluten-free diet. Quinoa grain thus represents a consumption alternative for patients with coeliac disease, given its high biological value and low prolamin concentration (≤ 7%), having a distant phylogenetic relationship with cereals that contain gluten. Nevertheless, there are only limited experimental data in the literature to support this recommendation (Zevallos *et al.*, 2012). However, some studies have adopted *in vitro* approaches to examine the feasibility of using quinoa for coeliac patients (Vincenzi *et al.*, 1999; Berti *et al.*, 2004; Bergamo *et al.*, 2011). On the basis of these studies, it was concluded that quinoa can be a safe component of a gluten-free diet; but none of the quinoa cultivars used in the evaluations were known. Only Bergamo *et al.* (2011) reported the immunological non-reactivity of quinoa on the basis of duodenum biopsies taken from coeliac patients.

Recently, Zevallos *et al.* (2012) evaluated the immune effect of 15 quinoa cultivars by testing the proliferation of T/interferon-γ (IFN- γ) cells obtained from the small intestine of coeliac patients, and the production of IFN- γ/IL-15 following the cell culturing of duodenum biopsy samples. The test results suggest that quinoa grain is safe for patients with coeliac disease. Nevertheless, a high degree of variability was observed in the immune effect of quinoa proteins, depending on the cultivar analysed, as previously described for oats (Comino *et al.*, 2011). It was also reported for the first time the activation of T-cells by proteins obtained from two quinoa cultivars (‘Ayacuchana’ and ‘Pasankalla’) at similar levels to those for gliadin, causing secretion of cytokines from cultured biopsy samples. Some peptides that are toxic for coeliac patients probably do exist among the quinoa proteins derived from these cultivars, but the small quantity of these epitopes may be clinically irrelevant. However, given the absence of *in vivo* data, it is difficult to anticipate the effect of quinoa consumption in coeliac patients. Accordingly, further studies – including the amino acid profile (proline and glutamine), subfractions of prolamin, and *in vivo* studies of quinoa – are required to confirm the safety of quinoa for coeliac patients and facilitate its integration in the gluten-free market (Zevallos *et al.*, 2012).

Until now, the biomedical literature has recorded only one case of allergy to quinoa (Astier *et al.*, 2009). This was reported in a 52-year old man who developed a serious systemic reaction, including dysphagia, dysphonia and generalized urticaria and angioedema following the ingestion of quinoa accompanied by fish and bread. The symptoms were resolved with intravenous corticosteroids and antihistamines. The patient’s clinical history included a previous allergic reaction to quinoa and seasonal rhinitis caused by grass pollen. The cutaneous allergy test using ground quinoa seeds was positive (an irritation diameter of 15 mm was observed, compared to just 5 mm in the control), while the results for fish and bread were negative. Quinoa protein extracts (both raw and cooked) and serum from allergic and non-allergic patients were analysed using immunoblotting. The analysis indicated reactivity for immunoglobulin E in the serum of the allergic patient, via the detection of a 35 kDa band, for extracts of both raw and cooked quinoa protein; and the band did not appear in control samples of non-allergic patients. Interestingly, the main storage proteins in quinoa seeds include type-11 S globulins known as “chenopodins” and a cysteine-rich 2S fraction. Two heterogeneous groups of polypeptides in a range of 30–40 kDa (acid subunits) and 20–25 kDa (basic units), joined by disulfur bonds in the original protein, characterize the type-11S proteins (Brinegar and Goundan, 1993). Thus, the band close to the 35 kDa displayed using immunoblotting could belong to the chenopodin A acid subunit class – which is of particular interest for the development of functional foods based on highly soluble quinoa seed proteins (Astier *et al.*, 2009).

3.4 Anti-cancer activity

Cancer is a multistage process, emerging from various cellular and molecular (e.g. genetic and epigenetic) alterations, with asymptomatic and latent properties (Hanahan and Weinberg, 2000; Li *et al.*, 2005; Su *et al.*, 2013). In this context, diet is considered a contributing factor for cancer, as well as other chronic diseases. Dietary phytochemicals present in various food products have displayed chemopreventive effects against cancer, both in preclinical models using animals and in epidemiological studies on humans (Wang *et al.*, 2012; Lee *et al.*, 2013). Numerous studies report the potential preventive activity of certain types of food against different types of cancer, identifying bioactive compounds responsible for this preventive activity in phytochemicals: apigenin (parsley), carotene (carrot), curcumin (turmeric), cyanidin (cherries), delphinidin (pomegranate), 3,3'-diindolylmethane (brussel sprouts), epigallocatechin gallate (EGCG) (green tea), fisetine (strawberry), genisteine (soybean), lycopene (tomato), naringenin (orange), phenyl isothiocyanate (PEITC) (watercress), proanthocyanidins (berries), pterostilbene (cranberry), quercetin (onion), resveratrol (grape), retinoic acid/retinol (carrot), rosmarinic acid (rosemary), silibinin (thistle), sulforaphane (broccoli), vitamin D3 (mushroom), vitamin E (sunflower) and zerumbone (ginger), among others (Wang *et al.*, 2012). They are able to block or suppress multiple biological mechanisms related to carcinogenesis, including metabolism of cancer, DNA repair, cellular protection, apoptosis, regulation of cellular cycle, angiogenesis and metastatic processes (Lee *et al.*, 2013).

The broad chemical diversity of the compounds described in quinoa has led to renewed interest in research into these compounds, particularly as potential phytotherapeutic and chemotherapeutic agents. For example, Kuljanabhagavad *et al.* (2008) used the MTT colorimetric assay (3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide) to describe the cytotoxic activity of the following saponin compounds in HeLa cervical cancer cells: 3 beta-([O-beta-d-glucopyranosyl-(1→3)-alpha-l-arabinopyranosyl]oxy)-23-oxo-olean-12-en-28-oic acid beta-d-glucopyranoside (1); 3 beta-([O-beta-d-glucopyranosyl-(1→3)-alpha-l-arabinopyranosyl]oxy)-27-oxo-olean-12-eno-28-oic acid beta-d-glucopyranoside (2); 3-O-alpha-l-arabinopyranosyl serjanic acid 28-O-beta-d-glucopyranosyl ester (3); and 3-O-beta-d-glucuronopyranosyl serjanic 28-O-beta-d-glucopyranosyl ester (4), together with their aglycons: 3 beta-hidroxy-23-oxo-olean-12-en-28-oic acid (I); 3 beta-hidroxy-27-oxo-olean-12-en-28-oic acid (II); and serjanic acid (III). The cytotoxic effects of saponins 1 and 2 (described above) were very similar (IC50 > 100 µg/ml), while the aglycons I and II of these same saponins proved to have a similar IC50 value of 25.4 µg/ml. For its part, the hederagenin (VI) aglycon, with an IC50 of 15–23 µg/ml, proved more potent than the oleanic acid (IV), with an IC50 of 62–99 µg/ml, suggesting a similar cytotoxic effect in the cervical cancer cells between aglycons I, II and IV, due to the presence of an aldehyde in its structures (Kuljanabhagavad *et al.*, 2008). This study also reported the relation between apoptosis and the inhibitory effect on cellular growth of bidesmosidic saponins and their aglycons in colorectal adenocarcinoma (Caco-2). For this purpose, a flow cytometry analysis was performed on cells treated with 100 µg/ml of bidesmosidic saponins 1–4 and their aglycons I–III for 24 hours. The results showed that the levels of apoptosis induced by saponins 1–4 were 13.18%, 13.18%, 25.50% and 26.40%, respectively; and those induced by their aglycons I–III were 51.40%, 51.40% and 50.23%, respectively, thereby correlating the apoptotic effect in Caco-2 cells with the cytotoxicity test performed on HeLa cells. These results revealed significant differences in the relation between the structure and activity of bidesmosidic saponin isolates, depending on the nature and position of the functional groups in the structure of the aglycons (Kuljanabhagavad *et al.*, 2008). Recently, studies by Gawlik-Dziki *et al.* (2013) evaluated the nutraceutical potential of quinoa leaves by analysing their phenol content and combined bioactivity using an experimental model based on the cellular culture of two prostate cancer cell lines of rats (MAT-LyLu and AT-2), characterized by having different metastatic potential. Large quantities of phenolic compounds with anti-oxidant activity (Tables 2 and 3) were observed in the quinoa leaf extracts, and were related to the effect of inhibiting cellular proliferation, migration and invasion in prostate cancer cell lines. It was shown that both the chemical extract and that obtained after *in vitro* simulated digestion exerted an inhibitory effect on the activity of the lipoxygenase enzyme, which at the same time related to the chelating, anti-oxidant and free- radical-reducing activities of these extracts. These observations revealed that the phenolic compounds of quinoa leaves can also exert a chemopreventive and anti-cancer effect, with the intervention of intracellular signalling mechanisms dependent on oxidative stress and reactive oxygen species (ROS), through synergistic effects. These results confirm the nutraceutical potential of quinoa leaves – relevant not only to the development of cancer, but also to other diseases related to oxidative stress – and they highlight new perspectives for introducing quinoa leaves into a normal diet, at least as a supplement (Gawlik-Dziki *et al.*, 2013).

4. Final remarks and future prospects

Quinoa has recently gained worldwide importance due to its nutritional benefits. The nutritional value of its grains has been widely recognized for their high quality protein (particularly rich in essential amino acids) and for their carbohydrate, oil, mineral and vitamin content. Quinoa is also considered a good source of dietary fibre and other bioactive compounds, such as polyphenols and triterpenoids. In this context, various studies adopting different biological approaches have confirmed that the bioactive components present in both seeds and leaves of quinoa possess hypocholesterolemic, anti-oxidant, anti-inflammatory and anti-cancer effects, as well as being safe for consumption by patients with coeliac disease.

Quinoa, therefore, offers great potential for use in complementary and alternative medicine. With the emergence of new technologies in the area of chemical research, molecular biology and pharmacology, the use of quinoa as a nutraceutical agent is increasingly gaining recognition. Nevertheless, further work is needed to gain a better understanding of the pharmacological potential of these phytochemicals, including their pharmacokinetic and pharmacodynamic behaviour, metabolic profile, toxicity, interaction with other compounds, stability of formulations and dosage regime, as well as potential polymorphisms which could affect therapeutic efficacy.

In short, the information presented in this chapter supports the potential of quinoa as a food supplement that could enrich the normal diet as a source of functional compounds important for reducing chronic disease risk factors and opening new perspectives for using quinoa in biomedicine.

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CHAPTER: 3.6

Quinoa, coeliac disease and gluten-free diet

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Abstract

Quinoa is an Andean crop with multiple agronomical, nutritional and industrial applications. Coeliac disease (CD) is a condition characterized by an inappropriate immune response to dietary gluten leading to histological damage of the small intestine, and an effective treatment is a lifelong gluten-free diet (GFD). Quinoa contains low concentrations of gluten and has a distant phylogenetic link with gluten-containing cereals (wheat, rye and barley). This prompted consideration of quinoa as a naturally gluten-free product, suitable for patients with CD, although evidence is scant. The present chapter aims to review the current scientific literature pertaining quinoa and coeliac disease.

Initial *in vitro* studies examined the suitability of quinoa for patients with CD using the agglutination activity of undifferentiated myeloid leukaemia cells (De Vincenzi *et al*., 1999), measuring the concentration of coeliac-toxic peptides (Berti *et al*., 2004) and analysing immune reactivity in T-cell-proliferation studies and organ culture explants (Bergamo *et al*., 2011). The main limitation of these studies was the use of one non-described cultivar. Recently, cultivars have been identified with putative toxic quinoa peptides that seem to elicit immunological activation of gliadin-specific CD4+ T-cells, as well as elicit secretion of cytokines when cultured with coeliac duodenal biopsies (Zevallos *et al*., 2012). *In vivo* studies are almost absent – with the exception of a retrospective review of dietary history of patients eating quinoa (Lee *et al*., 2009) and our feeding study (Zevallos *et al.*, 2013) – which show that short-term consumption of quinoa is well tolerated among individuals with CD and has a mild hypocholesterolemic effect that could also be relevant for a non-CD subject at risk of obesity.

To conclude, results from *in vitro* and *in vivo* studies indicate that some quinoa cultivars have small amounts of gluten-like proteins which can stimulate immune cells *in vitro*, but that do not exacerbate coeliac disease when eating as part of a GFD. However, further studies evaluating the long-term effects of quinoa consumption are needed. On the basis of current literature, it is anticipated that quinoa can be safely consumed by CD patients.

Key Words: quinoa, coeliac disease, gluten-free diet

1. Introduction

Coeliac patients are effectively treated with a gluten-free diet (GFD), which entails the strict avoidance of dietary wheat, rye and barley. Compliance to the GFD facilitates the recovery of damaged intestinal mucosal, but gluten dietary transgressions cause activation of immunopathological mechanisms that prevent mucosal healing.

Gluten-free products are manufactured mainly with maize, rice, potato and millet flours, having a significant impact on palatability, availability, nutritional value and cost. New products that can improve any of those parameters are a welcome addition to the GFD, providing that there is enough evidence to support their suitability for coeliac patients. This chapter explores the scientific evidence to support the incorporation of quinoa as part of the GFD.

2. Coeliac disease

Coeliac disease (CD) is a multi-organ autoimmune disease that affects mainly the villous architecture of the proximal small intestinal in genetically predisposed individuals. CD was first described by Arataeus in 200 A.D. as a malabsorptive syndrome with chronic diarrhoea (Thomas, 1945). CD is characterized by an inappropriate immune response triggered by the ingestion of amino acid sequences found in seed storage proteins of wheat, barley and rye. Typical gastrointestinal symptoms include diarrhoea, bloating, vomiting and abdominal pain. The only effective treatment is to follow a strict gluten-free diet (GFD). Compliance with the GFD facilitates the recovery of damaged intestinal mucosal, but gluten dietary transgressions cause activation of immunopathological mechanisms that prevent mucosal healing.

2.1 Prevalence

The prevalence of CD in European adults is approximately 1 in 100, compared with 1 in 133 in the United States of America (Fasano *et al*., 2003), while in Australia, New Zealand and Oceania, figures are similar to Europe (Logan and Bowlus, 2010). Other regions (Japan, China) where CD is historically rare have started to report new CD cases (Wu *et al*., 2010). In North Africa, children of Arab-Beber origin living in the western Sahara have a 5.6% prevalence of antiendomysial antibodies (Catassi *et al*., 1999) and 2.6% anti-tissue transglutaminase in Mexico, indicating that CD could be under-recognized in these territories (Remes-Troche *et al*., 2006).

2.2 Pathogenesis

Practically all coeliac patients have the heterodimeric HLA class II alleles encoding for HLA-DQ2 or DQ8. These molecules expressed on antigen-presenting cells such as macrophages, dendritic cells and B cells, present dietary gluten to CD4+ T cells in the lamina propria. This presentation activates the T helper 1 pathway which then up-regulates the secretion of interferon-γ (INF-γ) that contributes to the expansion of cytotoxic T-cells, fibroblasts and the release of metalloproteases to degrade the extracellular matrix of enterocytes at the lamina propria (Daum *et al*., 1999; Sollid, 2002).
Furthermore, these effects can be enhanced by peptide de-amidation, which is a process mediated by the enzyme tissue transglutaminase (TG2) that increases the number of negatively charged residues by converting glutamine into glutamic acid (Molberg *et al*., 1998). The number of available negatively charged anchor residues are crucial for the HLA molecules which have peptide binding pockets specifically designed to accommodate these types of residue. Moreover, the position of amino acids affected by de-amidation and the presence of proline residues resistant to digestive enzymes also contribute to an increase in antigenicity (Shan *et al*., 2002; Vader *et al*., 2002).

The innate activation of monocytes, macrophages and dendritic cells by gluten or non-gluten components via different pathways (perforin, granzyme and Fas/FasL) facilitates the cytotoxic activity of intraepithelial lymphocytes (IEL) in duodenal epithelium (Junker *et al*., 2009), induces the expression of non-classical class I molecule MICA on the intestinal epithelium which acts on natural killer cells and T-cells (Salvati *et al*., 2005), and up-regulates epithelial interleukin-15 (IL-15) production. IL-15 has a pivotal role in activation of innate and adaptive immune responses, is an important growth factor for IEL, blocks immunosuppressive pathways and could act together with IL-21to reinforce innate immunity (Maiuri *et al*., 2001).

2.3 Clinical manifestations

The typical clinical manifestation of CD in adults includes generalized malabsorption, weight loss and diarrhoea which can be continuous, intermittent or alternated with periods of constipation. Atypical signs that are becoming more predominant include body mass indices (BMIs) above 25, absence of diarrhoea, vague lassitude, abdominal pain, bloating and tiredness (Dickey and Bodkin, 1998; Lo *et al*., 2003). In children, classical symptoms such as anorexia, vomiting and anaemia are observed during the early months, followed by loss of appetite, failure to thrive, behavioural problems, abdominal distension, small stature, muscle wasting and enamel defect in the early years (Aine *et al*., 1990). Moreover, milder cases characterized by partial absence of symptoms are also becoming increasingly common (Rodrigues and Jenkins, 2006). CD is a complex condition that is associated with many diseases, mainly autoimmune diseases, but also reproductive, neurological and dermatological disorders.

Unresponsiveness to a GFD in CD patients after thorough dietary and histological review is an early indication of refractory coeliac disease (RCD). Its prevalence is unknown, but it is likely to affect 5% of the CD population (Tack *et al*., 2010). RCD can be divided into type I with phenotypically normal intraepithelial lymphocytes (IELs) and type II with abnormal IELs, characterized by expressing cytoplasmic CD3, but lacking surface expression of the T-cell markers CD3, CD4, CD84 and T-cell receptor (Verbeek *et al*., 2008), which can develop into an enteropathy-associated T-cell lymphoma (EATL).

2.4 Diagnosis

Mild gastrointestinal symptoms and genetic predisposition could be an initial indication of CD, which can be evaluated with serological screening. The diagnosis is usually made on the basis of an abnormal duodenal biopsy on a gluten-containing diet and subsequent histological improvement after a GFD. However, the wider clinical spectrum of CD requires careful examination and willingness to review diagnosis later in the light of clinical progress and research.

The current serological gold standard is the IgA endomysial antibody (EMA) and the anti-tTG IgA ELISA with high specificity (95–99%), sensitivity (90–93%) and good correlation with the degree of mucosal damage (Stern and Working Group on Serologic Screening for Celiac Disease, 2000). Recently, antibodies to de-amidated gliadin peptides (DGP) were shown to be of diagnostic value (Volta *et al*., 2010), although a meta-analysis suggested that the tTG antibody test outperforms the DGP (Lewis and Scott, 2010). However, by using multiplex immunoassay (MIA) to measure a panel of tTG, DPG, both IgG and IgA tests could reduce time and cost, particularly involving IgA-deficient CD patients. Further investigation is required to determine effectiveness in a clinical setting.

Histological assessment of the small intestine remains the gold standard in the diagnosis of coeliac disease. The coeliac lesion predominantly affects the mucosa of the proximal duodenum with less damage occurring towards the distal part. Multiple biopsies using standard size forceps are needed due to the patchy nature of the villous changes (Bonamico *et al*., 2002; Siegel *et al*., 1997). In 1992, Marsh classified the histological progressing of CD according to its severity in five stages, namely pre-infiltrative, infiltrative, hyperplastic, destructive and hypoplastic (atrophic) lesions (Marsh, 1992). Examination of biopsies provides a clear diagnostic advantage over serological tests, but problems could arise when measurements are taken from poorly oriented biopsies, particularly in undiagnosed predisposed patients with quasi-normal mucosal structure.

3. Gluten-free diet

The current effective treatment for CD since 1950 is to follow a strict lifelong gluten-free diet (GFD). This maintains the normal structure of the intestinal mucosa and reduces the risk of complication and mortality rate in coeliac patients (Nachman *et al*., 2010; West *et al*., 2004). The GFD includes naturally occurring gluten-free foods, such as fruit, vegetables, unprocessed meat, fish and poultry. Products such as pasta, bread, breakfast cereals, crackers and snack foods, that are usually manufactured with gluten-containing cereals (wheat, barley and rye), are replaced with flour from gluten-free cereals (e.g. maize, rice, millet, buckwheat and sorghum). Adherence to the GFD depends mainly on factors such as palatability, availability, age and cost. Foods containing “hidden” sources of gluten (e.g. wheat thickeners and malted barley) and the existence of ambiguous labels also reduce compliance to the GFD (Lerner, 2010).

3.1 Gluten

Gluten is a generic term to identify the storage proteins of cereals such as wheat, barley, rye and oats. Wheat kernels can be separated into bran, germ and endosperm. Protein within the endosperm part of the wheat kernel, can be subdivided according to their solubility in water, salt, alcohol and alkalis into albumins, globulins, prolamins and glutenins, respectively (Osborne, 1907).

Prolamins are alcohol-soluble storage proteins characterized by their higher content of proline and glutamine with a molecular weight of 10–100 kDa (Hilu and Esen, 1988). Prolamins are storage proteins because they are a significant source of nitrogen, sulphur and carbon for the developing embryo. They are named according to the cereal of origin, namely prolamins from wheat are known as gliadins, from barley as hordeins and from rye as secalins. According to their decreasing electrophoretic mobility, gliadins can be divided into α, β, γ and ω gliadins. Based on the N-terminal amino-acid sequences, gliadins can be categorized into α, γ and ω.

Glutenins consist of alcohol-soluble subunits which form alcohol-insoluble polymers. These subunits are stabilized by interchain disulphide bonds and have been classified according to their electrophoretic mobility into high molecular weight (HMW), medium molecular weight (MMW) and low molecular weight (LMW) groups, the latter being the main group (Shewry and Tatham, 1990; Wieser, 1994). The proteins of these groups can be further divided into two different types on the basis of sequence homologies, the LMW group and the HMW group containing HMW glutenin subunits (HMW-GS) (wheat), HMW secalins (rye) and D-hordeins (barley). The HMW-GS are the main contributor facilitating dough formation and this group of proteins is further subdivided into subfractions 1Dy10, 1Dx5, 1Dx4 and 1Dy9. Rye and barley have equivalent HMW glutenin subfractions, but they are less suitable for dough formation.

3.2 Cereal toxicity

Coeliac-toxic cereals are part of the Gramineae family, also known as Poaceae due to its association with the genus *Poa.* This family includes 12 subfamilies, two of which are more relevant to coeliac disease. The Pooidae (including wheat, rye and barley) are considered toxic for coeliac patients and the Panicoidae (including maize, millet and sorghum) are considered suitable for the GFD (Bracken *et al*., 2006; Kasarda, 1994).

Extensive studies have focused mainly on wheat; testing for rye, barley and other plants has been rather minimal despite the likelihood of them having similar storage proteins. Instead, taxonomical classification of plants has been used as a guide to separate safe grains from unsafe grains (Kasarda, 1994). Monocotyledoneous plants, members of the Gramineae family and the Triticeae tribe (wheat, barley and rye), are considered coeliac-toxic, while those from other tribes (maize, rice, tef) are generally assumed to be safe for coeliac patients. Hence quinoa, a dicotyledoneous plant from the Chenopodiacea family, is also considered safe for coeliac patients.

4. Gluten-free diet and quinoa (*Chenopodium quinoa* Willd.)

The nutritional adequacy of the GFD has been challenged on several occasions, because the elimination of wheat and other coeliac-toxic cereals reduces the dietary intake of minerals and vitamins, increasing the risk of nutritional deficiency in treated coeliac patients (Gray, 2006). Other studies have found that the vitamin content of gluten-free cereals may be leaving CD patients vulnerable to folate deficiency (Thompson, 2000b), and that the traditional GFD is nutritionally deficient compared with a regular diet, with negative long-term effects on vitamin status (Hallert *et al*., 2002).

The growing demand for alternative food products and ingredients to supplement the nutritional value of the GFD has directed patients and the industry towards Andean grains, potential new crops with multipurpose agro-industrial applications. Nutritionally, these crops contain high amounts of carbohydrates, protein, vitamins and minerals. The main Andean grains are quinoa (*Chenopodium quinoa* Willd.), kiwicha (*Amaranthus caudatus*) and kañiwa (*Chenopodium pallidicaule*).

Adaptability to different environments is another advantage of Andean grains. For example, moderate yield was obtained when cultivated in Europe, with production levels of 2–3.8 tonnes/ha in field trials, suggesting that they could become a sustainable alternative crop for European agriculture (Aufhammer *et al*., 1995; Herencia and Alia, 1999; Jacobsen *et al*., 1994).

Quinoa is emerging as a potential novel crop in various regions of the world due to its agronomical, nutritional and industrial applications. One of the characteristics that highlights its value from a nutritional point of view is the quantity and quality of proteins. Quinoa has a higher protein content than most cereals with a balanced amino acid profile (Drzewiecki *et al*., 2003; Ruales and Nair, 1992). Quinoa proteins can be classified into albumins, globulins, prolamins and glutenins after sequential extraction in various solvents (Prakash and Pal, 1998). The main types of protein are albumins and globulins (44–77%), with prolamins the least prevalent (0.5–7%) (Lindenboom, 2005).

Based on the distant toxonomical relation with wheat and low levels of prolamins, it has been assumed that quinoa is unlikely to damage the intestine of coeliac patients and therefore should be a safe addition to the GFD. However, on the basis of current knowledge, this assumption is not supported by strong experimental data, prompting the question of whether quinoa prolamins may indeed not exacerbate coeliac disease or be a safe addition to the GFD.

Furthermore, until these questions are satisfactorily answered, quinoa and other Andean grains will not be recommended as part of the GFD (Farrell and Kelly, 2002; Nicolas *et al*., 2003). Moreover, a survey of 63 coeliac organizations and 42 physicians showed that there is great concern that insufficient research has been conducted on the safety of novel crops such as quinoa (Thompson, 2000a). For these valid reasons, controversy and misunderstanding persist among health professionals, and quinoa continues to be excluded from the various lists of recommended products for coeliac patients. This will be the case until solid scientific evidence is presented.

5. Immunochemical evaluation of quinoa prolamins

The limited number of plants with similar properties to coeliac-toxic cereals (wheat, rye and barley), has prompted a search for alternative products to replace those cereals. The hypoallergenic nature of quinoa and its "organic" image has led to it being recommended as a source of vegetable protein for special dietary purposes such as a gluten-free diet (GFD). The safety assessment of new additions for the GFD requires strict tests that can confirm suitability. However, to facilitate the incorporation of new products, a relatively simple and quick safety assessment was used to determine that quinoa was "gluten-free" and could be added to the GFD (the level of gluten was measured and the taxonomic origin of the plant in question assessed).

Nevertheless, the level of prolamins within quinoa is highly variable, depending on the cultivar and the environment in which the plant is cultivated (Herencia and Alia, 1999). The taxonomic classification is only a guide, originally based on the taxonomic origin of monocotyledoneous plants, and since quinoa is dicotiledoneous, this guidance may be less reliable.

5.1 Immunochemical properties of quinoa proteins

Berti *et al*. (2004) recognized this lack of information and, therefore, assessed the amount of gliadin-like proteins in quinoa flours as well as the immunochemical reactivity of the protein fractions extracted from quinoa samples. Quinoa grains were obtained from the *Asociacion Nacional de Productores de Quinoa* (Anapqui, Bolivia) and proteins were extracted by sequential flour extraction. The protein profile of each extract was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, immunoblotting with serum from coeliac patients or rabbit antigliadin polyclonal antibodies, and gliadin content was measured by enzyme-linked immunosorbant assay (ELISA).

The immunoreactivity of the quinoa proteins extracted was very low both with commercial antigliadin antibodies and with serum of a coeliac subject, and was comparable to that of proteins in gluten-free flour. The only weak positive band detected by human serum antibodies (either IgA or IgG) could be attributed to cross-reactivity towards a protein that was present also in gluten-free flours.

The gluten content in quinoa flour was measured using a commercial ELISA kit based on monoclonal antibodies against heat-resistant ω-gliadins (Skerrit and Hill, 1991). The content of gliadin-like proteins was evaluated also on flours obtained from other grains (soybean, buckwheat, oat and maize).

A wheat sample was the positive control. Gliadin standards covered the range of 2–20 ng of gliadin. Soybean and maize showed a gluten content below the assay detection limits. The gluten content in quinoa (1.6 ± 0.6 mg/kg) was less than half that of buckwheat (4.2 ± 0.2 mg/kg). Berti and collaborators concluded that quinoa could be a safe choice for the production of gluten-free products, at least from an immunochemical point of view. However, they only included one unknown cultivar and tested this sample using monoclonal antibodies against heat-resistant ω-gliadins.

5.2 Quinoa prolamins in 15 quinoa cultivars

The level of quinoa prolamins with gluten-like epitopes was determined *in vitro* (Zevallos *et al*., 2012), using a library of anti-gliadin and anti-high molecular weight glutenin subunits (HMW-GS), murine monoclonal antibodies (mMAbs) using dot immunoassay and double sandwich ELISA. Prolamins were extracted from 15 identified quinoa cultivars from the germplasm bank at INIA, Peru (National Institute of Agricultural Research).

Four mMAbs were used, namely, PN3 (anti A-gliadin 31-49), CDC5 (anti α-gliadin 56-75), CDC3 and CDC7 (anti HMW-GS 1Dy10). *Invivo* toxicity studies have demonstrated that the A-gliadin 31-49 peptide, a 19-mer peptide, and the α-gliadin 56-75, the immunodominant gliadin T-cell epitope (Anderson *et al*., 2000), are responsible for destruction of villous architecture (Fraser *et al*., 2003) and that HMW-GS 1Dy10, important dough-forming HMW-GS, are also likely to be implicated in epithelial damage.

Fifteen quinoa cultivars were compared against wheat starch standards of known gluten content (A, B and C) and negative controls (sorghum and millet). The wheat starch standards were graded according to colour intensity as A (-), B (++) and C (+++). Eight quinoa samples were completely negative and were equivalent to the wheat starch acceptable for CD patients (A). Cultivar 'Witulla' gave a slight reaction (+) to antibodies raised against gliadin peptides. Cultivars '03-21-1181', 'CICA-17' and 'Blanca de Jujuy' gave a slight reaction (+) to antibodies raised against HMW-GS, and cultivars 'Ayacuchana', 'LP-4B' and 'INIA-Pasankalla' gave a slight reaction (+) to both gliadin peptides and HMW-GS antibodies. Also, 'Ayacuchana' was the only cultivar that showed a moderate reaction (++) to anti-gliadin antibody CDC5 (anti α-gliadin 56-75) (Table 1).

The prolamin concentration of 15 pure quinoa cultivars was also determined using mMAb PN3, raised against the toxic A-gliadin 31-49 peptide. The highest concentration was observed in cultivars 'Ayacuchana' (2.56 mg/kg), followed by (in descending order) 'Witulla', 'LP-4B' and 'INIA-Pasankalla'. The results indicate that all quinoa cultivars have a gluten content below the maximum amount of gluten (20 mg/kg) suggested for foods that may be labelled gluten-free (FAO and WHO, 2008). Seven cultivars showed quantifiable levels of toxic prolamins with maximum values of 2.56 mg/kg ('Ayacuchana') and a minimum of 0.48 mg/kg ('Rojo Achachino'). To conclude, 15 quinoa cultivars from the Andes have low levels (< 20 mg/kg) of prolamins with a similar structure to known toxic gluten epitopes and therefore can be labelled gluten-free.

6.1 Adaptative and innate immunostimulatory response to quinoa prolamins

The adaptative immune system plays an important role within the immunopathogenesis of CD. Tissue transglutaminase II (TG2), located in the intestinal mucosa, de-amidates gliadin peptides which can then be presented to lamina propria CD4+ T cells by antigen-presenting cells (DQ2+ or DQ8+). Activated CD4+ T cells following the T helper (Th)1-type pathway initiates the production of interferon gamma (IFN-γ) and subsequent mucosal deterioration (Nilsen *et al*., 1998; Sollid, 2002). However, other studies suggest that some gluten peptides can induce mucosal damage through the direct activation of innate immune mechanisms such as interleukin-15 (IL-15) secretion (Maiuri *et al*., 2003). Therefore, in order to test coeliac toxicity of novel gluten-free products, it is necessary to consider *in vitro* systems that can address both innate and adaptive immune response.

6.1 Cellular agglutinating activity of quinoa prolamins

De Vincenzi *et al*. (1999) examined the suitability of quinoa for coeliac patients by extracting alcohol-soluble proteins from quinoa grains and testing the extracts on K562(s) cells, a line of chronic myeloid

Table 1. Level of coeliac-toxic epitopes in quinoa cultivars, negative and positive controls analysed by immunobinding assay and non-competitive ELISA. Samples with non-detectable amount of toxic epitopes are represented by a minus sign. HMW-GS, high-molecular-weight glutenin subunit; mMAb, murine monoclonal antibodies; ND, not determined.

Method	Dot immunobinding assay					ELISA
mMAbs	Anti-gliadin		Anti-HMW-GS			Anti-gliadin
	PN3	CDC5	CDC3	CDC7		PN3
Quinoa cultivars						
Illpa INIA	-	-	-	-		-
Kamiri	-	-	-	-		-
03-21-1181	-	-	+	-		-
Salcedo	-	-	-	-		-
Kancolla	-	-	-	-		-
03-21-0386	-	-	-	-		-
Rojo Achachino	-	-	-	-		-
Chullpi Rojo	-	-	-	-		-
Blanca de Jujuy	-	-	+	+		-
CICA-17	-	-	-	+		-
Ayacuchana	+	++	+	+		2.56
LP-4B	+	-	-	+		1.38
INIA-Pasankalla	+	-	+	+		1.21
Witulla	+	+	-	-		1.64
Rojo Coporaque	-	-	-	-		-
Wheat Starch A	-	-	-	-		ND
Wheat Starch B	++	++	++	++		ND
Wheat Starch C	+++	+++	+++	+++		ND
Sorghum	-	-	+	+		-
Millet	-	-	-	-		-

leukaemia tissue, measuring the level of cell agglutination. The total PT-digest was also resolved on a sepharose-6B-mannan column and two fractions were obtained: fractions A (representing 94% of the total loaded proteins) and fractions B (1%). In addition, the authors measured the amount of potential coeliac toxic epitopes by commercial ELISA based on an mMAb that recognizes ω-gliadin.

The results revealed low levels of toxic prolamins in the quinoa samples (0.003 g/100 g of whole flour). The results could possibly be underestimated, due to limitations of the ELISA system and the amino acid composition of quinoa prolamins which have lower levels of proline in comparison with coeliac toxic prolamins. Peptic-tryptic digest of quinoa prolamins did not agglutinate K562(s) cells, even when tested at high concentrations. Fraction A was not active in agglutinating K562(s) cells, showing the same behaviour as the total PT-digest, but fraction B was very active in agglutinating K562(s) cells.

Moreover, this activity was inhibited when combined with fraction A, suggesting that whole PT-digest quinoa prolamins were not able to agglutinate the K562(s) cells, potentially because peptides in fraction A were interfering with those in fraction B. The results from this experiment provide very important information about quinoa prolamins. However, CD is a T-cell mediated disease affecting mainly the small intestine epithelium; it seems more appropriate to use gliadin-specific T-cells lines from duodenal biopsies to understand the effects of quinoa prolamins in CD.

6.2 Immunological evaluation of prolamins from potential gluten-free grains

Bergamo *et al.* (2011) analysed the immune activity of prolamins from tef, millet, amaranth and quinoa, comparing them with wheat gliadin using intestinal T-cell lines (iTCLs), cultures of duodenal explants from HLA-DQ2 CD patients and HLA-DQ8 transgenic mice (tg).

Intestinal T-cells from CD patients were isolated and incubated with four de-amidated prolamin extracts (tef, millet, amaranth and quinoa) and compared against de-amidated gliadin. INFg production was increased in de-amidated gliadin but was undetected in the other tested samples. Furthermore, samples were tested in 24-hour organ culture intestinal biopsies, and CD25 activation in the lamina propria and increased density of CD3 IEL was observed in biopsy specimens cultured with PT-gliadin. However, there was no significant difference in CD25/CD3 levels compared with samples incubated with medium alone.

The authors also tested the prolamin samples gliadin-sensitized HLA-DQ8 tg mice, immunized with PT-gliadin that induced a significant response in cells that were stimulated *in vitro* with PT-gliadin from both the spleen and the mesenteric lymph nodes (MLNs). No significant cell-mediated proliferation was observed among testes samples, even at higher concentrations. In conclusion, tef, millet, amaranth and quinoa did not show immune cross-reactivity with DQ2- and DQ8-restricted gliadin epitopes, or induction of innate immunotoxicity, and could be considered suitable for use in the diet of patients with CD. However, there was only one non-described cultivar used for each sample.

6.3 Adaptive and innate immune response of quinoa prolamins in coeliac disease

The authors investigated the ability of quinoa prolamins to stimulate the adaptative and innate immune response in coeliac patients by activation of gliadin-specific T-cell lines, level of INF-γ in cell lines supernatants and innate immune activation of duodenal biopsies from coeliac patients using the organ culture system (Zevallos *et al*., 2012).

Briefly, gliadin-specific CD4+ T lymphocytes derived from duodenal biopsies from ten diagnosed coeliac patients were isolated and proliferation assays performed by incubating T-cells with and without antigen, measuring cell tritiated thymidine uptake in counts per minute and calculating the stimulation index (SI). Cytokine INF-γ was also measured from cell supernatants cultured with and without antigen. Moreover, duodenal biopsies from eight diagnosed coeliac patients were cultured overnight *ex vivo* with and without antigen. Cytokine (IL-15 and INF-γ) secreted from cultured biopsies with and without antigen were measured by ELISA and results were corrected for weight of biopsy and volume of supernatant before comparison using a non-parametric test.

Positive SI ($SI \geq 2$) was observed in all ten T-cell lines cultured with gliadin ('Rektor'), with stimulation indices ranging from 2.1 to 24.1. Two out of ten T-cell lines (C and G) cultured with prolamins from cultivars 'Ayacuchana' and 'Pasankalla' showed positive SI. In T-cell line C, 'Rektor' gliadin induced an INF-γ secretion of 108 pg/mL, whereas an equal concentration of 'Pasankalla' prolamins induced a lower concentration (20 pg/mL). In T-cell line G, no detectable values were obtained (Table 2).

The presence of gliadin-stimulated T-cell responses restricted by the HLA-DQ2 or HLA-DQ8 in coeliac patients supports the evidence that the adaptive immune response to gliadin is directly responsible for the inflammatory mucosal lesion (Lundin, 1993; van de Wal *et al*., 1998). If this information is extrapolated to the results obtained in this study, it could be argued that some quinoa prolamins may cause duodenal mucosal lesion in coeliac patients.

However, it could equally be argued that all T-cell lines stimulated with quinoa prolamins have an $SI < 3$, which, according to other studies, would be considered negative (Vader *et al*., 2002). Furthermore, it is possible that some PHA (e.g. mitogenic glycosylated amino glycans) present in quinoa prolamins (de Vincenzi *et al*., 1999) remain in the final sample, despite the inclusion of methods (ultrafiltration) aimed to eliminate those molecules. Moreover, the PHA-like hypothesis would not explain the effects of TG2 on increased antigenicity. It is clear that further investigation regarding the properties of quinoa prolamins in coeliac disease is needed.

Therefore, prolamins from these two quinoa cultivars ('Ayacuchana' and 'Pasankalla') were extracted

Table 2. Stimulation indices of each T-cell line against de-amidated prolamins from wheat and four quinoa cultivars ('Rektor', 'Ayacuchana', 'Pasankalla', 'Witulla' and 'LP-4B'). * indicates positive stimulation indexes (ie. ≥ 2).

T-cells	Stimulation Indices (SI)				
Lines	Rektor	Ayacuchana	Pasankalla	Witulla	LP-4B
A	2.9*	<1	<1	<1	<1
B	6.5*	<1	<1	<1	1
C	3.5*	1.4	2.4*	1.4	1
D	6.2*	1.4	1.3	1.2	1
E	4.8*	1	<1	1	1.2
F	24.1*	1.5	1.4	1.4	1.4
G	2.1*	2*	2*	1.5	1.6
H	4.9*	1	<1	<1	1.1
I	4.9*	<1	<1	1.2	<1
J	20.2*	1	1.1	1.1	1.4

Table 3. Concentration of INF-γ and IL-15 in 8 organ culture experiments. [1]Mean +/- SE (all such values). [2]Significant difference from medium, $p < 0.05$(Wilcoxon's signed ranked test for paired data).

Biopsy	Concentration of INF-γ / IL-15 in pg/mg tissue				
	Medium	Ovalbumin	Rektor	Ayacuchana	Pasankalla
A	0.6 / 0.2	0.3 / 0.2	1.7 / 0.8	1.4 / 0.7	0.9 / 0.4
B	0.2 / 0.1	0.6 / 0.4	0.3 / 0.4	0.5 / 0.2	0.2 / 0.2
C	0.3 / 0.2	0.4 / 0.1	0.4 / 0.3	0.5 / 0.2	0.2 / 0.2
D	0.2 / 0.2	0.4 / 0.2	0.3 / 0.2	0.3 / 0.2	0.3 / 0.3
E	0.4 / 0.3	0.5 / 0.3	0.4 / 0.3	0.5 / 0.3	0.5 / 0.3
F	0.3 / 0.2	0.3 / 0.2	0.3 / 0.3	0.5 / 0.2	0.3 / 0.2
G	0.5 / 0.3	0.2 / 0.2	0.6 / 0.2	0.3 / 0.4	0.3 / 0.2
H	0.4 / 0.3	0.5 / 0.4	0.8 / 0.4	0.5 / 0.6	0.9 / 0.7
8	0.37[1] (0.04[2])/ 0.22[1] (0.02[2])	0.39[1] (0.03[2])/ 0.26[1] (0.02[2])	**0.60[1] (0.11[2])/ 0.37[1] (0.05[2])**	**0.57[1] (0.08[2])/ 0.35[1] (0.04[2])**	0.46[1] (0.07[2])/ **0.31[1] (0.04[2])**

and cultured with small intestinal biopsies from eight diagnosed coeliac patients. Biopsies were cultured without antigen and with ovalbumin (negative control), gliadin (positive control) and quinoa prolamins (test sample). Cytokine INF-γ and IL-15 were measured by ELISA and statistically compared between concentrations in media from biopsies cultured without an antigen and biopsies cultured with an antigen (gliadin or quinoa prolamins). Furthermore, prolamins from 'Ayacuchana' stimulated the secretion of both INF-γ and IL-15, while prolamins from 'Pasankalla' were able to stimulate secretion of IL-15 when compared against a positive control ('PTG', 'Rektor') and a negative control (ovalbumin) (Table 3). These results confirm the potential cell activation of the innate immune system in a multicellular context by quinoa prolamins. However, the range of values was highly variable, suggesting that the immune system of some patients will be more activated than others by the presence of quinoa prolamins.

This study reported for the first time activation of two gliadin-specific T-cell lines by prolamins from two quinoa cultivars ('Ayacuchana' and 'Pasankalla') following TG2 de-amidation, which could suggest an analogy with a situation seen in oats where a small proportion of coeliac patients were affected, probably due to the presence of an epitope with similar characteristics to gliadin epitopes (Arentz-Hansen *et al*., 2004). It is conceivable that coeliac-toxic peptides exist within quinoa prolamins from these two cultivars.

In addition, the effects of gliadin on IL-15 secretion may not be exclusive to coeliac patients, rather a common innate mechanism in the general population (Bernardo *et al*., 2008). However, coeliac patients seem to be more sensitive to the effects of IL-15 and develop a secondary inflammatory response involving the secretion of INF-γ which does not occur in non-coeliac patients. Cultivar 'Ayacuchana' seems to elicit that initial and secondary response. However, further *in vivo* investigation of quinoa's amino acid profile, prolamin subfractions using CD4+ T-cells as a marker of toxicity, and the effects of quinoa as a whole composite food is required to confirm these findings.

7. In vivo feeding studies

Dietary gluten can cause inflammation and histological deterioration of the small intestine in genetically predisposed individuals. An effective treatment for coeliac patients is to follow a strict gluten-free diet (GFD), but gluten-free cereals are less available and less palatable and can contain fewer nutrients than their gluten-containing counterparts. Alternative gluten-free products that can improve any of these qualities are a welcome addition to the GFD. However, it is possible that traces of known toxic peptides within the prolamin fraction of these alternative products can exacerbate coeliac disease. Guidance about toxicity can be obtained from taxonomic classification (Kasarda, 1994), but ultimately, confirmation is required through feeding studies, particularly if there are traces of known toxic peptides within the prolamin fraction as observed in some quinoa cultivars (Zevallos *et al*., 2012).

7.1 Nutritional effects of alternative grain on the gluten-free diet

This study evaluates the effect of substituting alternative grains on the nutrient profile of the standard gluten-free dietary pattern (Lee *et al*., 2009). The alternative grains were selected on the basis of their nutrient profile, availability and cost. The nutritional intakes of 50 randomly selected patients were retrospectively reviewed using a 3-day usual dietary intake record.

A meal pattern includes a serving from each of the groups: protein, dairy, fruit or vegetable, and grain. A snack consists of at least two choices from: protein, dairy, fruit or vegetable, or grain. The consumption patterns from the 50 diet records were used to create one average intake pattern. The "alternative" gluten-free dietary pattern was developed by substituting only the grain or starch portion of the standard menu pattern with alternative gluten-free grains or grain products. The alternative diet used cereal at breakfast (oats), bread at lunch (high fibre brown rice bread) and a starch side dish for the evening meal (quinoa). The content of protein, fat, carbohydrate, fibre, thiamine, riboflavin, niacin, folate, iron and calcium formed the basis of the nutrient comparison between the two menu patterns.

Results indicate that the standard gluten-free diet pattern did not meet the USDA recommended number of 6–11 grain servings per day. The study population omitted a grain portion at a meal 39% of the time, and rice was used as the grain in 44% of meals, followed by potato (8%), oats (5%) and corn (4%). Buckwheat and quinoa were each used for only one meal. The dietary records indicate that 44% of the meals were rice based and 55% of the total snacks comprised commercially prepared snack foods, such as chips, pretzels and gluten-free cookies, donuts and cakes.

The standard gluten-free diet did not meet the recommended intake for fibre, thiamine, riboflavin, niacin folate, iron or calcium. The change in dietary grains significantly increased selected nutrient levels in the diet: protein (20.6 vs 11 g), iron (18.4 vs 1.4 mg), calcium (182 vs 0 mg) and fibre (12.7 vs 5 g). The "alternative diet" provided an improved nutrient profile compared to the standard gluten-free diet ($P = 0.0002$). Therefore, the substitution of alternative grains positively impacts the nutrition profile of the GFD (fibre, thiamine, riboflavin, niacin, folate and iron); furthermore, the substitute grains are widely available and less expensive. Although no gastrointestinal effects were reported, it was assumed that patients tolerated well the addition of quinoa to their diets in at least one portion a day during the study period.

7.2 Gastrointestinal effects of coeliac patients eating quinoa

The authors examined the clinical, histological and immunological response of treated coeliac patients eating 50 g of quinoa daily for 6 weeks as part of their usual GFD (Zevallos *et al*., 2013). Nineteen patients participated in the study, 2 males and 17 females with a median age of 59 years, BMI of 23 kg/m^2, 9 years on a GFD and all were HLA-DQ2 positive. The study was approved by the Ethical Committee at St Thomas' Hospital, London, and all patients gave written informed consent before participating in the study. All participants were diagnosed adult coeliac patients and on a gluten-free diet for at least 1 year. Participants were excluded if they had any medical condition considered sufficiently serious to interfere with the study or to constitute an unacceptable risk to them. Participants were given a card to record gastrointestinal symptoms (diarrhoea, abdominal pain, increased bowel sounds and vomiting) throughout the study period. In addition, ten treated coeliac patients provided duodenal biopsies for morphometric measurements at the beginning and at the end of the study.

Gastrointestinal symptoms were graded daily (0 none, 1 mild, 2 moderate and 3 severe). Ten patients did not report any symptoms. Nine patients reported symptoms ranging from mild to moderate during the first 2 weeks of the study. Most of them were mild abdominal pain, followed by mild increased bowel sounds and diarrhoea. This might be due to an increase in dietary fibre as reported in other feeding studies (Storsrud *et al*., 2003). Duodenal biopsies from ten patients were assessed randomly and blindly before and after eating quinoa using three morphometric parameters (VH:CD, SECH and IEL). Results indicate that the mean values of VH:CD went from slightly below normal levels (2.8:1) to normal levels (3:1). The same occurred in SECH from 28.76 to 29.77 µm. IEL decreased from slightly abnormal (30.3) to just below normal (29.7). Although a positive trend was observed (increased VH:CD and SECH, decreased IEL), no significant difference was achieved in any of the measurements

The mean values of VH:CD (3:1) and SECH (29.77 µm) at the end of the study were at the lower end of the normal range (3:1 to 5:1 and 29 to 34 µm, respectively), which is relatively expected in a group of coeliac patients with a wide range of time on a GFD (1–33 years), as it can take over two years from the start of GFD to achieve normal or quasi-normal morphometric parameters (Wahab *et al*., 2002). The other morphometric parameter, IEL count, was

Table 4. Morphometric measurements of 10 patients before and after quinoa challenge. The normal values for VH:CD ratio vary between 3:1 and 5:1, for SECH measurements from 29 to 34 µm and the normal percentage of IEL, counts vary between 10 and 30. No significant difference was found when samples were compared.

Patient	Ratio of villous height to crypt depth		Surface enterocyte cell height		Intraepithelial lymphocyte count	
Number	VH:CD		SECH (µm)		IEL (%)	
	Pre	Post	Pre	Post	Pre	Post
1	2.3	2.8	30.34	34.08	27	30
2	1.9	1.5	23,20	27.25	34	41
3	2.1	2.7	23.90	28.76	60	44
4	2.9	2.8	32.11	29.81	20	15
5	2.0	2.2	22.85	29.02	39	45
6	3.2	3.9	26.40	34.23	12	10
7	3.3	3.8	35.46	29.33	20	35
8	2.9	3.2	31.80	28.07	36	40
9	5.2	4.5	33.60	27.70	15	12
10	2.3	2.4	27.91	29.44	40	25
Mean (SD)	2.8 (0.98)	3.0 (0.89)	28.76 (4.56)	29.77 (2.45)	30.30 (14.49)	29.70 (13.5)

conversely on the higher end of normality (10–30%) after quinoa consumption (29.7%). Nasseri-Moghaddam *et al*. (2008), in a review to determine the range of normality of IEL in coeliac patients, indicated that < 35% on slides stained with immunohistochemistry (ICH) could be considered normal, 36–39% borderline and > 39% increased. Moreover, the author indicated that normal IEL counts should take into account environmental exposures, ethnic background and regional differences.

All median values for blood tests at the beginning and at the end of the study were within the appropriate normal range, with the exception of total cholesterol and LDL, which were slightly higher than the recommended 4 and 2 mmol/L, respectively (NICE, 2010). The total cholesterol in the study population was reduced from 4.6 to 4.3 mmol/L, LDL from 2.46 to 2.45 mmol/L and HDL significantly reduced ($p \leq 0.05$) from 1.8 to 1.68 mmol/L after eating quinoa. This mild reduction in cholesterol is in agreement with an early study in which induced hypercholesterolemia on mice was strongly alleviated by quinoa (Konishi *et al*., 2000). Although the cholesterol values were still slightly higher than the recommended level and there was a reduction of HDL, it is clear that patients could benefit from eating quinoa.

To conclude, the addition of quinoa to the gluten-free diet of 19 adult coeliac patients did not cause exacerbation of the disease, and gastrointestinal symptoms were mostly absent during the study, which could be interpreted as an early indication that quinoa is well tolerated among coeliac patients. However, further study is needed to determine whether this positive trend towards mucosa recovery and cholesterol reduction would continue over a longer period of time.

Conclusions

To conclude, there is scientific evidence from *in vitro* and *in vivo* studies indicating that some quinoa cultivars have small amounts of gluten-like proteins which can stimulate immune cells *in vitro*, but that do not exacerbate coeliac disease when consumed as part of the GFD. However, the evaluation and safety assessment of quinoa as an ingredient within the gluten-free industry will require further investigation.

Results from T-cells and organ culture studies indicate that prolamins from two quinoa cultivars ('Ayacuchana' and 'Pasankalla') could induce immune activation. Further experiments involving generation of quinoa-specific T-cell lines/clones, morphometric measurements after organ culture and evaluation of other markers of innate immunity will complement the current data. Furthermore, the positive trend towards mucosal improvement, mild hypocholesteroleamic effects and reduction in gastrointestinal symptoms after eating quinoa, would ideally require a long-term double blind randomized cross-over study. Although, further studies are still needed, on the basis of the current literature, we can suggest that quinoa can be safely consumed by CD patients.

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Section 4
Social and Economic Aspects

CHAPTER: 4.1

INTERNATIONAL QUINOA TRADE

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Abstract

Quinoa has become one of the fastest growing commodities in world trade in recent years. The quinoa trade is so dynamic that in 2012, a special tariff subheading was created for the grain. The outlook for quinoa supply and demand points to continued growth for the international quinoa trade. This paper presents the characteristics of the international quinoa trade, identifying the main exporters and importers, market access conditions and the key determinants of global supply and demand for this product. It analyses the outlook for global supply and demand of quinoa, examines the characteristics of its value chain and highlights public policy challenges to strengthen the productive sector and achieve a more efficient value chain.

Introduction

In recent years, there has been a systematic increase in international demand for quinoa quinoa-derived products, reflected in the rapid increase in cultivated area. The major quinoa-exporting countries are Bolivia and Peru, but there are also other countries in the international market interested in raising production, such as Ecuador and to a lesser extent Chile, Colombia and the United States of America. There are many reasons to explain this increase in demand, including but not limited to the high nutritional quality of quinoa and its derivatives, the trend of healthy eating habits, renewed interest in ancestral cultures, and the fact that quinoa is a product grown by small-scale peasant farmers and most of the production is organic. International demand is therefore expected to continue increasing in response to structural processes. The conditions for quinoa's access to international markets, both regional and global, are also favourable, since the crop benefits from low tariff protection levels and few phytosanitary barriers. This paper examines the positive outlook for the quinoa international market and looks at the challenges faced by public policies to enhance this outlook. It analyses production, post-harvest management, processing and distribution, and considers how to ensure that most of the benefits of this expansion cycle can be retained by producers and their organizations.

Quinoa world trade

The world trade of quinoa has grown significantly in recent years. Since 2006, there has been a sharp increase in exports from Latin America, the region of the three Andean countries that account for over 80% of global exports. Due to this phenomenon,

[1] This article is an adaptation of the FAO-LAIA (2013b) study carried out by the same authors on "Tendencias y Perspectivas del Comercio de la Quinua" (Quinoa trade trends and outlook).

the World Customs Organization has introduced a tariff opening for quinoa, subheading 10.08.50.

Quinoa exports amounted to approximately USD131 million in 2012, with high concentrations in both origin and destination. For example, 84.2% of global exports originate from Bolivia, Ecuador and Peru, 10% from the United States of America, and 6% from the European Union.

On the other hand, the United States of America accounts for 53% of quinoa imports, followed by Canada (15%). The remaining exports are destined to France (8%), the Netherlands (4%), Germany (4%), Australia (3%) and LAIA group member countries (3%).

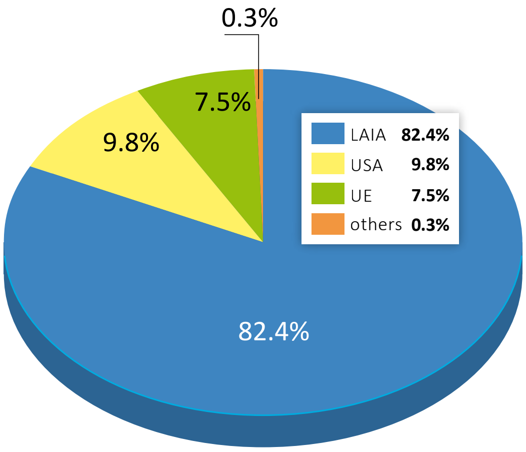

Figure 1. Global world exports in quinoa (2012).
Source: Comtrade and LAIA

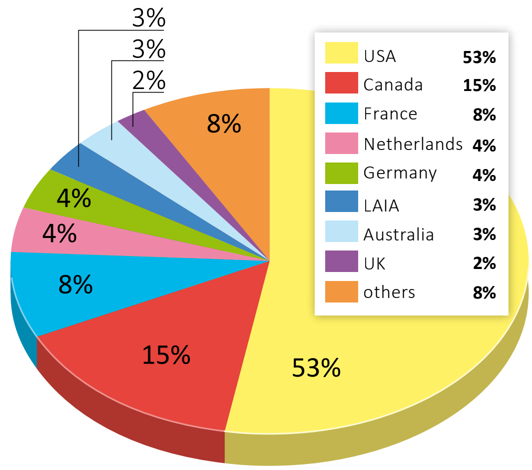

Figure 2. Leading global importers of quinoa (2012).
Source: Comtrade and LAIA

Although there are no statistics available on the historical evolution of the global quinoa trade, it is clear that regional exports of quinoa – taking into account the combined external sales of Bolivia, Ecuador and Peru – have grown steadily in the last 20 years. They grew from USD700 000 in 1992 to USD111 million in 2012, an average annual increase of 28.8%.

It should be stressed that the growth rate of quinoa exports has accelerated in recent years. For example, sales increased fourfold between 1992 and 2002, while they increased 39 times between 2002 and 2012. Quinoa exports have also increased markedly in terms of volume: from 600 tonnes in 1992 to 37 000 tonnes in 2012, representing an average annual growth of 22.8%.

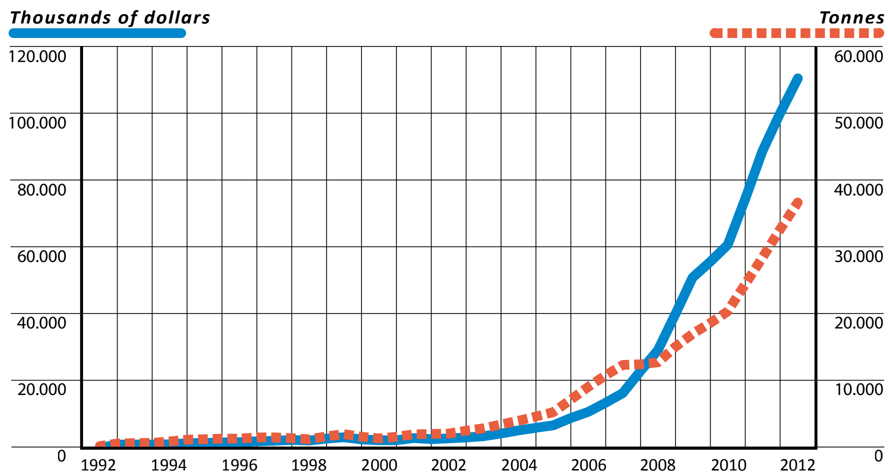

Figure 3. Regional exports of quinoa: 1992–2012 - Source: LAIA

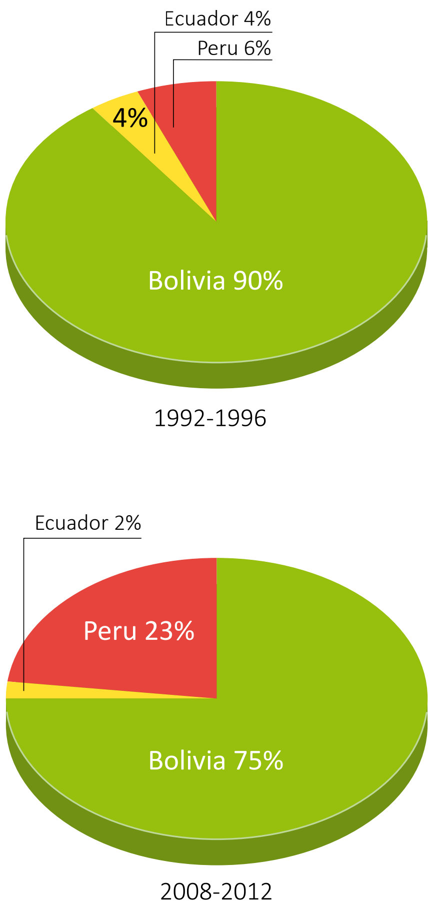

Figure 4. Origin of the regional exports of quinoa. Source: LAIA

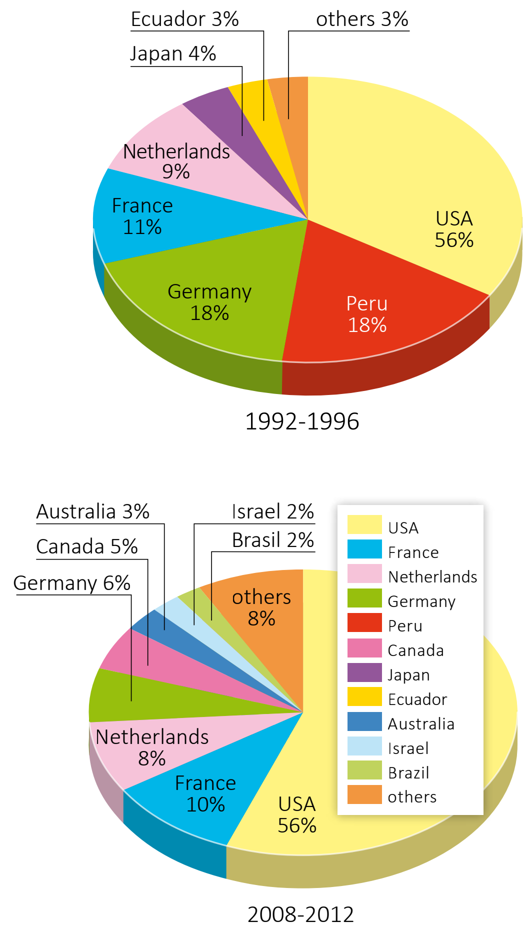

Figure 5. Destination of regional exports of quinoa. Source: LAIA

The breakdown of regional quinoa exports according to origin has changed slightly in the last 20 years, with Bolivia continuing to be the leading exporter, (although its participation has fallen from 90% to 75%), followed by Peru (participation has increased from 6% to 23%), while Ecuador has decreased in relative importance.

The breakdown of regional quinoa exports according to destination market has also changed slightly in the last 20 years, as new markets emerge and existing markets are restructured. However, the major concentration of sales has remained the same.
In the last 20 years, the United States of America has grown as a destination market, accounting for 56% of imports. At the same time, new important markets have appeared, including Canada (5%), Australia (3%), Israel (2%) and Brazil (2%).

Meanwhile, European markets – Germany, France and the Netherlands – have lost their relative importance as destination markets, and Japan, Peru and Ecuador do not even rank among the leading buyers. This is the direct result of their domestic production, which has increased to meet both domestic and international demand.

Note that despite the reduction in relative terms of exports to the European Union, this reduction has occurred in a context of a general rise in the volume traded on the international market. Therefore, in absolute terms, exports to European markets have increased significantly.

The United States of America is the leading market for quinoa exports, and it accounts for over half the exports from Bolivia (54%), Ecuador, (55%) and Peru (61%).

In terms of the breakdown of the remaining exports according to destination, there are some differences. Bolivia's significant buyers included France (13%) and the Netherlands (10%). Ecuador's remaining sales were concentrated in Germany (30%), while Peru's remaining exports were spread between Germany, Canada, Israel, Australia and Italy, each accounting for < 10%.

Lastly, it should be noted that Peru currently reaches the largest number of markets in terms of quinoa sales, with sales in 51 countries during the last 5 years. Meanwhile, Bolivia and Ecuador accessed 36 and 17 markets, respectively.

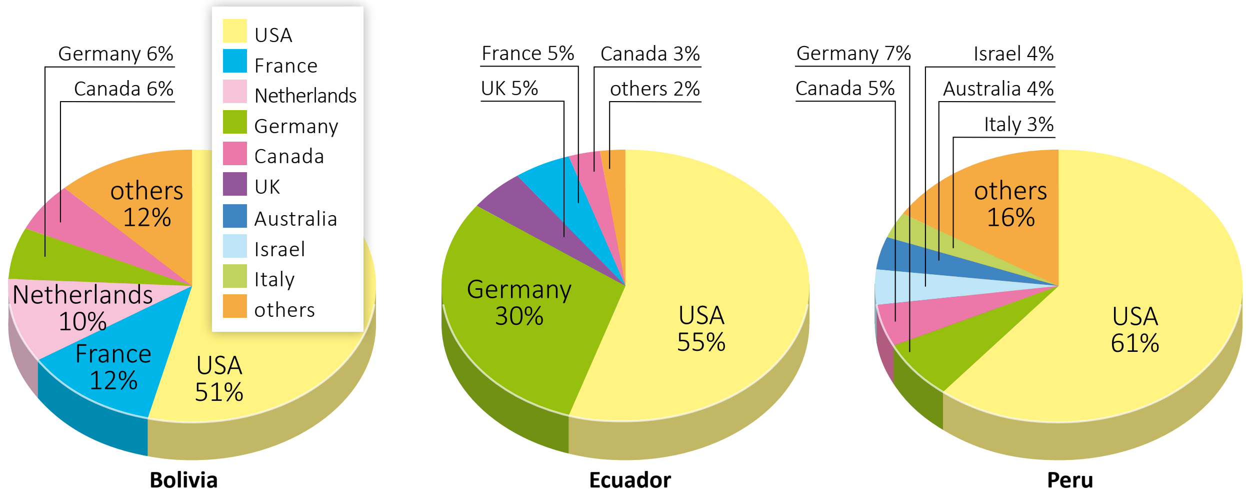

Figura 6. Destination of quinoa exports for each country: 2008–2012 Source: LAIA

The commercial exchange between member countries increased in the last 20 years, from barely USD21 000 in 1992 to USD3.5 million in 2012 – a significant increase, albeit a minor proportion of the total value traded on the international market. The volume traded inside the Latin American region rose from 22 tonnes in 1992 to 1 382 tonnes in 2012 – again, a small proportion of the total volume traded internationally.

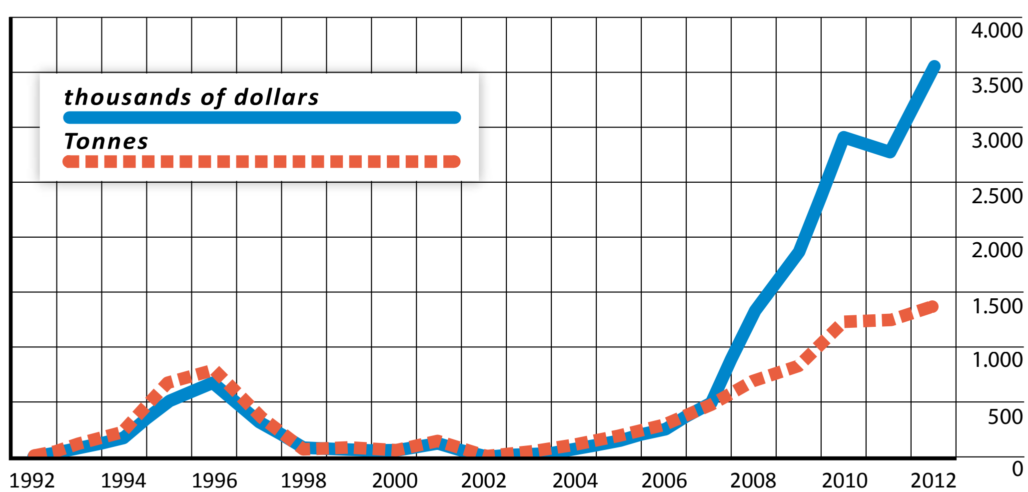

Figura 7. Intraregional quinoa trade (LAIA): 1992–2012 Source: LAIA

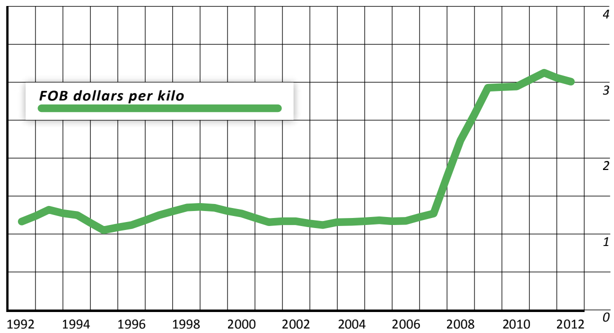

Figure 8. Trend of quinoa export prices: 1992-2012

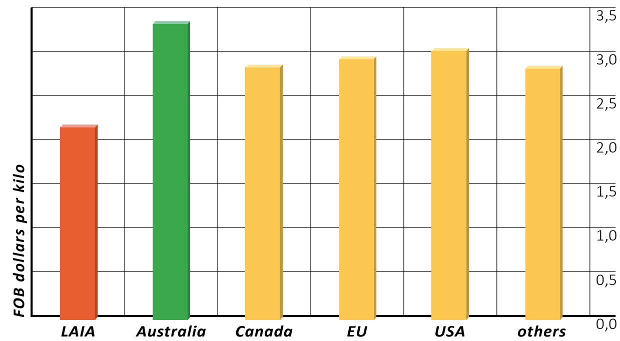

Figure 9. Prices according to destination market (2012)
Source: LAIA

Trend of international prices

Available figures show that the international price (FOB) of quinoa was relatively stable between 1992 and 2007, at USD 1.11.3/kg. In the subsequent 2 years, the price rose sharply to around USD2.9/kg in 2009, to then settle at around USD3/kg.
This rapid increase in price reflects the high demand in the international market. Indeed, prices have stayed high despite the increase in cultivated area leading to greater available supply.

Market access conditions

Quinoa exports face low tariff protection in the major destination markets. Indeed, the United States of America has a non-preferential tariff for WTO member countries of barely 1.1%. Meanwhile, the European Union levies a specific tariff of EUR37/tonne on this product – the equivalent of a value-added tariff of approximately 1.6% and, therefore, low protection. The other major markets, including Canada, Japan, Australia and Israel, have fully exempted quinoa imports from taxes.

With regard to other major economies with market potential, even if they do not rank among major importers, Russia has a moderate levy (5%) and while China has a tariff of 3%, except for seeds, which are fully exempt from tax.

In addition to these low tariff protection levels, the main exporting countries enjoy preferential mechanisms in many of the importing countries. In particular, exports from Ecuador and Peru to the United States of America are totally exempt from tax as a result of the tariff preferences granted under the ATPA (Andean Trade Preference Act) and in the United States of America–Peru Free Trade Agreement, respectively.

Access to the European Union market is also free of customs barriers for the region's three quinoa exporters. Bolivia and Ecuador benefit from the tax exemption laid down in the General System of Preferences, while Peru receives identical treatment under the Free Trade Agreement with European countries. Lastly, it should be mentioned that Peruvian sales of quinoa to China are also free of customs barriers in accordance with the China–Peru Free Trade Agreement.
In short, it is clear that the tariffs and preferential mechanisms in force do not pose a significant barrier to quinoa's access to major world markets.

Conditions for access to regional markets

In most LAIA member countries, taxes are levied on quinoa imports. However, at the same time many countries in the region have tax-free measures for quinoa purchased for sowing. These countries include Argentina, Brazil, Ecuador, Paraguay, Peru, Uruguay and Venezuela.

Quinoa imports are not subject to tax in Peru. Ecuador has the highest protection for external purchases with a 25% tariff, followed by Bolivia, Colombia and Panama (10%). MERCOSUR member countries apply a lower tax (8%), while Chile and Cuba have the lowest tariffs.

Table 1. Tariffs and preferences in different markets

Preferential tariff/levy	Importing country							
	USA	Canada	EU	Japan	China	Russia	Australia	Israel
NMF tariff	1,1%	0%	37 €/t	0%	Seeds 0% Other 3%	5%	0%	0%
Exporter	Preferential rate							
Bolivia	None		SGP 0 €/t		None	None		
Ecuador	ATPA 0%		SGP 0 €/t		None	None		
Peru	TLC 0%		TLC 0 €/t		TLC 0%	None		

Source: WTO. Years used for the table: 2011 for Australia and Russia, 2012 for the United States of America, Japan, Israel, European Union and Canada.

Note: the grey area indicates that there is no preferential treatment, because the MFN tariff is zero

Table 2. Quinoa tariff in LAIA countries

Country	MFN% tariff	
	Quinoa for sowing	Quinoa. Other
Argentina	0	8
Bolivia	0	10
Brazil	0	8
Colombia	0	10
Cuba	0	3
Chile	0	6
Ecuador [1]	0	25
Mexico	7	7
Panama	10	10
Paraguay	0	8
Peru	0	0
Uruguay	0	8
Venezuela	0	8

Source: LAIA

[1] In addition to the MFN Tariff, there is a 0.5% levy (Development Fund for Children)

Although there are tariffs in most countries in the region, the intraregional quinoa trade is mostly exempt from tax thanks to the tariff preferences stipulated in current commercial agreements.

Therefore, although there is still some room for further liberalization of the intraregional quinoa trade, this option has limited potential because of the depth of the agreements already established.

Table 3. Quinoa tariff preferences between LAIA countries (%)

Beneficiary	Grantor												
	Arg.	Bol.	Bra.	Col.	Cub.	Chi.	Ecu.	Mex.	Pan.	Par.	Per.	Uru.	Ven.
Argentina	*	100	100	100	12	100	50	20	12	100	90	100	100
Bolivia	100	*	100	100	100	100	100	100	34	100	100	100	100
Brazil	100	100	*	100	12	100	50	20	12	100	90	100	100
Colombia	100	100	100	*	20	100	100	100	20	100	100	100	20
Cuba	28	100	100	20	*	20	No pref.	28	20	12	No pref.	No pref.	100
Chile	100	No pref.	100	100	20	*	100	100	100	100	100	100	100
Ecuador	100	100	100	100	100	100	*	40	28	100	100	100	100
Mexico	20	100	20	100	12	100	No pref.	*	12	8	100	50	12
Panama	28	No pref.	28	20	20	100	No pref.	28	*	12	100	No pref.	20
Paraguay	100	100	100	100	34	100	86	100	34	*	100	100	100
Peru	100	100	100	100	No pref.	100	100	100	100	100	*	100	100
Uruguay	100	100	100	100	20	100	84	100	20	100	100	*	100
Venezuela	100	100	100	20	100	100	100	28	20	100	100	86	*

Source: LAIA

Non-tariff restrictions

The international trade of agricultural commodities and food is governed by phytosanitary regulations and by various norms and standards, and quinoa is no exception. Given that there are few or no tariff barriers, non-tariff barriers constitute the critical factor for access to major world markets for the regional production.

At present, quinoa exports do not face limitations preventing them from gaining access to the more demanding markets, such as the United States of America and the European Union – once the health certification regulations have been met with the competent authorities. It should also be noted that the higher the processing level of the product, the lower the associated health risks, which means that quinoa-derived products have easier access to world markets.

In addition to meeting health requirements, exporters have to comply with food safety guarantees, including measures concerning the maximum tolerable levels of chemical and other residues.

Furthermore, a common requirement from importing countries, and one that is becoming increasingly important, is product traceability, applicable to both the primary productive process and to the other stages in the productive chain.

Similarly, organic production must comply with standards and procedures and obtain formal certification recognized in the destination markets.

Maintaining the favourable market access conditions enjoyed by quinoa and quinoa-based products will depend, to a large extent, on the level of compliance with health control and food product safety requirements commonly applied in international markets.

These requirements have increased in number in recent years and are likely to become stricter, and, therefore, must not be considered lightly by public agencies responsible for maintaining and promoting the phytosanitary heritage of producing countries.

Outlook for Supply and Demand

Although there are no formal projections on the surface area used to grow and produce quinoa, various estimates report that in 2011, the total sown area in Bolivia, Peru and Ecuador totalled 101 527 ha (equivalent to 80 241 tonnes of quinoa produced), and these three countries represent approximately 90% of the sown area worldwide (FAOSTAT).

According to unofficial estimates published in various communication media in Peru and Bolivia, in

2013, the cultivated area for quinoa reached 50 000 ha in Peru and 80 000 ha in Bolivia. The Bolivian Foreign Trade Institute reports that in 2013 the cultivated area in Bolivia exceeded 95 000 ha, i.e. production of > 55 000 tonnes and exports of around USD100 million.

The Peruvian Ministry of Agriculture, for the inauguration of the Encuentro Nacional de Granos Andinos (12 August 2013), reported that Peru's quinoa exports would reach USD45 million that year and that Peru hoped to become, by the end of this decade, the world's leading exporter of quinoa.

The authorities in charge of the farming sector in Andean countries have, therefore, been strongly supporting the increase in cultivated area, production and exports. In order to meet the official projections of the two leading global producers and exporters, cultivation area and production will have to double by the end of this decade, and more producers will need to come on board. Today, there are an estimated 70 000 and 60 000 quinoa producers in Bolivia and Peru, respectively, , the vast majority of whom work on small-scale farms and in precarious conditions in terms of their access to factors of production.

In an analysis of the historical production trends and export volumes in the region, FAO–LAIA (2013) took annual data (1992 to the present) and used a temporal variable to estimate econometric regressions. The exercise showed that the trend growth for exports was 19%, much higher than the trend growth for production (5%), which indicates that in the future, the rate of regional production growth must increase markedly in order to meet demand.

Table 4. Econometric estimate of the region's quinoa production trends and exported volumes

Dependent variable	Period	Constant	Trend	R-squared
Production Log	1992-2011	10.3 ***	0.05 ***	0.83
Export Log	1992-2012	6.20 ***	0.19 ***	0.95

(***): 99% significant. - Source: FAO-LAIA, 2013

In this scenario, it is reasonable to hope that there will be a corresponding increase in the international price of quinoa, which in turn will lead to an increase in production. Everything indicates that quinoa is currently on the verge of penetrating international Markets. Therefore, moderate price increases can be expected in the short and medium term, with more stable prices in the long term as the supply and demand curves tend to converge.

Figure 10 shows that by the end of this decade, exports will reach 100 000 tonnes, i.e. practically twice the current volume; this means that at current prices, exports will amount to around USD300 million.

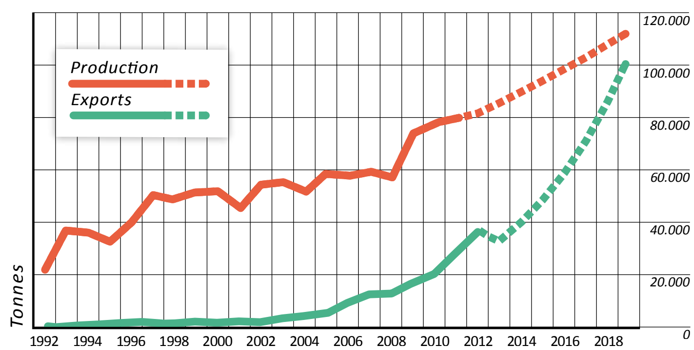

Figure 10. Projections: regional production and exports of quinoa
Source: FAO-LAIA, 2013.

Consumption outlook

As mentioned above, demand has grown rapidly in recent years, especially from high-income countries such as the United States of America, Canada, France and Germany.

Moreover, in some of the traditional quinoa- producing and consuming countries, such as Bolivia, Peru, Ecuador and to a lesser extent Chile, Argentina and Colombia, there is also renewed interest in the production and consumption of quinoa and quinoa-derived products. The reasons behind this boom in demand appear to be structural, and some are stressed below:

Demand for healthy foods

The increase in demand for quinoa and processed quinoa products in high-income countries is linked to broader trends and changes in consumer eating habits. Western consumers seek foods that offer healthy nutritional value and health and safety guarantees, and which are associated with special characteristics (e.g. organic farming) or convey cultural traditions of recognized value.

Recent studies classify the value traits of processed foods in five major groups: Pleasure, Health, Fitness, Convenience and Ethics (Gautier, 2010). The possibility of combining one or more of these characteristics is the key to adding value to foods and guaranteeing dynamic demand in international markets.

Quinoa contains at least two of the above characteristics, due to its status as a healthy food and the ethical characteristics associated with its cultural history and tradition. For this reason, the dissemination of the specific characteristics of quinoa and its nutritive qualities would consolidate its position as a product capable of meeting the increasing expectations of consumers and helping them access healthy foods.

New uses and forms of consumption

Until a few years ago, quinoa was primarily grown to feed the grower's family, mostly peasants and small-scale producers in Andean countries. However, new forms of consumption have now emerged.
The main use of quinoa consumption of the grain in a wide range of ways: toasted or ground, or transformed into flour and incorporated into various mixtures and food preparations. This is the most common application for those who grow the grain to feed their families.

In the case of production for export, the grain is generally sent to the destination market, where it is subject to agro-industrial transformation processes, which produce prepared foods using quinoa flour (e.g. various kinds of biscuits and pastries). According to the estimates of the Bolivian Government, 80% of Bolivia's production is intended for export markets. The export records relate to the quinoa grain, which has a specific tariff classification; any exports of food products with some quinoa content have no special tariff classification (they are classified under "other") and therefore there are no records that allow us to speculate on the magnitude of such exports.

Given quinoa's status as a functional product with special characteristics that appeal to niche markets, it is likely that the forms of consumption will evolve to include a range of food preparations, since its high nutritional content gives it added value. In this respect, Alarcón (2012) reports that quinoa's physical characteristics make it particularly suitable for agro-industrial processing, and in the future, it is consumption of processed quinoa that is destined to increase and become more widespread.

The Andean populations attributed medicinal properties to the consumption of quinoa grains and flour, thanks to the high content of vitamins, mineral salts and various trace elements.

Recent research confirms its use as an alternative for patients suffering from disorders linked to coeliac conditions. Quinoa can easily replace wheat flour and other products affecting coeliac patients, satisfying all the nutritional requirements usually met by wheat consumption.

Furthermore, there is ongoing research that may prove that consumption of quinoa and quinoa-based products has positive effects on diabetes patients, thanks to the high fibre content and presence of easily digestible carbohydrates. The confirmation of these properties will boost progress in medicinal uses of quinoa for the specific treatment of this disease, opening up a market with enormous prospects.

Researchers are also working on uses of quinoa and its derivatives in the cosmetics, beauty and personal care industries.

Products (e.g. body soaps and creams) have been made based on some of quinoa's biochemical properties, and they compete well against other products of similar uses but less natural organic value.

There is no guarantee that these alternative uses of quinoa will ever be adopted on a large scale. Nevertheless, examples exist of products being successfully associated with healthy lifestyle, leading to the development of innovative industries recording high growth rates at global level. For example, New Zealand companies associated honey production (promoting it as healthy) with a powerful cosmetics and beauty products manufacturer.

Quinoa consumption

Although exports to high-income countries will continue to be the driving force behind the increased demand for quinoa, we must not underestimate the potential impact of policies that foster local consumption, such as ongoing broadcasting policies and public purchasing programmes aimed at improving the nutritional condition of the population. In this respect, it is worth noting that in Bolivia, the authorities have begun to incorporate quinoa into school meals served to low-income populations.

Figure 11 reveals that quinoa consumption in the importing countries is still well below the levels observed in Bolivia and Peru. However, the importing countries are very likely to raise current consumption levels, even if they do not reach the same levels as Bolivia and Peru.

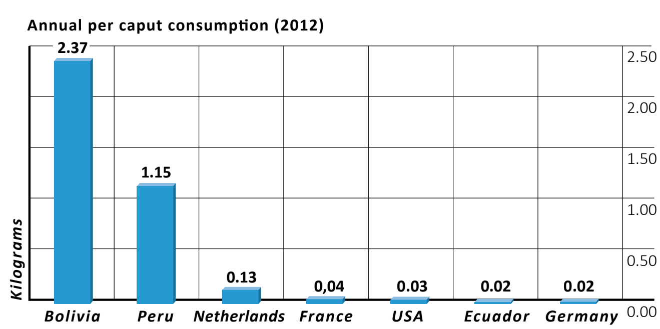

Figure 11. Annual per caput consumption (kg). Source: Author's chart, based on the estimate of apparent consumption, with data from FAOSTAT, LAIA, Trade Map and World Bank.

Even though external demand appears robust and sustainable, it is important to develop actions aimed at strengthening domestic demand. In Bolivia, 80% of production is geared towards export, while in Peru this proportion is around 25%, although rising rapidly, since the increase in production recorded in recent years is mainly driven by access to international markets.

Both Bolivia and Peru have launched policies to stimulate quinoa consumption through school food programmes and promotion of healthy eating, including quinoa as a key component.

Production outlook

Increases in quinoa production volumes are based primarily on the cultivated area, rather than on increases in physical productivity per hectare. Indeed, in the case of Bolivia, quinoa yields reached 0.645 tonnes/ha in 2000, while in 2011 an average of 0.59 tonnes/ha was recorded. The 10-year series shows stagnation with slight upward or downward fluctuations, probably linked to climatic conditions.

In the case of Peru, the situation is slightly different, since quinoa yields reached 0.97 tonnes/ha in 2000, and 1.16 tonnes/ha in 2011, representing a modest increase in productivity measured in physical terms.

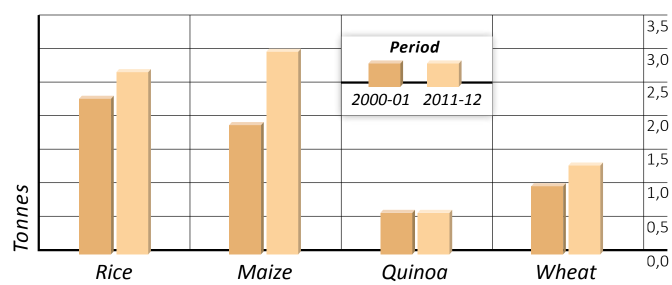

Figure 12. Yield trend for four types of cereals (tonnes/ha) in Bolivia.

Source: Author's chart, based on data from *Encuesta Nacional Agropecuaria, Estado Plurinacional de Bolivia,* 2008.

Figure 12 shows how in Bolivia, other grains (wheat, maize and rice) have increased their yields, while quinoa has stayed the same. Increasing productivity is, therefore, vital in future, because: first, it is a direct way of improving producer income; and second, expanding the cultivation area under quinoa to new geographical regions is not a variable that can be indefinitely extended.

This stagnation in yields is due to a range of factors, the most significant of which are described below:

a) Constraints affecting small producers (who represent the majority of quinoa producers) and preventing them from accessing basic production inputs, such as credit, technical assistance and water resources.

b) The wide genetic variability and low quality of the seeds used, affecting yields and product quality. Seed improvement is critical for raising productivity, and the research carried out by agricultural research institutes and non-profit private institutes is currently not sufficient to bring about significant technological change. Furthermore, there are no institutional arrangements for the dissemination of already-existing technological developments.

c) Substantial post-harvest losses, also associated with the limited resources of small producers (e.g. storage and processing facilities).

d) Lack of efficient processing infrastructure adoption of rudimentary methods, leading to high post-harvest losses.

e) Use of degraded or marginal soils for quinoa cultivation (despite its great agro-ecological adaptability, the crop still requires basic fertile conditions to develop).

In conclusion, in traditional quinoa-producing countries, there is still immense scope for increasing production: by increasing the cultivated area (a process underway in recent years); and by optimizing the potential for increased yield by overcoming the difficulties and limitations affecting small-scale farming. However, it is unlikely that the increase in production of the Andean countries will be sufficient to cope with global demand. Other countries may take advantage of this situation and increase their quinoa production; however, it should be noted that, depending on which countries enter the quinoa production race, the effect on the Andean producers may vary. They could find themselves at a disadvantage in areas of finance, technology and productive resources.

Characteristics of the value chain

Quinoa is almost entirely produced by peasant producers, which means that supply is very fragmented. In Bolivia alone, it is estimated that there are at least 70 000 small producers of quinoa, which means that on average they grown about 1 ha of quinoa per farmer – this is central to the nature of the market channels established for quinoa flow to local, regional and export markets.

Even though these channels may vary slightly depending on the final destination of the product, their basic structure is similar and adapted to dealing in quantities traded in small volumes and highly heterogeneous in nature in terms of quality and physical characteristics.

Moreover, quinoa is not a product that can be consumed directly, and this also conditions marketing and distribution: middlemen are inevitably involved to implement a range of preliminary processes, such as drying, dehulling and saponin removal.

International demand has resulted in higher standards in terms of product quality and homogenization. This in turn reduces transaction costs and makes it easier to guarantee product quality and safety. There are no detailed studies available for quantifying how resources are distributed between the different linkages in the value chain. However, as with other value chains, the bulk of the income is most likely retained by food industry traders and processors. For example, in a comparison of retail prices in United States supermarkets in July 2013 prices were USD14-25/kg for pearl quinoa (direct research by the authors), while the export price FOB is USD3/kg.

Key professionals in the value chain

The value chain comprises a wide range of professionals with varying levels of economic power. They adopt different kinds of technology and the ties formed with other linkages in the value chain also vary. The structure of quinoa marketing circuits is similar to that of other Andean grains and other products with strong peasant roots: the weakest link is the primary production (FAUTAPO Foundation, 2012).
The major stages in the quinoa value chain are described below and summarized in Table 5 (IDEPRO, 2012).

Primary production

Primary production is dominated by small individual producers, although they are sometimes organized in cooperatives and other associations, both formal and informal. There is no basic infrastructure for storage, drying and dehulling, and income levels are low. As a consequence, they have little bargaining power and are the weakest link in the value chain. There are a small number of exceptions, such as producer organizations members of CABOLQUI (Bolivian Chamber of Exporters of Quinoa and Organic Products), who have obtained as much as 70% of export prices FOB.

Storage and basic processing

This stage mainly involves small (including micro- and individual) companies that have recently joined forces as producer cooperatives and associations, establishing small-scale facilities to improve the drying, winnowing and saponin removal processes. Storage centres are generally located locally, and the production is then sent to regional and international markets.

Industrialization

This process may include part of the basic primary processing, but entails above all the grinding of the grain and its preparation, either for direct consumption as flour or for incorporation in further transformation processes involving quinoa. This stage mainly involves small and medium-sized companies, including a limited number of cooperatives and producer associations.

The companies basically collect, extract and industrialize organic or conventional quinoa, ultimately selling the product at its final destination, whether national or international.

Marketing for the domestic market

Local domestic markets or markets intended for small rural populations are mostly supplied by the same small producers, who sell their products on farmers' markets organized weekly in different locations.

Regional markets and markets in major urban centres are primarily supplied by wholesalers working with the processing industry. When the processing facilities are also located in an urban centre, direct transaction can take place between the grain brokers and the processing industry.

Marketing for the foreign market

Quinoa production intended for external markets must meet higher standards of presentation, uniformity and safety. It should be noted that most quinoa for export is produced and certified as organic quinoa, and therefore passes through specialized marketing channels dealing directly with the importers at the end destination. The importers are generally medium or large companies with the administrative structure and financial support necessary to carry out the formalities and meet the requirements associated with international trade.

Table 5. Main components of the value chain

LINKAGE	VARIANTS
Marketing	Marketing for export. Specialized export companies. Medium to large size.
	Marketing for domestic market. Medium-sized companies, generally part of the processing industry.
Industrialization	Processing. Medium or large-sized industry, located at regional level or in major urban centres.
Primary transformation	Industrial beneficiary. Local or regional level and medium-sized grain brokers .
	Non-industrial beneficiary. Local level small grain brokers
Production of the quinoa grain	Production of conventional quinoa. Micro and Small producers and associations
	Production of organic quinoa. Small producers and associations

Source: Compiled by authors based on data from IDEPRO, 2012.

Peasant organizations and associations that have successful dealings with foreign markets tend to be backed by a public institution or an NGO.

Requirements for improved operation of the value chain

The structure of marketing channels is changing as a result of both the rapid increase in external demand and the increase in demand from densely populated urban centres.

In order that the economic benefits of rising demand and higher international prices reach small peasant producers, it is necessary to improve the brokerage and processing channels. To do so, public support policies must focus on finding solutions to existing major problems:

- Support is required to enable small producers to form associations. Technical, financial and institutional backing is needed to allow associations access to small-scale storage facilities and primary treatment facilities for selection and preparation of the grain for industrial transformation. Small producers would thus represent a stronger link in the value chain.
- Programmes can be implemented to improve producers' position as suppliers to the processing industry. Such initiatives have already been successfully experimented in several countries.
- Government programmes to stimulate domestic consumption of quinoa should be associated with mechanisms of direct public purchase from producer cooperatives and organizations, thus shortening the marketing chain.
- Detailed studies on the quinoa value chain and its transformation process should be conducted in order to better target public policy requirements and priorities and improve policy implementation.

Challenges for public policies

The positive outlook for quinoa production and its placement in regional and international markets is forecast to continue.

Quinoa cultivation therefore represents a valuable opportunity to promote the development of small-scale family farming. However, if the benefits are to reach small producers at the end of the chain, specific, appropriately targeted public policy is required. Priority areas include:

- Increasing productivity, incorporating technological innovations (especially with regard to seed quality and crop management) and promoting technical assistance and technological transfer programmes.
- Developing lines of research that allow production with improved levels of standardization and uniformity, without damaging the vast and rich biodiversity present in this crop.
- Promoting the development of associations to increase the scale of operations in small peasant production, including basic production and processing, industrialization and marketing.
- Improving storage and drying facilities, in order to minimize post-harvest losses, while giving small producer associations greater bargaining power in their relationship with the other links in the value chain.
- Conducting studies on new uses of quinoa, aimed at increasing supply and responding swiftly to new types of market demand.
- Promoting campaigns to enhance knowledge of the product on international markets, especially campaigns focusing on nutritional characteristics and ethical and cultural values.
- Monitoring markets, especially foreign markets, in order to prevent mismatches between supply and demand with a negative impact on prices.
- Maintaining public policies to promote domestic consumption, for example, incorporating quinoa in the catering services provided in schools and colleges, and implementing other promotion and distribution measures.

It should be stressed that the implementation of public policies to support quinoa production requires: the participation of the various professionals in the value chain; continuity over time to allow an effective impact; and targeting to ensure that the effect is felt where it is most needed.

Concluding remarks

The growing demand on international markets will continue to be the main factor driving the development of quinoa cultivation in coming years. There is a high level of interest in the crop due to: its health-promoting properties; the values and traditions associated with its production; and the wide range of preparation and consumption options offered by the grain and its derivatives.

The projected growth of demand and supply in both developing and developed countries indicates that prices will remain stable, or even increase, at least until the end of this decade. Finally, the cultivation of quinoa will continue to be a valid alternative to improve the income of small producers, especially those in the Andean region. Stimulating the production of quinoa should be considered a powerful public policy tool for fighting rural poverty and improving the food and nutrition of low-income populations. However, authorities must ensure that higher prices do not result in vulnerable populations who are not traditional quinoa consumers being denied access to this nutritious food. Government public purchase programmes implemented in Bolivia, Peru and Ecuador aim to avoid such an outcome.

The development of quinoa cultivation also requires support policies specifically geared towards overcoming the principal problems affecting productivity, especially considering that developed countries will increasingly enter the market as producers. It is essential to continue research and to disseminate technological packages tailored to the different agro-ecological conditions under which the crop is grown.

Similarly, strengthening the value chain, improving bargaining conditions for small producers and equipping organizations with basic storage and primary transformation facilities, should all be areas of priority concern for public authorities. Otherwise, there is the risk that the economic prosperity and benefits of high quinoa prices may never reach small producers.

Lastly, the interest and coordinated efforts of public organizations in producer countries, as well as NGOs and international organizations linked to the agrarian sector, can raise awareness about the qualities of quinoa, thus consolidating its status as a healthy food, associated with traditional values and cultures that are valued on international markets.

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CHAPTER: 4.2.

Quinoa trade in Andean countries: opportunities and challenges for family

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Abstract

Quinoa's revival has roused much interest in Andean as well as in European and North American countries. This Andean product, formerly denigrated and destined only for self-consumption, has made its way into the diet of the urban populations of Andean countries and has now spread to the United States of America, Europe and other parts of the world. In the Andes, farmgate prices have gone up and the quinoa sector has become attractive to investors. A wide range of products based on this *Chenopodium* have appeared in shops – from breakfast cereals to healthy snacks, noodles, beverages, beer and even ice cream. These products are well positioned in niche quality markets such as the nutraceutical, organic and fair trade markets. Against this backdrop, various other commercial channels also: from the most traditional (barter) to the most modern (online sales), through to contract farming with modern processing plants with organic certification and fair trade labelling.

This chapter presents the changes in how quinoa is marketed quinoa in specific segments of this market, through a review of the literature of case study research in Bolivia, Peru, Ecuador and Chile.

Introduction

Quinoa can no longer be considered a food staple intended primarily for the self-consumption by the indigenous populations of the Andean highlands. The revival of quinoa has roused much interest in Andean as well as in European and American countries. In the last decade, the quinoa supply has diversified in terms of both varieties and products available. Today, in addition to the basic pearl quinoa (with the saponin removed, ready for consumption), there is a wide variety of quinoa-based products, such as breakfast cereals ("pipocas", quinoa flakes etc), biscuits, healthy snacks, noodles, instant soups, beverages, beers and ice creams.

The commercial "boom" of quinoa and market segmentation at national and international level have resulted in the creation of new value chains. Quinoa is established in specific quality markets, such as nutraceutical, organic and fair trade markets (Cáceres, 2005). While traditional trading systems still exist, such as barter, it is the surge in quinoa sales in supermarkets and the export boom which have altered both trading and production systems. Certification processes for organic quality and fair trade have developed, primarily for the international market. Initiatives to promote the specific qualities of quinoa have also appeared through new short food supply chains, especially in Ecuador and in Chile, but also in Peru with the gourmet market.

Conventional chains

Bolivia and Peru: the two largest players in Andean quinoa

Most producers from the Andes sell quinoa on weekly farmers' markets where the measuring unit used is the "arroba" (11.5 kg). Some areas continue to practise bartering (in exchange for vegetables or bread), for which the measuring unit is the "puñado" (Image 1).

Local farmers' markets or larger markets (e.g. the Challapata farmers' market in Bolivia or the Manco Capac market in Juliaca, Peru) mostly bring together wholesalers who handle large volumes and supply the urban markets and processing plants. These middlemen buy quinoa at the weekly farmers' markets or directly from the communities; they assume the transport costs of the grain. As in many other chains, it is the middlemen who have the true bargaining power and control over the quinoa sector, since they deal in large volumes. Nevertheless, their market power is not the same everywhere. According to Risselborn (2011), competition is now fierce in the southern Altiplano of Bolivia and the middlemen have lost market power. In many communities, producers now have several options: sell to middlemen, to local companies or to cooperatives. These choices are linked to complex socio-economic models, as illustrated by the work of the ethnographer Ofstehage (2010, 2011, 2012) for the case of the San Agustín community in Bolivia.

Image 1: Quinoa barter for bread in the Peruvian Altiplano. © Aurélie Carimentrand (July 2012).

Most of the quinoa market flow is recorded in the harvesting months. However, due to the fragmented sale strategies characteristic of small producers, a good portion of the production also flows to the market throughout the year. On local farmers' markets, producers do not usually handle standardized products; rather they sell a mixture of quinoa varieties. With the marked rise in urban and international demand for quinoa, new channels have been created which handle standardized products. This trend has impacted the organization and governance of these value chains, through the development of contract farming with high potential producers. Agro-industrial companies and exporters seeking to meet the market demand for uniform and large grains, encourage producers to sow improved quinoa varieties (e.g. 'Salcedo INIA' in Peru). This phenomenon represents a risk for the biodiversity of cultivated quinoa, as cultivation tends to be increasingly homogeneous, leading to limited varieties. Nevertheless, white quinoa is no longer the only variety sold: the market for coloured quinoa (red, black etc.) is also developing.

In Bolivia, the bulk of production is 'Quinoa Real' from the southern Altiplano of Bolivia (departments of Oruro and Potosí). Its revival began in the 1950s on informal markets, where it was destined for both domestic consumption and export to Peru, through networks of intermediaries formed by merchants in the region and the Peruvian Altiplano (Laguna, 2002). The emergence of peasant organizations (*Organizaciones Económicas Campesinas* – OECAs), in particular the *Central de Cooperativas Operación Tierra* (CECAOT) in 1975 and later the *Asociación Nacional de Productores de Quinua* (ANAPQUI) in

1983, with the support of the *Confederación Sindical Única de Trabajadores Campesinos de Bolivia* (CSUTCB) facilitated the marketing of 'Quinoa Real'. These organizations were backed by foreign NGOs. Their objective was to improve the living conditions of quinoa producers. They aimed to obtain better prices and add value at the various levels of the quinoa supply chain, by taking care of collection, hulling, partial processing and marketing. These second-level organizations brought together several local organizations (Ayaviri *et al*., 1999; Healy, 2001; Hellin and Higman, 2003; Laguna, 2011). Competition from private companies arrived soon after with Saite y Irupana in 1987, Jatariy in 1997, Quinuabol in 1998, Andean Valley in 1999, Quinua Food in 2003, etc. There are 62 small-scale, semi-industrial and industrial quinoa plants in the country (Figure 1).

The first recorded export of quinoa in Bolivia was in 1983, when CECAOT shipped 200 tonnes to the Quinoa Corporation in the United States of America. According to data from the Bolivian Institute of Foreign Trade (IBCE), in 2012, around 26 252 tonnes of quinoa were exported for USD80 million. Quinoa exports have increased sharply since the 1990s. A large number of private firms, as well as various support institutions, have followed suit. However, there have been other increases besides exports.

For example, consumption on the domestic market has also increased threefold in the last 4 years, from 4 000 to 12 000 tonnes in 2012 (even though the annual per capita consumption is still low at approximately 1 kg). In the 2012 crop year, approximately one-quarter of production was destined for the domestic market, one-quarter was "smuggled" to Peru and the remaining half was exported to the international market (Gout *et al*., 2013).

In Peru, the leading quinoa producers' organizations are in Puno, Ayacucho, Cusco and Junín. However, they have neither followed the same development trend nor have the same impact as the Bolivian organizations. Peru's history of weak union movement dates back to the period of the dictatorship. None of these organizations are structured from the grassroots to the national level as they are in Bolivia. Moreover, even if there is a strong cooperative movement, it is concentrated in the lowlands and in commodities such as coffee, cocoa or tropical fruits. Furthermore, the majority of quinoa producers are individual farmers and are not necessarily part of a cooperative or association. However, the formation of associations, backed by local NGOs and regional governments, is spreading (see the 2013 directory of quinoa value chain). Peruvian law 29972 (2012) on the "inclusion of agrarian producers through cooperatives" strengthened this perspective. At present, these organizations do not have their own processing plants, except for the COOPAIN cooperative (San Román province, Puno) which comprises 15 organizations (> 500 members cultivating some

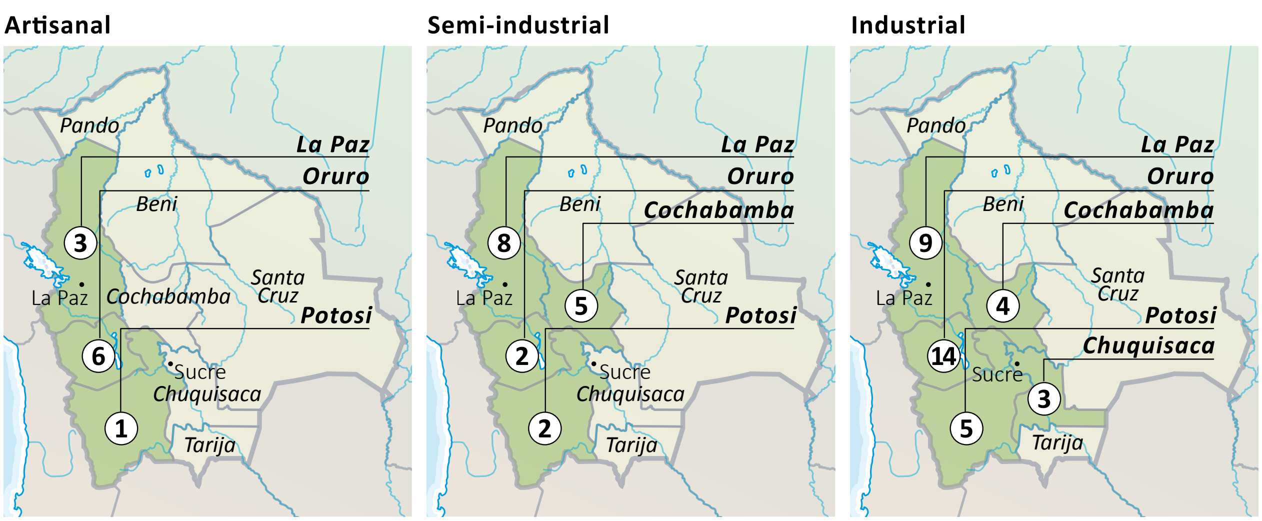

Figure 1: Quinoa processing plants by department and type in Bolivia. Source: IBCE/Foreign trade No. 210, p. 13, March 2013.

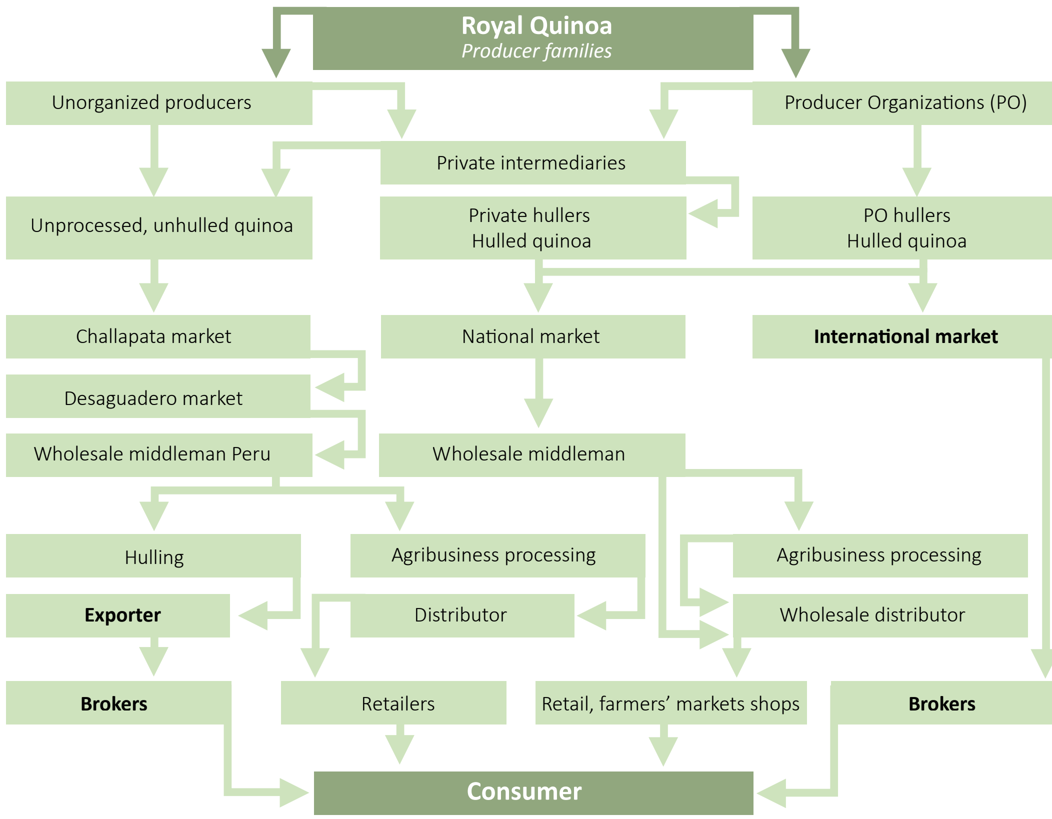

Figure 2: Economic actors in the 'Quinoa Real' value chain (southern Altiplano of Bolivia)
Source: Gout *et al*. (2013) from Soraide (2008).

520 ha of quinoa). It is the leading organization of quinoa producers with its own plant in Puno. The other quinoa processing plants are private firms.

Quinoa exports in Peru began in 2005. In 2011, the country exported around 7 991 tonnes of quinoa to 36 countries for a value of USD25 million (SUNAT). This value rose to more than USD30 million in 2012. The main market for Peruvian quinoa is the United States of America. Sierra Exportadora, a public organization, actively promotes quinoa and fosters relations between the different actors in the chain. In 2011, the leading firm in quinoa exports was "Organic sierra y selva" with a value of USD10 million (40% of the country's quinoa exports). The company runs a very modern plant (automatic washing and drying) in the Lurín district in southern Lima. Another major company is the "Grupo Orgánico Nacional"; it too operates a plant in the south of Lima (Chorrillos). There are four factories in Puno which process quinoa for export: the Altiplano SAC, founded in 1994, and Agroindustrias CIRNMA, ASAIGA and the COOPAIN cooperative since 2010. Altiplano SAC attempted to export quinoa directly but following a series of difficulties, it preferred to deal with a Lima-based broker who coordinated the transportation and handled the customs formalities.

At national level, the authorities hope to raise the annual per capita consumption of quinoa, which is currently 800–1 000 g. The national market for new quinoa-based products is developing at the same rate as Peru's growing middle class.

With the Peruvian gastronomic "boom", neo-Andean chefs are promoting the consumption of quinoa, using it in modern dishes. The APEGA-Peruvian society of gastronomy, organizer of the Mistura Food Festival, is working to create an alliance between chefs and farmers including quinoa producers.

From 2007 to 2011, the farmgate price increased threefold, moving from a value of PEN1.22 (nuevos soles) per kg to a value of PEN3.68/kg (MINAG – OEEE, Figure 3).

Ecuador

In Ecuador, a substantial portion is sold to traditional middlemen; the rest is directly purchased by private sector representatives, such as Inagrofa, or "socially-responsible" firms, such as the *Fundación Mujer y Familia* (FUNDAMYF) or Sumak Life, the main intermediaries dealing in large volumes. The middlemen have contacts with merchants and grocers. The main ones are located in the city of Ambato and their operations include purchase of Ecuadorian quinoa, and storage and distribution of large quantities of Peruvian or Bolivian quinoa smuggled into the country. However, given the sharp increase in quinoa prices in Ecuador since 2012, the supply chain is likely to undergo changes. There may be at least a temporary reduction in the activity of the wholesalers and retailers, who only supply the domestic market. This would benefit agrifood companies which focus on exports. Even if export prices are high, there is no impact on consumption, at least in the United States of America. Sales to associations and farm cooperatives occur on two levels.

- The first level involves peasant organizations specialized in quinoa and targeting special export markets with certification, such as Coprobich. Otherorganizations may not be fully autonomous in the commercial process, which continues to be managed by external entities with variable legal status (foundations, socially responsible companies, private firms).
- The second level involves less specialized peasant organizations, supporting a variety of producer activities and intervening on a small scale in the artisanal transformation of quinoa and other Andean grains, as in the case of Unopac in Cayambe or Mushuk Yuyay in Cañar.

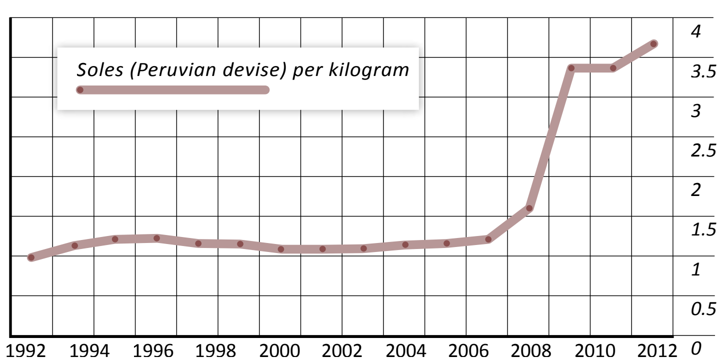

Figure 3: Trend of quinoa farm prices in Peru.
Source: Based on data from MINAG-OEEE.

Two years ago, the company Inagrofa attempted contract farming, supplying seeds and technical assistance to producers. This experiment was in the provinces of Imbabura and Carchi, but was short-lived, as only a small number of producers could sell through the company and they were left without a market. In 2013, the company sought again to work with producers, but from other sectors, given the lack of motivation of the producers who previously worked with this firm.

With regard to exports, an Ecuadorian consortium was created in early 2013, comprising three private companies, Cereales Andinos, Urcupar and Rogetore y Franco, with two foundations, FUNDAMYF and *Maquita Comercializando Como Hermanos* (MCCH).

Public purchases of quinoa and its derivatives occurred for just 1 year, in 2010. It involved a public procurement order for 260 tonnes under the food supply programme from the Coprobich organization and producers in the northern mountains. Due to the difficulties meeting processing deadlines (since private plants prioritize quino processing for their own market rather than that of the organization), Coprobich lost money in this sale to the state. The rules of public procurement were later changed and to date, there have been no quinoa purchases by the state, at least not in any significant proportion.

A relatively small proportion of the quinoa is sold directly at fairs, be they peasant, socially-responsible or agri-ecological. For example, at the farmers' markets of the northern mountains, of around 100 points of sale, only two or three sell quinoa. Sales are just 10–20 kg/week. At the socially-responsible famers' markets in the northern highlands, accompanied by the NGO AVSF (5 fairs and 600 producers), around 2.5 tonnes of quinoa were sold in 2012. Organizations, such as FICI, CCM, Unorcac or agri-ecological associations in the southern mountains, promote and manage farmers' markets. These markets contribute, albeit on a small scale, to the direct sale of quinoa by farmers, at fairly accessible prices (more or less half the quinoa price applied in large-scale

Image 2: Quinoa producer and Chimborazo quinoa leader, Ecuador © Jean-Philippe Noel

retail outlets such as supermarkets). Even though barter is still practised on various producer markets, in particular the farmers' markets of the northern mountains, where peasant groups declared 2013 the international year of barter, it is gauge how important this practice is for quinoa. Although the crop is present at producer fairs, it accounts for a very small proportion of the goods on sale.

Current prices are high in Ecuador – as in other parts of the world – for both producers and consumers. Currently, producers sell on average 1 Spanish quintal of 46 kg of raw quinoa (unwashed, unscarified) for between USD80 (USD1.74/kg) and USD120 (USD2.5/kg). The consumer price is USD2.2–3.3/kg at local farmers' markets and USD5.5–6.6/kg in urban markets and supermarkets. Unlike in Bolivia, these high fluctuating prices are a new phenomenon in Ecuador. In 2009, 1 Spanish quintal was worth more or less USD40, in 2010 USD90 and it fell again in 2011 to around USD30–40, to then rise in 2012 to USD80–100.

There is high demand from importers for quinoa, but production does not meet demand, resulting in increasingly high prices: from USD3 000/tonne FOB in 2012 to USD3 500–4 000 (or, in some cases, USD5 000/tonne FOB) before the end of 2013.

Chile

The main quinoa production area in Chile is located in the Iquique region, at an altitude of 3 800 m asl in the Chilean Altiplano. Production is mainly carried out by elderly farmers, since young people have abandoned farming and migrated to the large cities. In this region, quinoa has its roots in the Aymara culture. In Norte Chico (region of Coquimbo), some producers are striving to (re)introduce quinoa with the primary goal of producing healthy food. In central Chile (between San Fernando, Curicó and Linares, Libertador Bernardo O'Higgins region), quinoa is grown at sea level (< 800 m asl) by small elderly producers on small plots. Quinoa is traditional in this area, and for some producers, it is a crop with interesting economic potential, once the issue of marketing is resolved. In the southern part of Chile (around Temuco, in the Araucania region), quinoa is linked to the Mapuche culture and is found growing in the gardens of Mapuche women (Bazile, 2013).

In numerous studies about Chilean quinoa, reference is often made to a growth boom on the national market. It is, however, extremely difficult to find evidence to back this claim. In the absence of proof, increase in supply is usually considered the same as market growth. It appears that quinoa self-consumption continues to grow and that sales often pass through informal markets. However, surveys (Bazile *et al*., 2012) show that farmers sell an increasingly large amount of their production on both informal and formal markets (middlemen, cooperatives etc.), in addition the different regional markets: > 25% in the south, > 50% in the north, > 85% in the centre.

In the 1980–90s, there was a shortage of quinoa for national consumption, especially in the Tarapacá region where more than 90% of Chilean quinoa is currently produced and where the majority of the indigenous Aymara people live. Several factors explain this quinoa shortage, including bioclimatic factors, prices on international markets, migration of young Aymaras, and competition with and overlapping of Bolivian production. This shortage had a huge impact on quinoa production in the Chilean Altiplano.

The commune of Colchane is one of eight rural communes in the Tarapacá region (260 km northwest of the city of Iquique, regional capital of Tarapacá) with a total of 23 Aymara communities. In this commune, approximately 1 200 ha is devoted to quinoa cultivation, but only 250–350 ha actually produce quinoa, since the community still practises the tradition of crop rotation, leaving the land fallow or idle for 2 years.

Since 2000, the Altiplano producers have regained interest in the cultivation and marketing of quinoa, thanks for the main part to high international prices and access to projects and financial resources. They have also begun to organize themselves at regional level to optimize the production and sale of quinoa and its by-products, with the aim of conquering local, national and international markets.

In this context, two quinoa processing organizations were created in 2000 and 2007, respectively, in the commune of Colchane: Juira Marka (NGO) and QuinoaCoop (cooperative). Juira Marka was created in 2000, with the intention of bringing together and organizing the 160 quinoa producers from over 20 Aymara communities in the commune of Colchane. The plan was that they unite and join forces so as to collectively cope with the technical and economic changes required to establish their position in the global market. QuinoaCoop was created in 2007 within a single Aymara community, the Ancovinto community.

Self-consumption varies from family to family; it is nevertheless estimated that 30% of the quinoa harvested every year goes to self-consumption and the rest is sold. The main markets are: Bolivia, through the Pisiga-Bolivia bimonthly farmers' market on the Chilean border; and direct sale of small quantities (with personal networks or through the markets) in Iquique, Alto Hospicio, Putre or Pozo Almonte. In 2009, Colchane producers sold unprocessed quinoa at the Pisiga market for between CLP450[1] (pesos) and CLP800/kg. The same quinoa, processed and packaged, can be sold as much as CLP3 000/kg to Chilean consumers in the cities of Iquique, Alto Hospicio, Pozo Almonte and Arica.

Between 2000 and 2009, the price of unprocessed quinoa rose sharply, reaching more than USD2,100 (CLP 54 000) per quintal (45 kg). In the post-harvest period (April–August) the purchase price fell slightly (due to increased supply) to USD80/quintal. In Pisiga, Bolivian buyers no longer apply different prices to the different grains, and all ecotypes are sold at the same price. The colour does not have much impact on the value, as in previous years (Arar, 2009).

Support from the Government and non-governmental institutions is important for the development and commercialization of quinoa. Quinoa producers are in contact with the professionals of these institutions; they present projects through which they can obtain economic or material resources for production or marketing. The main institutions intervening at commune level are:

- Prodesal: agreement between the Municipality of Colchane and INDAP (*Instituto de Desarrollo agropecuario*), technical support for the cultural management of quinoa and camelids (llamas, alpacas).
- Origenes: programme of CONADI (*Corporación Nacional de Desarrollo Indígen*a), evaluation and allocation of resources to collective and individual projects of producers on three issues: organization, production and culture (for example, the UMA project [*agua* in Aymara] which concerns access to water and field irrigation).
- FIA (*Fundo de Innovación Agraria*): financing of collective projects with productive goals.
- UNAP (*Universidad Arturo Prat*): survey of production systems and varietal improvement of plant species cultivated in the Altiplano.

[1] Based on an exchange rate of 1 USD = 500 CLP approx.

The two organizations, Juira Marka and QuinoaCoop, are entering the market and are striving to position themselves. Juira Marka, despite the close ties between this organization and the municipality of Colchane, and after a good start, has been struggling to survive, and to build on and participate in innovations at territorial level. The difficulties stem from the different situations of the 136 members, and the main problem has been existing conflicts between communities. In the case of QuinoaCoop, the vision of its young leader is to "modernize production" and processing, and alliances have therefore been formed with the *Universidad Arturo Prat* in Iquique (*Cátedra del desierto*). It currently has 14 members, all from the Ancovinto community (southern sector Cariquima) (Bazile *et al*., 2011).

Juira Marka began to sell quinoa in 2000 under the *Grano del Sol* brand, in the form of various quinoa products and by-products, in local and national supermarkets (e.g. the Roxy chain). It even sells to private companies that supply airline companies (Skychef). In 2004, it decided to use the regional funding it had obtained to buy a processing plant (five machines) and a storehouse to process its own grain. But production and transformation stopped a few years ago for several reasons, but mostly due to competition from the Bolivian market. Bolivian buyers at the Pisiga market or in small communes on the other side of the Chile–Bolivia border, offer a good price and pay cash for unprocessed quinoa. For this reason, many Chilean producers prefer to sell their unprocessed production directly to these buyers.

The QuinoaCoop was initially founded with the goal of giving a different focus, a more commercial legal framework, to an organization which already existed as the *Indígena Aymara de Ancovinto* community. Its primary goal is to produce and sell quinoa on a larger scale under its own trademark and name, conquering new national or international markets. Producers still work in traditional organized groups or *Ayne,* as they are known in the indigenous Aymara tongue. They deliver part of their production to the cooperative, which sells various quinoa products under the QuinoaCoop trademark. With the support of institutional projects (FIA), it acquired a processing plant, machinery and storehouse for processing, transforming and packing quinoa. The *Universidad Arturo Prat* (UNAP) provides technical assistance. The cooperative recently began to market its product on the local, national and international quinoa market in different forms: grain, white and toasted flour, "pipoca", biscuits.

Agriculture in the valleys of central Chile is typical of the type of export agriculture backed by Chilean public policies since the early 1980s. They are mostly export monocultures (vine and fruit trees), grown on fertile soils, with access to high technology and substantial capital. In the "dry coastal" region, a tiny isolated farming region, the poor, depleted soils are a serious constraint in family farming. It is here that quinoa is grown on small plots. Quinoa is closely linked to the identity and social history of these impoverished peasants; it is associated with a special gourmet culture intertwined with an entire sociotechnical background. On these farmlands characterized by an inhospitable climate, the cooperative movement has had a powerful social role. The *Cooperativa Las Nieves* was created at the end of the 1960s, right in the middle of the agrarian reform in Chile. The neoliberal economic model, introduced during the Pinochet dictatorship, destroyed nearly all social networks linked to agricultural cooperatives in Chile. In the 1990s, the search for a means to save the cooperative led the economic stakeholders of the time to propose quinoa as the springboard for local rural development. In 2004, the *Agrícola Las Nieves* company was formed. It comprised seven members, including *Cooperativa Las Nieves,* which at that time yielded its name to the major producers in the zone. They joined forces in order to export their quinoa to the North American and European markets. Quinoa then evolved from a self-consumption product (> 80%) to an economic commodity (> 90% sold). Thus, the local social structure depends on the ties between one stakeholder with a powerful position in the sector, *Agrícola Las Nieves*, and all the other stakeholders in this rural territory. *Agrícola Las Nieves* has positioned itself as the only large-scale transformation and marketing company; it is today the promoter of all public-funded quinoa projects. The huge difference in prices paid to producer-shareholders (8–10 ha on average) and to isolated small producers (0.25–1 ha) gave rise to repeated conflicts until the recent disappearance of the company. The small producers in the central zone have now formed a cooperative (*Cooperativa de Productores de Quinua* *del secano, COOPROQUINUA)*; rather than focusing on export, the association concentrates on getting the production to the domestic market, in particular the Santiago market, 200 km away.

Value chains of organic and fair trade products

Fair trade quinoa from Bolivia

The bulk of exported quinoa is organic and/or covered by fair trade labels. There is also a domestic market for organic products. For the international market, organic quinoa is certified according to the standards of the importing countries. Quinoa certification is handled by national and foreign certification firms, such as Biolatina or Imo Control.

National standards have existed for the domestic market since 2006: the Bolivian technical standard (law 3525/06) and technical regulations for organic producers in Peru (Supreme decree 044-2006-AG). In Bolivia, the association of Bolivian ecological producer organizations, *Asociación de Organizaciones de Productores Ecológicos de Bolivia* (AOPEB), actively strives to promote the national consumption of organic products. In Peru, quinoa can be purchased through new short organic food chains, such as the weekly organic produce markets (Bioferias) held in Lima.

There are a range of fair trade initiatives for quinoa (Carimentrand, 2008, 2011). Most fair trade importers resort to the use of Fair trade labels. The most popular is the FAIRTRADE label by Fairtrade International (previously known as FLO), which was adapted to quinoa in 2004. It guarantees a minimum price – recalculated in 2012 to reflect the price increase and the sustainability problems faced by quinoa channels. The current minimum price for processed quinoa is USD2 250 per tonne for conventional quinoa and USD2 600 for organic quinoa (Fairtrade International, 2012, Table 1). It also guarantees a fair trade premium of USD260/tonne.

Quinoa fair trade began in the Altiplano of Bolivia in 1989 with the first contracts signed by quinoa-growing OECAs with fair trade European importers, thanks to the support and contacts received through international technical cooperation. At that time, there were no labels for fair trade quinoa. Quinoa was sold in world shops that sought to support small producers “from the South”. From 2004 onwards, the FAIRTRADE certification for quinoa meant that fair trade quinoa could be sold in supermarkets, especially in Europe, through fair trade brands such as Alter Eco or Ethiquable in France. Fair trade draws attention to the biodiversity of quinoa by offering a range of coloured quinoa grains (black quinoa, red quinoa or mixed quinoa).

In the early days, fair trade focused on promoting the organization of producers; in other words, producer organizations handled the collection, washing and hulling of the quinoa, and they were in direct contact with import businesses to get the produce out of the country. The producers therefore played a major role in adding value to the final

Table 1: Changes in fair trade prices with the FAIRTRADE label for quinoa

	Existing prices in USD/tonne	New prices in USD/tonne
Geographic scope	Bolivia, Ecuador, Peru	South America
Product form	Raw quinoa	Processed quinoa
Price level	Farmgate	FOB
Minimum fair trade price for organic quinoa	861	2 600
Minimum fair trade price for conventional quinoa	771	2 250
Fair trade premium	85	260, of which at least 78 to be invested in environmental sustainability

Source: Fairtrade International (2012)

product through their organizations. In this case, the communities were more than suppliers of the raw material: they had significant control over the production chain and accordingly more extensive bargaining power.

In 2013, there were five FAIRTRADE certified producer associations in Bolivia: ANAPQUI, the "*Asociación Ayllus Productores de Quinua y Camélidos*", the "*Asociación de Productores Comunidad Cayñi*", the "*Asociación de Productores de Quinua Salinas*" and the "*Asociación Integral de Productores Orgánicos Capura* – AIPROC". Another important label for Bolivian quinoa sold in France is the "bio-équitable" label used by a company called Jatary and based on the Fairtrade standard (ESR) of the Ecocert organic certification organization. In recent years in Bolivia, exports from producer organizations under the FAIRTRADE label have lost some ground to fair trade exports by private firms (Gout *et al*., 2013). With the new fair trade standards allowing certification of contract farming, a debate has been sparked. On the basis that in "the medium term, capacities shall be transferred to the producers", the door was thrown wide open for the fair trade marketing system. Until then, it had been concerned with promoting value added for producers; now it was to become something more conventional (Gout *et al*., 2013).

In Peru, there is only one FAIRTRADE certification organization: the Coopain Cabana cooperative in the province of San Román near the city of Puno (Image 3). It has been observed that organic agriculture certification bodies tend to propose their own fair trade labels (e.g. the FAIR CHOICE label of Control Unión). Often, certification takes place at

Image 3: COOPAIN-CABANA Cooperative (Department of Puno, Peru). © Aurélie Carimentrand, July 2012.

the same time. In Peru, the two largest exporters of quinoa are both FAIR CHOICE certified. Moreover, the largest exporter also has the FAIR FOR LIFE label from IMO Control (a Swiss certification organization with its head office in Lima).

On the way to organic certification

In Bolivia, organic certification emerged soon after fair trade, with the implementation of the quinoa natural production programme (PROQUINAT) in 1992 at the level of ANAPQUI. It was in response to the demand for quality from fair trade consumers, confirmed by a market study conducted by IICA (IICA/PNUD, 1991; Laguna, Cáceres and Carimentrand, 2006). ANAPQUI and CECAOT organize the training and collective certification of their members, organic quinoa producers in the Bolivian Altiplano. They also collect, process and export organic quinoa. Private sector competition soon arrived in the shape of Bolivian companies, such as Saite, Jatary, Quinuabol, Andean Valley, Quinua Food – members of the Bolivian chamber of organic quinoa producers and exporters (CABOLQUI). They source organic quinoa through contracts with producers who have organic certification. Meanwhile, quinoa sales continue to grow in both organic/health food stores and European and North American supermarkets. Organic quinoa is also delivered to various other countries, including Japan, Australia, and China. Ecuador and Peru have certainly followed the Bolivian trend.

In Peru, organic certification began in the 2000s. Producers aimed to access markets that are more lucrative than the domestic market, and more open than national institutional markets such as the national food assistance programme (PRONAA). In the department of Puno, which accounts for roughly 80% of Peru's quinoa production, certification was promoted mainly by NGOs, the Juliaca urban-rural promotion centre (CPUR) and the natural resources and environment research centre (CIRNMA) of Puno. Thanks to the technical and financial support of CPUR and CIRNMA, organic certification was obtained by 300 producers from various districts in the provinces of San Román (Caracoto, Vilque, Manazo), Chucuito (district of Juli) and Azangaro. These channels transform and export organic quinoa through the "commercial arms" of the NGOs: El Altiplano SAC for CPUR and Agroindustrias for CIRNMA. Other initiatives worthy of mention include the Puno-Cusco brokerage (financed by FIDA) and Pronamachcs (with APROMIC).

Organic certification impacts the way transactions occur in these channels, with collective certification carried out on behalf of NGOs or private companies, such as "Organic Sierra y Selva" and "Grupo Orgánico Nacional". These companies sign contracts with the producers; they provide technical assistance and, in some cases, seeds (Carimentrand, 2008).

The experience of small producers from Ecuador

In Ecuador, the differentiated quinoa markets, i.e fair trade and organic, are primarily managed by foundations handling both community development projects andquinoa trading. The fair trade and organic quinoa market accounts for around 500 tonnes/year, the bulk of which is exported, with only small volumes sold on the domestic market.

In Ecuador, only one peasant quinoa certification organization exists with both the FAIRTRADE and the SPP (small producer) label. SPP is a proprietary label of the producers and is managed by FUNDEPPO (*Fundación de Pequeños Productores Organizados*). It includes "Bio Taita Chimborazo" (Coprobich), the association of organic producers and traders. Its sales on the foreign market vary between 20 and 100 tonnes, depending on orders from its two leading customers (Ethiquable and Inca Organics) and on its quinoa processing capacity. Since it does not have its own plant, processing is done by leasing the plants owned by Sumak Life and FUNDAMYF. However, with the support of AVSF and funding from the Caders project of the Ecuadorian Ministry of Agriculture, Coprobich is now building its own plant and aims to become the leading Ecuadorian organization producing, processing and directly exporting quinoa to the fair trade and organic market. With this infrastructure, the organization intends to sell quinoa grains and its derivatives on the domestic market, while promoting the SPP fair trade label managed by Latin American fair trade producer organizations.

Image 4: Quinoa plant of the Coprobich organization under construction in 2013 © Jean- Philippe Noel

Various foundations have created commercial arms for quinoa exports under the fair trade principles of the WFTO (World Fair Trade Organization). They include, in particular:

- The *Fundo Ecuatoriano Populorum Progressio* foundation (FEPP), whose commercial arm is the Camari network of retailers on the domestic market, dealing in socially responsible produce including quinoa. Camari sells around 18 tonnes/year, especially on the domestic market.
- The Maquita Cushunshic foundation MCCH (*Maquita Comercializando Como Hermanos*), with a small quinoa processing plant. It sells on the domestic market and exports about 8 tonnes.

The above players produce or process mostly organic quinoa and BCS is the main certification company. In addition to these three leading fair trade companies, there are other players in the marketing of organic quinoa, including:

- FUNDAMYF, with its Ramdipak trademark quinoa, is the only company retailing organic quinoa in Ecuadorian supermarkets. Between 2007 and 2011, it exported 46–135 tonnes of quinoa, with significant fluctuations from year to year.
- The *Escuela Radiofonicas Populares del Ecuador* foundation (ERPE) owns the Sumak Life quinoa processing and export company. Sumak Life is the leading exporter of quinoa at national level, and also of organic quinoa with an average 200 tonnes/year exported between 2007 and 2011.

In this context of a socially responsible and solidarity economy, the organizing processes and control of the chain continue to be in the hands of external players, rather than of the producers themselves. This is contrary to the desired goal of empowering and offering development opportunities to the families of quinoa growers, the very principles at the core of fair trade. This confusion surrounding the commercial functions and support functions of the foundations has led to the emergence of related activities. Quinoa growing, therefore, does not represent the sole source or even main source of income for families. The conflict between helping families and building the capacity of producers' organizations, has led to divisions among the quinoa producers from the province of Chimborazo – the leading quinoa-producing zone in the country, with some 2 000 quinoa-producing families, and an annual production of 500–1 000 tonnes. Thus, the indigenous peasant organization, Coprobich, which had successfully united most of the producers from Chimborazo and comprised 1 600 members, decided to break away from the ERPE foundation and its Sumak Life company, in order that producers could certify, process and export their own quinoa. This proposal was not accepted by the ERPE foundation, which wanted to continue providing technical assistance, and handling processing and marketing on behalf of the producers. The result was a split, with around half the members staying with Coprobich, and the other half forming the Sumak Tarpuy organization within ERPE.

In this debate about management models for the quinoa chain and agrifood chains in general, few players understand the the independent development of peasant organizations. The challenge lies in managing the key stages of processing and marketing, while guaranteeing the fair trade proposal of creating short trading channels and fostering a more direct and fairer relationship between producer and consumer.

The experience of Mapuche producers from southern Chile

In the south of Chile, quinoa – or *dawe* as the Mapuches call the grain – is a secular plant grown by women in their gardens. It is grown together with other local horticultural species using traditional farming techniques. The NGO, CET-SUR, spent more than 15 years helping the Mapuches identify, harvest and disseminate local varieties, exchange knowledge and techniques, and recover traditional uses. Thus CET-SUR drew up, in collaboration with communities, a self-certification protocol for short chains, guaranteeing the authenticity of Mapuche quinoa on local and regional markets and among culinary chefs. The association of stakeholders or interested producers, Mapuche communities, municipal employees, local tour operators, researchers etc., established a new approach. The Centre for Innovation and Mapuche Entrepreneurship (*Centro de Innovación y Emprendimiento Mapuche* – CIEM) is following the same direction: the project's steering committee involves Mapuche communities working alongside NGOs. The Mapuche experience highlights the fact that territorial construction must be built on social (mutual assistance, barter etc.), cultural (cosmogony, rituals, culinary traditions etc.) and agronomic (adaptation of varieties, association of species in shifting cultivation, biological control, fertility management etc.) values – the very values included in Mapuche agri-ecological practices. Support for communities, initially in the form of technical and economic assistance, has evolved into the recognition of a product marked by the Mapuche identity and related practices.

Outlook and Concluding remarks

The different ways in which producers organize market relations underline their adaptability and capacity for change, with an increasing number and variety of players in the quinoa value chain: new products, new quality labels, new governance models, new alliances and new institutional practices.

The Bolivian, Peruvian, Ecuadorian and Chilean experiences presented in this chapter concerning the quinoa regeneration process have much in common, but there are differentiating features. The export trend which began in Bolivia in the 1990s spread to the other countries. In Bolivia, as well as in Peru or Ecuador, the socially responsible solidarity model (producer cooperatives and associations that manage the quinoa value chain from processing to export) competes with the capitalist model, associated with corporate social responsibility and the implementation of social programmes in parallel with their contractual strategies.

Faced with the development of the commercial growth of quinoa in non-traditional zones of the Andean countries (e.g. the Peruvian coast) and in foreign countries (e.g. the United States of America and France), the Andean producers have found new ways of improving and protecting their products: protected designation of origin for quinoa (developing rapidly), socially responsible trade, and new short food chains. In this perspective, it is vital to: i) safeguard the cultivation biodiversity of quinoa in relation to the management of these innovating chains ii) adopt appropriate governance of quinoa-growing territories.

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Section 5
Quinoa crops in Andean countries

CHAPTER: 5.1.a

Quinoa in Bolivia: The PROINPA Foundation's Perspective

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Abstract

Historically, there has only been limited investment in the development of technology for quinoa, a major crop in Bolivia. In spite of this, there has been major progress in the preservation of genetic resources and the selection and generation of varieties. Issues such as integrated pest management, fertility and mechanization have received little attention, while significant investments have been made in processing plants to meet international standards and capacity levels.

The last decade has been called the Bolivian quinoa boom on account of the incredible growth of quinoa production and its rapid penetration of international markets. Between 2000 and 2013, production and exports grew from 1 000 to 40 000 tonnes and from USD1.164 million to USD140 million, respectively. Once a local crop, quinoa has gone global, generating major profits for thousands of Bolivian producers who have been lifted out of poverty. In many cases, their annual income has increased from less than USD1 000 to over USD15 000.

It is no surprise that farmers who had been living in poverty for several generations would seize the opportunity offered by quinoa. The crop's success has led to intensive farming that does not always take into account geographic conditions such as fragile soils with low levels of organic matter and susceptible to erosion. As a consequence, traditional farming practices, which balanced quinoa production with the raising of llamas while respecting community norms, have been disrupted. Several public and private initiatives are currently attempting to reverse this situation.

There are concerns that quinoa consumption by growers in the southern part of the Altiplano has decreased, threatening the nutritional balance of their diet. On the other hand, recent studies have shown that although quinoa consumption has diminished, increased income has led to a more varied diet with increased consumption of fruits, vegetables and meat. At national level, despite high prices, per capita quinoa consumption is increasing thanks to the government's promotion policies.

This chapter describes the main quinoa-producing areas in Bolivia, from traditional areas such as the Altiplano and valleys, to new areas such as the Puna (high cold dry plateaus) and low areas in the east, which could become decisive in terms of domestic consumption.

Presentation of the general quinoa context in Bolivia

According to Gandarillas (1982), the mountain ranges of Ecuador and the Andean tableland, comprising the Peruvian and Bolivian Altiplano and Inter-Andean valleys, were the cradle of great civilizations and the birthplace of several Andean crops. One such crop was quinoa (*Chenopodium quinoa* Willd.), which was central to the development of these civilizations. During the domestication process of wild species growing in the valleys, the first spontaneous hybrids between two diploid species (*C. petiolare* and *C. hircinum*) appeared (Gandarillas, 1984). Their progeny generated considerable genetic variation, which, subject to the pressure of natural selection and human actions, was gradually modified and became the cultivated quinoa that we know today (see Chapter 1.3 "Domestication and Prehistoric Distribution" and Chapter 1.4 "Dynamics of the global expansion in quinoa production"). Settlers from the ancestral cultures travelled great distances between ecologically different areas, bringing with them seeds that, through subsequent natural hybridization among quinoa strains from the valley and the Altiplano, gave rise to genetic variation. These strains were then skilfully exploited for agricultural use, and varieties such as 'Quinoa Real' were selected (Gandarillas, 1982).

Gandarillas points out that the greatest variation in cultivated quinoas is found around Lake Titicaca, in an area ranging from the Cusco in Peru to Lake Poopó in Oruro, Bolivia. He also suggests that quinoa is not a legacy from the Incas, but from earlier cultures. Indeed, Bruno (2005) reports evidence of the domestication of quinoa by the Chiripa culture in around 1500 B.C.

Historically, quinoa has always had an important place in Bolivian culture. It is used and eaten by families in rural areas. Given its excellent nutritional value, there have been many initiatives to increase its consumption. While the results have been impressive, they have not produced significant changes in the national diet.

Nevertheless, quinoa production has seen a steady increase in the last 20 years, especially in the southern part of the Altiplano. Quinoa is practically the only commercially viable crop able to adapt to the characteristics of this area, one of the driest in the country. Indeed, at 3 750 m asl, average annual precipitation is 200 mm with around 200 days of freezing temperatures a year (Gandarillas, 1982; Orsag, 2011).

Until the 1980s, quinoa in the southern Altiplano was grown almost exclusively in the foothills and on the slopes of hills and mountains. A traditional production system was used featuring community plots (*mantas*), biannual crop rotations (cover crops/quinoa), fallow fields, use of manure and other techniques that generally prevented deterioration of the land and the environment. Raising camelids, sheep and other animals was an important activity in the region, and it produced manure – fundamental for the fertility of agricultural soil (Orsag, 2011).

In the Altiplano, farming families who had been poor for generations had few opportunities for change. With high rates of migration to the cities and abroad, the international success of quinoa represented an opportunity that was too good to miss. The situation inevitably resulted in dramatic changes to traditional methods of growing quinoa, to social structures and to income levels, particularly in the southern Altiplano.

Since the 1980s, quinoa farming has moved from the hillsides to the plains, where soils are highly susceptible to wind and water erosion because they are fragile, sandy and contain low levels of organic matter. The situation has been further aggravated by the cultivation of large expanses of land and the introduction of agricultural equipment when farmers lack the necessary experience to use it under fragile soil conditions. Moreover, the llama population has decreased, because raising llamas is perceived as being too much work and not as profitable as farming quinoa; the result is a reduction in manure. The introduction of tractors has encouraged the spread of quinoa production into the plains, and global warming has also played its part as there is decreasing risk of frost. Large expanses of land continuously sown with quinoa are giving rise to ecological disequilibrium and there are more frequent attacks by pests and diseases. Furthermore, this rapid growth has given rise to social problems: traditional farming in community plots is abandoned; or children of ex-farmers return to their communities from the city and claim their rights to the land, undermining ancestral practices and communal organization (Vieira, 2012; Winkel, 2013).

Given the favourable context for quinoa, in the 2010s the Government of Bolivia began to promote various aspects of the grain: production, consumption, processing and export (Ministerio de Desarrollo Rural and Tierras, 2009). As a consequence, the United Nations declared 2013 the International Year of Quinoa. However, there is much concern and criticism regarding the rapid development of quinoa production in Bolivia, which has resulted in soil degradation, high quinoa prices and low domestic quinoa consumption (Jacobsen, 2011; Winkel *et al*., 2012). Experts tend to agree on possible solutions, but many of them are difficult to implement over large areas of land.

History of quinoa research

Past research

Formal and systematic research into quinoa in Bolivia began in 1965–1971 and was based at the Patacamaya Experimental Station[1] through the Andean Crops Project co-funded by the Government of Bolivia and OXFAM FAO Bolivia II (Gandarillas, 2001). The main technical experts were the engineers, Argos Rodríguez, Humberto Gandarillas, Segundo Alandia and, as advisor, Dr Martín Cárdenas. During this period, the foundations for genetic improvement, preservation of genetic resources, commercial production and pest management were established.

Given the wide genetic diversity of quinoa, some 1 000 accessions were collected, mostly from Bolivia and southern Peru. Donations were later received from Oruro Technical University (UTO) and the Inter-American Institute for Cooperation on Agriculture (IICA) in Peru, and a total of 1 375 accessions were finally collected (Rojas *et al*., 2001). Following the evaluation of this material at the Patacamaya Experimental Station, 17 varieties of quinoa were established and described (Gandarillas, 1968). This material formed the basis of the Bolivian seed bank, the largest in the world today.

The project focused on increasing domestic quinoa consumption and concentrated on sweet varieties for two reasons: eliminate the laborious task of removing saponins, and decrease processing costs. In 1967, the 'Sajama' variety was successfully obtained and distributed. It was the first variety in the country achieved through directed hybridization of sweet quinoa and 'Quinoa Real', followed by selection. It can withstand temperatures as low as -6°C (Gandarillas, 2001).

To determine which improvement technique should be used for quinoa, the following aspects were studied: types of flower, duration of flowering period, reproduction modes, percentage of cross-pollination, self-fertilization and its effect on the vigour of the progeny, crossing techniques and methods of analysing the progeny of crossing techniques (line breeding, pedigree breeding and mass selection).

With regard to quinoa production, studies were carried out on: the need for fertilizing substances, fertilizer dosage, sowing times density in irrigated and non-irrigated land, and pests and diseases affecting quinoa and the respective control methods.

The *Instituto Boliviano de Tecnología Agropecuaria* (IBTA – Bolivian Institute of Agricultural Technology) was created in 1975. Within this institutional framework, a joint project between IBTA and the International Development Research Centre (IDRC) of Canada was carried out between 1978 and 1991. During this period, samples of quinoa germplasm were taken and analysed with a focus on genetic improvement, identification of inheritance mechanisms for various qualitative traits, cytoplasmic male sterility, crop agronomy, pests and diseases, nutrition, extension and industrialization (Gandarillas, 2001).

The project produced the 'Huaranga', 'Chucapaca', 'Kamiri' and 'Samaranti' varieties, the first three by means of hybridization and selection and the fourth through selection. 'Chucapaca' is still produced, mainly in the central Altiplano. It has high yield, large grain size and resistance to temperatures as low as -6°C.

Later, also under the aegis of the IBTA, a joint IBTA–World Bank project (1992–97) was implemented. It included the main ingredients of food security in Bolivia: potato (part of the PROINPA research programme on potato), wheat, quinoa, legumes, maize, livestock and forage plants. The project made a significant investment in human resources, and for the first time, more than 30 Bolivian professionals left the country to do master's and doctoral degrees at universities in the United States of America, Europe and Latin America.

During the IBTA–World Bank project, the 'Sayaña', 'Ratuqui', 'Robura', 'Santamaría', 'Intinayra', 'Surumi', 'Jilata', 'Jumataqui' and 'Patacamaya' varieties were obtained and released (Espindola and Bonifacio, 1996; Rojas and Ordoñez, 1998). Another very important result was the homogenization by means of mass selection of local varieties from the southern Altiplano, including 'Real Blanca', 'Toledo', 'Utusaya', 'Rosa Blanca', 'Kellu', 'Pandela', 'Chillpi', 'Achachino', 'Manzana', 'Toledo Amarilla', 'Real Elba', 'Rosada' and 'Lipeña'.

When the IBTA closed in 1997, the programmes fell into the remit of the prefectures. The quinoa programme and the seed bank based at the Patacamaya Experimental Station came under the control of the *Servicio Departamental Agropecuario* (SEDAG – Departmental Agricultural Service) at the La Paz Prefecture. The prefecture authorities lacked both judgement and commitment to the national agricultural sector, resulting in mismanagement of the Patacamaya Experimental Station and the loss of the seed bank and genetic material in the quinoa improvement programme following the violent interference of members from a neighbouring area.

In the final years of the IBTA, the PREDUZA project (*Proyecto de Resistencia Duradera de la Zona Andina* – Project for the Lasting Resistance of the Andean Region), coordinated by the University of Wageningen, agreed to work on the long-term resistance of quinoa to mildew (*Peronospora variabilis* Gaum). The project involved the study of the whole collection of quinoa for resistance to mildew during the 1997/98 and 1998/99 farming seasons. A plot was allocated to evaluate 100% of the accessions at the Patacamaya Experimental Station. Two replicates of 50% were also established at the Belén and Choquenaira experimental stations, in areas of higher rainfall used by the Higher University of San Andrés (UMSA). In the light of the events at Patacamaya and the loss of the seed bank, researchers from the former IBTA set out to reconstitute the seed bank, beginning with the material being analysed.

Current state of research

When the IBTA was closed in 1997, the Foundation for the Promotion and Research of Andean Products (PROINPA) was founded on the initiative and with the support of the Swiss Agency for Development and Cooperation (SDC), the International Potato Center (CIP) and the Bolivian Ministry of Agriculture. The aim was – without any political influence – to support technological development in the country and maintain the capacity to respond quickly to requests from farmers. Initially, PROINPA limited its research to potato, but, given the lack of institutionalized national agricultural research on a crop as important as quinoa, in 1999 its directors decided to incorporate quinoa. The best and most experienced researchers from the former quinoa research programme at IBTA went to work at PROINPA. Their contribution was vital to avoiding the loss of years of work and the genetic heritage that the quinoa seed bank represented for the country. The National Bank of High-Andean Grains was later established by PROINPA.

Once the bank was constituted, international support and cooperation came from the Danish Ministry of Foreign Affairs through its development programme, DANIDA, and from the International Plant Genetic Resources Institute (IPGRI) (now Bioversity International). Subsequently, the Ministry of Agriculture, through the National System of Genetic Resources for Food and Agriculture (SINARGEAA) (2003–08), confirmed PROINPA's role as keeper of the National Bank of High-Andean Grains . In June 2008, the Government of Bolivia created the National Institute for Innovation in Agriculture, Livestock and Forestry (INIAF). The National Bank of High-Andean Grains was transferred to INIAF in July 2010, together with all genetic material, documentation and equipment (Rojas *et al*., 2010).

At the PROINPA Foundation, the researchers could no longer depend on stable funds from Bolivia's national treasury, and instead had to compete for national and international resources. They had to be much more competitive, contact researchers around the world and undergo annual audits. Precious long-term funding was obtained from the McKnight Foundation, and researchers from Brigham Young University (BYU) provided advice, in particular with regard to modern molecular biology tools.

With subsequent funding for germplasm banks through SINARGEAA and later from INIAF (2008–10), the project was able to strengthen the management and preservation of the National Bank of High-Andean Grains (2001–10). The bank included quinoa (*Chenopodium quinoa* Willd.), *cañahua* (*Chenopodium pallidicaule* Aellen), amaranth (*Amaranthus caudatus* L.), saltbush (*Atriplex* sp.), *cauchi* (*Suaeda foliosa* Moq) and wormseed (*Chenopodium ambrosioides* L.). The storage spaces were renovated for better preservation of the seeds; centralized and decentralized collections were added; agromorphological and molecular characterization and analysis were carried out; the structure of the core collection of quinoa was examined with traditional and molecular tools; and nutritional value was determined (Rojas *et al*., 2010b).

New currents in international research on neglected underutilized species (NUS) have also reached Bolivia. Since 2001, important projects have been conducted in coordination with Bioversity International to strengthen capacities in the *ex situ* conservation of germplasm collections of quinoa, *cañahua* and amaranth. In addition, the first *in situ* conservation work was carried out in communities in the Altiplano, near Lake Titicaca and in the Cochabamba highlands. Conservationist farmers were encouraged to use promising quinoa accessions directly. Research focused on the richness of quinoa varieties, local uses, the roles and responsibilities of family members, and using agrobiodiversity to access high-value markets (Rojas *et al*., 2010a).

With funding from the McKnight Foundation, priority was given to looking for varieties that were better adapted to the effects of climate change (late rains concentrated in short periods). Researchers worked on improving quinoa's mildew resistance, drought tolerance and precocity by incorporating multicriteria selection (i.e. including other grain characteristics such as colour, size and industrial quality) (Bonifacio *et al*., 2006). In addition, the molecular markers SSR and SNP were developed and used with the valuable support of Brigham Young University (De Jarvis *et al*., 2008; Maughan *et al*., 2012; Jellen *et al*., 2011). Six varieties were released during this period: 'Jacha Grano', 'Kurmi', 'Blanquita', 'Qusuña', 'Aynoqa' and 'Horizontes'. Seeds from these varieties, together with those released earlier, were multiplied and widely distributed. These seeds (1 500–2 000 kg annually) were in turn multiplied by producers. Thanks to genetic improvement, quinoa obtained for conditions in the central Altiplano has the grain size of 'Quinoa Real' – a trait much appreciated by national and international markets. Recent studies have examined its agro-industrial characteristics (starch, amylose, amylopectin, starch granule diameter, reducing sugars and liquid), in order to open up new avenues of research to meet specific requirements from the processing industry in Bolivia (Vargas, Bonifacio and Rojas, 2013).

With mounting concern about the monoculture of quinoa and the indiscriminate expansion of planted areas, leading to the unsustainability of production, several institutions, including PROINPA, have begun research into cultivation of shrub species in the Altiplano. Experiments have involved the collection, sowing and transplanting of bushes and grasses, in particular of native legumes (IBCE, 2013).

There are other major players in the technological development of quinoa in Bolivia. They include universities, such as Oruro Technical University (UTO) and the Higher University of San Andrés (UMSA), as well as the Centre for the Promotion of Sustainable Technology (CPTS) and the FAUTAPO foundation.

Since 1995, UTO, through its Faculty of Agricultural and Veterinary Science, has conducted research projects through thesis work on a range of subjects: improving agricultural mechanization, adaption to climate change, environmentally friendly soil management, ecological pest management, managing agrobiodiversity, irrigation systems, and production of bushes, pastureland and grasses. In 2008, the Quinoa Research Centre was established in Salinas de Garci Mendoza (Barrientos *et al*., 2013).

The Faculty of Agronomy at UMSA has carried out various projects to increase quinoa yields through application of layers of organic fertilizer and water at critical moments in the developmental phase, good crop management and pest control (Orsag *et al*., 2013). The QUINAGUA–SUMAMAD project has involved studies on proper soil and climate management for quinoa, on the basis that water and fertility constitute the building blocks for crop development (García *et al*., 2013).

The Centre for the Promotion of Sustainable Technology (CPTS) has very successfully resolved one of the main constraints in commercialization of quinoa, namely processing to eliminate saponins and impurities without losing nutrients. In over a decade of research, significant advances have been made in machine design to improve efficiency in the consumption of water, electric energy and thermal energy during the drying process and to reduce costs substantially. More than ten companies currently use CPTS technology in their processing lines (www.cpts.org). CPTS has also designed technology and machines for the ecological production of quinoa, including sowing machines, threshing machines, watering equipment, spraying equipment and combine harvesters.

FAUTAPO, through its COMPASUR programme (*Programa Complejo Productivo Altiplano Sur*), has done much to increase the competitiveness of the southern Altiplano, thanks to 'Quinoa Real', ranching and rural tourism (Fundación FAUTAPO – Compasur, 2013). The programme includes activities related to sustainable production, soil fertility, promotion of organic production, strengthening of growers' organizations, and transformation and industrialization of 'Quinoa Real'.

Founded in June 2008, the National Institute for Innovation in Agriculture, Livestock and Forestry (INIAF) (www.iniaf.gob.bo) established the Quinoa Programme in 2012 within the framework of its national research programmes and with the strategic partnership of UTO and PROINPA. Funding was received through international cooperation and multilateral organizations researching quinoa in Bolivia, including the International Development Research Centre (IDRC) of Canada, the Inter-American Development Bank (IDB), the World Bank, the McKnight Foundation, the United States Agency for International Development (USAID), the United Nations Development Programme (UNDP), the International Fund for Agricultural Development (IFAD), the Government of Netherlands through DANIDA.

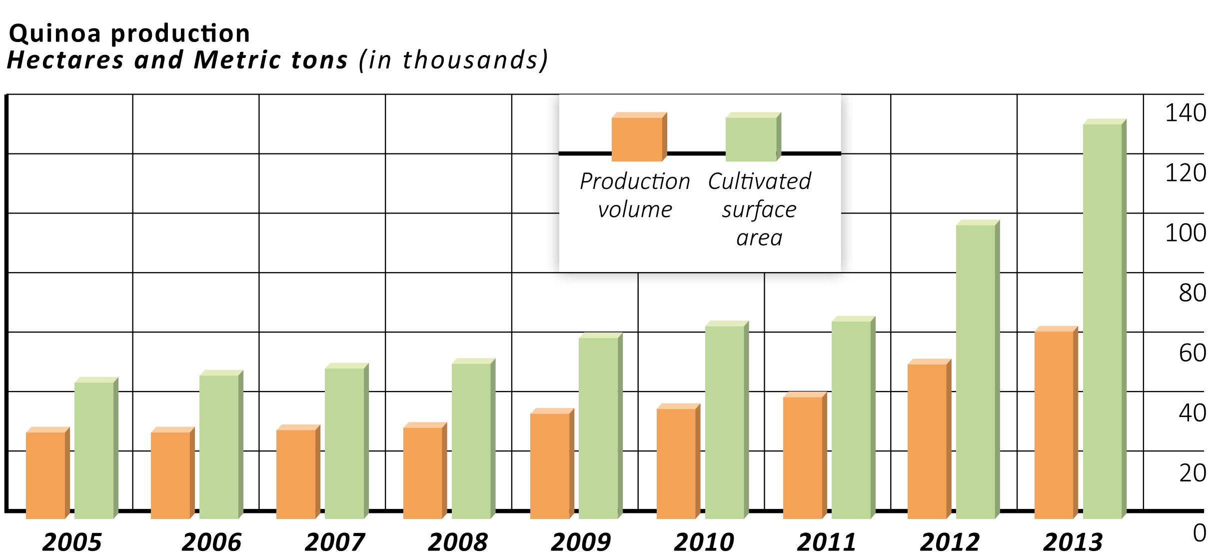
The importance of quinoa in Bolivia

This section focuses on the growth of quinoa in the southern Altiplano, where quinoa production has increased significantly and where 'Quinoa Real' is grown for export.

Data are presented on changes in cultivated surface area and production volumes (Figure 1), volumes and value of exports and their destinations (Figures 2 and 3), gross income for farmers (Figure 4), destination of production (Figure 5) and yields (Figure 6).

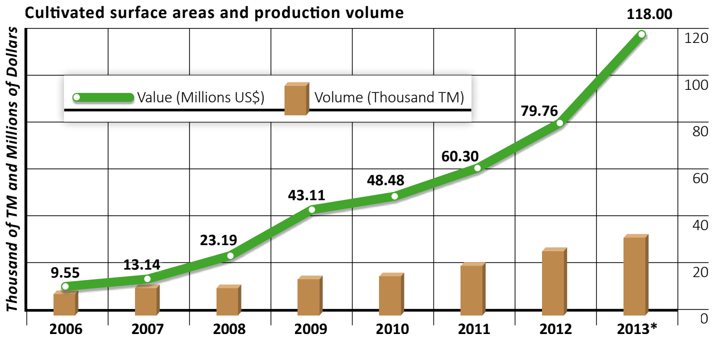
Figures 1 and 2 show the significant growth in quinoa between 2000 and 2007, but the "boom" was in 2007–2013. Cultivated surface areas increased from 50 000 to > 120 000 ha, production volume from 28 000 to 60 000 tonnes, and exports from USD12 million to > USD100 million. The highest demand was from the United States of America, Europe and Canada (Figure 3). Quinoa – once present only in fair trade markets – arrived on the shelves of every supermarket. These developments coincided with a global trend towards healthier, more

Figure 1: Cultivated surface areas and production volumes



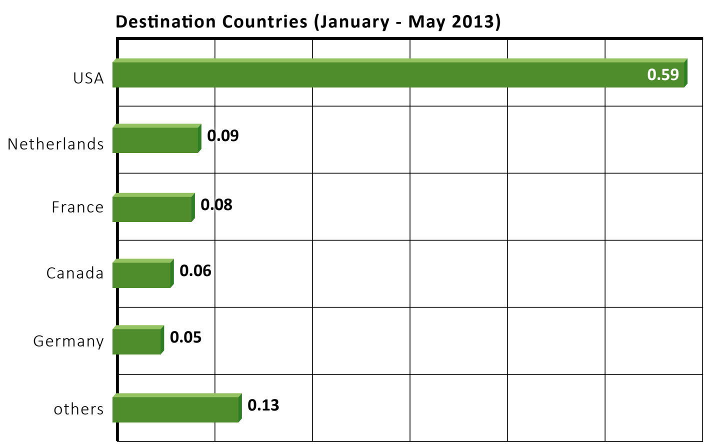
Source: IBCE/ SISPAM/INE

Figure 2: Export performance (pearled quinoa)



Source: CABOLQUI/IBCE

Figure 3. Export performance (Jan.- May 2013)



*Australia, Brazil, Israel, United Kingdom and others
Source: MDRyT/ INE

nutritious and organic eating, as well as a growing coeliac population (intolerant to the gluten present in grains). The situation represents an exceptional opportunity for the "golden grain" of the Andes. Quinoa meets all requirements and boasts an exotic history as a millenary crop growing from salt flats to the snow-capped mountains of the Bolivian Andes.

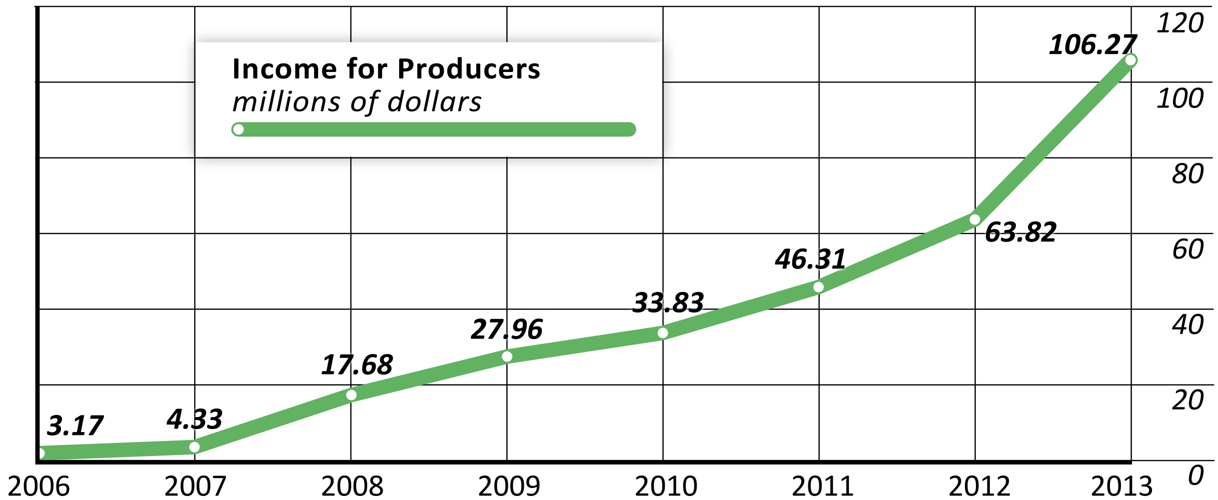
Given the great international opportunity presented by quinoa, the Government of Bolivia has encouraged major investments. Considerable support has been provided and private initiatives launched, with international cooperation from Denmark, the Netherlands, the United States of America and other countries. The largest association of quinoa producers (ANAPQUI) is becoming stronger, and several companies and processing plants organized around the Bolivian Chamber of Quinoa Exporters (CABOLQUI) are being set up. Bolivia takes part in major events and organic agriculture fairs around the world to promote the exceptional nutritional qualities of quinoa, open up markets and attract large foreign trading companies.

The International Year of Quinoa in 2013 represented another important turning point. Activities took place throughout the world, promoted by the governments of Bolivia and Peru, FAO, international organizations, European countries, the United States of America and others. The result was global promotion of quinoa. Demand for and the value of quinoa increased dramatically, as can be seen in the data for 2013 (Figure 2), when Bolivia exported 6 000 tonnes more than in 2012. The price of a tonne of quinoa rose from USD3 000 in 2012 to more than USD3 600 in 2013. Demand for quinoa is expected to continue rising in the coming years: a genuine boom. However, it will be circumstantial and unsustainable, since consumers are unwilling to pay such high prices and eventually prices will drop and find a resting point. Another important effect of the International Year of Quinoa is that not only consumption, but also production, is becoming global. Countries on every continent are evaluating materials and preparing their own production fields, some of which will be successful and produce large crops, giving rise to a greater international offering and more affordable prices.

One extraordinary effect of the quinoa boom has been the achievement of a long-awaited national goal: lifting Bolivian families out of poverty. Quinoa has achieved this for some 20 000 families. Just 15 years ago, the annual family income was < USD1 000; now, families can earn > USD15 000 a year through quinoa.

Unlike other crops in Bolivia such as soybean, much of the profits (an estimated 60–70%) generated by quinoa exports goes directly to farmers. In 2013, this amounted to > USD100 million (Figure 4). It should also be kept in mind that the informal market for Bolivian quinoa is huge. Indeed, surplus quinoa, which goes mainly to Peru, accounts for approximately 28% of national output (Figure 5) (Vásquez,

Figure 4: Income for producers



Source: IBCE / INE / FAOSTAT

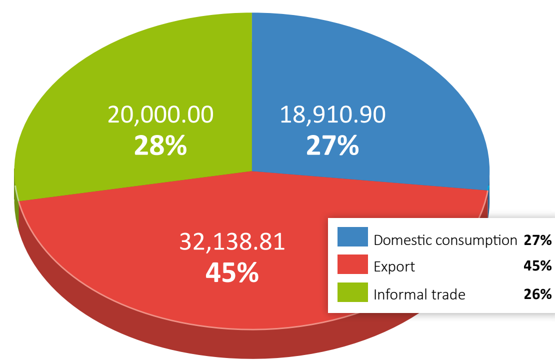
2013) and is estimated to generate USD20 million, most of which ends up in farmers' pockets.

A recurring topic of debate is the decreasing consumption of quinoa by farmers and the problem of malnutrition. A survey conducted by PROINPA during the 2012/13 farming season in four southern Altiplano provinces (Ladislao Cabrera, Nor Lipez, Daniel Campos and Antonio Quijarro) found that in a sample of 85 families quinoa consumption had indeed decreased. Quinoa used to be eaten on a daily basis, but now 16% of families consume it 5–7 times a week, the vast majority (74% of families) 2–4 times a week, and 10% once a week. The survey also found, however, that that the consumption of meat, grains, milk, fruit and vegetables has increased.

Farmers now have increased income and access to credit, and they can decide how to spend or invest their resources. Common investments include education for their children (all children and young people have access to elementary, secondary and higher education); housing, in both rural areas and urban centres; basic services (electricity, water etc.); and economic activities, such as transport and business, which in turn generate other sources of direct and indirect employment.

International prices obviously influence domestic prices, making quinoa less accessible to the general population. Nevertheless, quinoa consumption in Bolivia is gradually increasing: from 0.35 kg per capita in 2008 to 1.11 kg in 2011 and 2 kg in 2013 (Figure 5) (Vasquez, 2013). This rise in consumption has been encouraged through campaigns promoting quinoa as a healthy food and through various government policies, including quinoa subsidies for

Figure 5: Destination of production



Source: SISPAM

Year	Per capita consumption (kg)
2008	0.35
2012	1.11
2013	2.00

pregnant and breastfeeding women and for school breakfasts. Given that the population of Bolivia is 10 million, per capita consumption accounts for 12 013 tonnes. Since quinoa is starting to be cultivated in the valleys and plains as part of conventional agriculture, it is expected that local consumption will increase, costs will decrease and national consumption will be bolstered.

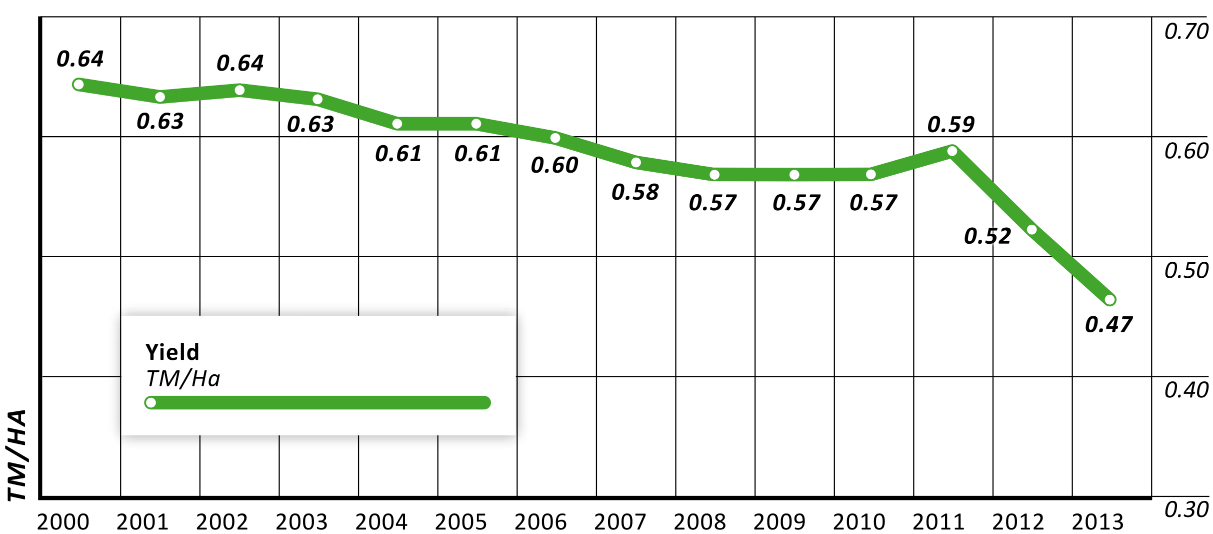
Growing international demand for quinoa and the resulting elevated prices have generated high expectations among farmers. In their eagerness to seize this opportunity, various unsuitable practices have been introduced into the very fragile agro-ecosystem of the southern Altiplano, where the soils are characterized by extremely low levels of organic matter (< 1%) and low moisture retention. They are predominantly sandy and susceptible to wind erosion. Average annual rainfall is 200 mm, which means there is very slow replacement of plant cover. Single crop farming is often practised, and the agricultural frontier is being expanded, thus reducing grazing land for llamas. Other problems include a lack of hedgerows between farming plots and inappropriate use of agricultural equipment. These constraints, combined with a lack of technically and economically viable technologies that can be adapted by farmers, have led to a decrease in yield per unit area (Figure 6). The "Prospects for quinoa in Bolivia" section of this chapter presents a brief description of technologies – existing and under development by PROINPA – that can reverse this trend. An important national goal is increase in yield per unit area, in order to lower prices without affecting farmers' revenues and to make quinoa more accessible in Bolivian society.

Areas of quinoa production in Bolivia

As a consequence of the current high economic value of quinoa, insatiable market demand and aggressive promotion by the Government of Bolivia have generated much interest in the cultivation of quinoa throughout Bolivia.

The main production area in the country is the Altiplano, particularly the southern part of this plateau, where large swathes of land are cultivated for export and where agro-ecological conditions make it impossible to grow other crops. The largest croplands are in the central Altiplano, while the northern Altiplano has smaller cultivated areas with greater crop diversity. Other significant areas of expansion are the Inter-Andean valleys, where the soil is more fertile and yields better crops, although organic quinoa is still very hard to produce. A new area of interest for farming quinoa is the arid and semi-arid Puna (Liberman, 1992; Ibisch and Mérida,

Figure 6: Yield performance



Source: IBCE/FAOSTAT/INE/SISPAM

2003). Another area is the eastern plains, where it is hoped that varieties can be adapted for winter crops to be rotated with soybean.

Gandarillas (1982) divided quinoa production areas into zones based on soil characteristics, climatic factors, and the possibility of raising livestock and farming. Bonifacio (in press 2014), suggests readjusting this division of production areas to reflect changes occurring in Altiplano production systems and the role of municipalities in rural development. On the basis of these criteria, a description of the quinoa production areas in Bolivia (Map 1) and of the predominant varieties in each is provided below. A glossary of terms and their English meanings, including the names of varieties in Quechua and Aymara – the ancestral languages of Bolivia – is presented in Appendix 1.

- Northern Altiplano
- Central Altiplano
- Southern Altiplano
- Inter-Andean valleys
- Puna
- Eastern plains

Northern Altiplano

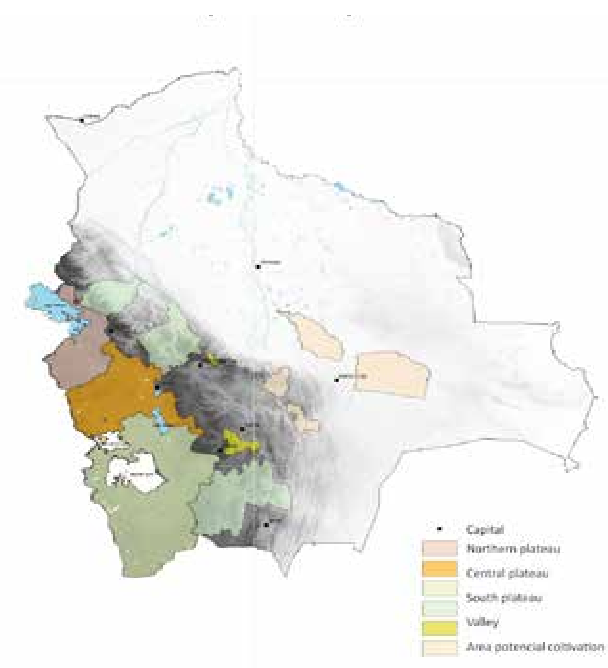
According to Gandarillas's subdivision (1982), the northern Altiplano is the most densely populated area of the country, not only because intensive agriculture is practised there, but also because the people living around Lake Titicaca fish as well as farm. According to Bonifacio (2013), Gandarillas's classification (1982) should take into account the municipalities in each province, since these entities are currently establishing autonomy statutes to become territorial administrative units for productive activities. The northern Altiplano comprises the municipalities of Copacabana and Tito Yupanqui in the province of Manco Kapac; Achacachi, Huarina and Ancoraimes in the province of Omasuyos; Viacha, Tiahuanaco, Laja, Taraco, Guaqui, Jesús de Machaca and San Andrés de Machaca in the province of Ingavi; Pucarani, Batallas, Puerto Pérez and Laja in the province of Los Andes; Escoma, Puerto Acosta and Puerto Mayor Carabuco in the province of Camacho; and Caquiaviri in the province of Pacajes.

The soils in the north are damp and rich in organic matter. Some municipalities feature wetlands that are used to raise alpacas, a species associated with damp soil conditions. In recent years, cattle-raising has been introduced as well as alfalfa cultivation, giving rise to large dairy-producing areas. Further south, in the area surrounding Lake Titicaca, soils are heavier owing to their alluvial and lake origins. On the slopes of the mountains, soils vary from medium to fine textured. To the southeast of the lake, soils are stony and finer. Some areas feature saline outcrops that are especially visible in the winter.

In this area, as in the rest of the Altiplano, the rainy season lasts from September to March, with an annual average precipitation of around 500 mm. Over the last decade, however, the rainy period has been beginning in November or December, resulting in delayed sowing times for quinoa and potato. Several authors (Arana *et al*., 2007; Saavedra and Garcia, undated.; Andersen and Mamani, 2009; Thibeault *et al*., 2010; Valdivia *et al*., 2013) agree that the rainy season has become shorter, with more intense rainfall in a shorter period. This new rain pattern is leading to demand for early varieties.

Under the influence of Lake Titicaca, the annual average temperature in the area is around 7°C,

Map 1: Quinoa production areas in Bolivia



lower than in the rest of the Altiplano. This may be attributed to the effects of cloudiness and the snow-capped mountains flanking the northern Altiplano. The maximum average temperature reaches 14.2°C, while the minimum average temperature between April and July is 4°C (Figure 6).

The main crops are potato, barley, broad bean and quinoa, followed by smaller tubers such as oca, *papalisa* and *izaño* and, lastly, *cañahua* and lupin. On irrigated land, potato and broad bean are the preferred crops. Potato crops are frequently exposed to freezing temperatures, except in some microclimates under the influence of Lake Titicaca. In recent years, forage species, including barley, alfalfa, oats, fescue and orchard grass, have become more prevalent because of the increase in livestock farming.

Alpacas are raised in the pampas of Ulla-Ulla and on the slopes of the Cordillera Real mountain range. Llamas are raised in areas with dry soil. Sheep farming is combined with agriculture in the rest of the area because, as well as grazing in community meadows, these animals eat crop stubble.

Figure 7 presents the precipitation and temperatures in the town of Cachilaya, representative of the northern Altiplano.

Improved varieties that adapt to conditions in the northern Altiplano are 'Kurmi', 'Blanquita' and 'Jacha Grano', while suitable native varieties are 'Phisanqalla' and 'Janqu Jupa'. The area is relatively wet and mildew is a potential problem, therefore varieties grown there must have a high degree of resistance to the disease. In addition, if quinoa is being grown for the market, grain size must be ≥ 2 mm.

Central Altiplano

Topographically, the central Altiplano is mostly flat, located between the Cordillera Oriental and Cordillera Occidental mountain ranges. The area covers the northern part of the Oruro department, the provinces of Aroma and Gualberto Villarroel and part of the province of Pacajes in the department of La Paz. Central Altiplano municipalities include Challapata, Pazña, Machacamarca, Toledo, El Choro, Corque, Sabaya, Caracollo, Eucaliptus, Totora, Choquecota, Turco and San Pedro de Curahuara in the department of Oruro; and Sica-sica, Patacamaya, Umala, Callapa, Curahuara de Carangas, Calamarca,

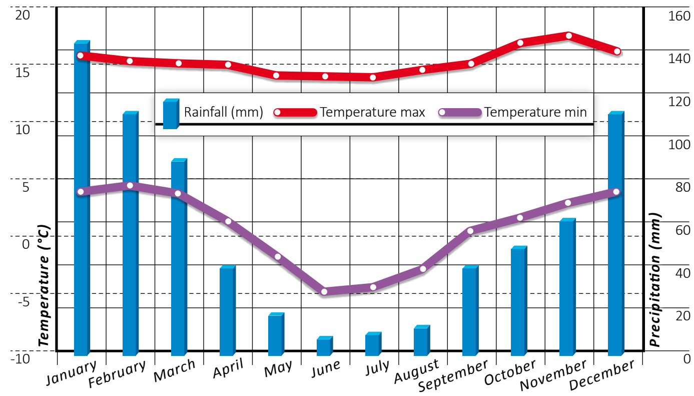


Figure 7: Precipitation and temperatures in Cachilaya, northern Altiplano

Colquencha, Collana Norte, Corocoro, Caquiviri and Calacoto in the department of La Paz.

Soils covered with tola (*Parastrephia lepidophylla*, *P. lucida*, *P. quadrangulare* and *Bacharis tola*) are slightly acidic, while soils where grasses predominate are saline or alkaline, depending on soil content and chemical composition. Soil is mainly medium textured and stony. Sandy soil is also common, covering vast areas of land where the vegetation consists of fescue (*Festuca ortophylla*) and Peruvian feather grass (*Stipa ichu*).

The recorded rainfall decreases from north to south and from east to west. For example, it rains about 400 mm a year in the city of Oruro and < 300 mm in Sabaya.

Rainy years are infrequent, but are very favourable for farming. In the more common dry years, quinoa is one of the plants that can still produce crops.

The annual average temperature is around 8.7°C, slightly higher than in the cloudy area adjacent to Lake Titicaca. The maximum average temperature reaches 17.7°C, while the minimum average temperature drops to -2°C between August and November and to -4°C between April and July. In general, > 200 days of freezing temperatures are recorded annually and every month has at least one frost. Figure 8 presents the precipitation and temperatures in the city of Patacamaya.

Improved varieties adapted to the central Altiplano are 'Sajama', 'Chucapaca', 'Jacha Grano', 'Horizontes', 'Aynuqa Waranqa', 'Kamiri', 'Sayaña', 'Patacamaya', 'Surumi', 'Intinaira', 'Santa Maria', 'Jumata-

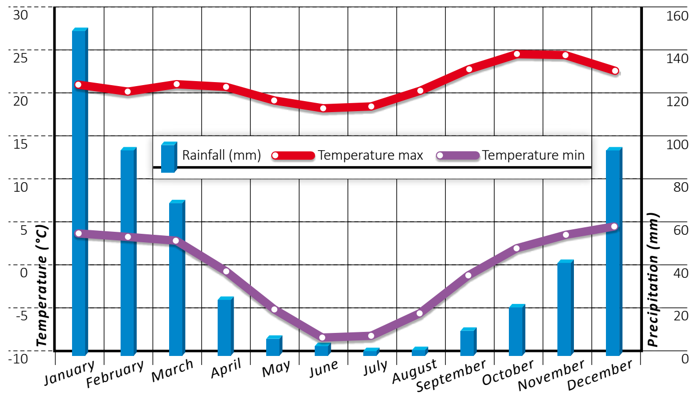


Figure 8: Precipitation and temperatures in Patacamaya, central Altiplano

ki', 'Jilata', 'Samaranti', 'Amilda' and 'Robura'. These varieties are suitable to the area because of their intermediate cycle and medium-to-large grain size. When introduced in other areas (e.g. the southern Altiplano), they have adaptation problems and extend their production cycles.

Southern Altiplano

The southern Altiplano encompasses the provinces of Daniel Campos, Antonio Quijarro, Nor Lipez, Sud Lipez and Enrique Baldivieso in the department of Potosí, and the provinces of Ladislao Cabrera, Eduardo Avaroa and Sebastián Pagador in the department of Oruro. The whole area is extremely arid, containing the salt flats of Uyuni and Coipasa that cover vast areas. Against a backdrop of varying climatic factors and, in particular, the adaptation of 'Quinoa Real', new municipalities have been created in the southern Altiplano. The Potosí department now comprises the towns of Colcha K, San Agustín, Tomave, Llica, Tahua, San Pedro de Quemes, Uyuni and Coroma, whilethe Oruro department includes the communities of Salinas, Pampa Aullagas, Santiago de Andamarca, Santiago de Huari, Belén de Andamarca, Challapata, Santuario de Quillacas, Chipaya, Coipasa and Sabaya.

The extreme west and southwest are practically desert. The mountains are rocky and mineralized, unsuitable for agriculture or livestock. Nevertheless, in some areas characterized by volcanic hills, farmers have developed very specialized techniques to grow quinoa manually.

This area is the driest in the country, and the rainy season begins as late as January. Recorded annual rainfall in the area, which borders the desert in Chile, varies between 50 and 200 mm.

The annual average temperature is 5.7°C. During the growing season (Dec.–Mar.), the average temperature is 11°C, as in the central Altiplano. In the most recent period on record, the maximum average temperature reached 18ºC and the lowest average temperature was -11ºC in the period April–July.

The soil is sandy and coarse, and the predominant vegetation is tola. In some areas, wind erosion led to the formation of dunes. The soil type and scarce precipitation mean that grasses for grazing are scant.

Some areas have become specialized in quinoa production, such as Salinas de Garci Mendoza, Llica and Colcha-K in the agro-ecological area between the salt pans. They are suited to the cultivation of the large-grained 'Quinoa Real' variety, thanks to interaction between the genotype and the environment.

The southern and western parts of the southern Altiplano are more arid with fewer grasslands, except along the rivers or in poorly drained land where alpaca-raising is an important activity – for example, on the wetlands in the Azanaques mountains.

Raising llamas was once important. However, with the introduction of tractors and the extensive cultivation of quinoa, it has become a secondary activity. Quinoa production, which used to be concentrated in the area between the salt pans, has now spread into the municipalities of Uyuni in the east, Chipaya in the west, and Santiago de Huari and Andamarca in the north. These areas are mostly flat and suitable for tractor tilling, which means that the sustainability of quinoa production and camelid husbandry could be jeopardized unless action is taken to manage soil and plant resources. Figure 9 presents the precipitation and temperatures in the town of Uyuni, representative of the southern Altiplano.

For the southern Altiplano, the improved varieties are 'Qusuña' and 'Horizontes' and the selected varieties are 'Mañiqueña' and 'Qanchis Blanca'. In addition, more than 20 local varieties are grown, the most popular of which are 'Real Blanca', 'Chaku', 'Pandela', 'Toledo' and 'Phisanqalla'. When these varieties are moved to more humid areas – for ex-

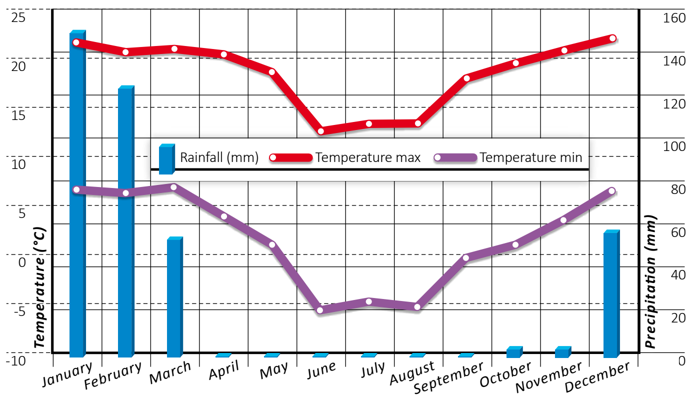


Figure 9: Precipitation and temperatures in Uyuni, southern Altiplano

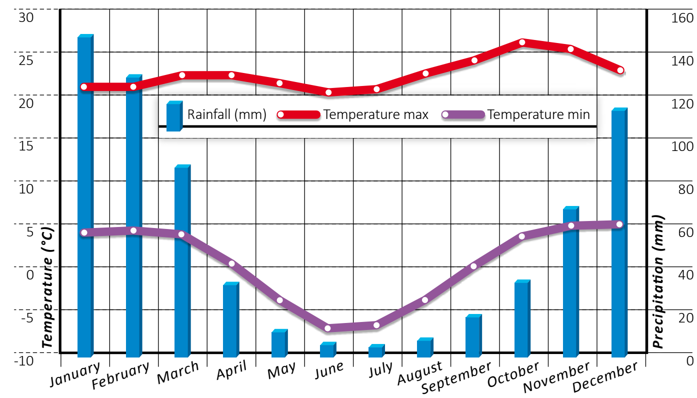


Figure 10: Precipitation and temperatures in Valle Alto, Cochabamba, Inter-Andean valleys

ample, in the centre and north, where there is more precipitation – they suffer severe attacks by mildew.

Inter-Andean valleys

The Inter-Andean valleys cover the departments of La Paz, Cochabamba, Potosí, Chuquisaca and Tarija. The northern valleys are located to the north of the Real and Tunari mountain ranges, and include the Sorata, Inquisivi, Independencia and Morochata valleys, as well as those in the Cochabamba department. The central valleys are in the departments of Potosí and Chuquisaca. The southern valleys are in Tarija, and in the provinces of Nor Cinti and Sur Cinti in Chuquisaca, as well as in the provinces of Nor Chichas and Sur Chichas in Potosí.

The soils in this area range from heavy and medium to light. They are mostly stony on rolling terrain, with the exception of the open, irrigated valleys of Cochabamba and Tarija.

The climate is also quite variable as a result of the topography and the proximity to several mesothermal areas. Frost is frequent during the dry season (May–July). In those valleys where this phenomenon is not observed, early potatoes and vegetables are grown.

Rainfall is variable, ranging from 350 to 700 mm. In the Lequezana pampas in Potosí, the average annual precipitation is around 400 mm, while in Tarija it reaches 700 mm and in Cochabamba 500 mm.

The most common crop cultivated in valley bottoms is maize. Wheat is grown at intermediate altitudes, while potatoes and barley are cultivated at 3 000 m asl. On irrigated lands, there is considerable production of vegetables and fruits. Quinoa is traditionally cultivated in all valleys, also in proximity to maize and potato crops. In recent years, interest in quinoa has increased, and farmers in Valle Alto in Cochabamba have successfully grown quinoa using improved varieties such as 'Kurmi', 'Blanquita' and 'Jacha Grano'. Figure 10 presents the precipitation and temperatures in Valle Alto in Cochabamba.

At present, no improved varieties exist for the Inter-Andean valleys. However, the 'Kurmi' and 'Blanquita' varieties have performed well, mainly because of their partial resistance to mildew and the high commercial quality of the grain.

Quinoa in Puna

The areas of Puna (high cold dry plateaus) and Cabeceras de Valle (heads of valleys) were not classified as quinoa production areas by Gandarillas (1982). Nevertheless, in recent decades, quinoa production has reached high-altitude areas, such as Puna, where yields are destined mainly for the local market. Examples of these areas in Puna include the towns of Betanzos and Villazón in Potosí, Colomi and Tiraque in Cochabamba, and Iscayachi and Yunchará in Tarija. These areas are located at an altitude of 3 000 m asl; annual precipitation is > 600 mm and average temperatures are 15°C during the growing season.

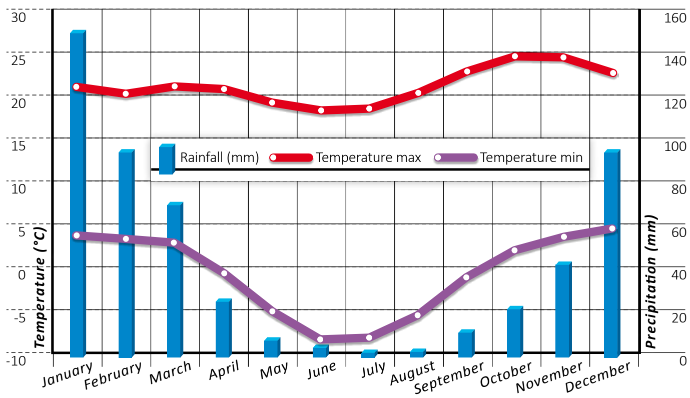


Figure 11: Precipitation and temperatures in Puna, Potosí, Puna region

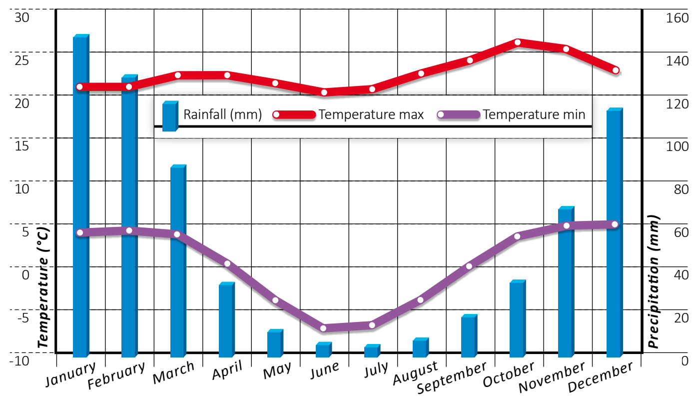


Figure 12: Precipitation and temperatures in Chane, Santa Cruz, eastern plains

In the three most recent farming cycles (2010–13), the performance of seven quinoa varieties was evaluated through a participatory approach in different areas of Puna in Tarija. The best-performing varieties were 'Sajama', 'K'ellu' and 'Pasancalla', with yields exceeding than 950 kg/ha. These results demonstrate quinoa's capacity to adapt to such areas. Further expansion of quinoa production would require the development of farming techniques suitable to the specific conditions of the area (Martínez *et al.*, 2013). Figure 11 presents the precipitation and temperatures in the town of Puna in Potosí.

Eastern plains

The high prices of quinoa have attracted the attention of growers in eastern Bolivia, where a highly mechanized form of industrial agriculture (specialized in soybean, maize, cotton, sugar cane and winter wheat) is practised. The eastern plains are at an altitude of around 400 m asl, annual precipitation is about 1 000 mm, and winter temperatures range from 15° to 25°C. The precipitation and temperatures in the town of Chane in Santa Cruz are presented in Figure 12.

In the past 5 years, the National Association of Oilseed and Wheat Producers (ANAPO), has expressed interest in introducing quinoa in the tropical zone of Bolivia – a phenomenon known as the "tropicalization" of quinoa. Dr Alejandro Bonifacio from PROINPA and Marín Condori, an engineer from a private company in Santa Cruz, are currently conducting initial evaluations of quinoa sown in these areas, focusing on valley varieties. The first results reveal evidence of morphological and genetic variations due to stress caused by high temperatures. Grains have nonetheless been obtained and seeds harvested. At morphological level, variation occurs in the growth habit, resulting in more branching and the formation of limp panicles. There is a certain degree of instability at genetic level, with variation due to rearrangements leading to genetic segregation. In terms of reproduction, the high temperatures cause flower abortion, resulting in incompletely developed grains. In the event of rain, the grain runs the risk of germinating on the panicle itself or becoming dark because of fungal contaminations. Inheritable variation in quinoa is being exploited to select material with the potential for adaptation.

Prospects for quinoa in Bolivia

An area of major concern with regard to the rapid growth of quinoa production in Bolivia is the downwards trend for yields (Figure 6). Technology has not kept pace with growth, in particular in terms of management of soil fertility and irrigation, mechanization adapted to agro-ecological conditions, use of pastures and forage, and ecological pest management.

This section presents a brief overview of the progress made by the PROINPA Foundation.

In the field of integrated pest management, work has focused on the taxonomic identification of implicated species and on the development of technologies for organic production, such as eco-insecticides comprising extracts of different species and sexual pheromones (Saravia, Bonifacio and Aduviri, 2011; Figueroa *et al*., 2013; Saravia *et al*., 2013). Recent research has examined breeding, evaluating parasitoids, predators and entomopathogens. In fertility management, significant progress has been made in the use of micro-organisms as growth promoters, resistance activators, phosphorus solubilizers, nitrogen fixers etc. (Ortuño *et al*., 2013).

Pest control is hindered by the fact that farmers still use fumigation backpacks. This leads to high labour costs, as well as inefficiency when applications cover cropland of > 5–10 ha. Work is underway to develop environmentally friendly products for application using spraying equipment mounted on tractors. Furthermore, efforts are being made to make integrated management common practice: implemented at the same time by all farmers.

To maintain the soil's productive capacity in an area of such slow natural plant replacement (soils used for agriculture take > 10 years to regrow their plant cover), it is essential to incorporate organic matter. There are numerous recommendations and initiatives for restoring native plant life and reintroducing the llama population in these areas. While fine on a small scale, such recommendations become difficult to implement on a large scale – as with the case of the thousands of hectares planted with quinoa. PROINPA is analysing various local species (grasses, bushes and legumes) that can adapt as well as or better than quinoa to the agro-ecosystem of the southern Altiplano. Native pulses (*Lupinus* ssp.) emerge as an excellent option for use as green fertilizers. They can provide nearly 8 tonnes/ha of dry matter. Nevertheless, numerous challenges remain, including: breaking seed dormancy, producing seeds in considerable quantities, acquiring technologies to incorporate green fertilizers and using efficient micro-organisms to accelerate decomposition.

Tola – a generic name grouping together five species native to the area – is an effective plant cover providing soil protection. Of the five species, priority has been given to *Supu thola* (*Parastrephia lepidophylla*), *Uma thola* (*P. lucida*) and Ñaka *thola* (*Baccharis tola*), because they produce an abundance of seeds without seed dormancy. Managing these species in semi-mechanized or mechanized systems can help restore plant cover, protecting the soil against erosion, producing organic matter and providing food and habitat to the natural enemies of pests that attack quinoa. These environmental functions are all critical for the southern and central Altiplano regions.

PROINPA has also made an important contribution by generating new varieties that provide better yields, resistance to mildew, and tolerance to drought and frost. Work in recent years has focused on generating varieties for industrial use, depending on the starch type and the amylose-to-amylopectin ratio. For example, for the production of crème caramel, desserts and instant custard mixes, quinoa varieties with more amylopectin are required, while varieties containing more amylose are better suited to making snack foods and noodles. In addition, the starch granule diameter of different varieties plays an important role in the production of popped and puffed products. New varieties produce high-quality seeds in terms of health, varietal purity and grain size.

The Government of Bolivia is planning significant investments to promote the production, processing, export and domestic consumption of quinoa in order to reach an annual cultivation area of 1 million ha by 2025. On the basis of a conservative estimate of 300 000 ha and conservative prices, quinoa revenues could total USD500 million in Bolivia, making it the country's leading agricultural export.

Conclusion

At present, quinoa offers a unique opportunity for Bolivia, generating more than USD100 million in revenue. Some 20 000 families have been lifted out of poverty and an important industry meeting international requirements has been created. The Government of Bolivia is in the process of implementing several policies to encourage the production, export and domestic consumption of quinoa, and aims to more than triple current production. In order that this long-term vision become a reality, however, immediate investments are required in several areas, including management of the whole quinoa system, which encompasses the native vegetation of bushes, grasses and legumes as well as the camelid population. Standards need to be implemented to make processes –in farmers' fields, soil and pest management, storage, commercialization, processing and export – more efficient, and to ensure that foreign markets are maintained and further developed. Harmonious solutions to social conflicts generated by the quinoa trade must also be found.

The International Year of Quinoa in 2013 generated huge expectations with regard to this crop, on account of its high nutritional value, its capacity to withstand harsh conditions and its very high prices. As a result, countries with greater potential for investment in technology are beginning to produce quinoa and enter the market, creating serious competition for Bolivia and jeopardizing its dominance. For this reason, policies and investments must to a large degree focus on aspects such as organic production. In Bolivia, unlike other countries, organic quinoa crops could cover thousands of hectares. In addition, gluten-free quinoa crops can be cultivated on vast areas of land in the central and southern Altiplano regions, where grains that could contaminate quinoa in other countries are unable to thrive. Furthermore, 'Quinoa Real' should be promoted at international level for its large grain size, taking care to apply a designation of origin attesting that its unique source is in the southern Altiplano. These measures are necessary to maintain and consolidate the current role played by Bolivia in the world quinoa market.

Quinoa is grown on a smaller scale in the valleys and on the high plateaus of Bolivia. The great genetic diversity of quinoa in the country means that it is possible to develop more productive varieties for each area, and to develop technology to improve crops and productivity. In such areas, the production of organic quinoa is not a realistic goal. Instead, it is important to move towards environmentally sustainable, technically feasible, and economically and socially viable production. These areas are key to increasing exports of Bolivian quinoa and, more importantly, to boosting local and national consumption.

The transition from the quinoa boom to a stable situation poses a major challenge for all stakeholders involved in quinoa cultivation and trade. Sustainability awareness and a vision of long-term prosperity must be collectively developed.

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CHAPTER: 5.1.b

The Southern Altiplano of Bolivia

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Abstract

Quinoa has been a staple food for Andean populations for millennia. Today, it is a much-appreciated product on the international health-food, organic and fair-trade food markets. Quinoa producers in the southern Altiplano of Bolivia initiated this change approximately 40 years ago. On high desert land, they succeeded in developing a thriving agricultural crop for export. Although they enjoy lucrative niche markets, quinoa producers are not specialized farmers, nor do most of them live year-round in the production area. These are some of the paradoxes that characterize quinoa production in the southern Altiplano of Bolivia. Following a description of the origin, diversity and biological traits of the 'Quinoa Real' ecotype, on which production in this area is based, this chapter explores the importance of quinoa in local agrosystems and in the systems of agricultural and non-agricultural activities managed by southern Altiplano families. Geographic mobility and pluriactivity are part of the ancestral lifestyle of these populations and have to date determined how territorial resources are used and producers are organized in the context of quinoa's commercial success. Quinoa production in the region is presenting signs of agro-ecological and social vulnerability; however, it has the capacities to respond and adapt accordingly. Key points for the sustainability of local agrosystems are: i) harmonization of communal and individual regulations concerning access to and use of land in socially equitable agrosystems with a balance between crops and animal husbandry, ii) international standards for the recognition of 'Quinoa Real' in export markets, iii) continuous updating of rules and regulations so that local agrosystems can adapt to unpredictable changes in the socio-ecological context on different scales of space and time.

Key words: social adaptability, family farming, Bolivia, ecotype, territorial rules and regulations, pluriactivity, 'Quinoa Real', socio-ecological system, agricultural sustainability.

Context and issues of quinoa in the southern Altiplano of Bolivia

World leadership: the result of 40 years of efforts

The southern Altiplano of Bolivia dominates the international quinoa industry, with production – depending on the year – accounting for up to 90% of world exports (Aroni *et al.*, 2009; Rojas, 2011). In the 1970s in the area of Lípez, on the southern edge of the Uyuni salt flats, quinoa production for export began to rapidly spread, and continued to do so in the 1980s, towards the west and north of the salt flats, a region known as the Intersalar (Figure 1). Production on a large scale was initially a response to the commercial demand from neighbouring Peru, which had a larger population with a significant proportion of city-dwellers ate large amounts of quinoa (unlike the situation in Bolivia at the time). A Belgian non-governmental organization (NGO) working in communities in the area donated tractors to boost the initial phase of production (Laguna, 2011). Southern Altiplano farmers were thus able to respond in a timely manner to rising commercial demand from North America and Europe for vegetarian, gluten-free and protein-rich foods in the 1980s. Markets soon opened up for fair-trade and organic products, sustained mainly by European demand. The new export markets in the Northern Hemisphere did not supplant the Peruvian market, which until recently accounted for over half of quinoa exports from Bolivia (albeit mainly in the informal economy) (Aroni *et al.*, 2009). Today, local quinoa farmers make the most of a variety of markets, offering conventional quinoa, certified organic quinoa and certified fair-trade quinoa for the domestic market, the Peruvian informal sector and markets in the Northern Hemisphere.

Four paradoxes of quinoa production in the southern Altiplano

The growing international demand for quinoa places producers and their organizations in a privileged position for negotiating with importers, usually foreigners. Despite their success in export markets, however, local producers have not chosen to become definitively specialized in quinoa farming. On the contrary, the majority of them simultaneously continue non-agricultural activities, often involving temporary migration (Vassas Toral, 2011). Cultiva-

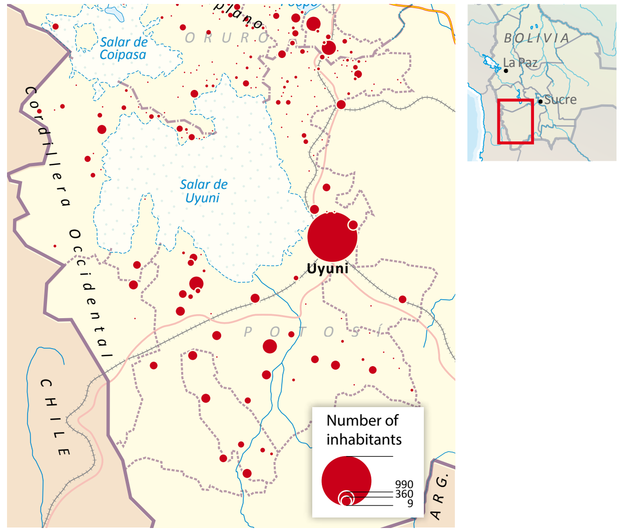

Figure 1: Map of communities in the southern Altiplano of Bolivia

tion of export crops by farmers not permanently resident in the rural area is just one of the paradoxes of quinoa farming in the southern Altiplano of Bolivia (Winkel, 2011).

The environment is characterized by extreme conditions – rocky or sandy soil almost permanently exposed to drought, frost, El Niño events, violent winds and intense solar radiation due to the high altitude – and it is surprising that an export crop has managed to flourish so successfully. To our knowledge, quinoa is a unique case worldwide: an export crop, produced practically without inputs, in an extreme environment of cold, arid high mountains. Growing areas, located at elevations of between 3 650 and 4 200 m asl, receive annual precipitation ranging from 150 mm in the south of the region to 300 mm in the northeast, with more than 200 days of frost a year (Geerts *et al.*, 2006). Quinoa has high tolerance to drought, but it is nevertheless unable to complete its vegetative cycle with only the rainfall received in an average year. For this reason, a 2-year fallow land system is adopted: the first year, precipitation accumulates in the soil; the second year, there is a full 1-year growing cycle (Michel, 2008).

Another paradox of quinoa production in this region is that, while being a healthy food grown by small producers, sometimes with organic and/or fair-trade certification, its cultivation could jeopardize the ecological and social foundations of the agrosystem (Michel, 2008; Vieira Pak, 2012). This situation goes against the desired benefits of family farming, which uses low levels of inputs and advocates ancestral roots and knowledge. Concerns about the sustainability of quinoa production were initially expressed, often in simplistic and alarmist terms, by journalists, businesspeople and researchers, who reported increasing soil erosion and highlighted the short-term vision and profit motives of some local producers and operators. Farmers and decision-makers in Bolivia are aware of the growing environmental and social vulnerability of their agro-ecosystem; therefore,, with the support of national and international institutions, they have begun to take initiatives to resolve emerging problems.

This leads us to another paradox of quinoa production, this time socio-economic. For three decades, the quinoa boom was essentially the result of sectorial and individual initiatives developed in an "organizational vacuum" (Félix and Vilca, 2009). In contrast, during the last 10 years, there has been an attempt to establish collective regulations at local, national and international level, involving community authorities, producers' associations, NGOs, rural development agencies, regional and central governments, and international food chains. While there are numerous cases worldwide of rural populations denied access to their own territorial resources, the southern Altiplano of Bolivia, in contrast, is an example of rural populations controlling the access to local land and seed resources, in addition to most export markets. By taking advantage of the growing global demand for grains, they are able to resist regulatory pressures from the outside.

This brief assessment of quinoa production in the southern Altiplano of Bolivia, will now examine the dynamics of the export market. The quinoa boom, beginning at the end of the 1970s, has yet to show signs of slowing down. Indeed, between 2000 and 2010, the value of exports increased fortyfold, totalling more than USD45 million. During the same period, average prices to the producer rose from USD1 200 to more than USD3 000 per tonne of standard quality quinoa (Rojas, 2011). These exceptional productive and commercial dynamics challenge the ecological, social and economic foundations of a sustainable agrosystem and present all the characteristics of a genuine agricultural revolution (Mazoyer and Roudart, 2006, 2009). While individual improvisation was initially the rule, initiatives are now emerging to renew the collective practices of local resource management.

The diversity, origins and uses of genetic resources

Dozens of local varieties, a single ecotype?

The southern Altiplano of Bolivia is the land of 'Quinoa Real'. Contrary to common belief, 'Quinoa Real' is not one variety of quinoa: nearly 50 local varieties fall under the generic name of 'Quinoa Real', each one identified by its common name and phenotype (Bonifacio *et al.*, 2012). These local varieties can be differentiated by the form of their panicles: amarantiform, glomerular or intermediate. Their leaves, panicles and grains also present very diverse and sometimes mixed colours, from green to yellow and purple for the leaves, and from white to pink, red, orange, yellow, violet, coffee and black for the panicles and the whole grains. The pigmentation in the grains is, however, generally unstable. Once the quinoa has been washed and the saponin removed, the grains of most 'Quinoa Real' varieties take on a white or cream colour. The grains of only a few varieties remain dark red, brown or black. Today, there is commercial demand for both types of grains: white and dark. The rarer coloured grains fetch a much higher price on the market: USD4 500/tonne against USD2 600/tonne for white grain quinoa (values as at May 2013, source: InfoQuinua.bo). The affirmation that the recent expansion of 'Quinoa Real' production has been detrimental to the diversity of quinoa cultivated in the region prior to the export boom is therefore erroneous.

Another common and unproven theory is that the local varieties of 'Quinoa Real' are distinct ecotypes, each one adapted to a specific microhabitat. If an ecotype is defined as a genotype within a species, that is different because of traits resulting from the selective action of local environmental factors (Zeven, 1998; Soraide Lozano, 2011; Bonifacio et *al.*, 2012), there is currently no evidence that the distinct varieties of 'Quinoa Real' can be differentiated by means of this ecological criterion. Indeed, during the recent period of expansion, the same varieties have occupied mountainsides and plains, regardless of the microclimate, topography or soil type of these different habitats. The capacity of each variety of 'Quinoa Real' to grow in ecologically diverse environments within the region is an essential adaptive feature in a very unpredictable mountainous environment, where specialization limited to a specific habitat or microclimate would be extremely risky and counterproductive. Such a wide adaptive capacity has been called "ecological versatility" by Zimmerer (1998), who, in a study of potatoes in the Peruvian Andes, demonstrated the preservation of agrobiodiversity and sustainable production in agrosystems using few artificial inputs. This ecological versatility does not mean that the notion of ecotype has no relevance in quinoa; rather, the ecotype is defined on a much larger scale than that of local variety and microhabitat. Accordingly, all the local varieties of 'Quinoa Real' which are very productive in the Bolivian southern Altiplano are vulnerable to mildew when planted in the Lake Titicaca area where the air is more humid and temperate than in their home region (Danielsen *et al.*, 2003). On the other hand, northern Altiplano varieties can barely withstand the cold drought conditions around the Uyuni salt flats. Further detailed studies are required to determine the optimum growing areas for the many local varieties of quinoa. In particular, ecophysiological analyses need to be conducted to understand how varieties adapt to different soil types, since this may constitute a factor of ecological differentiation within the large agroclimatic zone of the southern Altiplano. Recent studies comparing 'Quinoa Real' (Salare ecotype) and the Chilean Coastal ecotype reveal distinct capacities for exploring and exploiting the soil (Álvarez-Flores, 2012; Álvarez-Flores *et al*., 2014; see Chapter 2.8). Until more precise data are available, however, the quinoa ecotypes must be regarded as corresponding to the large agroclimatic regions of their area of distribution: central Altiplano, arid Altiplano, dry valleys, humid valleys and the coast. This wide ecotypic differentiation – without a specific microhabitat – matches the main genetic types of quinoa identified in the pioneering work by Wilson (1988) and largely corroborated with respect to Bolivia by Rojas (2003), Bertero *et al.* (2004) and Del Castillo *et al.* (2006). In this regard, the 'Quinoa Real' varieties correspond as a group to the "arid Altiplano" quinoa (Salare ecotype).

Ancient, and as yet unaltered, genetic resources

With regard to the origins of quinoa in the southern Altiplano of Bolivia, a comparative study based on molecular markers in the genome of ancient quinoa grains found in archaeological sites and of modern grains collected in the region, has revealed an almost perfect match between genotypes during a period of more than 650 years (Grasset, 2011, Programa ECOS-Sud Arqueoquinoas, unpublished data). This similarity suggests a pre-Incan origin for the local varieties still cultivated today in the area of the Uyuni salt flats. It also shows the absence of genetic erosion in quinoa germplasm, despite the many social and environmental changes in the region through time: the pre-Incan era, the Inca and Spanish conquests, the Little Ice Age, the colonial and republican periods, and the current expansion of export crops.

The absence of any appreciable impact on the genetic diversity of quinoa during the recent boom, as pointed out by Del Castillo *et al.* (2007), has at least two explanations. First, different kinds of quinoa have continued to be used locally for a wide range of food preparations (see the section below on food uses in the area), as well as for medicinal and ritual uses. Second, the commercial product, 'Quinoa Real', is identified with a set of diverse varieties which were traditionally cultivated and which have now found a market: white grain quinoa, dark grain quinoa and quinoa for puffed grains (*pipocas*). White grain quinoa has the greatest share of sales and it is also the quinoa with the largest number of local varieties: 44, according to the catalogue published by Bonifacio *et al.* (2012). Dark grain varieties and those used for puffed quinoa are marginal commercial products that nevertheless allow very special varieties to be maintained within the 'Quinoa Real' group. There are seven varieties of dark grain quinoas, two of which – *phisanqalla amarantiforme* and *phisanqalla hembra* – are suitable for puffed quinoa.

This diversity of genetic resources satisfies producers, buyers and consumers of 'Quinoa Real'. Despite the efforts of research laboratories and public institutions, improved varieties and certified seeds have not created much interest among farmers (Baudoin-Farah, 2009). When counterproductive goals are not being pursued – for example, the removal of bitterness from the grains of some varieties ("counterproductive", because the bitterness was actually an effective protection against birds and other animal pests) – genetic improvement research sometimes runs into genuine biological obstacles. For example, resistance to mildew has been linked to agronomic characteristics, such as small grain size and a long vegetative cycle, which are unsuitable for a commercial crop (Gamarra *et al.*, 2001). In managing genetic 'Quinoa Real' resources, there is a fine line between genetic improvement and participatory plant breeding, between uniformization of seeds and preservation of agrobiodiversity, between private interests and collective heritage.

From production certification to designation of origin

Rather than pursuing seed certification, quinoa growers are interested in certifying grain production. Whether organic or fair trade, certification of 'Quinoa Real' is an established process, encouraged since the beginning of the 1990s by the National Association of Quinoa Producers (ANAPQUI) with the support of European NGOs (Laguna, 2011). According to local estimates, 25–40% of today's 'Quinoa Real' production in the region is marketed as "organic". Exports to Europe and North America comprise almost exclusively this type of quinoa (MDRyT and CONACOPROQ, 2009; Aroni *et al.*, 2009).

With regard to the use of genetic resources, the Government of Bolivia, faced with growing competition in international markets, issued a general policy document indicating that "an indispensable and pending task [is] to obtain the quinoa designation of origin [*Denominación de Origen*], for legal and commercial purposes" (MDRyT and CONACOPROQ, 2009). In Bolivia, the "Quinoa Real" designation of origin was approved in 2002 by the National Intellectual Property Service (SENAPI), and a technical document was published in 2011 to promote the distinctness of the product and to protect its geographic and cultural origins (Soraide Lozano, 2011). Similarly, farmers in the area of Lípez (to the south of the Uyuni salt flats) began a designation of origin process in 2009 for their own local crops (Laguna, 2011; Ofstehage, 2012). Nonetheless, on the international scene, the lack of consistency in the many rules and regulations regarding the legal management of plant genetic resources hinders the sovereignty of states and the rights of farmers over these resources (Chevarría-Lazo and Bourliaud, 2011).

Importance of quinoa in the agrosystem and systems of family activities

An agricultural landscape in profound transformation

The majority of the crops that make up the richness of Andean agriculture – Andean tubers and grains, broad beans, green vegetables, forage plants etc. – can only be grown in areas with sufficient access to water. In most of the cold and arid southern Altiplano, the options are restricted to growing potatoes (sweet and bitter) and quinoa. Even before the recent success of export crops, and despite the very harsh environmental conditions, growing potatoes and quinoa was generally sufficient not only for families' personal consumption, but also for supplying local markets and, in particular, mining camps (Franqueville, 2000; Laguna, 2011).

Traditionally, agricultural plots were located on mountainsides: they are less exposed to night-time frost than the plains, while the plains were mainly used for grazing llamas and sheep, which can withstand the cold better than crops (Pouteau *et al.*, 2011). To this day, the pasturelands are owned and used collectively, while the farming plots, although belonging to the communities, are used individually and are generally passed down within the family (Félix and Vilca, 2009; Vieira Pak, 2012). As international demand for quinoa emerged in the 1970s, cultivation extended into the plains and tractors were used to increase production. It should be noted that in this region, on both mountainsides and plains, quinoa is grown on non-irrigated lands, sown in holes – not in furrows as in the rest of the Bolivian Altiplano.

Given the subsidies for rice and wheat consumption granted by international food aid programmes since the 1960s (Franqueville, 2000), and considering the lack of major livestock markets, local producers decided to limit potato crops to family consumption and to convert an increasing share of pastureland for quinoa crops. Figure 2 shows how quinoa crops expanded in a community near the Uyuni salt flats. Between 1963 and 2006, the cultivated area grew by 360%, spreading mainly to the plains, although the mountainsides were still cultivated. An independent study conducted in three towns in this area shows that between 1975 and 2010 the cultivation of quinoa increased by 70–300% on flat land and

Figure 2: Expansion of cultivated areas between 1963 and 2006 in a community in the southern Altiplano of Bolivia. Source: Jean-Rémi Duprat. CNRS – UMR 5175, EQUECO Project, 2008.

decreased by 16–32% on mountainsides (Medrano Echalar *et al.*, 2011). This expansion has led to the uniformization of the agricultural landscape. There are vast monocultures of quinoa and fallow plots while the native vegetation – grasses and bushes that make up the *tola* – is increasingly relegated to marginal, rocky land or mountainsides that cannot be worked by machines (Michel, 2008).

Quinoa in the family system of activities

These changes to the local agrosystem have occurred in a socio-economic context in which agriculture and animal husbandry are part of a system comprising a range of agricultural and non-agricultural family activities. In an arid region that for a long time had a marginal role in the national economy, pluriactivity and temporary migration have been part of families' strategies to adjust to environmental and economic risks (Saignes, 1995; Vassas Toral, 2011). Making the most of their proximity to contrasting ecoregions, such as the Pacific coast to the west and the Inter-Andean valleys and tropical grasslands to the east, the inhabitants of the southern Altiplano have over the centuries developed a way of life based on trading natural resources between these distinct regions (Platt, 1995; Flores Ovando, 2008). Wool, llama leather and meat, potatoes, quinoa, salt and medicinal grasses were traded for maize, coca, firewood, fruit, oil and other goods from neighbouring regions.

Today, lorries have replaced the llama caravans of the past, but the system combining agricultural and non-agricultural activities has been maintained. Non-agricultural employment now includes urban business or artisan jobs, the civil service, mining and tourism (Figure 3) (Vassas Toral, 2011; Winkel, 2013). The new aspect is the growing – even predominant – share of family income generated by local agricultural production, thanks to the expanded international quinoa market. Although there are no regional statistics on the composition of family income, a survey conducted among 36 families in the area of the Uyuni salt flats shows the wide diversity of income depending on social status and, in particular, non-agricultural activities (Acosta Alba, 2007). For these families, annual earnings from quinoa production averaged nearly USD3 500 and reached a maximum of USD18 000, accounting for up to 70% of family income (ibid.). These figures are from 2007 – before the price of quinoa doubled in 2008. An independent survey in 2010 of 35 families in another community reported that most producers had an annual income of USD13 000 and that 11% of farmers with extensive farmland (> 30 ha) had an annual income of USD45,500 (Medrano Echalar *et al.*, 2011). Overall, the success of quinoa has meant that, in local farmers' household budgets, quinoa cultivation has supplanted animal husbandry in its traditional function of providing savings and insurance. Moreover, unlike livestock, quinoa does not require a continuous human presence in the production area; this facilitates the di-

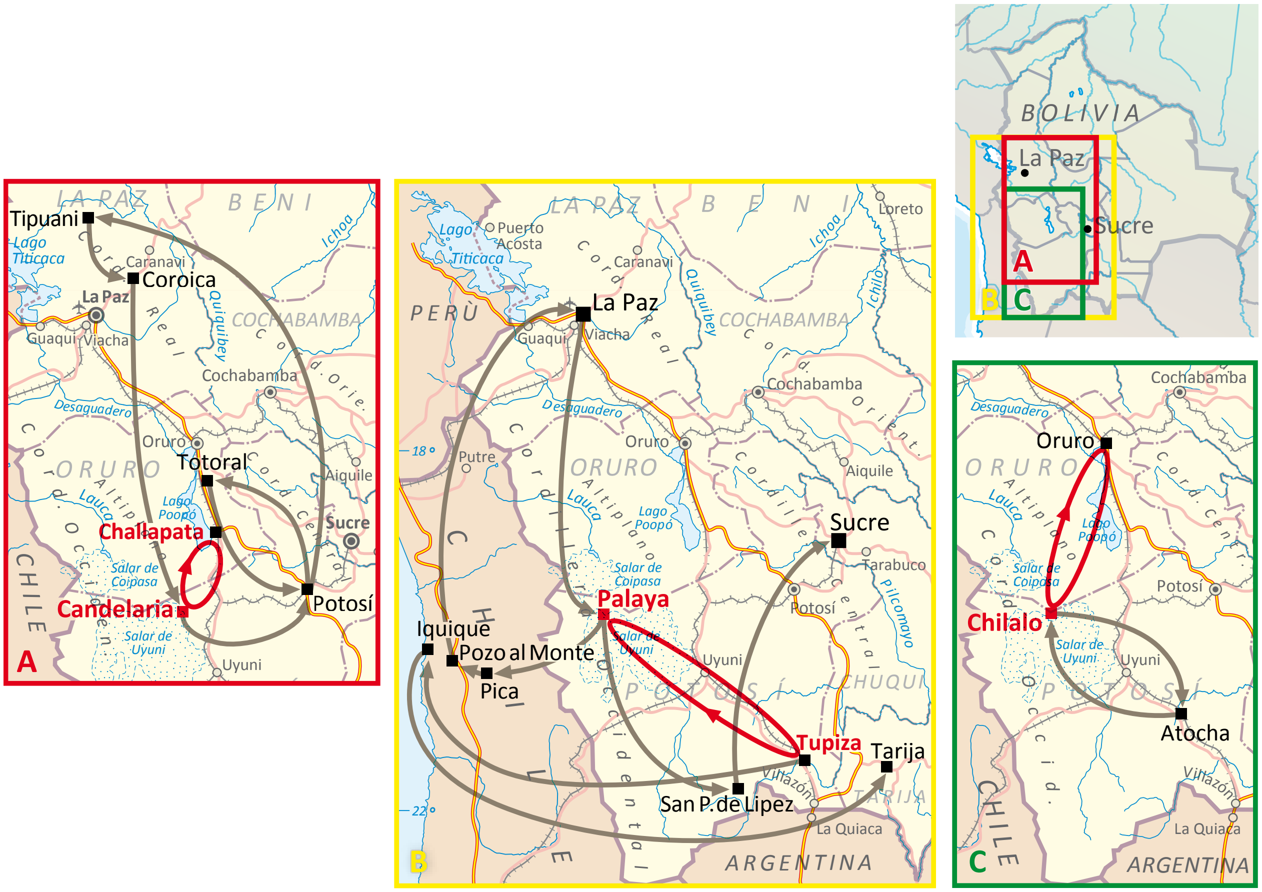

Figure 3: Examples of migratory paths for three quinoa producers in the southern Altiplano. Source: Vassas Toral (2011)

versification of family income through mobility and pluriactivity outside the production area (Chaxel, 2007; Vassas Toral, 2011).

The success of commercial quinoa production contributes to the integrated development of the region. The rural communities where the crops grow are linked with nearby cities where the producers settle with their families and where they invest most of their farming income: in the education of their children, in business or artisan activities, in the construction of houses or in the purchase of vehicles (Laguna, 2011; Vassas Toral, 2011). A comprehensive assessment of quinoa's economic contribution to the development of the southern Altiplano must be carried out, taking into account not only grain sales, but also revenue from industrial processing, related activities (e.g. agromechanics, transport), reinvestments, taxes etc. The revenue generated in this region solely through the sale of quinoa has been estimated at BOB360 million (bolivianos, approx. USD50 million) (2008 data, Aroni *et al.*, 2009).

Quinoa's current situation and prospects

For almost three decades, the development of quinoa production in the southern Altiplano received little support from official institutions. In contrast, during the last 10 years the 'Quinoa Real' boom has attracted the attention of numerous national and international support programmes and projects. Given the growing interest in a product emblematic of vigorous Andean agriculture, a series of working documents has been published by the AUTAPO and PROINPA foundations and are available online, in particular: a synthesis by Aroni *et al.* (2009) on the situation of 'Quinoa Real' in the region, and a more general report by Rojas (2011) in support of the declaration of 2013 as the International Year of Quinoa. An atlas of 'Quinoa Real' production has been published with satellite maps and statistics showing the main biophysical and socio-economic indicators in ten municipalities of the region (Fundación AUTAPO, 2012). The large amount of technical, social and economic data available in these documents cannot be detailed here. However, some of the information from the above-mentioned synthesis is presented below, followed by specific comments on the environmental and social challenges posed by the 'Quinoa Real' boom.

Quinoa in a few figures

In the southern Altiplano, quinoa is grown by 6 300 on-site farmers and 8 000 producers, whose primary residence is outside the community. Nearly 70% of production takes place on the plains. Sowing is mechanized in 76% of cases, while harvesting is almost exclusively manual. Indeed, only 2% of farmers use string trimmers. During the 2007/08 farming season, production totalled 28 000 tonnes from a cultivated area of approximately 49 000 ha, to which can be added 46 000 ha of fallow land. In Bolivia, domestic demand for quinoa is estimated at 7 000 tonnes per year. The value of reported quinoa exports has increased almost fortyfold in the last 10 years. Export volumes officially went from 1 400 tonnes in 2000 to 10 400 tonnes in 2008, and rose to 26 000 tonnes in 2012. After the United States of America (USD10.1 million for 4 095 tonnes in 2008), France is the second largest importer of 'Quinoa Real' (USD3.7 million for 1 700 tonnes in 2008). Bolivia has 62 quinoa processing plants, both artisanal and industrial, which contribute to the added value of quinoa within the country.

An agrosystem reaching its territorial limits

Annual quinoa production in the southern Altiplano requires a 2-year precipitation cycle and, therefore, two growing areas: the area where the crop is growing, and the area tilled for sowing in the following cycle. As cultivated areas are extended and concentrated, it is difficult for the natural *tola* vegetation to recolonize fallow plots, because its natural seed banks quickly become impoverished. The bare soil in fallow fields and in yet-to-be-planted plots remains exposed to the wind, which is especially strong in the Altiplano. Given the very slow regrowth of the native vegetation (Joffre and Acho, 2008; Medrano Echalar *et al.*, 2011), the conversion of large areas of grasslands into croplands constitutes an almost irreversible change in plant cover and hastens the process of wind erosion (Michel, 2008). Moreover, the areas recently converted into cropland are concentrated in low, flat areas that, because of cold air drainage at night, are more susceptible to frost than the surrounding hillsides (Pouteau *et al.*, 2011). Indeed, the frosts in 2007 and 2008 revealed the vulnerability of the quinoa agrosystem in these new production areas. Despite this, given the high selling prices of quinoa, farmers are willing to accept the economic risk of growing it in the plains.

Loss of soil fertility in land mechanically cultivated for quinoa is often cited mentioned as a constraint, as the main cause of a supposed decrease in quinoa yields and proof that the agro-ecosystem has exceeded its capacity (Cossio, 2008; Félix and Vilca, 2009; Jacobsen, 2011). A recent study of soil fertility in the area of greatest quinoa production indicates that 88% of soils have low to moderate fertility (Cárdenas and Choque, 2008). There are no data, however, to assess the impact of quinoa production on these fertility levels. The same study does not find any link between the quinoa yields in ten communities in the area and the average duration of land use (30–50years). In general, the "evidence" of accelerated soil degradation in the region lies in national statistics on grain yields. It should be noted that these data are aggregated at national level and cannot adequately characterize a local phenomenon such as soil fertility. Furthermore, they do not reveal a statistical trend indicating a decrease in quinoa yields over the last 50 years, including during the recent production boom, although a comparison with the previous period is possible (Winkel *et al.*, 2012). More importantly, grain yield is not an appropriate indicator of potential soil degradation, because the annual yield of a crop is the result of several concomitant factors aside from soil fertility: climate, agricultural practices and possible attacks by pests. In the case of quinoa in the southern Altiplano, the mediocre results of mechanized sowing (compared with manual sowing) and the frequent cultivation of crops on plains exposed to wind, frost and pests, are factors that may contribute to loss of soil fertility and explain the relatively low yields (500–700 kg/ha) usually obtained on the plains, when compared with the higher yields (1 000–1

500 kg/ha) obtained on mountainsides carefully farmed by hand and less affected by weather and pests (Winkel *et al.*, 2012).

Given the uncertainty regarding agronomic indicators of soil quality and their relation to grain production, the most tangible indicator of the agro-ecological limit reached by the current production system is the surface area of land converted to quinoa crops. In most communities, the land that can be worked by machine and converted into farming plots has already reached its limit. This has caused rising tensions among families about land access (Vieira Pak, 2012) and natural plant cover has been reduced (Michel, 2008). Aroni *et al.* (2009) estimate that, of the 145 000 ha of potentially arable land in the southern Altiplano, one-third is being farmed, one-third is lying fallow and the rest is “virgin land” (reserve areas, pastures, steep slopes etc.). Most producers do not keep areas of native vegetation for animal husbandry because it is not financially profitable. However, it is important to factor in the ecological benefits of such land, as these areas act as natural barriers to wind and water erosion, habitats for the natural predators of quinoa pests, and sources of uncultivated resources (e.g. firewood and medicinal plants). To reap these environmental benefits and ensure the sustainability of the agrosystem, it is recommended to maintain hedgerows and sow quinoa in beds or strips with the native vegetation (ANAPQUI, 2009; Michel, 2008).

As a result of current changes in the use of territorial resources, the quinoa socio-ecosystem is susceptible to inequitable land access and uniformization of the landscape. Aware of these vulnerabilities, farmers, peasants’ organizations and the authorities in charge of land management have begun local consultation processes to implement new rules and regulations on the use of territorial resources.

Regulations needed for production and commercialization

Since the 1952 agrarian reform, which had only minimal impact on the southern Altiplano – an environmentally inhospitable region disregarded by large landowners – a myriad of rules and regulations on land access and use have been created. Local rules and customs, transmitted and enforced by aboriginal authorities, coexist with national laws passed by the Government. Collective rules, the product of public consultations, compete with conditions of power or oversight by companies or certification organizations. However, rules are rarely applied in a uniform way across the region, either because of a lack of consensus in the local population or due to a dearth of resources for their implementation.

In this context, the NGO, Agronomists and Veterinarians without Borders (AVSF, formerly VSF-CICDA), in coordination with the National Association of Quinoa Producers (ANAPQUI), set out at the beginning of the 2000s to establish new collective rules on territorial management adapted to recent changes in the agrosystem in several communities in the area (Félix and Vilca, 2009). After a long process of raising awareness and consultation with local stakeholders, technical and regulatory recommendations (both individual and collective) were proposed with the aim of achieving sustainable quinoa production in the southern Altiplano (ANAPQUI, 2009). A gradual participatory methodology was implemented, whereby local stakeholders seeking to overcome disagreements and conflicts could reach a consensus on the rights and obligations required to manage communal lands equitably and sustainably.

Uses and markets

The many traditional or novel uses of quinoa

The exceptional nutritional value of quinoa is well documented. In addition to its high content of proteins and balanced amino acids, the grain has high levels of minerals, anti-oxidants, unsaturated fatty acids and dietary fibre (Rojas, 2011; Soraide Lozano, 2011). Quinoa also offers multiple non-food applications: medicinal and ritual in its traditional forms, as well as chemical, pharmaceutical and cosmetic in its contemporary and industrial forms.

The versatility of quinoa makes it suitable for 35 different traditional food preparations, including soups, main dishes, pastries and drinks (Rojas, 2011). The populations living in the southern Altiplano eat quinoa in various forms: pearled, pilaf, ground, toasted and fermented (as the traditional drink called *q’usa*). Miners and peasants in the Altiplano use quinoa grains as food in rituals. Quinoa leaves are also consumed, for example in *yuyu*, a ritual soup prepared by regional stockbreeders for the llama festivities held between New Year’s Eve and Carnival, when quinoa leaves are still tender. Quinoa stems are burned to ashes and mixed with other substances to make *lejía,* a kind of paste used to activate the alkaloids in the traditional consumption of coca leaves. Quinoa has a wide range of uses in medicine, which exploits all the plant parts (Rojas, 2011): it is used in plaster to treat bone fractures and is a recommended part of the diet during convalescence (Bonifacio *et al.*, 2012).

Non-traditional uses of 'Quinoa Real' have been encouraged in Bolivia since the beginning of the 1970s; events organized by Oruro Technical University have promoted Creole cuisine (Iñiguez de Barrios, 1977). Today, quinoa – in the form of flour, flakes or puffed grains – is an ingredient in numerous industrial products, including noodles, biscuits, energy bars and cereals manufactured inside and outside the country. These products are included in state school lunch and family food subsidy programmes. They also respond to the growing international demand for gluten-free food.

Domestic quinoa consumption has been the subject of various articles in the international press reporting an alarming loss of food security for local populations because of high prices and the profit motives of producers and exporters (Sherwin, 2011). The arguments presented, however, do not hold in an in-depth analysis of local eating habits, and they lack the historical perspective to assess changes in quinoa consumption dating back to before the current commercial boom (Banks, 2011; Winkel *et al.*, 2012). In particular, they are based on a comparison of the amounts of quinoa, noodles and rice eaten by local populations – a common comparison (e.g. Montoya, 2009; Borja and Soraide, 2007), but an inadequate one in terms of nutrition, because it is important to take into consideration quinoa's specific characteristics, notably its high protein and dietary fibre content. Local consumers know that they can be satiated with just a small quantity of quinoa (Rojas, 2011), and they therefore add only moderate amounts to soups and other dishes (Banks, 2011). Therefore, to compare quinoa consumption on a quantitative basis with consumption of other grains does not make sense: the two types of food do not have the same nutritional value or the same function in the human diet.

Important non-food uses of quinoa include applications employing saponin, a by-product from bitter quinoa grains, such as the varieties that make up 'Quinoa Real'. The detergent and cosmetic properties of saponin have long been known to local populations and are now recognized by industry. This by-product also makes a powerful bioinsecticide and, in pharmaceutical applications, an antibiotic and an effective adjuvant for the intestinal absorption of some medicines (Rojas, 2011). Lastly, some uses of quinoa for animal food and health should also be mentioned: as forage and to relieve altitude sickness in cattle (MDRyT and CONACOPROQ, 2009).

A diversity of markets and forms of commercialization

Although 'Quinoa Real' farmers and their families never stopped eating the "golden grain", the paradox of quinoa is that it was valued as a commercial resource outside the country many decades before it regained its lost recognition at home. The market dynamics for 'Quinoa Real' did not, therefore, follow a typical pattern, since exports grew before the grain regained its domestic market.

During the last 40 years or so of resounding commercial success, demand for 'Quinoa Real' has continued to grow. New kinds of demand have not replaced the old ones, but have simply been added to them. Production increases have kept pace with market diversification and evolving commercialization channels. The contraband market coexists with formal commerce, conventional quinoa with organic quinoa, and individual sales with private or group distribution. This situation prevents quinoa producers in the southern Altiplano from falling under the control of just a few trading companies, a common occurrence for other farmers in the world. Currently, more than 20 producers' associations and private companies store, process, transform and sell 'Quinoa Real' in Bolivia (Aroni *et al.*, 2009). These organizations and even the *rescatiris* – rural middlemen often stigmatized for taking advantage of the humblest farmers – have their operating methods and play their role in the economic system of 'Quinoa Real' (Ofstehage, 2010, 2012).

Aroni *et al.* (2009) describe in detail the various distribution systems operating in the southern Altiplano. 'Quinoa Real' is commercialized as follows: "some 43% through the informal sector, the Challapata open market or intermediaries who sell quinoa on domestic or contraband markets, and the remaining 57% is stockpiled by organizations to be sold to processing companies and exporters". More than 95% of quinoa exports are in the form of grain, most of which has organic certification and a smaller proportion fair-trade certification. There is also a growing demand for quinoa derivatives, such as flour, flakes, pastries, puffed quinoa, cereals and chocolate quinoa bars. This demand has enabled the development of a national industry capable of contributing to the expansion of the domestic market. Several products from this industry have been incorporated into government food subsidy programmes.

Questions and problems

'Quinoa Real' farmers in the southern Altiplano were forerunners and are now leaders in the cultivation of quinoa for export. After 40 years, their success is the result not only of continuous effort, but of constant adjustments to growing and ever-changing demand, thus demonstrating their great capacity for adaptation and social learning. Given the duration of the quinoa boom and the number of stakeholders involved, the questions and problems raised today have already been addressed in various studies: some focus on a specific subject such as genetic resources or soil fertility (Arce, 2008; Bonifacio *et al.*, 2012, Cárdenas and Choque, 2008; Michel, 2008); others seek to organize the available technical, social and economic information (Aroni *et al.*, 2009; Soraide Lozano, 2011); while others reflect more inclusively on socio-environmental issues and possible solutions (Cárdenas and Choque, 2008; Félix and Vilca, 2009).

Of all the subjects examined by researchers, land use is the most important and also the most widely debated in the media. Mechanization, insufficient fallow land and lack of fertilizer are presented as the causes of accelerated soil degradation, fuelling a vicious cycle of farming areas expanding to the detriment of pastureland. Nevertheless, there has to date been no published research demonstrating a clear, short- and long-term relation between quinoa production and soil fertility in the region. This lack of scientific research has not halted the discussion on soil exhaustion and the agrotechnical solutions to solve the problem. Many experts recommend systematically incorporating manure from camelids or sheep. This practice would undoubtedly foster animal husbandry and thus restore balance between agriculture and stockbreeding in the agrosystem. The impact of manure on soil fertility, however, appears very uncertain. Indeed, as Cárdenas and Choque point out (2008, p. 64): "the nitrogen content of manure is very poor and dynamic; also, the phosphorus and potassium are lost or are retained by mineral fractions [...]. The carbon-to-nitrogen ratio in manure is very high, so the degree of mineralization is very low, and it is difficult for the humus present to mineralize substances that accumulate in the soil." These conclusions are confirmed by Miranda Casas (2012) in his in-depth study showing a limited response of quinoa to manure fertilizer in non-irrigated plots, possibly due to nitrogen immobilization mechanisms in the soil. The author does, however, point out that the advantages of organic fertilizer may be indirect and the result of, for example, improvements to some physicochemical properties of soil, such as resistance to erosion and permeability. The potential benefits need to be evaluated under the agro-ecological conditions of the southern Altiplano.

In a similar vein, although recommendations to have hedgerows or to sow seeds in strips and beds for the prevention of soil erosion seem sensible, the effectiveness of such measures is yet to be demonstrated. Moreover, it is not certain that farmers, who do not all have the same access to land or the same economic capacity, would accept them. The option of returning to the ancestral system of *mantas* (the practice of alternating between farming and animal husbandry on communal land) seems too far removed from the current importance of quinoa production in the area and local economy. Indeed, the current circumstances cannot be compared to the situation that reigned before the commercial success of quinoa, when *mantas* helped to regulate soil fertility and maintain the equilibrium between farming and animal husbandry.

With regard to the excessive extension of cultivated areas, one solution is to intensify irrigated crops: farmers could produce greater yields in smaller areas and reduce the rate of expansion (CTPS, 2008). However, in a region with very scarce and often saline water resources, irrigation represents a serious threat to agricultural sustainability (Geerts et *al.*, 2008). Moreover, maintaining irrigation infrastructure in a region exposed to frequent frosts is technically challenging; ensuring equitable access to such costly infrastructure would lead to socio-economic problems. As with other innovations related to agricultural practices, systematized and comprehensive studies are needed, taking into account the local environmental conditions and the growers' specific socio-economic situations, with a view to the social and ecological sustainability of the agro-ecosystem (Cárdenas and Choque, 2008). On the whole, investments in intensive quinoa production in a region with environmental restrictions as severe as in the southern Altiplano are deemed very risky (Michel, 2008).

While the subject of land use has sparked great interest among various stakeholders (including promoters of lucrative agrotechnical solutions), the issue of land access was pushed into the background for a long time. The absence of formal rules led to an upsurge in quinoa farming, as inequalities among community members widened in terms of access to land; in some cases, conflicts were even generated within and between communities. As mentioned earlier, NGOs and producers' associations have addressed this sensitive matter, creating rules to achieve sustainable agricultural production. These communal rules on territorial management must be disseminated and effectively implemented to ensure a legal framework for land ownership and access. It would be interesting to examine how the usufruct of cultivated plots in a system of community land ownership has helped to protect community members against outside interests, contributing to the sustainability of the local agrosystem.

Similarly, the 'Quinoa Real' designation of origin process should be extended to protect local producers' access to their own seed resources. In this respect, a minor but important factor is the practical recognition of the diverse local varieties. Although their different food uses are appreciated, their agroecological characteristics are little known. Different "ecotypes" are mentioned without reference to the various responses of varieties to ecological factors such as soil and climate. This lack of knowledge hinders an understanding of the potential interactions between genotypes and the environment that have been observed in quinoa (Bertero *et al.*, 2004); this in turn invalidates efforts to differentiate the physicochemical characteristics and nutritive value of the varieties.

In addition to the problems related to land ownership, two other social issues should be highlighted. First, temporary migration, a very common practice among quinoa farmers, is sometimes blamed for irresponsible behaviour by some migrant producers who exploit and extract territorial resources. As demonstrated by Vassas Toral (2011), the social realities are more complex, and it is worth examining how the quinoa producers' system of mobility and pluriactivity contributes to the social sustainability and rural-urban development of the region. Moreover, given the farmers' capacity for adaptation and social learning, it seems pertinent to examine whether their responsiveness and adaptability would be affected by a proliferation of territorial and agrocommercial regulations. The key lies in the bargaining power and propositional force of the producers when they organize and take part in representative entities capable of self-transformation (Young, 2010). They are in apposition to prevent the excessive centralization of regulations and the privatization of their common property (Ostrom, 1990).

Conclusions

While gaps remain, there is now enough knowledge about quinoa production in the southern Altiplano of Bolivia to propose explanations for the four paradoxes characterizing the grain's commercial success. In some cases, these explanations can guide towards solutions to the challenges arising from the agricultural revolution experienced in the region over the last 40 years.

"A very special grain grown by unspecialized farmers": although quinoa benefits from niche markets, some of them very demanding and sophisticated, its producers continue to rely on agricultural and non-agricultural pluriactivity and mobility – two indispensable conditions for the economic sustainability of family farming in a regional context of great agroclimatic and economic uncertainty. Family strategies of pluriactivity and mobility are developed at the expense of animal husbandry. On the other hand, they foster integrated development between rural communities and medium or large cities in the region, thus reducing the need to migrate to distant urban centres.

"A productive crop in a harsh environment": recent studies have revealed some of the specific ecophysiological adaptations of 'Quinoa Real' varieties, for example, the vigorous root system enabling plants to explore the soil quickly and deeply and efficiently harness scarce water and nutrient resources. The aptitudes of these plants are complemented by the local practice of non-irrigated farming, resulting in farming methods that use very few artificial inputs (chemical fertilizers, fuel and pesticides). It is known, for example, that the 2-year fallow land system allows the soil to accumulate over 2 years the precipitation required to complete a quinoa farming cycle in this arid region. In contrast, the practice of sowing in holes has not yet received much attention in terms of its potential agro-ecological interest. The potential benefits are numerous, stemming from the optimization of the plants' water and nutrient use, resistance to wind, and tolerance of frost and pests.

"An organic, potentially unsustainable crop": it cannot be denied that, in some cases, unsustainable growing methods are practised in the region. This situation arose from an initial scarcity of knowledge about how to run large-scale commercial farming in an extreme environment with limited use of artificial inputs. Today, organic fertilizer, hedgerows and sowing in strips and beds are recommended strategies. Although their effectiveness is yet to be proved, these partial solutions should not be rejected. On the contrary, the problems they claim to solve must be considered on another scale. The structure of the rural landscape – i.e. the organization of the physical space encompassing crops, grazing land and natural areas – should be considered in terms of its multiple functions: preserving soils, controlling pest populations, balancing agriculture and animal husbandry, and providing benefits that are not strictly agricultural (e.g. firewood, medicinal plants).

"Sectorial dynamics requiring collective regulations": the development of quinoa agriculture began with a sectorial vision of the production chain of the crop that disregarded animal husbandry and natural areas. Over time, given the need for a consensus on equitable and sustainable land management, community members and quinoa producers' associations initiated various participatory processes to define new communal rules on land use. These same local rules were included in the 2012 revision of Fairtrade International standards to promote the sustainable production of quinoa, illustrating a successful example of bottom-up regulation: from grassroots to international bodies. As for the 'Quinoa Real' designation of origin, the regulatory process remains uncertain because of the complexity of the international procedures to ensure the rights of states and farmers over their plant genetic resources.

If the spatial structure of the rural landscape is considered together with the social aspects relating to land access, territorial organization and management emerge as the issues most requiring innovative solutions to deal with the unprecedented transformations in agriculture and local societies brought on by the quinoa boom of the last four decades. These organizational and social innovations should receive as much attention as agrotechnical ones on the agenda for the sustainable development of farming in the southern Altiplano.

Finally, it should be pointed out that the various solutions for the adaptability of the 'Quinoa Real' agrosystem in the southern Altiplano of Bolivia are related to the following:

- Plant material with an exceptional capacity to adapt to the environment and high intra- and intervarietal diversity. These qualities may be essential for an agrosystem requiring few inputs and providing a model for dry-farming in other mountainous and arid regions of the world. The biological characteristics of this plant material must therefore not be altered, nor should this collective heritage be taken from the control of farmers. The designation of origin could serve as a framework for this protection in the context of the accelerated spread of quinoa cultivation outside its Andean birthplace.
- A responsive and proactive local society exhibiting a high level of social learning based on several mechanisms for social cohesion: vigorous community customs and traditions, active associations and adherence to community landownership rules. Care should be taken not to fall into black and white judgements opposing migrant producers and on-site producers, farmers and stockbreeders, *rescatiris* and intermediaries, Lípez and Intersalar etc. Mechanisms for cohesion and social

learning enable the local society to adapt to climate and market changes undreway in the region long before the quinoa boom (Banks, 2011). These factors also allow society to resist command-and-control trends and the agrotechnical and financial "packages" that are usually presented when an agricultural crop of economic interest emerges (Holling and Meffe, 1996; Briggs, 2003). These factors for social cohesion are the key to agricultural and food sovereignty for local populations and the country as a whole (De Schutter, 2011).

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CHAPTER: 5.2

Peru

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Abstract

Quinoa is one of the food grains grown in Peru since time immemorial and is one of the staple crops – together with maize, potato, and Andean roots and tubers – dating back to the pre-Columbian era. By 500 years after the Spanish Conquest, the area under quinoa had shrunk significantly, particularly in the valleys of the central and northern Andes. However it continued to be cultivated in the Puno region of the Altiplano, using ancestral systems of cultivation known as *aynokas*, which allowed its genetic diversity to be preserved. In the rest of the Andes, the cultivation of quinoa declined, both genetically and culturally.

During the first decade of the twenty-first century, a stable market for quinoa developed at both domestic and international level, and the prices were sufficiently high to make quinoa farming economically important. As the value of quinoa has increased, so has its planted area (70% is located in Puno, 20–30% elsewhere in the Andes, in valleys and elevated areas). It has begun to be cultivated in coastal areas, with yields of over 7 000 kg/ha.

As crop areas and the market grow, there is increasing research into quinoa cultivation, its improvement and processing technologies. Peru is currently one of the primary producers and exporters of quinoa. In 2012, the area under quinoa cultivation reached 38 495 ha, with an average domestic yield of 1 149 kg/ha and a total production of 44 210 tonnes.

The Domestication of Quinoa

Approximately 10 000 years ago (around 8000 BC), human populations became established in the Lake Titicaca basin at an elevation of around 4 000 m asl (Aldenderfer, 1998; Rigsby *et al*., 2003). They developed crops and learned how to manage the richness of the existing resources, despite adverse climate and soil conditions, by adapting cultivation systems dominated by potato, small tubers and the genus *Chenopodium* (Pearsall, 1992).

Archaeological evidence indicates that the process of domestication began around 5000 BC in Ayacucho, in the central Andes of Peru (Uhle, 1919; Lumbreras *et al.*, 2008). The botanical remains found at archaeological sites in the Peruvian Andes very often include traces of plants in the *Chenopodium* group, primarily *C. quinoa*, *C. pallidicaule* and *C. quinoa* var. *melanospermum*.

Nordstrom (1990) notes that species of *Chenopodium* were domesticated in Peru before 3000 BC. His studies were based on two very clear traits separating wild forms from domesticated ones, focusing on the thickness of the testa or seed–coat. He studied collections from four archaeological sites in the province of Junín: Cueva de Panaulauca, Pancan, Tragadero and San Juan Pata (3000 BC – 1300 AD). The samples comprised seeds of wild modern forms: *C. salinum* ("quita") and *C. ambrosoides* ("paiko"), as well as seeds of domesticated varieties of Andean *Chenopodium*: *C. quinoa* ('Jauja'), *C. quinoa* ('Bolivian') and *C. pallidicaule* ("kañiwa"). It was found that the samples of modern domesticated varieties had larger diameters (1.4–3.1 mm) than both the wild samples (< 1.0–1.8 mm) and the modern varieties of domesticated kañiwa (1.0–1.4 mm). The thickness of the testa was 0–28 µm in modern domesticated varieties, and 15–80 µm in modern wild varieties. Of all the archaeological samples, 89% were in the thin size range of the domesticated forms and they were well represented in the collection. The samples from the four archaeological sites ranged in thickness from 1.6 to 63 µm.

On the basis of studies carried out at the archaeological sites of Camata and Quelcatani in the western Lake Titicaca basin of Peru, Eisentraut (1998) notes that the domesticated forms of *Chenopodium* were already present during the Late Archaic/Early Formative. The degree of domestication was determined by measuring the thickness of the seed-coat, the shape of the margins and the patterns on the surface of the testa in wild, weedy and domesticated forms. Eisentraut compared the thickness of the testa and classified the seeds as follows: domesticated < 20 µm; wild 20–25 µm and weedy > 40 µm. An analysis of the three types revealed the following:

- The Camata collection comprised seeds of wild, weedy and domesticated varieties, while the Quelcatani collection comprised domesticated and weedy forms.
- The Camata samples had a seed-coat thickness of 4.4–54.1 µm, and the Quelcatani samples 4.3–23.7 µm.
- In the Camata collection, the seeds of domesticated forms had truncate margins and slightly undulating surface patterns, those of weedy varieties had rounder margins and smooth to undulating surface patterns, and the seeds of wild samples had rounded edges and relatively smooth surfaces. In the Quelcatani collection, the seeds had truncate margins and the surface patterns were smooth or slightly foveate.

Murray (2005) carried out studies on the domestication of *Chenopodium* at Jiskairumoko (Ch'amak Pacha archaeological project), a pre-Columbian archaeological site (approximately 3000–1400 BC) located 54 km southeast of the Puno region of Peru. The site is at 4 115 m asl, in the Aymara community of Jachacachi, close to Lake Titicaca. Among the plant remains found at the site, there is a wide range of forms of *Chenopodium* – indication of a strong dietary dependence on this group of plants. Given that the Altiplano was home in the past to an abundance of forms of quinoa it is still grown there today, it can be deduced that quinoa was domesticated in this region. A criterion for distinguishing between wild/weedy and domesticated varieties is testa thickness: domesticated < 20 µm, wild/weedy > 20 µm. In the collection of 38 *Chenopodium* seed samples, 97% were classified as domesticated (testa thickness < 20 µm), while only one seed was classified as wild/weedy (> 20 µm). In another analysis of 20 seeds, 95% had truncate margins (a characteristic of domesticated varieties) and a smooth testa surface pattern. Domesticated varieties of *Chenopodium* were found in the oldest layers of the archaeological site – indication that some of these forms had already been domesticated at the time of settlement.

The repeated manifestation of a thin testa and truncate margins reveals an adaptive response to selective pressures for the reduction of germination dormancy and seedling vitality (Smith, 1992), a response to human manipulation that affects a plant's life cycle.

During the era of the Spanish Conquest, quinoa was a valuable crop and this was well documented at the time (Tapia, 1979). Such was quinoa's importance to the Incas that it was considered sacred and was called *chisiya mama*, meaning "mother grain" in Quechua (National Research Council, 1989).

Production and Destination of Quinoa in Peru

Historical Data Series: Area, Yield and Production

Following the Spanish Conquest, the cultivation of quinoa diminished significantly for a variety of reasons, but in particular because the Spaniards introduced crops important to them, such as barley, wheat, oats, beans and peas.

Table 1: Area, yield and production of important food and industrial crops in Peru, 2011

Crop	Area (ha)	Yield (kg/ha)	Production (tonnes)
Cotton (raw)	45 811	2 664	122 047
Rice (paddy)	359 612	7 298	2 624 458
Peas (dry)	48 933	993	48 590
Peas (green)	27 285	3 697	100 876
Cacao	84 174	671	56 499
Coffee	367 096	903	331 547
Sweet potato	16 532	18 091	299 080
Sugar cane	80 069	123 455	9 884 936
Kañiwa	6 338	781	4 953
Barley (grain)	148 062	1 359	201 218
Onion	19 785	36 746	727 016
Asparagus	33 144	11 836	392 306
Cowpeas	16 056	1 421	22 817
Beans (dry)	78 918	1 113	87 853
Broad beans (dry)	52 003	1 243	64 646
Broad beans (green)	13 339	4 802	64 050
Kiwicha	1 796	1 679	3 016
Maize (dry yellow)	27 7388	4 543	1 260 123
Maize (flour corn)	198 263	1 289	255 651
Maize (sweetcorn)	43 212	8 516	367 994
Oil palm	33 324	10 797	359 784
Potato	296 440	13 738	4 072 455
Plantain	148 657	13 239	1 968 051
Quinoa	35 475	1 161	41 182
Wheat	145 484	1 472	214 141
Cassava	94 280	11 833	1 115 593

These crops prosper at altitudes of > 3 000 m asl, using simple, low-cost farming methods, and they have been part of the Peruvian diet ever since their introduction. The introduced crops are grown on 435 106 ha, and, given the characteristics and location of the cultivated area, it is likely that it was planted with quinoa during the Inca period (Table 1) (Ministry of Agriculture – Office of Economic and Statistical Studies, 2013).

Table 2: Historical data series: area harvested, production and yield of quinoa in Peru, 1951–2011

Year	Area (ha)	Yield (kg/ha)	Production (tonnes)
1951	47 200	900	42 500
1961	28 610	786	22 494
1971	15 035	426	6 405
1981	18 384	592	10 880
1991	21 007	735	15 439
1992	7 874	503	3 960
2000	28 889	976	28 191
2001	25 600	870	22 267
2002	27 852	1 091	30 374
2003	28 326	1 062	30 085
2004	27 676	975	26 997
2005	28 632	1 138	32 590
2006	29 949	1 016	30 428
2007	30 381	1 047	31 824
2008	31 163	958	29 867
2009	34 026	1 158	39 397
2010	35 313	1 163	41 079
2011	35 475	1 161	41 182
2012	**38 495**	**1 149**	**44 210**

FAOSTAT (2013), Peruvian Ministry of Agriculture (2013)

Table 2 shows the cultivated area, yield per hectare and domestic production between 1951 and 2012. During this period, the cultivated area was at its lowest in 1992, when it was just 7 874 ha. In contrast, it was 47 200 ha in 1951 and hundreds of thousands of hectares during Inca times. During the first decade of the twenty-first century it grew steadily, reaching 38 495 ha in 2012 (Ministry of Agriculture, 2013; FAOSTAT, 2013). According to the Peruvian Ministry of Agriculture (2013), the last nine crop years (2004/05 to 2012/13) saw the area planted with quinoa grow at an average annual rate of 5.8%.

Table 3 shows the distribution of quinoa cultivation in the various regions of Peru during 2011.

Table 3: Distribution of Areas under Quinoa Cultivation in Peru. Year: 2011.

Region	Production (tonnes)	Area (ha)	Yield (kg/a)
Amazonas	2	4	686
Ancash	140	132	10.559
Apurimac	1.262	1.094	1.153
Arequipa	1.013	498	2.034
Ayacucho	1.444	1.952	740
Cajamarca	141	151	934
Cuzco	1.796	1.866	963
Huancavelica	429	472	910
Huanuco	293	356	824
Ica	41	18	2.300
Junin	1.448	1.191	1.216
La Libertad	354	328	1.080
Moquegua	25	35	724
Puno	32.740	27.337	1.198
TACNA	52	42	1.238

Historical Data Series - Statistical Compendium (Peruvian Ministry of Agriculture, 2013)

According to information released in 2013 by the Office of Economic and Statistical Studies (OEEE) of the Peruvian Ministry of Agriculture, in 2012 about 68% of Peru's domestic production was concentrated in Puno, followed by Ayacucho, Cusco, Apurímac, Junín and Arequipa, which together accounted for 27%. The 2011/12 crop year experienced growth of 10.5% over the previous year. The Puno region had the largest planted area with 30 330 ha, an increase of 6.9% over the preceding crop year. It was followed by Ayacucho, with 4 308 ha and an increase of 54.2%. Cusco had a planted area of 2 216 ha but reported a decrease of -3.9% compared with the preceding crop year. Apurímac showed 1 331 ha (increase of 1.1%) and Junín 1 436 ha (18.6%). Together, these five regions accounted for 94.2% of all the quinoa cultivated area in Peru.

From 1951 to 2012, the yield per hectare of quinoa revealed a downward trend, decreasing from 900 kg/ha in 1951 to 426 kg/ha in 1971. In 2000, a slight upward trend began, and in 2012 a yield of 1 149 kg/ha was recorded (Ministry of Agriculture, 2013; FAOSTAT, 2013). According to OEEE data (2013), the regions with improved yields are Arequipa (2 034 kg/ha), Junín (1 216 kg/ha), Puno (1 198 kg/ha), Apurímac (1 153 kg/ ha) and La Libertad (1 080 kg/ha). For

2012, the average national yield was around 1 148 kg/ha, with a decrease of 1.1% compared with the same period in 2011. At approximately 2 834 kg/ha, the yield of the Arequipa region is the best in the country; other regions that have maintained yields above the national average are Apurímac, Tacna, Junín, La Libertad and Ayacucho. Puno, the primary producer in the country, had an average yield of 1 110 kg/ha (Ministry of Agriculture-OEEE, 2013).

Destination of Quinoa Production

For several decades, the diet of Altiplano farmers consisted almost entirely of quinoa. However, in recent years, due to the increasing profitability of its sale and export, the consumption of quinoa by its producers has decreased markedly as the crop and is replaced by less nutritious foods such as rice and noodles.

Table 4 shows the distribution of Peru's quinoa production: 12% is consumed locally, 47% is sold domestically and 41% is sold internationally (Estrada, 2012).

Table 4: Destination of Quinoa Produced in Peru (%) 2011

	Local consumption	Domestic market	Foreign markets
Puno	15	35	50
Cusco	10	70	20
Junín	15	35	50
Ayacucho	15	60	25
Arequipa	5	35	60
National	12	47	41

Domestic Trade

Consumer Prices

From January 1995 to February 2013, consumer prices increased continually at an average annual rate of 0.5%. In January 1995, the average consumer price was PEN3.19/kg. By February 2013 it had risen to PEN9.87/kg (Ministry of Agriculture, 2013). This increase in the cost of quinoa has made it less accessible to low-income populations, who require a more nutritious diet in order to combat the high levels of malnutrition among children and pregnant women.

Producer Prices

In 2008, the average national price in *nuevos soles* was PEN1.60/kg (1 PEN = 0.34 USD), and in 2009 the price increased to PEN3.36/kg, a significant jump of 110%. Since that year, prices have had an upward trend, reaching PEN3.88/kg in 2012, an increase of 6.2% over 2011. Prices varied at regional level. The highest price was in Tacna (PEN4.85/kg), followed by Ancash (PEN4.74/kg), Moquegua (PEN4.57/kg), La Libertad (PEN4.44/kg), Huánuco (PEN4.12/kg), Junín (PEN4.10/kg) and Puno (PEN4.01/kg) (Ministry of Agriculture, 2013).

Agricultural Production Chain

In 2012, the quinoa production chain accounted for 0.14% of the farming industry's GDP and 0.23% of the agricultural subsector, with a contribution of 30.1 million Peruvian nuevos soles – an increase of 7.35% over 2011 (Ministry of Agriculture–OEEE, 2013).

External Trade

Exports of quinoa saw sustained growth from 2003 to 2012 (Ministry of Agriculture, 2013). Quinoa exports reached a peak in 2011, with 7 992 tonnes valued at USD25 375 000 free on board (FOB), i.e. an increase of 70.7% over 2010. It is important to emphasize that exports increased every year from 2007 to the end of 2011. The number one destination for Peruvian quinoa is the United States of America, accounting for 59.3% of exports, and in 2011, 5 011.3 tonnes, valued at USD15 290 300, entered the market. Germany accounted for exports of 507.6 tonnes (valued at USD2 052 600 FOB), Israel 184 tonnes (USD434 200 FOB), Canada 400.3 tonnes (USD1 366 900 FOB) and Japan 116.5 tonnes (USD316 700 FOB). Exports to these countries represented 78.9% of the quinoa sold outside of Peru (Ministry of Agriculture–OEEE, 2013).

Characteristics of Quinoa Cultivation

An Involved Population

More than 70 000 farmers plant quinoa. They are for the main part independent producers with plots of < 3 ha or belonging to organized associations with > 100 ha in the provinces of Junín, Puno and Ayacucho. In Puno – the top-producing region in Peru, according to the Operative Plan for Quinoa (Peruvian Ministry of Foreign Trade and Tourism, 2006) – it is estimated that there are some 9 465 farmers grouped into 130 organizations at provincial level, and one association at regional level. In the 2011/12 crop year, it was reported that quinoa production generated 2 659 575 paid working days, with a planted area of 42 074 ha (Ministry of Agriculture, 2013).

Table 5: Quinoa exports from Peru, 2003 - 2012

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Millions of US $	0.4	0.4	0.7	1.5	2	5.1	7.1	13.1	25.03	30.3
Thousands of tonnes	0.3	0.3	0.6	1.3	1.5	2.1	2.7	4.8	7.9	10.4

Agro-Ecological Zones

Quinoa is cultivated from sea level to an altitude of 4 000 m asl. The primary area of cultivation is between 2 500 and 4 000 m asl, in microclimates that, while varied, are generally cool to temperate, with frequent freezes, and where cultivation relies on rainfall. There are marked differences between the cultivation zones:

Suni/Altiplano

The Puno Altiplano is located in a region where temperatures are extreme, with major differences between day and night. In the Lake Titicaca basin, at around 3 800 m asl, the topography of the area is relatively even. The climate varies over the course of the year, with an average temperature of 7.3°C and an average yearly rainfall of 616 mm. Approximately 70% of the area under quinoa cultivation in Peru is located in this zone (Aguilar and Jacobsen, 2003; Mujica *et al*., 2004a, b; Mujica and Chura, 2012).

Quechua/Inter-Andean valleys

These valleys are located in the Quechua ecological region throughout Peru at 2 300–3 500 m asl. The climate is extremely variable, ranging between temperate and cold, depending on the altitude, latitude and time of year. Rains are heaviest from October to May. In the southern part of the zone, where the climate is drier, characterized by stark temperature differences between day and night, the primary areas of production are the Cusco and Apurímac regions. The main areas in the centre are Junín and Ayacucho. In the north, where the climate is more humid and where rainfall is more frequent, the main areas of production are Ancash and Cajamarca (Tejada, 1997; Tejada, 1998; Tejada, 2004; Wiener, 2006; Perez and Aguirre, 2012).

While lower altitudes are optimal for the cultivation of quinoa, frequent periods of drought or heavy precipitation can negatively impact various growth stages, and freezes and hailstorms during the ripening period affect yield.

Yunga

In recent years, quinoa has begun to be cultivated in the maritime Yunga, a transitional zone between the mountains and the coast at an altitude of 500 - 2 300 m asl. The climate is moderately warm and slightly humid, with sparse seasonal rain during the summer and sunny skies for much of the year. In Arequipa, quinoa is grown with some success in the irrigated fields of San Camilo in the La Joya district, Santa Rita de Siguas and Majes (Autonomous Authority of Majes [AUTODEMA], 2013).

Coastal Region

The coastal region ranges from sea level to an altitude of 500 m asl. This is a "new cultivation zone" for commercially grown quinoa, despite that fact that research into the adaptability of quinoa to the conditions of the central coast dates back to the 1990s (Apaza, 1995; Echegaray, 2003; Tapia, 2003; Mercedes, 2005; Barnett, 2005; Gómez and Gordon, 2012). There are reports of quinoa fields in Ica, Lima, Moquegua and Tacna. However, the high temperatures during the flowering period (when seeds are soft) are a constraint (Mendoza, 2013).

Cultivation Technologies

Traditional System

Traditional small-scale cultivation systems are used in the mountains, Altiplano and Inter-Andean valleys, mostly for growing bitter quinoas. The systems are characterized by the intensive use of manual labour (with or without the assistance of draught animals) from soil preparation to harvest. They are based on the rotation of crops grown primarily for family subsistence.

In the Altiplano zone, the cultivation of quinoa has a spatial and temporal distribution in traditional systems to take into account differences between ecological zones at various altitudes. Quinoa farmers adopt a range of practices in traditional systems, depending on the area and the time of year. Systems are designed for various purposes, including feeding the family, crop rotation, proper management of soil and pests, and conservation of germplasm *in situ*. In the Peruvian Altiplano, traditional systems are called *aynokas*, *mandas* and *laymes* (Ichuta and Artiaga, 1986). They are used to preserve at the same time quinoa and its wild relatives. Quinoa's wild relatives can be found in fields, along the edges of fields or at sacred sites (known as *Gentil wasi* or *Phiru*), and are tended to by farmers and used as food and for medicinal and religious purposes (Mujica *et al.*, 2000; Aguilar and Jacobsen, 2003; Mujica, 2008; Mujica, 2011).

In the Inter-Andean valleys, quinoa is cultivated in association with other crops and is typically planted in *shaywas* (the name given to a furrow or planted row separating one crop from another). In the lowest areas (Quechua Baja), it is combined with crops such as maize, beans and gourds (squash, pumpkin etc.), and in the highest areas (Quechua Alta and Jalca), with potatoes, broad beans, lupin etc.. One plot comprises various *shaywas* of quinoa, allowing farmers to diversify and reduce production risks, since quinoa is adapted to a variety of agro-ecological conditions and is particularly drought- and frost-resistant. A farming family growing 125 m of shaywas can achieve a yield of 20–50 kg of grain per year, most of which is consumed by the family itself (Tejada, 1997, 1998, 2004). *Takira* is another system of growing quinoa: colourful rows of quinoa panicles are interspersed with rows of other crops. Two important practices are Lent tillage and organic fertilization using liquid and/or solid manure from domestic animals. Lent tillage is the practice of preparing fallow land or turning the soil over 2–4 months before sowing: farmers plough the soil immediately after harvest (May or June), taking advantage of the moisture remaining from the rainy season, then leave it fallow until the beginning of the new crop year when the rains return (October or November). Organic fertilization is common practice among farmers, either through direct application of manure to the soil before sowing, or through *majadeo*, when livestock (cattle, donkeys, sheep etc.) are left tied to a stake or untied in pens from dusk until the following morning (12 hours per day) so that their solid and liquid waste is deposited on the soil surface to be incorporated through ploughing (Tejada, 1997).

Quinoa is cultivated in dryland farming systems. However, in areas where water is available, irrigation technologies may be used to supplement rainfall. Crop management techniques are rarely applied in the cultivation of bitter quinoa using traditional systems. It is generally cultivated without fertilizer, growing in conditions of high plant density (without thinning) and, most importantly, without weeding or hilling. Despite these conditions, the crop prospers and produces a harvest with an estimated productivity of 200-500 g/m2 (Tejada, 2004). The success of local quinoa is due to two important characteristics: tall plant height (2 m), which helps it compete against weeds and other crops, and long growth period (6-7 months), which allows it to develop and absorb nutrients for a substantial length of time.

However, in the last decade, monoculture has become widespread, following interventions by government, non-governmental organizations and trading companies. Consequently, a decreasing number of families now grow quinoa using traditional agricultural systems.

Modern System

In the irrigated fields of the Yungas, modern technologies are widespread and used intensively. Soil preparation is mechanized, irrigation technologies are adopted and there is high use of inputs. Prior to sowing, 20–30 tonnes of organic chicken fertilizer is applied. In addition, 300 kg/ha of nitrogen, 120 kg/ha of phosphorus pentoxide, 300 kg/ha of potassium oxide, 40 kg/ha of calcium, 20 kg/ha of magnesium and 1.5 kg/ha of zinc are applied via the irrigation system. The phytosanitary control of root rot and the preventive control of mildew are carried out by applying fungicides 20 days after sprouts appear, followed by four further applications at 10-day intervals. Insects are also controlled using chemicals; the precise chemicals used for insects depend on the type of insect and its impact. The cost of investment is USD5 500, but results in a production of 4 000–7 000 kg/ha (AUTODEMA, 2013). The 2012/13 crop year saw producer prices for quinoa fluctuate between USD3 and USD4, resulting in a significant profit for farmers in some regions.

Similar technologies are adopted in coastal cultivation. Fertilization and irrigation systems have a positive impact on the potential yield of various quinoa varieties, reaching 4 000 kg/ha in some fields in Piura, Lima, Ica and the coastal area of Arequipa.

Varieties

Table 6 presents the most common commercial varieties, along with several agronomical characteristics such as quality and range of adaptation. The varieties of quinoa shown are classified within the Altiplano and Inter-Andean valleys ecotypes.

The majority of varieties originating in the Altiplano are native cultivars of quinoa obtained by masal selection from traditional landraces' groups, such as kancolla, qoyto, chullpi, misa, witulla, pasankalla, cuchiwila, cheweca, chaucha, antahuara, hanqo jiura and aara/ajara. They are characterized by their tolerance of adverse climate and soil conditions, and have an average yield of 1 200 kg/ha. The commercial varieties selected from the Altiplano ecotype adapt well in Yungas and coastal environments (Mendoza, 2013), with high yields of 4 000–6 000 kg/ha. In contrast, in the Inter-Andean valleys, the Altiplano varieties ('Salcedo INIA', 'INIA-415 Pasankalla', 'INIA-420 Negra Collana') are not recommended for various reasons: low yield; excessive precocity (they ripen before the end of the rainy season); limited ability to compete against weeds (due to low height); and susceptibility to mildew.

The Inter-Andean valleys varieties include:

'Amarilla de Maranganí' and 'Amarilla Sacaca' – representative of the sacred Valley of the Incas (Vilcanota) in Cusco; 'Mantaro', 'Blanca de Junín', 'Blanca de Hualhuas' and 'Rosada de Junín' from the Mantaro Valley in Junín; 'Amozulca' and 'Namora' from the Cajabamba Valley in Cajamarca; 'Acostambo' from the Acobamba Valley in Huancavelica; 'Amarilla de Ancash' and 'Blanca del Valle' from Callejón de Huaylas in Huaraz; 'Roja Ayacuchana' from Ayacucho, and 'Blanca' from the Callejón area of Conchucos and from La Libertad. Yields vary between 2 500 and 5 000 kg/ha.

The quinoas belonging to the Inter-Andean valleys ecotype thrive throughout the Inter-Andean valleys of Peru. The sweet and semi-sweet varieties and cultivars of quinoa from the centre and south of the country and introduced in the north have done well, particularly 'Mantaro' in the Cajamarca region and 'Blanca Pindila' in La Libertad. Other varieties that have adapted well are 'Amarilla de Maranganí', 'Blanca de Hualhuas' and 'Rosada de Huancayo'. However, they generally do not adapt to coastal conditions, partly because they do not tolerate high temperatures during blooming, but also because their long life cycle and height are constraints on the coast, where farmers prefer earlier cultivars and short

Table 6: Important Commercial Varieties in Peru

COMMERCIAL VARIETIES	Year Released	POTENTIAL YIELD, kg/ha	Grain Colour	Saponin Content	Protein Content	Adaptation to Elevation
Kanccolla		3.500	White or pink	Bitter		Altiplano
Blanca de July		2.500	White	Semi-sweet		Altiplano
Witulla		1.200 -1.800	Black			Altiplano
Sajama		3.000	White Large	Sweet		Altiplano
Chewecca Amarilla		3.000	White small			Altiplano
Maranganí		3.500	Orange	Bitter		Cusco
Blanca de Junín		2.000 - 2.800	White	Semi-sweet		Inter-Andean valleys
Blanca de Hualhuas		2.500	White	Semi-sweet		Inter-Andean valleys
Rosada Huancayo		3.000	White	Semi-sweet		Inter-Andean valleys
Quillahuamán INIA	1990	3.500	Off-white	Low		0-3 500 m
Salcedo INIA	1995	Good	White & large	Sweet (0.014%)	14.50%	Valleys & Coast
Illpa INIA	1997	3.100	White & large	Sweet (0.02%)	16.14%	
INIA 415-Pasankalla	2006	4.500	Dark red	Sweet	17.40%	Altiplano & Coast
INIA 420-Negra Ccollana	2008	Good	Black	Sweet (0.015%)	17.85%	
INIA 427 - Amarilla Sacaca	2011	3.500	Yellowish-orange & large	High	14.83%	Inter-Andean valleys
INIA 431 - Altiplano	2013	Good	White & large	Sweet	16.90%	Coast & Mountains

Source: National Institute for Agrarian Innovation (INIA), Leaflet No. 1, April, 2013 plants to facilitate the harvesting process and to provide tolerance to lodging.

Factors Limiting Quinoa Cultivation

Biotic Factors

Diseases:

Of the fungal leaf diseases, mildew (*Peronospora variabilis*) is the predominant disease in all the quinoa cultivation areas in Peru, and it can affect quinoa at any phenological stage. Other secondary diseases frequently reported are leaf spots, caused by *Ascochyta hyalospora*, *Cercospora* sp. and *Macrophoma* sp.

One of the major diseases affecting the stem is brown rot (*Phoma exigua* var. *foveata*). Secondary diseases of the stem include eyespot (*Phoma* sp.) and sclerotia (*Sclerotium* sp.).

A common root disease is damping-off (*Rhizoctonia solani*); in some areas, root rot caused by a complex of soil fungi has also been observed (*Fusarium oxysporium, Phytium).*

Viral diseases reported include the Sowbane mosaic sobemovirus; bacterial diseases include the spot-causing *Pseudomonas*.

Insects:

Altiplano

Reports indicate the presence of cutworms that attack tender plants: *Feltia experta* (known locally as *tikuchi*), *Spodoptera* sp., *Copitarsia turbata* (armyworm) *and Agrotis ipsilon* (dark sword-grass, or *silwi kuru*). Insects that attack the leaves and grains include: *Eurysacca melanocampta*, *Liriomyza brasiliensis, Liriomyza huidobrensis*, *Hymenia recurvalis*, *Pachyzancla bipunctalis* (quinoa moth), *Perizoma sordescens* (inchworm or *kuarta kuarta*), *Epicauta willei*, *Epicauta latitarsis* (*padre kuru* or *chhallu chhallu*), *Epitrix subcrinita* (flea beetle or *piki piki*), *Myzus persicae*, *Macrosiphum euphorbiae*, *Frankliniella tuberosi* (thrip, also known as *llawa* or *kondorillo*), *Borogonalia* sp. (leafhopper) *Bergallia* sp. (leafhopper) and *Paratanus* sp. (leafhopper).

Coastal

Cutworms (*Feltia experta*, *Agrotis ipsilon* and *Elasmopalpus lignosellus*) and grubs (*Bothynus maimon*) are recorded. Leaf-eating insects include: *Delia* flies (*Delia* sp.), aphids (*Myzus persicae*, *Macrosiphum euphorbiae* and *Aphis gossypii*) and leaf and panicle caterpillars (*Eurysacca quinoae*).

Inter-Andean valleys

Cutworms (*Copitarsia turbata* and *Agrotis* sp.) are recorded, as well as leaf-eating larvae (*Scoteoborus*, *Adioristus* and *Epicauta latitarsis*), leaf- and grain-eating larvae (*Eurysacca melanocampta*) and *Astillus* sp. (panicle beetle and black beetle, known locally as *acchu*).

Nematodes:

In Peru there have been reports of quinoa-eating nematodes, in particular, *Nacobbus aberrans*, *Globodera pallida* and *Thecavermiculatus.*

Vertebrates:

Wild birds, whether alone, in small groups or in large flocks, are in competition with the people of the Andes for food to survive. The main species affecting quinoa are doves, which break apart the panicles and stems. This phenomenon is a particular problem in coastal environments.

Losses attributed to birds can reach 30–40% with sweet varieties such as ‘Sajama’, ‘Chewecca’ and ‘Blanca de Juli’. Varieties with compact panicles are not as targeted because the seeds are less exposed.

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CHAPTER 5.3.

Quinoa in Ecuador

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Abstract
Ecuador was the third country to undertake systematic and dynamic research into and development of the revival, promotion and use of quinoa in the Andean region. Efforts began in 1982, some 30 years after Bolivia and Peru, with the support of the *Instituto Nacional de Investigaciones Agropecuarias* (INIAP, the National Agricultural Research Institute), the Food and Agriculture Organization of the United Nations (FAO), the International Board for Plant Genetic Resources (IBPGR) and the Government of Canada (through the International Development Research Centre – IDRC).

Ecuador began by collecting germplasm at national level, exchanging with other countries and founding a national germplasm bank for Andean crops, presided over by INIAP. Following the characterization, documentation and evaluation of the germplasm, selective breeding was carried out and the first two high-yielding varieties, which have bitter grains, were released. The promotion of quinoa cultivation and consumption was thus initiated. At the same time, to establish a baseline, an analysis was performed of the crop's status within the country in terms of agriculture and socio-economics, and agronomic management, harvest and post-harvest technologies were developed. During that period there were estimated to be 1 000–1 200 ha under cultivation in the north central Sierra of Ecuador, while it was considered to have disappeared in the south. In 1992, the first "sweet" varieties were released – varieties with a low saponin content – with the objective of reducing water use and washing time while increasing urban consumption.

Beginning in 2000, new research into quinoa was undertaken by the National Programme of Andean Legumes and Grains (PRONALEG-GA) of the Santa Catalina Experimental Station. A new early variety was released with improved cold tolerance and low saponin content. This gave impetus to research and development in the areas of harvesting, post-harvesting and agro-industry. In the same period, several universities contributed to knowledge in the field with undergraduate theses, and the private companies involved grew in size and number. In 2013, attention focused on two types of production: certified organic and agro-ecological (conventional), and it is estimated that together they account for an annual cultivated area of around 2 000 ha. Most of this production is for export to the United States of America and Europe. Domestic consumption in Ecuador is still very low, and the Government

is promoting the consumption of quinoa through food programmes, aimed mainly at children. Quinoa production in Ecuador can be made more sustainable, as it can be grown in rotation with other crops, such as potatoes, peas, maize (planted alone or together with climbing beans) and pasture, at altitudes ranging between 2 400 and 3 600 m asl.

Introduction

For around 7 000 years, quinoa has been cultivated in the Andean region, where it has been valued for its nutritional qualities and its adaptability to difficult environmental conditions. In Ecuador, quinoa cultivation has been considered of secondary importance: the cultivated area is relatively small and per capita consumption is low (Jacobsen and Sherwood, 2002).

Pulgar Vidal (1954, cited by Tapia, 1979) believed that the Chibchas and other tribes of the Cundi-Boyacense plateau in Colombia grew quinoa intensively, and he also suggested that the ancient inhabitants of Cuyumbe (Huila, Colombia) assisted in the spread of quinoa southwards , which would explain its distribution in Ecuador.

However, Estrella (1998), on the basis of historical documents, maintains that, due to its nutritional and medicinal qualities, quinoa was highly valued by the indigenous peoples of Ecuador. For example, during Pedro Cieza de León's travels through the Andes in 1548, he found evidence of quinoa cultivation and recorded its value in the local diet. The Cañaris grew quinoa before the arrival of the Spanish, and at the end of the sixteenth century it was still a preferred food. In the Order of Tambo (*Municipal Acts of Quito*, 1934), issued by the City Council of Quito in 1549, it appears that quinoa was one of the foods that the inhabitants of Tambo would sell to travellers. In the eighteenth century, the historian Juan de Velasco identified two types of quinoa: the white form, that is "grown in large fields and is eaten like rice", and the red form that "can only be eaten toasted because it bursts, fluffs up and has a lovely flavour".

Tapia (1979), citing Cardozo (1976) and Romero (1976), states that in Ecuador quinoa persisted among farmers in the provinces of Carchi, Imbabura, Pichincha, Chimborazo and Loja. He also notes that quinoa plants are generally tall and produce small, very bitter grains. The estimated cultivated area during those years was 1 200 ha.

In 1967, INIAP reported the creation of the Programme for the Introduction of New Cash Crops in the Sierra. Observation and adaptation work was carried out with the aim of finding new sources of protein for human and animal consumption. In addition to work on rapeseed and *Lupinus mutabilis* (Alpine lupin or *chocho*), indigenous crops, such as quinoa, *Ullucus tuberosus* (melloco) and *Oxalis tuberosa* (oca), were observed and collected. This work was concluded in 1970 at the Santa Catalina Experimental Station.

The graduate thesis work of García (1984) involved the study of quinoa cultivation in eight provinces in the Ecuadorean Sierra. The principal findings were as follows:

The majority of quinoa producers are smallholders; they must seek other sources of income for their subsistence, since farming on its own does not meet their minimum needs.

Quinoa is generally grown in polyculture systems and very rarely as a monoculture. It is most frequently grown in association with maize, potatoes, oca and melloco. Very few farmers practise crop rotation.

The planting season varies from zone to zone and is associated with the rainy season. In the north it is in June - July, while in the centre and south it is in October–November. For late cultivars, harvest time is 7 - 8 months after sowing.

Weeding, hilling, thinning, fertilization and irrigation are not carried out for quinoa. However, it indirectly benefits when these tasks are performed for the primary crop. There is no pest or disease control.

Producers' yields range from 300 kg to 1 tonne per hectare. Production is intended solely for their own consumption, and it is rare for them to make exchanges or sell in the markets.

There is a total absence of institutional services and credit assistance. Consequently, farmers receive no information about the plant's agronomic and nutritional value.

Quinoa consumption is limited due to ignorance of its good nutritional qualities. It is also complicated by the need to wash the grain before it can be eaten.

Between the beginning of the 1980s and the end of the 1990s, significant advances were made in quinoa research and production, and the promotion of its consumption. Headway was made primarily through international cooperation and consultation with Bolivian scientists and, in Ecuador, through the work of INIAP, in conjunction with several universities and private companies. During this period: the national germplasm bank was established; four enhanced varieties (developed through selective breeding) were released; alternative technologies were developed for management, harvesting and post-harvest operations; food science studies and studies of agro-industrial applications were carried out; various ways of preparing and consuming quinoa were developed; and several private initiatives were created (with large and small producers) for production and commercialization, focusing on both domestic and international markets.

Since 2001, with the support of international partners (and, more recently, state funds), research into and development of quinoa and the other Andean grains has been resumed. International partners include the *Proyecto de Resistencia Duradera en Zona Andina* (PREDUZA, Durable Resistance Project in the Andean Zone), the International Fund for Agricultural Development (IFAD), the International Plant Genetic Resources Institute (IPGRI) and the McKnight Foundation. Through participatory processes, a new variety of quinoa is being released and the process of genetically enhancing it through hybridization (in consultation with PROINPA, Bolivia) is being initiated with the use of F6 lines and segregant populations in different descendants.

During this period, there has been a drive to increase the quality of selected grain, in conjunction with organizations of family farm producers. The use of threshing machinery has also been demonstrated, and various activities have been carried out to promote the nutritional value and diversify the consumption of quinoa and other Andean grains. In recent years, the Ecuadorean Ministry of Agriculture, Livestock, Aquaculture and Fisheries, with the support of FAO, has implemented quinoa development projects in varies parts of the country.

During the International Year of Quinoa, the Fourth World Congress on Quinoa and the Andean Grains First Symposium were held in Ecuador. In this context, the Ministry of Agriculture, Livestock, Aquaculture and Fisheries launched a quinoa cultivation development programme, including, among other things, expansion of the planted area, improvement of productivity, promotion of the use of certified seed (relying on the standard for the certification of quinoa seed), organizational strengthening of producers, establishment of production incentives (subsidies by way of inputs, training and machinery), provision of technical assistance and support for storage and marketing.

Quinoa Research in Ecuador

From the beginning through to the 1990s

In 1967, INIAP created the Programme for the Introduction of New Cash Crops in the Sierra with the aim of finding new sources of protein for human and animal consumption, including the collection and observation of indigenous crops, such as quinoa, melloco and oca. The initiative came to an end in 1970.

During the 1970s and 1980s, at the *Universidad Central, Escuela Politécnica de Chimborazo* and the *Universidad Técnica de Ambato*, several theses addressed the cultivation, agro-industrial production and use of quinoa (Peralta and Vicuña, 1981; García, 1984). In 1982, quinoa was included as one of the Andean crops studied within the curriculum of the School of Agricultural Sciences at the *Universidad Central*.

An important milestone at the Santa Catalina Experimental Station of INIAP was the creation of the Andean Crops Section and the Plant Genetic Resources Section (1982), affiliated with the Cereals Programme. Quinoa is one of a number of crops and foods of Andean origin that was forgotten or underused and heading towards extinction in Ecuador. The quinoa collection began to take shape as 271 accessions from every province of the Sierra were gathered together; by 1985 there were 334 entries. The selective plant breeding programme also began, with the key participation of the researchers: Carlos Nieto, Eduardo Peralta, Raúl Castillo, Jaime Tola and Alberto Ortega, and the Bolivian, Julio Rea. In 1986, the Andean Crops Programme was created.

In addition to the establishment of the germplasm bank, a guide was published on the management and preservation of plant genetic resources (Nieto *et al*., 1983) and the six ecotypes of Ecuador were identified and characterized (Gandarillas *et al*., 1989).

Currently, the germplasm bank of Ecuador, presided over by the National Department of Plant Genetic Resources of INIAP at the Santa Catalina Experimental Station, comprises 608 collections, of which 283 were gathered in Ecuador during the 1980s, while 325 came from the Andean countries and other donors (Mazón *et al*., 2002).

During the 1980s, INIAP released the first enhanced varieties of bitter grain quinoa, obtained through selective breeding: 'INIAP- Cochasquí' and 'INIAP-Imbaya' (Nieto *et al*., 1986).

In the same period, the first courses on quinoa cultivation were made available. In 1984, a course was offered to rural leaders of the Sierra, and in 1985 a technicians' course was organized with the support of engineer Humberto Gandarillas from Bolivia. In 1987, the Andean Crops Programme of INIAP published *Memorias de la Reunión Nacional sobre producción, uso y comercialización del cultivo de la quinua* (Proceedings of the National Meeting on the production, use and marketing of the quinoa crop).

Promotion also began of quinoa consumption. In 1984, INIAP presented 16 different quinoa-based dishes at the Traditional Food Contest organized by the Municipality of Quito and the Central Bank of Ecuador, winning first prize (Eduardo Peralta and Roxana Terceros). On the basis of this experience, the first quinoa cookbook was published in Ecuador: *La Quinua… un gran alimento y su utilización* (Quinoa… a great food and its uses) (Peralta, 1985).

In 1988, the Ecuadorean Institute for Standardization published the following quality standards: INEN 1671 for the identification of impurities and infestation levels in unprocessed quinoa grain; INEN 1672 for the identification of saponin content using the foam method; and INEN 1673, establishing the requirements that quinoa grain must meet. Carlos Nieto of INIAP was vice-president of the Technical Subcommittee.

In the mid- to late 1980s, efforts were made to organize quinoa production in Ecuador. In 1986, the *Asociación de Productores de Quinua* (PROQUINUA, the Association of Quinoa Producers) was founded – to be disbanded a few years later having been relatively successful. In 1988, the *Escuelas Radiof*óni*cas Populares del Ecuador* (now Fundación ERPE) in the province of Chimborazo began agro-ecological production activities, evolving in 1997 into the production of organic quinoa with small producers. In 1989, the Inagrofa company was founded to produce and sell quinoa; it does business to this day.

The Fundación ERPE made an important contribution. The 21 July 2012 issue of the newspaper, *El Comercio de Quito*, wrote in the agriculture and fisheries section that in Chimborazo, "quinoa is diversifying to find markets." It noted that, 15 years earlier, there had been little appetite for quinoa in rural and urban areas of Chimborazo, and that the situation had begun to change in 2000, when the producers of 90 municipalities in Colta, Guamote, Alausí and Riobamba decided to join forces to promote this Andean product, with the support of the *Fundación Escuelas Radiof*ónicas *Populares del Ecuador* (ERPE). The report noted that the foundation had started out with 200 producer families and 100 ha under cultivation, and that by 2003 there were 900 family groups and 430 ha. "Currently," the report stated, "1 700 families are growing [quinoa] organically" on 700 ha, adding that 400 tonnes/year were exported to Europe and the United States of America.

The promotion of quinoa consumption continued during the 1990s. In the first year of that decade, the Andean Crops Programme of INIAP compiled and published a cookbook with 92 quinoa recipes (Muñoz *et al*., 1990). That same year, Latinreco, Nestlé's research centre in Ecuador, published a book covering the five most recent years of quinoa cultivation and processing in Ecuador (Whali, 1990). In 1992, the 'INIAP-Tunkahuan' and 'INIAP-Ingapirca' varieties were released, both characterized by their low saponin content (Nieto *et al*., 1992). A study of the harvesting and post-harvesting of quinoa in Ecuador was also published (Nieto and Vimos, 1992).

Of the four varieties of quinoa released during that period, only 'INIAP-Tunkahuan' is still produced. This variety, which is of Ecuadorean origin (figures 1 and 2), was collected in the province of Carchi in

Figure 1. Cultivation of the 'INIAP-Tunkahuan' quinoa variety.

Figure 2. Seeds of the 'INIAP-Tunkahuan' variety.

1985. It is a semi-early (150–210 days) valley variety adapted to altitudes of 2 200–3 200 m asl. The plant height is 90–180 cm. It is green when young and pinkish-yellow at harvest. The grain is white and opaque, of medium size, round and flat, with a low saponin content (0.06%). The yield varies between 1.5 and 3 tonnes/ha (Nieto *et al.,* 1992; Peralta, 2010).

In 1996, the Ministry of Agriculture and Livestock published *Zonificación potencial del cultivo de quinua en el callejón interandino del Ecuador* (Potential areas of quinoa cultivation in the inter-Andean corridor of Ecuador), which stated that there were 86 856 ha where quinoa could be cultivated without climatic or soil limitations (Yugcha, 1998).

This phase came to an end in 1997 when the Andean Crops Programme was closed, and responsibility for quinoa was transferred to the Cereals Programme. In 1998, Ecuador contributed the 'INIAP-Ingapirca' cultivar and the 'ECU-420' landrace to the list of 25 samples that participated in the American and European Test of Quinoa, facilitated by FAO, *Universidad Nacional del Altiplano – Puno* (UNAP), *Centro Internacional de la Papa* (CIP, International Potato Center) and the Danish International Development Agency (DANIDA).

Quinoa research in the new millennium

In 2000, due to the demand from national and international institutions, INIAP resumed quinoa research within the framework of PRONALEG-GA, under the leadership of Eduardo Peralta.

It was necessary to identify quinoa accessions which satisfied consumer and producer expectations in terms of both morphological characteristics (plant type, colour and panicle) and agronomical characteristics (precocity, mildew resistance, grain quality, potential yield). The quinoa collection of INIAP was, therefore, characterized and the *Catálogo del banco de germoplasma de quinua del INIAP* (Catalogue of the quinoa germplasm bank of INIAP) (Mazón *et al*., 2002) was subsequently published.

With the materials selected from the germplasm bank, a process of evaluation and participatory selection of quinoa lines began at the Experimental Station and with farmers in different quinoa production zones of the Sierra in Ecuador. International support was provided by PREDUZA, FAO and the International Centre for Tropical Agriculture (CIAT), and students working on master's and doctoral theses also participated (Jácome, 2002; Guambuguete and Purcachi, 2003; McElhinny *et al*., 2007).

The 'INIAP-Pata de Venado' variety was subsequently released. It is of Bolivian origin (IBTA, E.E. Patacamaya, 1983, germplasm exchange) (Images 3 and 4), early (150–180 days), adapted to altitudes of 3 000–3 600 m asl (high, cold areas), with a plant height of 90–100 cm, and is green when young and pink at harvest. The grain is cream coloured, of medium size, round and flat, and has a low saponin content (0.05%). The average yield is 1.2 tonnes/ha (Mazón *et al*., 2007).

Figure 3. Harvesting of the 'INIAP-Pata de Venado' quinoa variety.

Figure 4. Washed seeds of the 'INIAP-Pata de Venado' quinoa variety.

This selection method did not result in varieties of quinoa with large grains, better mildew resistance etc. For this reason, in 2008, with the support of the McKnight Foundation (United States of America) and the expert advice of Bolivia's *Fundación para la Promoción e Investigación de Productos Andinos* (PROINPA, Foundation for the Promotion and Research of Andean Products), PRONALEG-GA/INIAP initiated a programme for the enhancement of quinoa through hybridization. The programme's main objectives were to develop new varieties of early quinoa with large grains, resistance to fungal leaf diseases, and high yield potential, adapted to marginal climate and soil conditions, and accepted both by farmers and in the markets. In 2013, F6 lines became available, with segregant populations in different descendants.

In order that farmers interested in producing quinoa and other Andean grains be aware of existing crop management alternatives, INIAP published the *Catálogo de variedades mejoradas de granos andinos: chocho, quinua y amaranto, para la Sierra de Ecuador* (Catalogue of enhanced Andean grain varieties: chocho, quinoa and amaranth, for the Sierra of Ecuador) (Peralta *et al*., 2012), as well as an additional catalogue of Andean grain varieties (Peralta *et al*., 2013). Fundación ERPE also published the *Manual de quinua orgánica* (Organic quinoa manual) (Raffauf, 2000).

The production of high quality selected seed, produced in non-conventional systems, is currently promoted in conjunction with organizations of family farm producers, and a guide has been published for the production and distribution of high quality seed (Peralta, 2010). On the basis of this experience, the Ecuadorean standards for the certification of quinoa seed were developed with the Ministry of Agriculture, Livestock, Aquaculture and Fisheries and with the support of FAO's "Andean Seeds" project.

In 2006, PRONALEG-GA/INIAP, together with the School of Biological Sciences of the *Pontificia Universidad Católica del Ecuador*, organized the Twelfth International Congress on Andean Crops. In 2013, the Fourth World Congress on Quinoa and the Andean Grains First Symposium were held in the city of Ibarra as part of the agenda of the International Year of Quinoa, in coordination with the Ministry of Agriculture, Livestock, Aquaculture and Fisheries, and the *Universidad Técnica del Norte*.

It is important to note that, within the framework of the International Year of Quinoa, and in view of growing national and international expectations, the Ministry of Agriculture, Livestock, Aquaculture and Fisheries is prioritizing quinoa, registering it in its list of strategic crops and giving it a strategic role in productive development and the makeover of the production matrix. Within the same context, the creation of new companies (e.g. URCUPAC) is being driven by the private sector. In addition, the *Consorcio Ecuatoriano de Exportadores de Quinua* (Ecuadorean Consortium of Quinoa Exporters) has

Figure 5. Current and potential geographic distribution of quinoa cultivation in Ecuador.

been founded; most of its members are Ecuadorean companies and organizations involved in quinoa cultivation: MCCH, *Fundación Familia y Mujer Andina* (FUNDAMYF), URCUPAC, *Cereales Andinos*, and *Rogetore & Franco*.

The Importance of Quinoa in Ecuador

In Ecuador, quinoa can be produced in the ten provinces of the Sierra (Figure 5), with a potential area of 100 000 ha (Yugcha, 1998).

The Sierra of Ecuador is traditionally the country's primary producer of foods for domestic consumption. Until the end of the 1990s, quinoa was not one of the most important crops, but it was a product intended for local consumption (communities, parishes or cantons in the same region) (Nieto, 1997).

According to Nieto (1997), the cultivation of quinoa was of secondary importance in Ecuador, not just because of the relatively small cultivated area, but also because of its low annual per capita consumption (< 1 kg) and the apparently limited interest in increasing its production and consumption. However, various institutions, researchers and Ecuadorean entrepreneurs, with the support of international organizations and ultimately the Government, have done much to revive and promote native crops traditionally not widely grown, among them quinoa. Successful outcomes include the recovery and conservation of germplasm, the production of high quality seed, the development of technological recommendations for quinoa cultivation and its industrialization, and the promotion of domestic and international use and consumption (Jacobsen and Sherwood, 2002).

According to García (1984), quinoa production centres were located in specific areas in six provinces in the Sierra. The most important – in terms of frequency and extent of cultivation – were Chimborazo, Imbabura and Cotopaxi, while Tungurahua, Pichincha and Carchi produced smaller quantities. In Cañar and Azuay, quinoa cultivation had disappeared. As of 1984, the area under cultivation was estimated to be only 900–1 000 ha.

During the 1980s, INIAP and Nestlé played a vital role in saving Andean crops, and quinoa was the priority. When Nestlé established the Latinreco research and development department, the only quinoa in Ecuador was found in furrows planted among other crops; it was basically not sold at all. In 1990, the Inagrofa company began to produce and sell conventional quinoa for domestic and regional markets, and organic quinoa for Europe and the United States of America. In 1999, ERPE began to promote the organic production of quinoa for export to the United States of America. In 2002, total production in Ecuador was estimated to be 2 000 ha. The output from 500 ha was earmarked for export as certified organic quinoa (Jacobsen and Sherwood, 2002).

While the quality of the quinoa produced in Ecuador is well below that produced in Bolivia and Peru, average yield is 30–50% higher. The future competitiveness of Ecuador may depend on the ability not only to increase the area under production but also to increase the productivity, quality and recognition of this output (Jacobsen and Sherwood, 2002).

On the basis of the results of the Third Agricultural Census, carried out in 2000, Junovich (2003) reported quinoa had 2 659 registered agricultural production units (APUs), cultivated on about 900 ha. Of these, 636 ha were harvested, producing 226 tonnes, of which 180 tonnes were sold. The average yield for the Sierra was 0.4 tonnes/ha. The average area planted with quinoa was 0.3 ha/APU. The provinces with the greatest number of APUs growing quinoa were Chimborazo, Cotopaxi and Imbabura; the main producer was Chimborazo, which produced about 80% of the total output in the census period.

In recent years, the area planted with quinoa in Ecuador fluctuated between < 500 ha and around 1 200 ha/year, producing volumes of no more than 500–600 tonnes (Figure 6).

After 2009, quinoa imports into Ecuador exceeded 500 tonnes/ year (Figure 7).

According to the Quinoa Development Project of the Ministry of Agriculture, Livestock, Aquaculture and Fisheries, quinoa exports from Ecuador do not exceed 500 tonnes/year (Figure 8). According to the Central Bank, Ecuador's exports of quinoa have shown fluctuations since 1987. An analysis of the 8-year period from 2004 to 2012 reveals a substantial increase in exports (from 41 tonnes in 2000 to 422 tonnes in 2008). Between 2004 and 2005, exports remained relatively steady, then in 2006 there was a 18% decrease compared with 2005. The FOB price also increased, reaching its highest value per tonne (USD1 870.80) in 2008 (Figure 9).

During the 2000–08 period, the primary destinations of Ecuadorean quinoa were the United States of America (53%), the United Kingdom (29%), France (6%), Germany (4%) and Spain (4%); other countries accounted for the remaining 4% of total exports (Figure 10).

Quinoa Production in Ecuador

The vast majority of quinoa farming in Ecuador takes place on family farms. According to the Third Agricultural Census (Junovich, 2003), during the census period, 2 659 APUs were registered, with a planted area of approximately 900 ha. The average area planted with quinoa in the Sierra region was

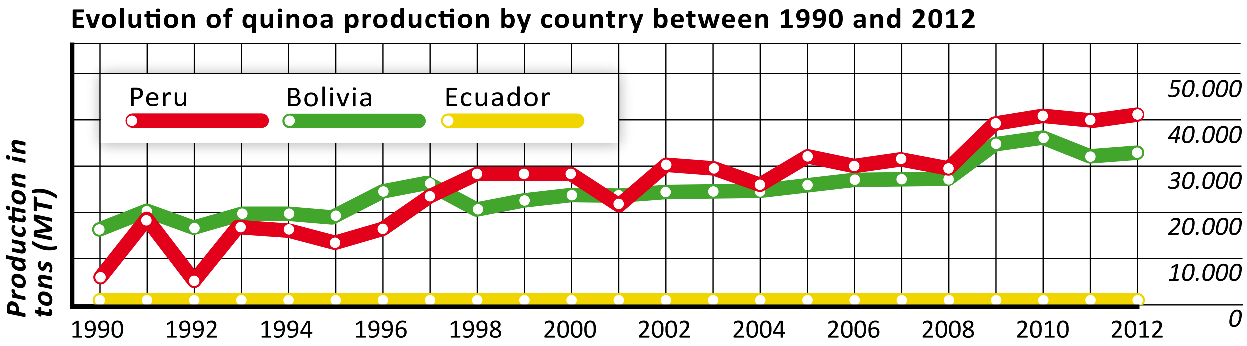

Figure 6. Evolution of quinoa production in Ecuador (Ministry of Agriculture, Livestock, Aquaculture and Fisheries: Project for the development of quinoa production in Ecuador, 2013).

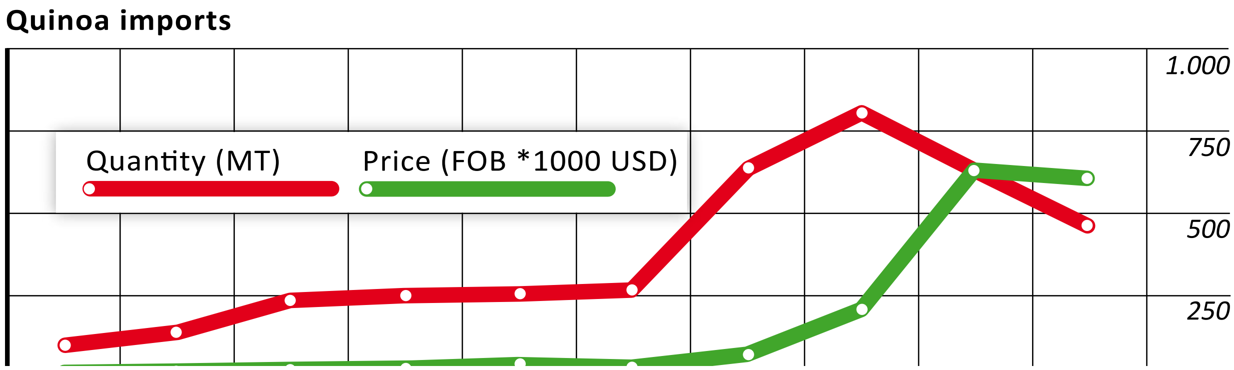

Figure 7. Imports of quinoa into Ecuador (Ministry of Agriculture, Livestock, Aquaculture and Fisheries: Project for the development of quinoa production in Ecuador, 2013).

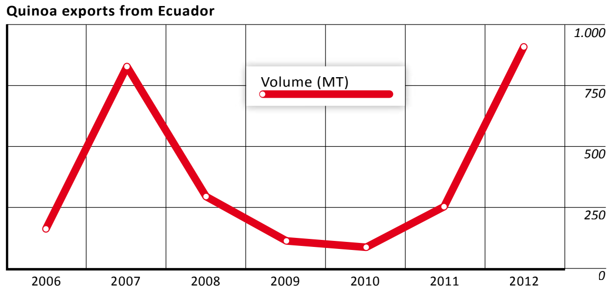

Figure 8. Exports of quinoa from Ecuador (Ministry of Agriculture, Livestock, Aquaculture and Fisheries: Project for the development of quinoa production in Ecuador, 2013).

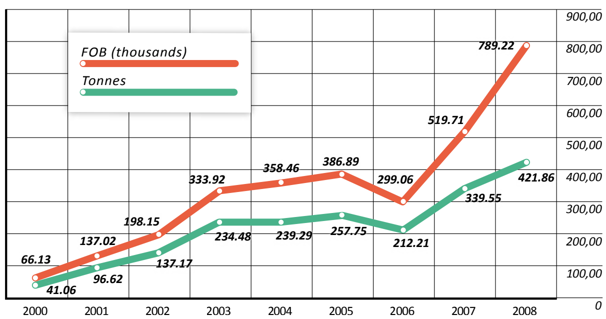

Figure 9. Annual demand for Ecuadorean quinoa, 2000–2008 (Central Bank of Ecuador, 2009).

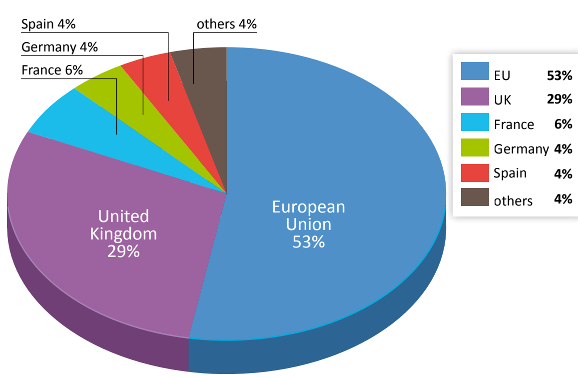

Figura 10. Primary countries of destination of Ecuadorean quinoa, 2000–08 (Central Bank of Ecuador, 2009).

0.3 ha/APU, indication that quinoa is cultivated on small farms. The provinces in which quinoa production is centred are Azuay, Cotopaxi, Chimborazo, Imbabura, Pichincha and Tungurahua. Of these, Chimborazo, Cotopaxi and Imbabura have the highest production.

In 2009, the areas under quinoa cultivation increased in the provinces of Chimborazo (mainly organic), Imbabura, Carchi, Cotopaxi, Bolívar, Cañar, Pichincha and Loja. It is estimated that > 60% of the quinoa cultivated in Ecuador is of the 'INIAP-Tunkahuan' variety.

During the past 15 years, the varieties planted in the provinces of Chimborazo and Bolívar in organic systems certified for export, and produced by ERPE and the *Corporación de Productores y Comercializadores Orgánicos Bio Taita Chimborazo* (COPROBICH, Bio Taita Chimborazo Corporation of Organic Producers and Traders), are mixtures of native varieties with bitter grains (and high saponin content), from red, pink, green and brown plants (Images 5 and 6). Some organic producers separate their crops by colour, and it is not rare to see fields of only red or green plants. These are varieties with medium-sized grains that are opaque white or cream coloured. They are produced primarily in the provinces of Chimborazo and Bolívar.

In Ecuador, around 90% of quinoa is planted as a monoculture; 10% is in polyculture systems in association with maize (planted alone or together with climbing beans), potatoes, beans, peas etc.

In conventional systems, crops rotated with quinoa include potatoes, maize and climbing beans, barley, peas, *chocho* (also known as *tarwi*) and small tubers such as melloco, oca and mashua.

The planting season is from November to February, with a density of 12–16 kg/ha.

In manual sowing (or using small seed drills), the distance between furrows varies between 40 and 60 cm. In mechanical sowing with a tractor, 80 cm are left between furrows to facilitate weeding and hilling (Peralta *et al*., 2012).

Organic Production

ERPE cultivates 400 ha of certified organic quinoa per year in five cantons of the province of Chimborazo, where climatic conditions vary. It mostly plants native varieties (with a variety of colours and growing cycles) and obtains yields of between 675 kg/ha and 1.35 tonnes/ha. The output is sold in the United States of America and Germany.

For family consumption, ERPE has internal rules of procedure governing producers, who must set aside 20–30% of their production for their own

Figure 11. Quinoa field with a mixture of native varieties.

Figure 12. Field planted with a native, pure-bred variety of quinoa.

consumption and use as seed. Depending on the climate, the average annual volume varies between 363 and 544 tonnes. ERPE sells all of the production in storage (363–456 tonnes). The remainder is for consumption by producers and their families (Juan Pérez, ERPE, personal communication).

COPROBICH produces 18–27 tonnes of certified organic quinoa in the province of Chimborazo. It is a corporation of producers legally recognized by Ministerial Accord No. 184 of 31 July 2003, issued by the Ministry of Agriculture, Livestock, Aquaculture and Fisheries. It is an autonomous, no-profit, private-law corporation providing services and social benefits to its members, who are Puruhá indigenous people from 86 communities in the cantons of Riobamba, Colta, Guamote, Guano and Penipe. At the time of writing, membership is ≥ 1 632 families. Since 2009, it has bought quinoa directly from them, exporting it as a fair trade product to France, Belgium, Germany and Canada.

FUNDAMYF cultivates an average of 400 ha/year of quinoa in the provinces of Chimborazo, Bolívar, Tungurahua and Cotopaxi, with a focus on certified organic produce. The variety grown is ‘INIAP-Tunkahuan’. The average yield is 998 kg/ha. Its production is sold in both domestic and international markets (María Eugenia Lima, FUNDAMYF, personal communication).

The *Fundación Maquita Cusunich* (MCCH) cultivates about 15 ha in Chimborazo, with an average yield of 680 kg/ha. It is also active in other provinces of the Ecuadorean Sierra.

Conventional Production

For the past 28 years, Inagrofa has been planting ≤ 600 ha/year with the ‘INIAP-Tunkahuan’ variety in the provinces of Carchi, Imbabura, Pichincha and Cotopaxi, with an average yield of 2 tonnes/ha. It also buys harvests in other provinces. It sells on the domestic market and exports to the United States of America (Rodrigo Arroyo, Engineer, Inagrofa, personal communication).

During the past 4 years, the Ministry of Agriculture, Livestock, Aquaculture and Fisheries has promoted the conventional cultivation of ‘INIAP-Tunkahuan’ quinoa in Imbabura, on 70–100 ha/year, obtaining yields of 1.5–2.5 tonnes/ha. It has worked with family farm associations and individual producers, offering training and technical monitoring; providing threshing services with stationary machinery; facilitating storage and processing operations; and promoting sales in the Ecuadorean and Colombian markets (José Manuel López, Engineer, Ministry of Agriculture, Livestock, Aquaculture and Fisheries, personal communication).

The Revelo Jara family conventionally produces an average of 80 ha/year of ‘INIAP-Tunkahuan’ in the province of Carchi, with an average yield of 1.13 tonnes/ha. Its reaches a national market and is used in government food programmes (Lourdes Revelo, economist, personal communication).

The *Asociación de Productores de Semillas y Alimentos Nutricionales Andinos Mushuk Yuyai* (APROSANAMY – Mushuk Yuyai Association of Seed and Nutritional Andean Food Producers) represents 14 members, 120 producers and 30 quinoa farmers. It agro-ecologically cultivates 7–11 ha/year in four cantons in the province of Cañar and one canton in the province of Azuay. Yield varies depending on climatic conditions.

The crop is produced on small plots with a relatively high number of families. They grow mainly the 'INIAP-Tunkahuan' variety and, to a lesser extent, 'INIAP-Pata de Venado'. They are losing interest in growing quinoa, due to the effects of climate variations on crop yield and also because no residue is generated for feeding livestock. The average yield from monoculture plantations is 1 350 kg/ha and the production is sold locally (at education centres, in towns and at community shops). The association promotes consumption by producer families and recommends that they store 10–15% of production for seed and their own consumption. Their average annual yield varies between 1.36 and 9 tonnes, depending on the climate. The producers sell 5.44 tonnes with added value per year, and they sell the remainder direct to consumers on local markets (Nicolás Pichazaca and the APROSANAMY technical team, personal communication).

CORPOPURUWA is a corporation with 62 founding members (47 men and 15 women) from the communities of San Miguel de Pomachaca, Asociación Mushuk Pakari, Pull San Pedro and Sacahuan Tiocajas in the canton of Guamote. It is legally recognized by Accord No. 38 of the Provincial Department of Agriculture of Chimborazo (Ministry of Agriculture, Livestock, Aquaculture and Fisheries, 2010). They produce small quantities of quinoa, in rotation with *chocho* and *tarwi*.

The Outlook for Quinoa in Ecuador

It is estimated that there are more than 80 000 ha in Ecuador with agro-ecological characteristics suitable for quinoa production: the country has the potential to intensify quinoa production and enhance productivity. In order to fulfil that potential, however, it must facilitate access to integrated crop management practices, quality seed, farm credit and threshing machines, while ensuring fair prices for producers.

Given the current agrarian structure in Ecuador, agro-ecological intensification is an option, especially for family farms where quinoa is a logical component of production (through crop rotations and associations) and where it is possible to gain access to alternative markets practising fair trade and selling organic products.

Given the international demand, the outlook for quinoa cultivation is favourable. For example, the *Consorcio Ecuatoriano de Exportadores de Quinua* (Ecuadorean Consortium of Quinoa Exporters) has brought together major corporations and development organizations to boost production, generate added value and realize sales on international markets.

The Ecuadorean Government, through the Ministry of Agriculture, Livestock, Aquaculture and Fisheries, is launching a quinoa production development project that includes distribution of certified seed and basic inputs, access to credit, introduction of sowing and harvesting machinery, and provision of technical consultancy.

The Government is also promoting the inclusion of nutritious, locally produced products (including quinoa) in school food programmes.

Nevertheless, there are further challenges in the agricultural domain that need to be considered. Fostering quinoa production while improving productivity using the systems available is hindered by a lack of technicians with sufficient academic and practical training. Moreover, young people are becoming increasingly uninterested in agriculture, leaving agricultural production in the hands of older adults; as a result, it is difficult to implement food production development programmes and projects, such as those involving quinoa, whether for domestic consumption or for international markets.

Within the context of climate change, it is difficult to plan when to sow crops, and the risk of losing harvests is increasing. For this reason, there has been an increase in livestock production, which is viewed by farmers as a sounder investment: it entails fewer risks from the point of view of climate and it gives a more reliable source of income.

Ultimately, the demand for quinoa is based on expectations of high prices on the international market; this could change from one day to the next as a consequence of the economic crisis in developing countries. In addition, the crop risks becoming overcultivated given the large number of countries and companies interested in quinoa production.

Conclusions

In Ecuador, in terms of area planted and per capita consumption, quinoa is a secondary crop; it is nevertheless very important for food security, especially for family farmers in the Sierra. Due to the high level of international demand and elevated market prices, many companies and organizations are now interested in increasing Ecuador's quinoa production. Initiatives are being backed by the Government through its productive development plan for quinoa; other government initiatives involve changes to the matrix of production and foreign trade.

Ecuador has the potential to meet this demand, since the agro-ecological conditions and technologies developed in the country (in terms of crop, harvest and post-harvest management, and the creation of added value) would allow the area under cultivation to be increased and productivity to be enhanced, thereby improving competitiveness in local, regional and international markets. However, challenges must first be overcome, the most important of which include: the poor partnership culture among producers; limited access to high quality seed and other inputs; difficulties in accessing machinery for soil preparation, and harvest and post-harvest operations; insufficient economic and infrastructure capacity for harvest storage; the dwindling interest in agriculture among rural populations, especially the young; and the need to develop technical assistance services, currently in their infancy.

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CHAPTER: 5.4

Quinoa in Chile

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Abstract

The biogeography of quinoa (*Chenopodium quinua* Willd.) provides a comprehensive view of a crop that is relatively minor in Chilean agriculture, despite growing in a large geographical area (18°–47°S). Quinoa's genetic diversity illustrates that it is a vital crop in the South American Andes region. It was domesticated in various geographical zones, which generated a wide variety of adaptative morphological and environmental features. Specific adaptations in each macrozone throughout the Andes have created five ecotypes, associated with subcentres of diversity. Two ecotypes are present in Chile – quinoa from the salt flats in the country's extreme north: Salare quinoa, and quinoa from sea-level areas in the central and south central regions: Coastal quinoa. Recently, these ecotypes have been associated with diverse production systems, depending on their biophysical, social and cultural features. Public policies and market relations also play a vital role in determining production system dynamics.

Key words: *Chenopodium quinua* Willd., Chile, biodiversity, geography, agro-ecosystems.

Introduction

In the current context of economic globalization, agriculture is vital to populations around the globe (Harvey, 2001, 2005). A variety of models based on use of natural resources, agriculture (FAO, 2006), social organization and cultural identity (Leff, 2005) may be applied to rural development and food production. Two production models are present in Chile today. One is export-driven and focuses on land concentration (particularly by increasing farm surface area), with resources and production chains highly dependent on external risk factors; the other is dominated by rural, family-run farms, and is limited to food production for local markets. These farms have adapted their practices to ecosystemic features that farmers have understood for generations. Both models have an important role in local development, and exist on a smaller scale in the case of quinoa crops. This chapter aims to use the specific case of quinoa in Chile to underscore the importance of (re)considering agricultural production system diversity in terms of biogeography, for the purpose of optimizing crop potential.

Quinoa was first domesticated over 7 000 years ago in southern Peru and northern Bolivia. It was adapted through a series of agro-ecosystems modelled by ancient civilizations, as it was transported from the north to southern Chile. This generated a high degree of genetic diversity, adapted to a broad ecological spectrum.

There have to date been no studies in Chile to substantiate the importance of traditional crops and farmers' varieties (landraces). Therefore, there is a lack of historical, anthropological, economic, geographical and/or strategic studies able to link quinoa's conservation to the country's development. If quinoa's diversity were considered a phytogenetic resource, the very perception of biodiversity could change from a biological and agricultural productivity standpoint. This in turn would lead to a change in agriculture's relationship with local ecosystems, giving impetus to a redefinition of agrobiodiversity, with globally recognized repercussions, such as agricultural system reproduction, the creation and maintenance of social ties and and the transmission of skills and patrimony from one generation to the next (Chevassus-au-Louis and Bazile, 2008; Kaine and Tozer, 2005). Building sustainability with a focus on agricultural biodiversity is linked to the territorial stakeholders' system. This constant gives meaning to the idea of biodiversity, described in geographical terms as a society's link with the diversity of living things, viewing it in terms of the "problems" (or benefits) it presents to that society. For Leff (2005), "Territory is the place where sustainability is rooted within ecological bases and cultural identities. It is the social space where social actors exercise their power to control environmental degradation and fulfill the peoples' needs, aspirations and desires that economic globalization is unable to satisfy."

At local level, the limitations and positive synergy of growth and agricultural development models are manifested in the spaces created (Zalabata, 2003). Environmental diversity translates to a variety of agrarian practices that do not easily fit into an agrarian systems analysis (Naredo, 1996). To identify sustainability through the ecological pressure or economic potential of crops, it is important to look towards the territory's structural diversity, reflected in the variety of soils, species, ecosystems, landscapes, and their uses and applications. These models describe mechanisms for organizing the territory in question. Additionally, this principle of representation or transcription must contribute to bridging the knowledge gap between rural and scientific cultures, so as to avoid confrontation and antagonism (Serrano, 2005) between traditional and innovative models (Hocde *et al.,* 2008).

The first section of this chapter focuses on the general history of quinoa in the Andes and in Chile, explaining its presence today in Chile's various agricultural contexts, from the far north to the valleys and mountains of the south. The second section presents the agricultural space and its various features: climate, soil and local varieties of quinoa. The third section addresses the characterization of quinoa producers in the Chile of today with respect to farming ecology (Parra, 2007; Rescia *et al.* 2002). The final part analyses the relevance of the features of the "quinoa territories" to explain the crop's high genetic diversity in Chile, and examines the importance of maintaining these features in terms of adaptation to different environments and markets, while offering specific products. In conclusion, despite its status as a minor crop, quinoa in Chile exhibits high ecological and production diversity, occupying a wide range of ecosystems. Quinoa presents new opportunities for agrarian development in Chile, given the potential social, ecological and economic interactions under sustainable development. Quinoa crop management should be planned in line with the dynamics of its broad biodiversity, with twofold vertical management over a wide geographical area extending from the Aymara to the Mapuche region, and horizontal, or local, management when territorial cohesion is required among local stakeholders: farmers and non-farmers; the public and private sector.

General history of quinoa in the Andes and Chile

In the Andean context, the information available indicates that quinoa was probably domesticated by ancient civilizations in different time periods and geographical zones, including the regions of Peru (5000 B.C.), Chile (3000 B.C.) and Bolivia (750 B.C.) (Kadereit *et al.,* 2003). Quinoa's presence in Chile today may be explained by cultural exchanges between ancient peoples, such as the Inca culture and other groups native to Chile, in various agro-ecological contexts from the Chilean Altiplano in the north (17°S) to the island of Chiloe and even further south (47°S, Puerto Rio Tranquilo). During the Spanish Conquest, quinoa was strongly discouraged as a crop, due to its important role in society and the fact that it was sacred to the indigenous peoples' religious beliefs (Ruas *et al.*, 1999).

As a result, quinoa was only retained as a crop in places devoid of agricultural modernization programmes, and it became the particular domain of rural and indigenous women. The current phenomenon of human migration from the rural regions of the Andes to urban centres exposes quinoa to the risk of genetic erosion. This process is consistent with a loss of genetic diversity (preserved *in situ* for thousands of years), caused by the disappearance of traditional farming practices.

Many historical documents describe quinoa as present in Chile, from the northern regions to the valleys and mountains of the south. The agricultural landscape was described early on by Pedro de Valdivia to King Carlos V during the sixteenth century: "… this land is fertile for livestock like Peru… abundant in the sustenance planted by the indigenous people for their subsistance, such as corn, potatoes, ***quinoa,*** madi, chili, beans…"

Later, the French botanist, Claude Gay, travelled to Chile during the nineteenth century and described quinoa as "…a plant native to the Americas and grown for some time in Chile. The Spaniards found it everywhere, from Copiapo to the island of Chiloe, where the inhabitants grew it along with corn and potatoes…" (Molina, 1810).

Juan Ignacio Molina (1810) documented quinoa's production system, with particular reference to the southern variety known as "Dahue", which produced "ashen leaves and white seeds. The black seeds are used to make a pleasant drink that settles the stomach, and the white ones, which swell to look like little worms when cooked, are prepared as a delicious soup; they even eat the leaves, cooked like spinach. Nearly three months before planting, they bring their livestock to sleep there, changing places every three nights; when the field has been well-fertilized, they plant the seeds on top of the grass and on top of the manure."

Quinoa crops had nearly disappeared by the mid-twentieth century, according to Looser (1943). Nonetheless, rural peasants were persistent and continued to grow it in the Andean region in northernmost Chile on the border with Peru and Bolivia (Lanino, 1976), in the central region south of Santiago, at sea level in Concepcion, and in Araucanía, where the Mapuche people knew it as *quinhua* or *kinwa* (Junge, 1978).

Quinoa ecotypes present in Chile

Quinoa grown in Chile retains the same morphological patterns and colours found at other latitudes (Gandarillas 1979; Bhargava *et al.*, 2005; Fuentes and Bhargava, 2011). However, specific adaptations to certain geographical regions throughout the Andes have generated five ecotypes associated with subcentres of diversity (Figure 1).

These subcentres are: (1) the Inter-Andean valleys (Colombia, Ecuador, and Peru); (2) Altiplano (Peru and Bolivia); (3) Yunga (Bolivia); (4) Salare (Bolivia, Chile and Argentina); and (5) Coastal areas at sea level (Chile and Argentina).

Figure 1: Distribution of quinoa ecotypes in subcentres of diversity: A. Inter-Andean valleys, B. Altiplano, C. Yunga, D. Salare, and E. Coastal. Source: By Francisco Fuentes Carmona

Quinoa crop in Chile is based on two quinoa ecotypes: Salare and Coastal (lowlands). The Salare ecotype is found in the regions of Tarapacá and Antofagasta in Chile's far north. Traditionally, these genotypes are grown by indigenous communities in the Chilean Altiplano, in saline soil, with rainfall of 100–200 mm/year occurring between December and February (Fuentes *et al.*, 2012). Several quinoa landraces in the northern region are closely related to varieties of quinoa from the Bolivian salt flats, where there is no natural border between the two countries. There is, however, evidence that some materials have been introduced to Antofagasta from Andean areas of Peru, to which the Atacama Desert acts as a natural barrier. Despite this, in most materials studied hitherto, the dominant morphology corresponds to Salare quinoa (Fuentes *et al.,* 2009a).

In the central and southern regions of Chile (the administrative regions of O'Higgins and Los Lagos) the Coastal quinoa is cultivated. It is suited to cultivation at 0–800 m asl under rainfed conditions (Fuentes *et al.*, 2012). In contrast to the dry conditions of Salare quinoa in northern Chile, the rainy season in the centre and south is concentrated in the winter months, with precipitation fluctuating between 500 and 1 900 mm/year, depending on the geographical zone, which includes the Libertador Bernardo O'Higgins, Los Rios and Los Lagos regions.

There is a marked and well-known difference between these two quinoa ecotypes grown in Chile, in terms of adaptation to altitude, drought and salinity tolerances, and sensitivity to day length. From an agronomic point of view, the Coastal ecotypes may be adapted to high altitudes through natural migration processes between regions as well as by natural or artificial crosses performed by breeders (Fuentes *et al.*, 2009b). Local varieties may also be adapted for other uses, for example forage or raw consumption (Fuentes and Bhargava, 2011).

Current distribution of quinoa in Chile and its associated climates

Distribution of quinoa in Chile may be analysed and understood considering three macrozones of ancestral (or relict) production and associated with subgroups of genetic diversity and agricultural production systems (Fuentes *et al*., 2009b, c, 2012) (Figure 2): the northern zone (administrative regions XV, I, II), the central zone (administrative regions VI, VII) and the southern zone (mainly in region IX, but also present in regions VIII, XIV, X, XI).

Figure 2: The three relict macrozones of quinoa production in Chile. Source: IMAS (ANR), 2009 (http://imas.agropolis.fr/ and http://www.quinua-chile.cl)

Northern macrozone

In northern Chile, the climate is influenced by the tropics, and quinoa growing is limited to higher altitudes (*puna*, as defined in terms of climate by Di Castri [1968]). Bioclimate differences are linked to summer rainfall typical of the region, which is influenced by low pressure from the eastern Andes (Lanino, 1976). Because of the high altitude, the temperature here is lower than in other tropical regions, and is heavily regulated by landscape microstructures. In Chile's northern region, quinoa is found exclusively in the Altiplano, in zones with an altitude between 3 000 and 4 500 m asl. The elevation of this high steppe climate (or "high marginal desert", according to the older classification – Köppen, 1931) has a direct influence on average temperatures, which are no higher than 5°C and vary significantly between day and night (Figure 3a). On average, there are 9–10 months per year with

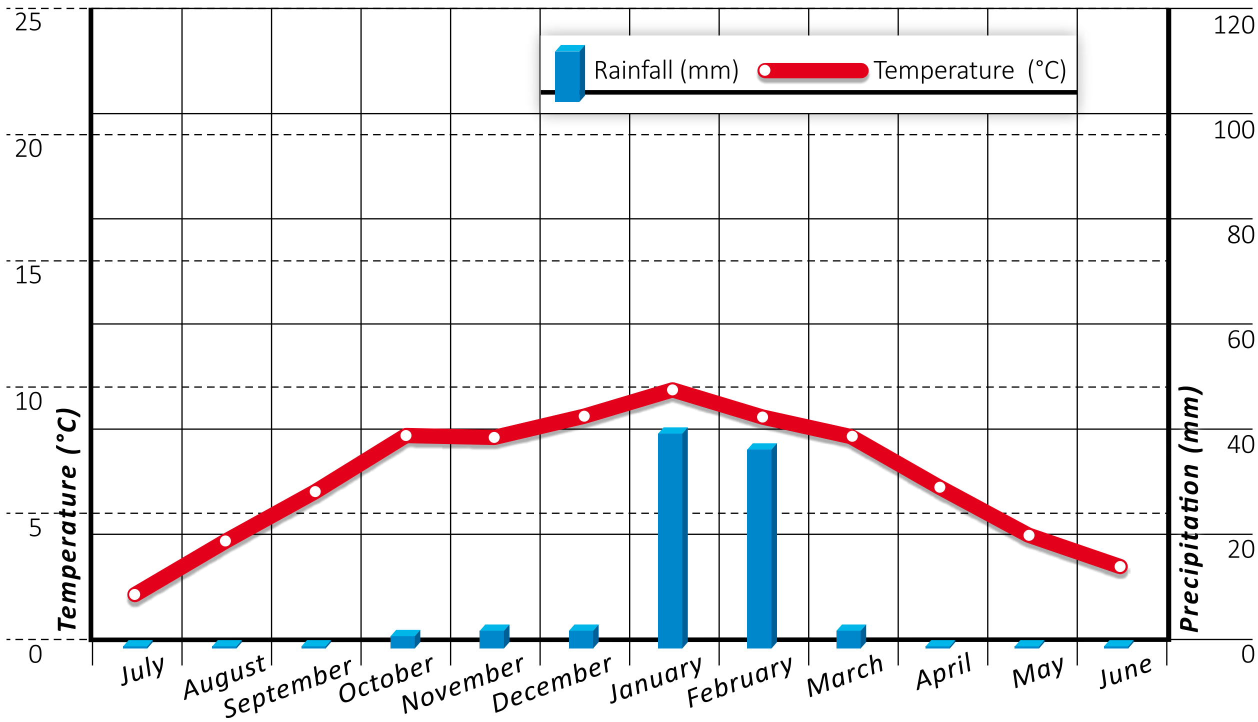


Figure 3a: General Climate Graph for Northern Macrozone using example of Ollagüe, *High Steppe Climate*

temperatures below 10°C, including four very cold months with an average annual temperature of only 4.5°C, an average high of 11.5°C and an average low of < 0°C.

Average annual rainfall is 120 mm. Most precipitation occurs during the summer and is convective in nature, stemming from clouds produced by rising air masses charged with moisture along the eastern hillsides of the Andes, originating from the Amazon Basin and the Atlantic. In some zones, rainfall may be greater than 400 mm/year, but these areas become progressively less common towards the south. Relative humidity tends to be low. Data collected by the weather station located in Vilacollo in the commune of Colchane (Tarapacá Region) for the 2005–06 season indicate maximum and minimum temperatures of 23.2° and -8°C, respectively, wind speeds of > 54 km/h, maximum solar radiation recorded of 1 218 W/m and annual rainfall of 147 mm (Arenas and Lanino, 2008; Delatorre *et al.*, 2008).

Central macrozone

Central Chile has a Mediterranean climate, with humidity progressively increasing from north to south (Di Castri, 1968). Quinoa production is concentrated in the so-called sub-humid Mediterranean climate region. Topographical orientation also influences the precipitation distribution, with higher rainfall in the western hillsides of the Andes mountain range and the coast than in the contiguous zones. In regions VI and VII there are two types of "warm temperate climate with prolonged dry season" – with or without heavy cloud cover. In addition, Köppen (1931) proposes "warm temperate climate with winter rainfall and heavy cloud cover" and "warm temperate climate with winter rainfall".

The climate with cloud cover is found in the coastal area of the northern part of this macrozone, including coastal plains and the western side of the Coastal Range. The ocean influences the climate, moderating temperatures and creating high humidity, which results in a higher number of cloudy days. Precipitation is frontal and concentrated in the winter, although the dry season may last 7–8 months, due to the influence of the Pacific anticyclone. Annual precipitation varies between 500 mm (region VI) in the north (Figure 3b) and almost 800 mm in the south (region VII). Approximately 80% of the annual rainfall occurs between the months of May and August.
The dry season lasts 7 months, with less than 40 mm

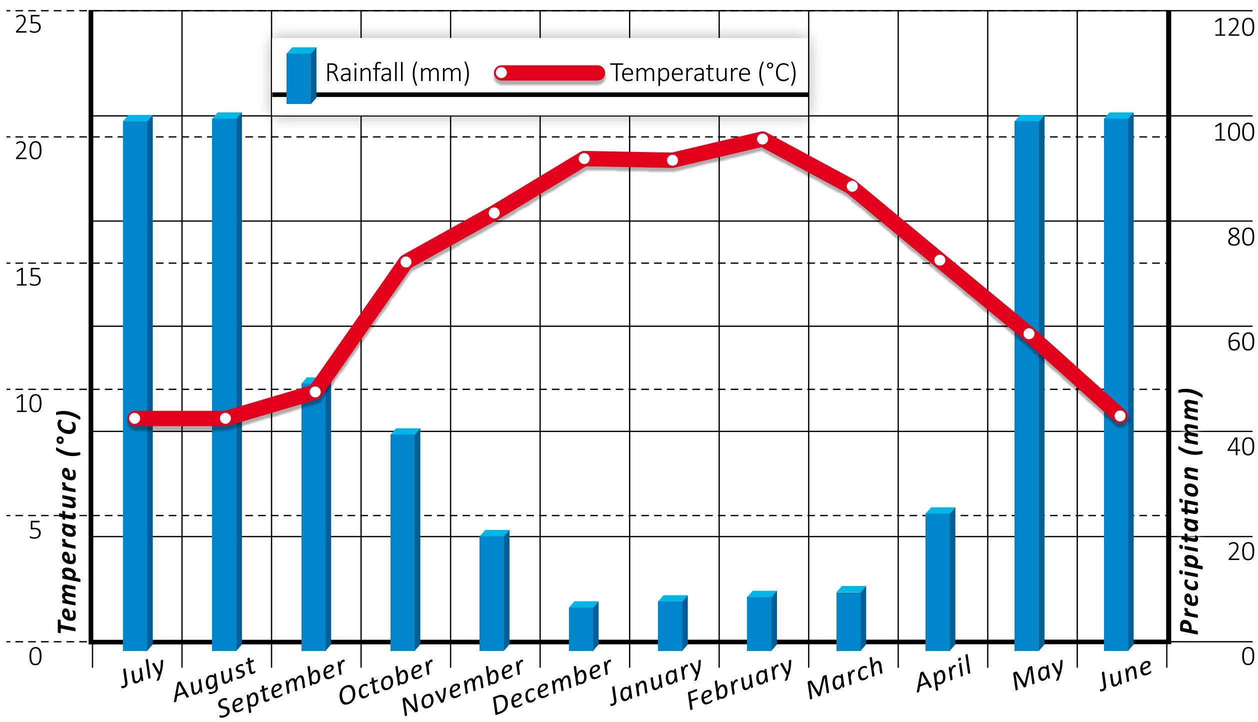


Figure 3b: General Climate Graph for Central Macrozone, using example of Paredones, Warm Temperate Sub-humid Mediterranean Climate with Prolonged Dry Season.

of precipitation occuring from October to April. Most quinoa crops from the central macrozone are cultivated near the coast, between the towns of Pichilemu and Iloca, in an area stretching 25 km inland, and influenced by the same climate (Olguin, 2011).
On the other hand, growing regions furthest from the coast, beyond the city of Santa Cruz and moving towards the mountains, have the same climate, but without the cloud cover. The regions located in the intermediate pressure zone or in the longitudinal valley of this region present Mediterranean climate conditions – hot, dry summers, and cool, damp, rainy winters. Rainfall is somewhat lower than on the coast, but average daily and annual temperatures are higher. The temperature difference between the warmest and coldest months is about 13°C in Rancagua, and only 8°C along the coast. From October to April, rainfall is less than 40 mm. The Chilean Coastal Range limits the maritime influence, and consequently there are more cloudy days than on the coast (Olguin, 2011).

General climate data for the central macrozone are as follows: average annual temperature 14.5°C, average maximum temperature 21.5°C, average minimum temperature 7.5°C; relative humidity 73%; rainfall 700 mm.

Southern macrozone

The southern quinoa production macrozone presents two types of climate. First, “warm and rainy temperate climate with Mediterranean influence”, is found from primarily the intermediate zone (38°S) to near Castro, on the Greater Island of Chiloe (region X, Los Lagos) (42°S). Average rainfall can reach 2 000 mm, with the monthly distribution highest in winter and decreasing in summer (Di Castri, 1968). Temperatures are characteristically moderate along the coast, rising in the mountainous area. Second, “warm temperate climate without a dry season”, is characteristic of the southernmost region. Rainfall is nearly continuous throughout the year, with an annual average of > 2 000 mm and significant monthly distribution from March to November. Temperature variation between night and day is moderate (≤ 5°C), with a recorded annual average of almost 12°C.

The temperate rainy climate with Mediterranean influence is present in the macrozone; it is influenced

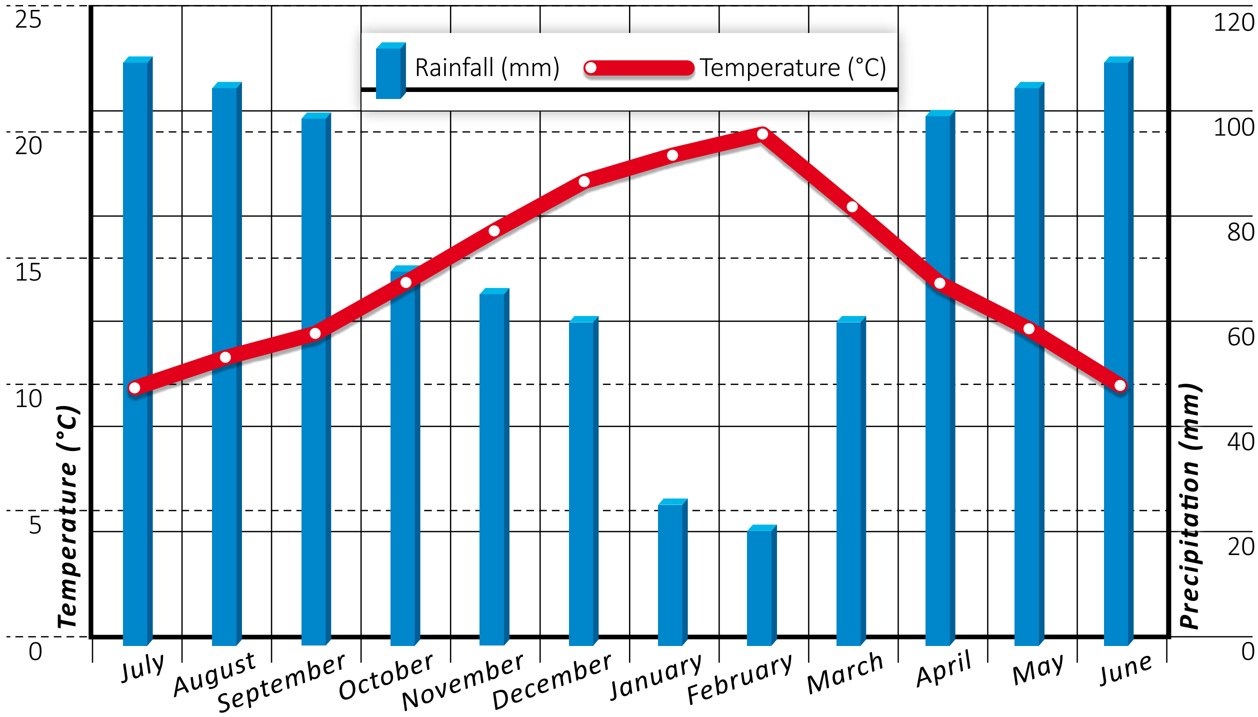


Figure 3c: General Climate Graph for Southern Macrozone, using example of Lebu (Coast), Rainy Temperate Climate with Mediterranean Influence.

by the ocean and there is moderate temperature variation in coastal zones (Figure 3c). In the longitudinal valleys and foothill regions, annual temperature variation is significant due to the distance from the coast, with stronger continental features. The average temperatrure is 11.5°C in the centre (12.5°C on the coast and 8.5°C in the foothills), with an average high of 17°C (16.5° and 16.5°C) and an average low of 6°C (9.2° and 1.0°C). Relative humidity varies between 75% and 85% in the foothills. Rainfall is distributed thoughtout the year, with a slight drop in monthly precipitation during the summer; annual rainfall is > 1 000 mm.

A warm temperate climate with a short dry season of < 4 months is found in the region's intermediate zone, located in the north and extending to around 39°. Moving southwards, temperatures progressively decrease. Precipitation is high and uniform throughout the year, with a slight decrease in the spring. Absolute precipitation values reach 2 050 mm and rainfall is never below 140 mm. The average annual temperature is 8.5°C, with a variation of 5°C. The coldest month (July) has an average temperature of 6°C and a maximum temperature of 12°C.

The state of quinoa production in Chile based on census analysis.

Evolution, 1997–2007

Over the last 15 years, quinoa crops have experienced immense growth in Chile's three macrozones. According to official data from the 1997 and 2007 agricultural censuses, the national cultivation area grew by 736%, from 175 ha in 1997 to 1 470 ha in 2007 (INE, 1997, 2007). Most of this increase occurred in the Region of Tarapacá, where over 92% of cultivation land is located. More specifically, the communes of Colchane and Pica have been at the centre of this increase: 749 ha in 2007 (compared to 163 ha in 1997), and 600 ha in 2007 (no planting was recorded in 1997). To a lesser degree, there was an increase in region VI during the same period: from 11 to 60 ha. Additionally, albeit with very small growing areas, regions such as Atacama, Coquimbo (north central zone), and Araucanía (southern zone) now appear in the census (Table 1 and Figure 4).
Furthermore, the number of quinoa producers doubled during the same period: from 119 in 1997 to

246 in 2007 (Table 1). This increase is proportionally smaller than the increase in area, and is due to the larger average farm size. In 1997 farms averaged 1.5 ha, compared with 6.0 ha in 2007, with a maximum of 46.2 ha per farm in the commune of Pica (northern zone).

In the methodology for data collection adopted, Chilean national agricultural statistics only refer to surface areas and farmers for whom quinoa is a major crop (farmers usually only declare areas of > 1 ha). It should, therefore, be mentioned that quinoa is traditionally grown in the central and southern zones of Chile, particularly Araucanía, by small producers (central) and small-scale Mapuche farmers (southern).

Given the small size of these holdings, they are not recorded in national statistics. Nonetheless, small-scale agriculture plays a vital role in the rural areas of these zones, motivating many farmers to preserve cultural features and implement seed exchange (Fuentes *et al.*, 2012).

Characterization of quinoa producers in Chile today.

A total of 868.5 tonnes of quinoa are produced at national level, 91% of which comes from the communes of Pica (59%) and Colchane (32%). The average national yield is 0.6 tonnes/ha. Although the highest yield is found in the regions of Araucanía (IX) and Libertador B. O'Higgins (VI), these averages are

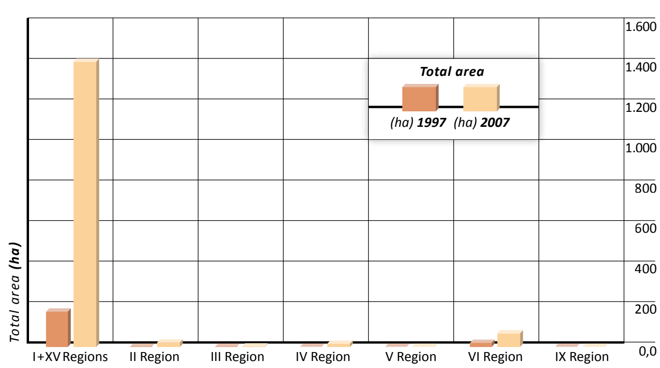


Figure 4: Evolution of total quinoa surface area (ha), 1997–2007.
Source: supplied by Qualitas AgroConsultores, based on *INE, 1997, VI Censo Nacional Agropecuario and INE, 2007, VII Censo Nacional agropecuario y forestal (National agriculture and forest Census, (Tn)*.

not representative, as they involve very small surface areas (recorded in the census). The highest yields are found in the region of Libertador B. O'Higgins, with an average of 1.2 tonnes/ha in the commune of Pichilemu; the lowest yields in Chile are found in the commune of Colchane, with an average recorded yield of 0.370 tonnes/ha (0.6 tonnes/ha at regional level) (Table 1) (INE, 1997, 2007).

When analysing the economic size of farms where quinoa is grown, we note their small size,[1] indication that in Chile, quinoa tends to be grown on family farms, with individual producers operating on a small scale.

In conclusion, the environments in Chile are in contrast to the extremes conditions found in other quinoa-producing countries in the Andean region. Thus, high altitude (3 500–4 000 m asl), arid conditions (100–300 mm per year), salinity and frequent frosts are characteristic only of the northern macrozone of the Chilean Altiplano. Quinoa growers in that region tend to be elderly, as young people abandon agriculture in search of new work and educational opportunities (Fuentes *et al.*, 2012). In the central macrozone (coastal areas and intermediate zones between San Fernando, Curico and Linares), quinoa is not unknown and may provide new economic opportunities for some producers. In the southern macrozone (region of Temuco), quinoa is found in 85% of small gardens grown by women.

Quinoa as part of agricultural systems

The methodology applied in the INE agricultural census did not reveal the true diversity of quinoa production systems throughout the Chilean territory.

These contexts are geographically diverse and generate a wide range of agricultural practices, while traditional selection processes lead to the emergence of landraces (genetically heterogeneous but agronomically stable populations), which explains the low variability (high stability) in annual yields. Field studies were performed over 4 years

[1] Qualitas AC has developed a model for estimating the gross production value for each registered holding, which has been used to categorize and classify holdings according to this variable. For more information, see INDAP, Qualitas AC, 2009, *Estudio de Caracterización de la pequeña agricultura a partir del VII Censo Nacional Agropecuarios y Forestal*.

Table 1: Evolution of quinoa producers, growing area, production and yield by commune and region, 1997–2007

Region	Commune	Number of Producers		Total Area (ha)		Production and Yield by Region 2007	
		1997	2007	1997	2007	PROD (t)	YIELD ($t \cdot ha^{-1}$)
XV Arica and Parinacota and I Tarapacá	Camiña	2.0	3.0	0.5	7.5		
	Colchane	101.0	153.0	162.7	749.0		
	Pica	-	13.0	-	600.0		
	Pozo Almonte	1.0	1.0	-	13.0		
	Huara	-	1.0	-	18.0		
	Putre	1.0	8.0	0.2	4.0		
	Regional total	105.0	179.0	163.4	1 391.5	800.8	0.6
II Antofagasta	Calama	1.0	2.0	0.1	1.0		
	Ollagüe	1.0	5.0	0.3	1.0		
	San Pedro de Atacama	3.0	13.0	0.7	7.0		
	María Elena	-	1.0	-	1.0		
	Regional total	5.0	21.0	1.1	10.0	6.9	0.9
III Atacama	Alto del Carmen	-	1.0	-	1.0		
	Regional total	-	1.0	-	1.0		
IV Coquimbo	La Serena	-	1.0	-	0.5		
	Paiguano	-	1.0	-	0.5		
	Ovalle	-	1.0	-	0.5		
	Monte Patría	-	1.0	-	1.0		
	Río Hurtado	-	1.0	-	0.5		
	Coquimbo	-	3.0	-	1.0		
	Regional total	-	8.0	-	4.0	12	1.2
V Valparaíso	Quilpue	-	1.0	-	0.1		
	Regional total	-	1.0	-	0.1		
VI O'Higgins	Pichilemu	8.0	15.0	10.6	34.0		
	San Vicente	-	1.0	-	0.5		
	Navidad	-	1.0	-	0.1		
	Chépica	-	1.0	-	2.0		
	San Fernando	1.0		0.5	-		
	Paredones	-	11.0	-	24.0		
	Regional total	9.0	29.0	11.1	60.6	58	1
IX Araucanía	Lautaro	-	2.0	-	1.0		
	Teodoro Schmidt	-	1.0	-	0.5		
	Curacautín	-	1.0	-	0.5		
	Vilcún	-	3.0	-	1.0		
	Regional Total	-	7.0	-	3.0	1.6	1.6
National total		119.0	246.0	175.6	1 470.2	868.5	0.61

Source: *Qualitas AgroConsultores*, from INE, 1997 and 2007.

(2008–2011[2]) in order to understand the reality of the rural context and traditional crop management (described below).

Farmers in each macrozone were interviewed, with the aim of understanding how important quinoa crops are in their holdings and how they manage their landraces. The sample analysed is representative of quinoa's importance in the three macrozones: 31 farmers in the northern macrozone (regions I and II), 26 in the central macrozone (region VI) and 34 in the southern macrozone (region IX). The data (quantitative and qualitative) were handled statistically, so as to describe quinoa producers and create decision trees by macrozone, underscoring the most relevant points of quinoa landrace dynamics.

Quinoa in the northern macrozone: managing diversity through tatas *(men over 60 years of age)*

Quinoa producers in northern Chile are mostly found in the commune of Colchane and in the town of Cancosa in the commune of Pica, and a minority are found in the town of Socaire in the commune of San Pedro de Atacama. The commune of Colchane has the largest area in the northern macrozone, and is home to 75% of the farmers in our study. Colchane is located at 3 800 m asl in the Chilean Altiplano, and is one of the eight rural communes in the first region of Tarapacá, located 262 km from the coastal city of Iquique, Tarapacá's regional capital. A total of 99% of its inhabitants (1 649) are indigenous (Aymara/Quechua), and they are organized into communities of neighbours (*ayllus*), which explains quinoa's traditional presence as a main crop, with potatoes in second place (INE, 2002). Since ancient times, the agricultural system in this zone has involved these two products and camelid livestock (llamas and alpacas) (Arar, 2009). Farming work is performed as part of community labour (*ayne*). Part of the traditional food strategy involves exchanging products with communities from other agro-ecological zones, such as vegetables in the foothills (Vidal, 2012).

Traditional methods are still used in quinoa cultivation, which is characterized by: an absence of chemical fertilization or pest and disease control (though this is not absolute, and products aimed at increasing production are beginning to appear); a low level of mechanization throughout the production process; and no cultivar selection.

Though quinoa is the region's most important agricultural product, the largest average farm measures only 3.6 ha in Colchane, 1 ha in Cancosa, and < 0.25 ha in Socaire. Traditionally, growing quinoa begins with soil preparation (November, December and January during fallow crop rotation, lasting until August or September). Seeds are planted in August/September (especially September, when moisture accumulation in the soil is generally more suited and frosts are less frequent). Crops are harvested earlier in Socaire (Jan-Mar.) compared with zones such as Colchane, where most activity occurs in April-May. Traditionally, seeds are planted manually and deep in the soil (sometimes up to 30 cm), to take advantage of the soil's moisture until seasonal rainfall can sustain the plants' growth (Lanino, 1976).

Soil recovers its fertility through crop rotation and the complementary raising of llamas. Llamas eat vegetable residue from the plant and provide organic fertilizer during fallow years (Arar, 2009). One of the most common practices is planting several types of quinoa, on the basis of the land parcels' relative exposure to the cold and frost (preferably red or pink varieties). These types of quinoa are recognizable by the colour of their seeds, and there is a secondary classification based on plant and inflorescence size. The most common types are: red (*lirio* in Aymara), pink (*canche*), white (*janku*), yellow (*churi*), brown (*chullpe*), dark red (*pandela*) and orange (*pera*).

All the subjects interviewed stated that they regularly consume quinoa as part of their diet, prepared in various ways. Quinoa plays an important role in the traditional cuisine of the Chilean Altiplano, as illustrated by its many culinary uses. Each type of quinoa has features that make it ideal for a specific dish: some varieties are better for stews or soups, others for grains, bread or toasted flour (*pito*), and some for desserts.

In 2008, each producer grew an average of 2.4 varieties of quinoa, out of the nine varieties found at the commune level. This means that most rural producers may plant varieties with the best features for their production, consumption or sales goals.

[2] Proyecto IMAS, ANR-Francia.
http://imas.agropolis.fr/ y http://www.quinua-chile.cl

Of the farmers interviewed, 70% grew more than one type of quinoa – further evidence that quinoa is a potentially important tool for limiting environmental risk. Nearly all farmers who grew only one type of quinoa planted white quinoa, for its colour and culinary features. For those farmers who grew two or more types, white quinoa was always one of the varieties grown.

The interview results made it possible to study quinoa's agricultural biodiversity in the Chilean Altiplano in greater depth (Table 2). It was possible to identify producers growing more than three varieties, and of these, 17% grew four or more varieties. These producers are considered a resource – because of theirknowledge of the local varieties they manage, and because of the seed dissemination they carry out within the territory via their social and professional networks.

To conclude, although producers have a broad knowledge of different types of quinoa, none of them grow or know every type available. Promoting the creation of mechanisms or spaces for the exchange of traditional knowledge is necessary to avoid later risk of genetic erosion and loss of quinoa germplasm conserved *in situ*. Additionally, growing quinoa may be further compromised by the advanced age of the farmers who operate in the region: the *tatas*, who use household labour (planting and harvesting). Quinoa's agricultural system undergoes continuous change in terms of management, especially considering that approximately 25% of young people remain in the rural zones of the Altiplano, with large numbers migrating to the

Table 2: Quinoa diversity management criteria according to production macrozones

	North	Central	South
Number of local varieties per holding	3-5	1	1-3
General characteristics of types grown	less or no photoperiod sensitivity in number of grains	photoperiod sensitivity	photoperiod sensitivity
Seed origin	passed down in families for generations within communities, and at Aymara fairs with Bolivia or Peru	passed down in families for generations, traded with neighbours, disseminated via the *Cooperativa Paredones* [Paredones Cooperative]	passed down in families for generations, *Trafkintu* (seed exchanges; trade), and agricultural modernization programs
Improvement	selection of red and yellow populations with a broad genetic base (UNAP)	Search for improved varieties by the *Cooperativa Agricola Las Nieves* [Las Nieves Agricultural Cooperative]	only registered improved variety in Chile: *Regalona* from private company *Semillas Von Baer*
Links between producers	strong communities, but competing over power, territorial conflicts	isolated	strong communities with links between regional sectors, conflict over numerous issues (forest management, water)
Public rural extension institutions (Prodesal/INDAP)	subsidies for livestock	basic technical support and fertilizer subsidies	dissemination of varieties, including *Regalona*
Producer organization	two cooperatives which are able to guide sales based on specific seeds: orientation according to market demand. New organizations are limited in their ability to offer producers a good price (now less than in Bolivia)	one cooperative which has conflicts of interest with some producers, as few are partners and the price varies between members and non-members. Solely export-driven (less biodiversity) with organic certificate bringing added value.	DAWE Project (with CET-Sur) for rural certification that retains the value of diverse seeds. Local market promotion and added value for seed diversity in restaurants.

Source: Proyecto IMAS (ANR), 2010

nearby cities of Iquique and Arica. This panorama endangers the workforce available for quinoa management. The exodus from rural communities influences the evolution of local customs and affects the traditional indigenous community structures that underpin traditional farming practices and landraces today.

Quinoa in the central macrozone: a product of ancestral and isolated rural growers

Quinoa is still grown in some areas of the coastal dry lands in region VI in B. O'Higgins and region VII in Maule. Despite a major reduction in cultivated land in the area – due to land use changes over the past decade with areas now dedicated to forest plantations (conifers) – some producers have maintained quinoa crops as a family tradition, in a region where the major crops grown are wheat, potatoes and legumes. The quinoa-growing area is often small and may measure just a few rows or be in a land parcel measuring 1–4 ha (Alfonso, 2008). Producers who plant large areas (around 10 ha) are landowners, while other producers rent most of their growing land, or have agreements in which they pay a percentage of their production (sharecropping). These producers may have another job, not on the holding (companies, agricultural industries etc.) in order to supplement their farming income. Most farmers are also elderly (average 65 years), and they only grow white quinoa. In 38% of cases, they acquire quinoa seeds through family members, while 46% obtain seeds from neighbouring farmers.

Chile's coastal dry lands in regions VI and VII have the country's highest poverty rates. Natural conditions limit agricultural development – rainfall is only 650 mm/year over a 5-month period, and those areas in the vicinity of estuaries are characterized by saline soil. As a consequence, farmers select landraces adapted to the challenging conditions, and a group of farmers in the communes of Paredones, Pumanque and Pichilemu (region VI) now have increased income from quinoa sales, thanks to the organization of an agricultural cooperative, *Cooperativa Agrícola Las Nieves.*

When describing the various crops planted in the region, it should be noted that wheat, corn, barley and oats are important to farmers, and take up a significant portion of individual farms. Quinoa crops represent the smallest share of farmland in the commune of Paredones, accounting for an average of only 34%, while in Pichilemu this figure is 61%, and in Pumanque 49%. Figures from the commune of Paredones indicate that farmers give greater importance to products yielding higher sales volume. Quinoa, on the other hand, is historically destined for family consumption, or for sale in small volumes to individual buyers. In the commune of Pichilemu, producers place greater importance on quinoa crops, despite having a smaller average growing area per farmer (3.03 ha).

The various tasks performed by quinoa producers in these regions take place as follows: soil preparation from August to November; seed planting in October and November; harvest between February and April.

Farmers for whom the most important crop in terms of area is quinoa (28% of producers) have an average area of 6.3 ha per farmer. Quinoa takes priority over other products grown, accounting for almsot 60% of the growing area. These farmers have close ties to the *Cooperativa Agrícola Las Nieves*, and also created the company *Agrícola Las Nieves Limitada*, with the aim of strengthening sales for export. The company may also acquire additional quinoa supplied by small-scale farmers in the region. For a second group (representing almost 21% of the producers interviewed), quinoa is an important crop and takes up half of their growing area. This group alternates quinoa on a biannual basis with grains (wheat or oats) or potatoes. Of this group, however, only 11% claim to have links with the *Cooperativa Agrícola Las Nieves*; this is in part due to the fact that they can obtain higher prices by selling privately.

A total of 51% of farmers dedicate less than 1 ha of their land to quinoa crops. These producers are usually landowners who inherited their farms. They produce primarily for their own family's consumption and occasionally sell their quinoa to tourists or neighbours, and therefore do not have ties to the *Cooperativa Agrícola Las Nieves*.

The information gathered was used to determine how the farmers identify their seeds, and it was noted that some of them only grow "white" quinoa. Several synonyms (white, golden, yellow) were used for what is, according to the farmers, the same type of quinoa. The perception among the farmers was that there was only one quinoa crop seed in the study area, and it was called by different names depending on the location. Agricultural practices were then analysed – from the planting period through to harvest – and it was demonstrated that there are several paths to selection among isolated groups of quinoa producers in the region. The dates of various agricultural tasks related to growing quinoa were identified for each producer in the three communes in the study area. The results revealed that the harvest period is concentrated in the period from February to April. In Pichilemu, 80% of farmers planted in August to harvest in January. The uniform nature of the agricultural practices adopted suggests that the farmers are growing the same type of quinoa. In the commune of Paredones, most planting is only done in November, although there are two major harvest periods. Approximately 33% of the crop is harvested in February and 50% in April. This suggests that there are at least two types of quinoa, early and late. On the other hand, in Pumanque, planting often lasts 4 months (July–Oct.), with homogenous distribution among the farmers (25% each month), although crops are usually harvested in April (80%). This suggests that there is one type of quinoa with a high photoperiod coefficient. In addition to considerations relative to crop management practices, it was also noted that some farmers select types of quinoa with higher tolerance for saline stress, as they cultivate land that is naturally penetrated by salty coastal waters near river mouths (Ruiz-Carrasco *et al.*, 2011). Therefore, management of farmer production systems and the dynamics of crop management in the central zone have led to a high level of quinoa type biodiversity, as revealed by molecular genetic analysis (Fuentes *et al.*, 2012). Nevertheless, recent high sales of seeds in the zone may have negative repercussions in terms of potential loss of genetic diversity, as seeds may become homogenized throughout the region to respond to potential market demands.

Quinoa in the southern macrozone: a tradition in Mapuche women's gardens.

Quinoa growing in southern Chile is currently practised on a small scale, performed mainly by Mapuche women, who grow it in small gardens near their homes, together with vegetables, as is the tradition in this region. Growing areas are usually less than 100 m2, and may reach 0.5 ha (Aleman, 2009). Such small growing areas are not generally registered in the Chilean National Agricultural Census (Table 1), which explains the fact that quinoa cultivation in the south is relatively unknown. Mapuche quinoa is always sown in animal pens or with large amounts of manure. This feature is uncommon in other regions, where quinoa is considered a crop requiring few or no inputs (fertilizers, pesticides etc.) for its development. Quinoa is often sown in gardens alongside corn, bean and potato crops to protect the latter from strong summer sun (Alfonso, 2008). The major difference between quinoa in the Altiplano and *kinwa* or *dawe* in the Mapuche language is that the latter is produced in regions with higher precipitation and lower altitude. It differs from quinoa from the central macrozone, in particular with regard to its adaptation to arid conditions, seed type (colour, size), higher productivity and photoperiod (Anabalon and Thomet, 2009). These contrasts are also the result of different management practices (crop density and planting depth) due to the low fertility and moisture. In the south, quinoa seeds are sown densely at a shallow depth via broadcast seeding (Thomet *et al.*, 2003).

The various types of quinoa found in this zone have been handed down in families for generations, and have been spared most agricultural modernization programmes (Thomet and Bazile, 2013). The traditional peasant/indigenous system in this region is characterized by highly diverse crops and landraces, and the wide range of uses for quinoa within families or communities – for example, it is used for human consumption, poultry feed, or preparing *mudai* (a traditional drink for Mapuche celebrations, recommended for pregnant women and medicinal purposes) (Aleman, 2009).

Descriptors have been developed to characterize landraces of quinoa, including the following features: inflorescence colour, seed colour, number of days between planting and harvest, size of seed and number of seeds per gram, inflorescence density, nutritional value and usability (Sepulveda *et al.*, 2003). According to the phenotype of quinoa in the southern macrozone, quinoa in the south can be classified as "early", with a period of 68–80 days between sowing and flowering (130–150 to harvest) (Figure 5).

Sowing dates depend on the features of the location and on theagricultural techniques adopted (mechanized or manual seeding). When this task is performed also depends on the depth at which seeds are sown, which in turn is associated with the moisture conditions (1–3 cm). In moist soil, seeds are sown later and at reduced depth. Given that yield decreases significantly with later sowings, this technique should only be adopted to avoid a late frost.

Interestingly, quinoa grown in the southern macrozone has reached a yield potential of 6 500 kg/ha in garden conditions, with organic fertilizer. This contrasts with the growing conditions of quinoa in the northern macrozone, where yield averages 180–640 kg/ha.

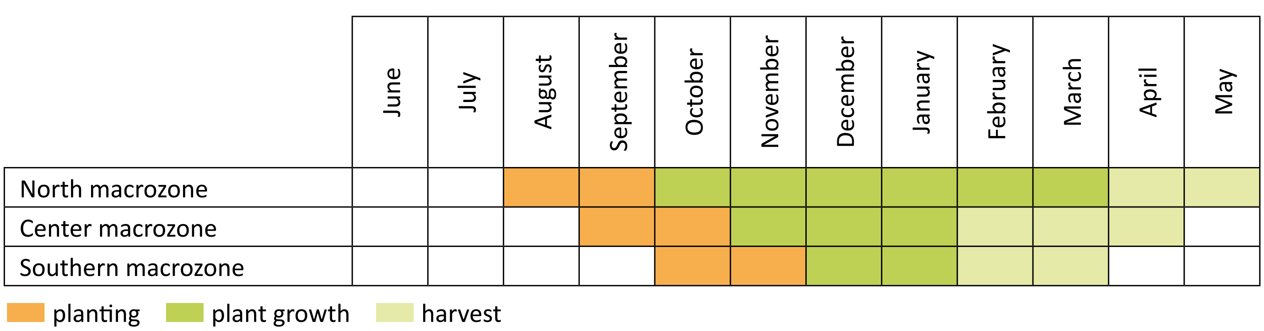
Genetic diversity in Chilean quinoa: *a treasure in farmers' hands*

The varied morphology presented by this crop in the major production macrozones has meant that the Andean farmers (Aymaras) on the arid coastline of the central zone and the Mapuches in the south have taken advantage of quinoa's many forms, using it as a food and for other purposes. For example, a range of plant and seed colours can be seen in quinoa fields, as well as differences in branching and/or plant architecture. Varied seed productivity and major phenological differences can also be observed (Fuentes and Bhargava, 2011; Fuentes *et al.*, 2012). Quinoa is an important genetic resource representing a challenge, as the genetic variables may influence features, such as seed production, seed saponin content, nutritional elements, tolerance to cold and disease resistance. It is necessary to understand these variables in order to increase opportunities for potential new uses.

Combined research has demonstrated evidence of quinoa's ancestral movement from southern Bolivia to the northern Chilean Altiplano, and then southwards. Analysis of various quinoa populations originating from the Andes and low altitude regions of southern Chile reveals that there are two different types of quinoa in the country: the Salare ecotype (found in the Altiplano) and the Coastal ecotype (found in the central-southern region) (Figure 1) (Fuentes *et al.*, 2009b; Miranda *et al.*, 2012; Fuentes *et al.*, 2012). Characterization of wild relatives has also demonstrated that there is a mixed system of self-pollination/cross-pollination in the Coastal ecotype, as well as an active complex crop weed (Fuentes and Zurita, 2013).

Recent investigations using molecular genetic approximations support hypotheses regarding quinoa's genetic relations in the South American Andes (Christensen *et al.*, 2007; Fuentes *et al.*, 2009b, 2012). One such hypothesis is that of Wilson, who points to quinoa's ancestral colonization of southern Chile, and suggests that Chilean quinoa populations came to the region via the Bolivian Altiplano. Our study confirms these hypotheses, revealing that quinoas from Chile's northern macrozone are closely related to Bolivian quinoa varieties (Salare ecotype) (Christensen *et al.*, 2007; Fuentes *et al.*, 2009b). There is also evidence that material from the Peruvian Andean zone was introduced to the Altiplano in the region of Antofagasta (north). Despite this, the dominant morphology in most materials studied in Chile's far north corresponds to

Figure 5: General characteristics of quinoa growing cycles in Chile's different production macrozones



Source: *Prepared by authors Bazile* et al.

Salare quinoa (Fuentes *et al.*, 2009b; Fuentes and Bhargava, 2011).

These genetic relations correspond to the concept of germplasm exchange – a practice that must have existed among pre-Hispanic people from the Altiplano in the north, extending to the central and southern lowlands in modern-day Chile, i.e. the Aymaras, Quechuas (Altiplano 18°–24°S), Diaguitas (30°S), Picunches (32°–34°S), Pehuenches (35°–39°S), Mapuches and Huilliches (40°S). This theory supports a north-south genetic relations model (Fuentes *et al.*, 2012).

Quinoa germplasm in the northern macrozone has been described using molecular genetic approximations that proved far more diverse than previously believed and reported (Fuentes *et al.*, 2009b). The greater diversity that exists in quinoa from southern Chile compared with quinoa from the northern macrozone may be explained by a cross-pollination system between coastal quinoas and *C. hircinum* weed populations. This hypothesis explains to a certain extent the difficulty faced by quinoa breeders in obtaining new, pure cultivars in Chile's central region (L. von Baer, personal communication).

Analysis of quinoa in northern and southern Chile has revealed the existence of shared microsatellite marker alleles. This confirms the theories of Wilson (1988) and Christensen *et al.* (2007), who both reported greater genetic similarity between quinoas in the southern Andean Altiplano and quinoas in southern Chile. Curiously, using the same molecular approximation, quinoas from northern Chile (Altiplano) present a greater number of unique alleles than quinoas from the south (coastal) (Fuentes *et al.*, 2009b). An analysis of existing genetic relations between *C. quinua* from the southern macrozone and wild relatives from the *Chenopodium* genus originating in southern and northern Chile, reveals similarities between *C. quinua* and *C. hircinum* at the nuclear DNA and chloroplast levels, supporting the hypothesis that under growing conditions in southern Chile, quinoa plants present a system of constant intra- and/or interspecific genetic information exchange. This represents the first natural evidence of an active complex crop weed in southern Chile (Fuentes and Zurita-Silva, 2013).

Recent analyses of intrafarm genetic diversity on farm plots in the different quinoa macrozones also compare the effect of seed selection by farmers (e.g. massal selection, the case of seed company AGROGEN). For the individuals sampled, three microsatellite loci (QAAT78, QAAT74, QCA57 – described by Christensen *et al.*, 2007) were used in order to observe an average allele per locus: 0.56 (values weighted by number of analysed plants) for the northern macrozone, 0.7 in the centre and 1.13 in the south (with just 0.2 in the massal selection group in the southern macrozone). This study confirms the concept of genetic diversity in Chilean quinoa based on molecular genetic approximation. Genetic diversity increases in the north and south of the country, and the seed selection process (as expected) revealed a decrease in populational genetic diversity (Martinez *et al.*, unpublished).

The importance of defining a collective *ex situ* conservation strategy: *Building a Chilean national quinoa collection*

Biodiversity data on Chilean quinoa highlight the national and global interest in defining a collective strategy in order to conserve the species' potential, achieve greater complementarity between *in situ* and *ex situ* conservation and lower the risk of losing the germplasm. However, farmers' rights must also be considered, with regard to long-term access to their ancestral heritage in seed banks. Farmers should be protected from the disadvantages of their seeds being reproduced annually outside their native ecosystems, and a diversity management scheme is required that includes traditional practices.

Ex situ conservation is usually carried out on seeds in germplasm banks at the *Instituto de Investigaciones Agropecuarias* (Institute for Agricultural Research) (INIA). The quinoa collection maintained by INIA's national germplasm bank, *Banco Nacional de Germoplasma*, includes a total of 377 accessions, and comprises material collected in 1994, as well as other materials from different sites collected by various national and international centres and organizations (Table 3) (Salazar *et al*., 2006, 2009).

The Semillas Baer company began collecting and conserving samples representing local landraces and quinoa populations grown in southern Chile in 1968, as part of the company's genetic breeding programme in that period (von Baer *et al.*, 2009). In 2001, as part of a cooperation agreement between INIA, the *Asociación de Municipalidades* (Municipalities Association) and the Semillas Baer company, 85 accessions were duplicated and sent to INIA for long-term conservation. In 2008, Baer Semillas sent 77 more accessions from southern Chile to INIA. The collection of the NGO CET SUR, which was begun in 2000, was added to the quinoa materials grown in southern Chile, for a total of 192 accessions, some of which are also currently stored by INIA's national germplasm bank (Madrid, 2011).

Collections made by institutions, such as the *Centro de Estudios Avanzados en Zonas Aridas* (Centre for Advanced Studies on Arid Zones, CEAZA) and the Arturo PRAT University (UNAP), between 2003 and 2006 have contributed to conserving samples representative of local varieties grown in the northern region of Chile (Altiplano ecotype), which, together with the materials conserved by INIA, add up to a total of 121 accessions (Madrid *et al.*, 2001).

Accessions of quinoa grown in Chile's central coastal areas are represented by samples obtained in two collection expeditions: one by CEAZA between 2005 and 2006, and the other by the same research centre, in cooperation with CIRAD (International Cooperation Centre of Agricultural Research for Development, France) in 2010, as part of an international scientific cooperation project, with a total of 64 quinoa accessions representative of Chile'snorthern, central and macrozones.

Most quinoa collections have been developed within the framework of studying and re-evaluating quinoa crops in Chile. For example, Semillas Baer's collection was created as part of the project "*Recuperación, Revalorización y Difusión del Cultivo y Uso de la quinua en cuatro Comunas de la Precordillera de la IX Región: Cunco, Melipeuco, Padre Las Casas y Vilcún*" (Recovery, Re-evaluation, and Dissemination of Quinoa Crops and uses in the Four Lowlands Communes of Region IX: Cunco, Melipeuco, Padre Las Casas, and Vilcun), financed with local funds from INDAP's PRODESAL and PRODER programmes and Semillas Baer. Collections have been made by Arturo Prat University as part of molecular genetic studies programmes (Fuentes *et al.*, 2009b) and programmes on selection and diversified crop use in the Altiplano and Pampa del Tamarugal (Fuentes *et al.*, 2009c; Fuentes and Bhargava, 2011), financed by national institutions such as the *Centro de Investigaciones del Hombre en el Desierto* (Research Centre of Man in the Desert, CIHDE) and *Fundación para la Inovación Agraria* (Foundation for Agrarian Innovation, FIA), as well as foreign institutions such as Brigham Young University (United States of America). Collections made by the CEAZA in the central zone, specifically in the regions of O'Higgins and Maule, were developed within the framework of the project, "*Cultivo doble propósito de Chenopodium quinua (quinua) para la Región de Coquimbo: Modelo de grano para consumo humano y follaje para ganado caprino"* (Two-purpose *Chenopodium quinua* [quinoa] crop for the Region of Coquimbo: Seed model for human consumption and for goat feed), financed by Innova CORFO (2006–08).

Today, all accessions are maintained at the INIA national bank in Vicuña, and 100% of the materials are kept in long-term conservation chambers. Of the samples, 92% have origin information (passport data); only materials donated by AGROGEN and UNAP have duplicates.

Chile does not have a national body regulating access to genetic resources conserved *ex situ.* Since 1995, following a ministerial mandate, INIA has acted as a national curator of Chile's phytogenetic resources, and can authorize access. However, because the authorization process is not mandatory, few institutions recognize INIA's powers in this area. Whether or not to distribute materials is usually decided by the researcher in charge. There is no common policy or coordinated procedure for access to material conserved *ex situ* between the various institutions that store plant germplasm, or between the various centres performing that task within a single institution (Manzur, 2003; Salazar *et al*., 2006). In the case of INIA, distribution depends on the type of material requested and who is making the request. The current system of exchange is rather complex. INIA formalizes germplasm distribution through a materials transfer agreement, which defines conditions for access. Generally speaking, a regulatory framework governing access to genetic resources is lacking, which limits the possibility of research cooperation between institutions that possess germplasm and those that do not.

Table 3: Building the national *Chenopodium quinua* collection conserved by INIA's *Red de Bancos de Germoplasma* [Germplasm Bank Network]

Number of Accesions	Region of origin (Comunes)	Collecting Institution	Collected by	Year collected	Year added to INIA system	Duplicated	Passport Data
73	Region of Tarapacá, Iquique	INIA	A. Cubillos	1994	1994	No	Yes
51	Central Zone, Chile	CEAZA	E. Martínez, E. Veas and P. Jara	2005-2006	2005 - 2006	No	Yes
25	Region ofCoquimbo	CEAZA	E. Veas		2006	No	Yes
85	Region of Araucanía (Melipeuco, Padre las Casas, Vilcún, Cunco)	AGROGEN-Semillas Baer[1]	I. von Baer	2001	2001	No?	Yes
77	Region of Araucanía (Villarrica)	AGROGEN-Semillas Baer	I. von Baer	2008		Yes	Yes
93 accessions of quinoa and 9 of wild relatives	Regions of Tarapacá and Antofagasta	UNAP	F. Fuentes	2003	2009	Yes (but not all)	Yes
13	Central Zone,Chile	CIRAD-CEAZA	D. Bazile	2010	2010	No	Yes
24	Region of Araucanía (Villarrica, Lumaco, Melipeuco)	CET Sur	M. Thomet	1999, 2005-2009	2010	?	?

[1]Now AGROGEN

Source: Prepared by authors Bazile *et al*.

Generally, non-profit conservation centres, such as germplasm banks, depend on state contributions, institutional funds, and national and international cooperation, usually through short-term projects. This does not guarantee any long-term funding. The germplasm bank network managed by INIA was created and opened in 1990 with support from the Government of Chile and the Japanese International Cooperation Agency (JICA). Other institutions create and maintain their collections using funding obtained through projects. Though many types of grants are available, these systems generally require product development which has high costs. Generally speaking, studying and conserving phytogenetic resources *ex situ* is not a priority for national funding (despite signed international agreements aimed at preserving biodiversity). As a result, resources for developing activities, such as surveying, characterizing and evaluating phytogenetic resources, are obtained indirectly through projects including some of these activities among their secondary objectives. It is important to highlight that in the case of quinoa, many research projects focus on *in situ* conservation and sustainable use of genetic resources. As a result, agricultural communities participate directly in the development of these proposals. Nevertheless, due to a government decision to support and develop plant species breeding programmes (*Chile potencial agroalimentario y forestal* – Chile food and forest potential), as well as programmes aimed at the survival and re-evaluation of traditional varieties and species, collection and characterization activities are more viable today.

Conclusion

Quinoa is important to territorial development in Chile, despite its status as a minor crop. Quinoa's ecological diversity is broad, and it is grown in various ecosystems. Therefore, quinoa crops offer new opportunities for territorial development in Chile. Quinoa may serve as a major source of supplementary income for family farms in the north, south and central regions of the country. Quinoa crops may also be useful for crop rotation to improve soil structure. However, against a backdrop of sustainable development, quinoa crop management should be designed in line with the dynamics of its broad biodiversity, while considering the vertical (national needs and cohesion) and horizontal (between local stakeholders) relations at play. A two-pronged vertical management scheme is required, taking into account the need to provide coherence to an extensive swathe of territory, from the Aymara to the Mapuche region. Horizontal or local management is needed, where territorial coherence must translate the need for sustainable territorial development into participation from all stakeholders: farmers and non-farmers, public and private (Bazile *et al*., 2012).

The high level of diversity in Chile's ecosystem, including photoperiod, soil and climate, has generated high genetic diversity in quinoa, as a result of adaptation to salinity and other stress factors. At country level, landraces and the diversity of farming practices today are the result of a process that began with the ancestral civilisations that lived in the southern Andes, thousands of years before the arrival of the European colonists. Quinoa's current diversity is the result of dynamics that were closely linked to the ecosystems and cultures present in those regions. This makes Chilean quinoa very important, as its broad ecological and geographical distribution bodes well for its growing potential in nearly every climate or condition around the globe.

Thanks to quinoa's hardiness, family farmers and stakeholders involved in breeding have a major opportunity to continue the process of creating local varieties and improving various agronomic and nutritional features (Lutz *et al*., 2013; Miranda *et al*., 2013; Schlick and Bubenheim, 1996).

This chapter on quinoa's genetic diversity model reveals major challenges for scientists, crop breeders and rural producers themselves. These challenges come from the development of initiatives aimed at increasing and maintaining collections of quinoa and its wild relatives' germplasms *in situ* and *ex situ*, while also increasing characterization of those resources, and thereby contributing to new genetic improvement programmes (classic and participative) and revealing the true power of current germplasm collections.

On the other hand, the global demand for quinoa is for organic quinoa, which is a major challenge in Chile if farmers truly want to access the international quinoa market, abiding by the certification criteria necessary to market their wares.

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CHAPTER: 5.5

Argentina

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Abstract

Although Argentina is not a major quinoa producer in the Andean region, quinoa crops have been present in the country for millenia. Archaeological evidence suggests quinoa was grown by hunter-gatherers in the Altiplano, in the province of Catamarca. Traces of seeds and stems of varying degrees of domestication have been found in various sites in the provinces of Salta, Catamarca, Tucumán, San Juan and Mendoza, and it is known that quinoa was once grown in the lowland provinces of Santa Fé and Córdoba. Today, however, quinoa is only found in the Andean region in northwest Argentina (NWA), and in part of the Andean Patagonia, in the provinces of Neuquén and Chubut. The recent interest in quinoa is twofold: commercial, given its current profitability, and cultural, as part of the effort to preserve the cultural heritage of the indigenous peoples in NWA and Patagonia. This interest has given rise to several projects focused on commercial production and crop preservation; projects in some communities are linked to the culinary demands of tourists.

Despite quinoa's narrow latitudinal (22°10'–25°14') and longitudinal (65°–67°31') range of cultivation in northwest Argentina – compared with its geographical distribution as a species – its altitudinal range is broad (2 334–4 012 m asl), with great climatic variation (from < 40 to > 700 mm/year of precipitation and an annual average temperature of 6–17°C). This context has led to substantial environmental diversity, linked to wide, strongly structured genetic and phenotypic variability, corroborated by both molecular studies and morphophenological characterization. These studies have detected four genetic groups: Altiplano, dry valleys, transition zone and wet eastern valleys. These groups are related to climatically similar environments in Bolivia, Peru, Chile, Ecuador and Colombia, which is an indication of the geographical-environmental continuity in their distribution. Studies also point to: i) a prolonged presence, allowing for genetic differentiation in local populations, and ii) high available phenotypic variability, providing high selection potential for various traits. Of these, development

duration is the most variable, and it explains the differential adaptation of genotypes.

Ancient quinoa in present-day Argentina

Quinoa has been grown in present-day Argentina for thousands of years. A decade of renewed interest in its study, together with pioneering research by Hunziker (1943a, b) and Hunziker and Planchuelo (1971), provide a more complete, though fragmented, snapshot of pre-Hispanic quinoa cultivation, processing and consumption. These studies led to the findings of sedes and stem fragments revealing various degrees of domestication, as well as microscopic plant remains on ancient tools throughout the country, from the extreme north in the province of Jujuy, to San Juan and Mendoza in Cuyo, passing through Salta, Catamarca, Tucumán and Córdoba. Most information comes from the mountain and valley environments of the (arid or semi-arid) Puna, although some data are available from the eastern forests and the neighbouring regions of El Chaco. The information gathered largely depends on the environmental or micro-environmental preservation conditions, and on the collection techniques used in archaeological studies.

The oldest evidence of quinoa use known to date comes from hunter-gatherer groups who lived in the Puna of Catamarca, approximately 3 500 years ago. These groups began raising camelids while developing horticulture for domestic consumption, at around 3 600 m asl. Findings of quinoa stems with morphological similarities to quinoa in the Peñas Chicas site 1.3 (Antofagasta de la Sierra) (Aguirre, 2007) show that dwellings, growing fields and areas where the crops were processed after harvest were very close together (Hocsman, 2006). After that period, stalk fragments found at Cueva Salamanca 1, El Aprendiz, Punta de la Peña 9 and Punta de la Peña 4 indicate that quinoa crops were present in the area until at least 1440 A.D. (Arias *et al.*, 2013; Rodríguez *et al*., 2006*).* These findings reveal a sustained local tradition of growing and using quinoa in the region, that lasted at least 3 000 years.

Food preparation areas in dwellings, waste pits, small plant storage areas and containers preserved in Antofagasta and other sites, such as Cueva Cacao 1 (Escola *et al.*, 2013; Rodríguez *et al.*, 2006; López Campeny, personal communication, 2012; Olivera, 2006; Pintar, personal communication, 2012), reveal quinoa seeds and other pseudocereals (*Amaranthus* and *Chenopodium* spp.) that correspond to the timeline of the stems found, between 1000 A.D. and the colonial era. In some cases, quinoa seeds have been recovered together with panicle fragments (infloresence branches, flowering stalks, with fruit still attached and wrapped in the parigonium). These are the ancient remains of quinoa prepared following the harvest (Escola *et al.*, 2013).

In various sites settled by established agropastoral groups in the southern Puna (Punta de la Peña 9, Punta de la Peña 12, Casa Chávez Montículos 1) between 300 and 800 A.D., large stone tools with blades – similar to those of modern knives – and scrapers have been found. Microscopic plant residue from quinoa, cañihua (*Chenopodium pallidicaule*) and amaranth (*Amaranthus* spp.) and their predatory moths have been found on the tools' edges, suggesting they may have been used for various agricultural tasks, including harvesting of pseudocereals (Escola *et al.*, 2013). Some of these tools have been found near places that may have been dedicated to growing quinoa at high altitudes, such as small and floristically diverse gardens, similar to those found today on low-lying terraces near small, permanent streams. Similar tools have been found in various sites in the Catamarca valleys of El Bolsón, La Ciénega, Hualfín, El Cajón and Yocavil; studies have not yet been conducted to determine whether they were also used for quinoa harvesting and processing, like those found in the Puna. The knife-scrapers and stem fragments suggest a common harvest process: people cut the stalks at a certain height rather than simply pulling up the whole plant. This would avoid soil loss and provide opportunities for post-harvest manipulation.

At least one pre-Colombian threshing structure, similar to those found in the Bolivian Altiplano, has been documented at the Punta de la Peña 9 site (López Campeny, personal communication, 2012; Escola *et al.*, 2013).

Besides the Catamarca Puna cases, the only known records of *Chenopodium* seeds in the Altiplano with morphological similarities to quinoa are carbonized seeds found in dwellings belonging to early agropastoral groups in the province of Salta. One such site is a waste pit in the village of Matancillas

2 (San Antonio de los Cobres), dating from around 1 A.D. (Muscio, 2004). The other is approximately 450 years older, and located in the living spaces of the Puente del Diablo cave (Lema, personal communication, 2013). More recently, quinoa has been identified in a shelter near the town of Angosto (department of Santa Catalina, Jujuy), close to the Bolivian border and south of the Cordillera de Lípez (Nielsen, personal communication). These exceptions aside, existing data for NWA come from valleys and ravines located at 2 500–3 500 m asl. Notable discoveries include *Chenopodium* spp. seeds dating from the first few centuries A.D., found in the living spaces of valley- and mountain-dwelling farming groups in Cardonal, Valle del Cajón, in the province of Catamarca (Calo *et al.*, 2012). One of the best-known pioneer cases in Argentina was initially studied by Hunziker (1934a) and dates from approximately 750 A.D.

The case involves quinoa, *Chenopodium* sp., *Amaranthus caudatus* var. *leucospermus* and var. *alopecurus* and *Amaranthus* spp. seeds found inside a container at a burial site in Pampa Grande, Serranías de las Pirguas, in the eastern forest (province of Salta). Later cases include: Alero Los Viscos in the El Bolsón Valley, Catamarca, where *C. quinoa* and *A. caudatus* seeds dating from around 1000 A.D. were found in a residential waste pit; and Las Máscaras Cave in the same valley (Korstanje, 2005) and Los Corrales Cave in the Tafí Valley (province of Tucumán), where wild *A. caudatus*, *Chenopodium quinoa* and other *Amaranthaceae* seeds have been found (Arreguez *et al.*, 2013). These represent the southernmost and easternmost enclaves where ancient quinoa seeds have been discovered in NWA. Many seeds resembling quinoa have been found in various sites in the north Calchaquí Valley in Salta (Puerto de La Paya, Cortaderas Bajo, Valdez and Portrero de Payogasta). These findings date from the era of Inca occupation in the area, 1520–1586 A.D. (Lennstrom, 1992), suggesting that sociopolitical circumstances neither interrupted regional use of pseudocereals nor restricted the access of social groups to them. Therefore, enclaves in the high valleys and eastern forests of the Puna in NWA where quinoa seeds and stems have been found outline a regional counterpoint sustained over time, along with early agricultural development, which continued well into the middle of the second millennium A.D.

Beyond the traces left on knife-scrapers and stem cuttings in the southern Puna, quinoa-growing sites in this region have been particularly non-conclusive and difficult to study; there is little documentation in archaeological records of farming structures or nearby dwellings. Nevertheless, there is microscopic evidence (pollen and other microfossils) of *Chenopodium* and *Amaranthus* dating from around 1000 A.D., indicating that quinoa may have been grown on the hillsides of Catamarca's high valleys, for example: at the site of Morro Relincho (Korstanje and Cuenya, 2008); in nearby Cueva Cristóbal in the dry Puna of Jujuy (Babot *et al.*, 2012); in the Chaco forest, near the eastern rainforests also in the province of Jujuy; and at the Moralito site, where elevation drops to 550 m asl (Echenique and Kulemeyer, 2001). Transitional environments like these would have been advantageous for diversifying plants, including quinoa. Numerous seed records point to a pre-Hispanic horticulture model characterized by wild–cultivated–domesticated complexes, dominated by associations of multiple pseudocereals (Lema, 2010). Beyond the geographical limits of NWA, pollen records indicative of quinoa and *A. caudatus* crops grown on small farms go back to the late pre-Hispanic settlements in the central Sierras of Córdoba, confirmed by seventeenth-century colonial records (Medina *et al.,* 2008).

Further south, Cuyo has sound archaeological evidence of quinoa seeds. There are more sites than in the northwest reporting accumulations of dried and thermo-altered seeds, presenting a degree of phenotypic variation. Settlements on the high slopes of the Frontal Cordillera in the province of San Juan, at 2 500–3 500 m asl, such as Gruta de los Morrillos de Ansilta, Vega de Los Pingos, Gruta Granero, Alero del Lagarto, Punta del Agua de Los Morrillos and Río Fierro, reveal early domestic agriculture including quinoa and beginning in approximately 500 B.C. (Lagiglia, 2001). The seeds date back as far as those recorded in the Puna; they were found in contexts corresponding to dwellings, burial sites or places of worship where quinoa was left as an offering or burial gift. At the same time, and as part of the same process of regional advancement towards domestic agriculture, approximately three sites in the valleys of Mendoza have revealed early records of quinoa or morphologically similar seeds, dating from 400 B.C. – 200 A.D. Agua de las Tinajas

I is a dwelling where *C. quinoa* var. *quinoa* and var. *melanospermum* seeds, inflorescences and stems, and *C. chilense* fruit (which may have been a weed) were collected (Castro and Tarragó, 1992; Bárcena, 2001). Similar findings at lower (i.e. older) levels may indicate quinoa's earlier – though uncertain – presence in northwest Mendoza from about 2000 B.C. (Bárcena, 2001). On the contrary, samples of *Chenopodium* sp., *C.* aff. *hircinum* and *Amaranthus* spp. described in Agua de los Caballos 1 have been interpreted as intrusive elements reflecting local flora (Hernández *et al.*, 1999–2000). The third early site in Mendoza is Gruta del Indio, studied in depth by Hunziker and Planchuelo (1971) (Lagiglia, 2001; Castro and Tarragó, 1992). In this sacred burial site, many seeds that may have been assigned to *C. quinoa* and *C. melanospermum*, *Amaranthus caudatus* and *Amaranthus* spp. were originally contained in bags made of plant fibres. It would appear that this group of seeds was kept in use by the farmers from Cuyo who occupied sites in Mendoza, including Reparo de las Pinturas Rojas, Reparo del Salto del Morado , Cave 1 in the Escorial Cerro Negro and Reparos del Rincón during the first millennium A.D., until as late as around 640 A.D. (Lagiglia, 2001; Bárcena, 2001). In the province of San Juan, there is material dating from 700–950 A.D. (Gambier, 2002). Farmers in the area continued to grow quinoa well into the second millennium A.D. after the Spanish colonization (Bárcena, 2011).

Traditional uses of quinoa during the pre-Hispanic era are rooted in practices that continue to this day in NWA. There is now a greater understanding of these activities thanks to the study of microscopic traces preserved on archaeological tools, often in the very sites in NWA where the quinoa seeds and/or stems originate. We therefore know that, as well as using the quinoa kernels, pre-Hispanic societies also took steps to remove the saponins from the grains. Quinoa was roasted, husked and ground to create flours, later used to create a dough or thickener – as revealed by microfossils similar to modern *C. quinoa* and *C. pallidcaule*, found on mills, mortars and pestles belonging to the inhabitants of the Catamarca and Jujuy Puna, from about 2700–2500 B.C. onwards (Babot, 2011; Babot *et al.*, 2012). The same preparation techniques were applied in hillside and valley environments, according to records of microremains similar to *Chenopodium/Amaranthus* found in mortar stones belonging to farmers in the Tafí Valley (Babot, 2009). In the Jujuy Puna, the various ceramic container fragments (in exceptional condition) found in the Cristóbal cave provide evidence of culinary preparations dating as far back as 1500–500 B.C.: quinoa as a sole ingredient or added to stews or thick soups with tubers and corn. Fresh leaves were probably used in soups or eaten raw. The presence of quinoa leaves suggests that the plants were grown next to the place where they were collected (Babot *et al.*, 2012). Microscopic remains of popped quinoa made by roasting kernels in ashes have been recorded in a container found in an offering in Antofagasta de la Sierra from around 550 B.C. (Babot *et al.*, 2012). Popped quinoa traces have also been recovered in sites in the Puna, such as Punta de la Peña 9 and Punta de la Peña 4, from as far back as 550 A.D., as well as agglutinated fragments of perisperm, suggesting residue resulting from food preparation in a humid environment. The first ceramic containers in NWA also suggest cooking with quinoa, revealing even older consumption patterns in the region. In addition to quinoa's culinary uses, ilipta – an additive used in the preparation of coca leaves – was also prepared and consumed as far back as the first millennium A.D.; the evidence comes from the remains of thermo-altered *Chenopodium/Amaranthus* starch mixed with bone char found in a small mill at the Los Viscos site in the El Bolsón Valley (Babot, 2009), and from microtraces of coca in the dental plaque of individuals buried in Antofagasta de la Sierra (Gonzalez, Baroni and Babot, 2013).

In summary, to date, records of quinoa seeds and stems have been found in a vast territory extending throughout central and northwest Argentina, from the Salta Puna in the north, to the El Bolsón Valley in Catamarca in the south; from Antofagasta de la Sierra near the Chilean border in the west, to Pampa Grande and the Tafí Valley in the east. If microscopic evidence is also taken into account, the territory can be said to stretch to the Jujuy Puna in the north, and most likely to areas in the Chaco region neighbouring the eastern forests in Jujuy in the east, where quinoa is still grown in environments similar to the Chaco Serrano in valleys to the east and southeast of Tarija, e.g. Abra de la Cruz, Yesera Sur and Yesera Centro (Daniel Bertero, personal observation), and – until 250 years ago – was grown in Sierras de Córdoba in central Argentina (Laura López, personal communication). This may represent a more accurate picture of the scope of ancient quinoa use in this part of Argentina, from its known beginnings around 1500 B.C. to the colonial era. This time frame may also be applied to San Juan and Mendoza in the Cuyo region, the eastern geographic centre of quinoa crops in the southern-central Andes. As studies continue to systematically recover the various parts of quinoa plants, this fragmented panorama will be completed.

In conclusion, we now have a better understanding of many features of quinoa's pre-Hispanic cultivation in the area which is today Argentina: time line, geographical distribution and processing and consumption methods. We are now better able to identify local selection processes and generation of varieties, when molecular and micromorphological methods can be applied to compare archaeological seeds. It was discerned that the selection process began with ancient seeds initially domesticated in the Bolivian Altiplano(Bertero *et al.*, 2013). Further research is required to ascertain how quinoa's early use and longevity are related to the applications of quinoa in NWA and Cuyo today, and to understand its role in the current distribution of modern and relict landraces of quinoa in Argentina. Studies also need to focus on the historical reasons for the near disappearance of quinoa crops in Argentine territory; in recent history and until today quinoa has become a marginal crop, despite its previously important place in daily life and rituals (Storni, 1942). Research is also required to: understand how different genes in varieties of quinoa were segregated; identify the phenotype features of ancient and transitional forms of the crop; and determine whether they were part of a greater network of plants that included other pseudocereals, such as ajara (*C. quinoa* spp. *melanospermum*), cañihua and amaranth. It is probable that quinoa was grown alongside such crops in a context of diversification, development and acceptance; this is contrary to the way it is cultivated today. Early agricultural practices may have been part of an effort to promote diversification (Lema, 2010). Quinoa was later adopted in a wide variety of culinary dishes, used as animal feed and in medicine, as well as for rituals; this may be linked to the shift in agricultural philosophy.

It is clear from evidence of seed offerings to the Pachamama and at burial sites, and of coca leaves chewed by travellers, that quinoa's symbolic importance transcends its nutritional value and dates back millennia in the eastern Andes.

Molecular features of quinoa's native germplasm

Several studies have been conducted on quinoa germplasm collections in recent decades. Initially, these studies were performed using morphological descriptors, and later with molecular markers. These tools are more effective than other evaluation systems: they can be used in any context, and applied to an unlimited number of samples, regardless of development stage.

Biochemical markers were the first molecular tool used to characterize quinoa germplasm (Wilson, 1988). Variations in electrophoretic patterns in 21 isoenzyme loci – together with morphometric data – were used to compare 98 populations from South America. Ruas *et al.* (1999) and Del Castillo *et al.* (2007) used random amplified polymorphic DNA (RAPD) to study the genetics and relationship between various accessions of *C. quinoa* and related species, as well as to evaluate the genetic structure of quinoa populations in the Bolivian Altiplano. Anabalón, Rodríguez and Thomet-Isla (2009) used amplified fragment length polymorphism (AFLP) markers and morphological descriptors to characterize local quinoa varieties in northern and central Chile. Following the development of the first group of microsatellite markers or SSR (simple sequence repeat) in quinoa (Mason *et al.*, 2005), more detailed studies on genetic variability became possible. These markers are appropriate for population studies, given their co-dominant nature and ability to detect a high degree of polymorphism. They have been widely used on various species to evaluate diversity and population genetic structure (Mondini *et al.*, 2010; Asfaw *et al.*, 2009; Naghavi *et al.*, 2010). They were also used to evaluate the genetic diversity of the quinoa collection of the United States Department of Agriculture (USDA) and that of the International Potato Center (CIP) and FAO. The two collections contain complementary entries from Peru, Bolivia, Ecuador, Argentina and Chile, and were evaluated using SSR markers (Christensen *et al.*, 2007). Entries were classified according to two major groups: one, those originating in the Chilean lowlands; the other, those from the Andean region. Many germplasm studies have been conducted, and numerous collections have been compiled in countries in the Andean region (notably in Bolivia and Peru). They do not, however, evenly represent all regions of origin; this is most evident in the case of entries from Argentina – since Argentina represents an extreme point in the Andean complex distribution, it was suggested that it may provide material with atypical adaptations (Wilson, 1988).

Today, quinoa in NWA is grown on small farms between the latitudes 22° and 27.5°S and the longitudes 65° and 67.5°S, either as a sole crop or interspersed with maize or potatoes (Brizuela, 2010). The region presents contrasting environments and landscapes. To the west, the subregion known as the Puna is characterized by high plateaus (average altitude of 3 500 m asl) and a dry climate with wide thermal amplitude, frequent freezes and low precipitation. The east is bordered by the Western Cordillera, which comprises two mountain chains: the western chain with high peaks (some permanently snow-capped) and a dry climate; and the eastern chain, with a lower altitude and wetter climate. They are separated by valleys, the largest of which is the Quebrada de Humahuaca, the main link between the Bolivian Altiplano and the eastern lowlands. Average monthly temperature in this zone varies between 7.5° and 16°C, and annual rainfall is 150–200 mm. To the south, there is a second dry valley known as Valles Calchaquíes, with a climate similar to the Quebrada de Humahuaca (Lorenzini *et al.*, 1999, inDiPPEC section, http://www.dippec.jujuy.gov.ar/clima.html; Curti *et al*., 2012). Long-term research and breeding programmes in countries such as Bolivia and Peru, and support from the United States of America, Europe and Japan (in particular, since the 1990s) have resulted in a major rise in international demand and commercial quinoa production (Risi *et al.*, 1984; Aroni, 1999). The growing interest in quinoa in Argentina is based on two elements: commercial, in the light of its current profitability; and cultural, as part of an effort to preserve the cultural heritage of NWA, including the conservation of quinoa crops in some communities, which is in part linked to the culinary demands of tourists.

It is a real possibility that quinoa will once again become a major crop for farmers in NWA. Faced with the simultaneous challenges of crop abandonment in some zones and the promotion of exotic varieties in others, it is imperative to understand quinoa – its uses and its value – in order to boost production. This critical situation led to the study of local germplasm and genetic diversity distribution, and collection efforts within the framework of a project financed by the Argentine Secretariat for Agriculture, Livestock, Fisheries and Food (SAGPyA) (Bertero, 2004). There have also been efforts to collect materials conserved in other countries (Bolivia, Peru and the United States of America), resulting in a collection of germplasm native to NWA. One such effort – Argentine Ministry of Science and Technology (MINCyT) Scientific and Technological Research Projects (PICT) for Andean Crops – approached the species from the perspective of three characteristics (morphophenological, nutritional and genetic) and investigated quinoa diversity in NWA using molecular markers.

Genetic variability structure of quinoa in northwest Argentina

The collection of quinoa native to northwest Argentina comprises approximately 90 accessions collected throughout the growing region, encompassing all previously mentioned environmental variability, and including cultivated quinoa (the majority), wild quinoa (ajaras) and intermediate forms without precise identification. Costa Tártara *et al.* (2012) described for the first time the genetic variability present in germplasm from NWA. From the native quinoa collection, 35 representative accessions were selected according to their native environment. They were characterized using 22 SSR markers chosen by Mason *et al.* (2005) and Jarvis *et al.* (2008), based on the level of success at amplification, the clarity of visualized patterns and the level of polymorphism detected in each marker. Results revealed high genetic variability within the collection – higher than that of the CIP–FAO collection (Christensen *et al.*, 2007) and the Chilean collection (Fuentes *et al.*, 2008) – with strong structuring. Analysis of molecular variance (AMOVA) clearly demonstrated the distribution of variability at different hierarchical levels (Figure 1).

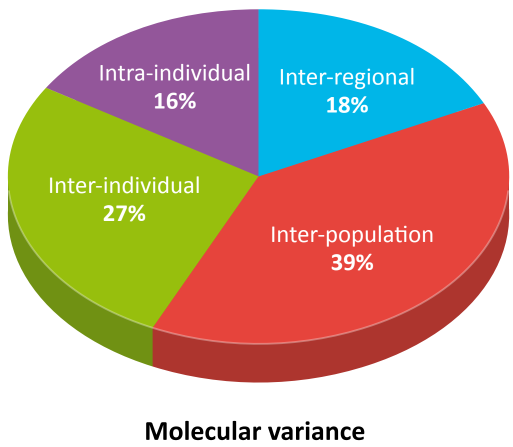

Figure 1: Percentage of molecular variance for each hierarchical level

According to fixation value indices, there is a degree of structure due to regional division (Frt = 0.18). According to the qualitative scale cited by Wright (1978), Fst = 0.57, there is major differentiation between populations. Fis (0.63) and Fit (0.84) values indicate a deficiency of heterozygous genotypes, compared with expectations in each subpopulation and for the total population, respectively (statistically significant F values, permutations test [p = 1 000]) (Peakall and Smouse, 2006). The high degree of differentiation between populations reflects a limited gene flow; this accentuates genetic drift processes and minor influence from activities related to recent exchanges. This is consistent with the hypothesis of a prolonged history of quinoa crops in the region, with seed conservation passed down by farmers for generations. The degree of genetic variability in local germplasm and its structure in the NWA region contradict a common argument among researchers and farmers who suggest that the germplasm found in Argentina stems from recent introduction from Bolivia. Seed exchange with Bolivia does exist, although no influence on local germplasm has been detected to date.

Analysis of local native population groups, on the basis of genetic distance between them, established four groups (Figures 2 and 3), representing the four main growing environments. The level of genetic variability revealed an increasing gradient moving from east to west, with populations from the Altiplano (G2) presenting greater diversity. The isolation and greater environmental homogeneity of the eastern valleys suggest processes accentuated by genetic drift; this is consistent with the lower degree of genetic diversity observed (Costa Tártara *et al.*, 2012).

Principle coordinates analysis (PCoA) obtained via molecular characterization corroborated the groupings obtained by UPGMA, confirming the order of those four groups.

Characterization and evaluation of germplasm collection of quinoa from NWA based on morphological and agronomic features

Evaluating and characterizing germplasm collections allows to understand the quantity and structure of genetic variability in the material studied, while at the same time identifying accessions with desirable attributes for later use in breeding programmes (Franco and Hidalgo, 2003). Characterization and evaluation of germplasm accessions are based on their various features (morphological, physiological, agronomic etc.). There follows a description of the progress made with the collection of quinoa germplasm native to northwest Argentina (NWA), on the basis of characterization and evaluation.

Scope and structure of phenotype variability

Native quinoa germplasm in NWA is highly diverse at the phenotypic level. This reflects variation in its environment of origin. Quinoa accessions from the NWA region present wide variability in their morphological and phenological traits. This is promising from a genetic improvement perspective, as these accessions may be used in breeding programmes to obtain varieties developed to avoid exposure during the most sensitive periods of frost and drought. These are two of the most important factors affecting local quinoa crop production (Geerts *et al.*, 2006; Pouteau *et al.*, 2011; Winkel *et al.*, 2011). On the other hand, variation of quantitative morphological features is relevant for future studies of new accessions of quinoa from NWA and other countries, as they are associated with the accessions' place of origin and altitude, and are consistent with variation observed in earlier characterizations of collections from Peru, Bolivia and Chile (Gandarillas, 1968; Risi and Galwey, 1989a,b; Ortiz *et al.*, 1998; Rojas, 2003; Rodríguez and Isla, 2009; Fuentes and Bhargava, 2010).

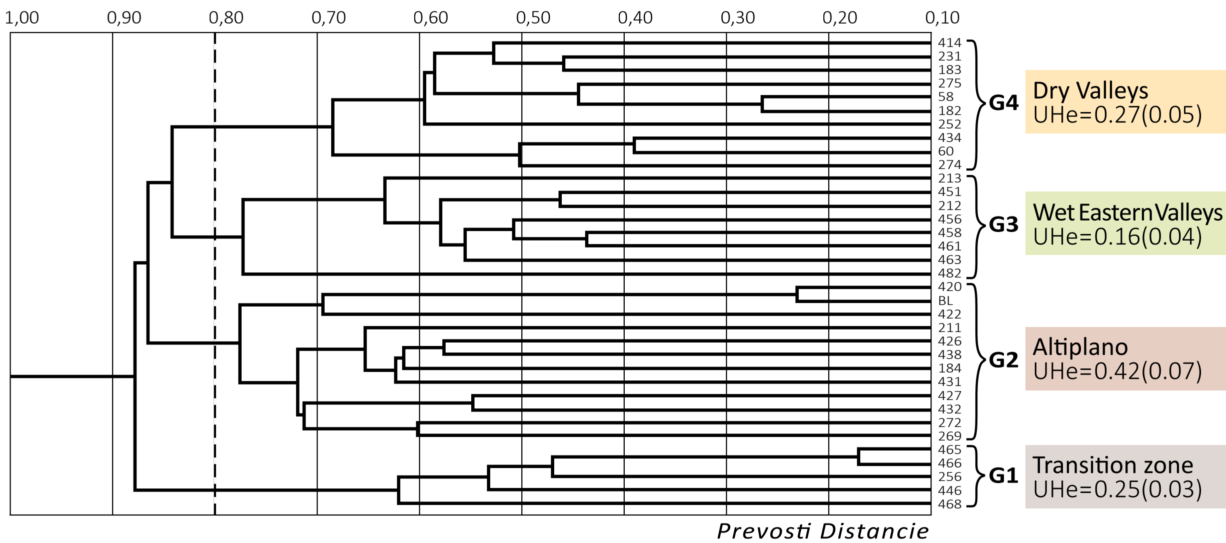

Figure 2: Analysis of native population groups (UPGMA), UHe genetic diversity, SE in parentheses (adapted by Costa Tártara *et al.*, 2012)

Results of a study on phenotype variability structure provided important ecogeographic information on the species, in a region for which there had previously been little information (Jacobsen and Mujica, 2002). In line with multivariate analyses on morphological features, native quinoa accessions from NWA were divided into four groups: transition zone (G1), Altiplano (G2), wet eastern valleys (G3) and dry valleys (G4). The groups are clearly differentiated and associated with their place of origin or altitude (Curti *et al.*, 2012), in line with the molecular characterization study via SSR (microsatellites), performed on the same set of accessions (Costa Tártara *et al.*, 2012). In this sense, the pattern of variability observed in the collection was similar to that of other quinoa germplasm collections (Risi and Galway, 1989a,b; Ortiz *et al.*, 1998; Rojas, 2003; Del Castillo *et al.*, 2008), and is a manifestation of the underlying genetic structure (Costa Tártara *et al.*, 2012). The four recognized groups may present similarities with those proposed by Rojas (2003) in accessions from the Bolivian collection originating in the Altiplano and humid valleys – e.g. G1 (southern Altiplano), G4 (northern Altiplano) and G7 (high valleys). This similarity points to similar genetic differentiation processes that may have affected quinoa throughout the southern Andean region, and indicates that the degrees of dryness and frost observed may be a major factor in ecotype differentiation (Curti *et al.*, 2012).

Characterization of temperature and photoperiod response in NWA quinoa accessions

Agriculture in the Andean region of NWA is exposed to several adverse climactic factors, including drought and frost. This presents a challenge to agricultural development in the region (Bianchi *et al.*, 2005). Most quinoa producers in NWA are small- or medium-scale farmers who traditionally use

Figure 3: Geographical location of 35 quinoa populations native to NWA. Different symbols and colours represent the four main genetic groups, representing four contrasting environments within the region: (circles) Altiplano (G2); (hexagons) dry valleys (G4); (triangles) transition zone (G1); and (squares) wet eastern valleys (G3).

few agricultural inputs on their crops. Therefore, the crop's capacity to ensure local food security is largely dependent on its agro-ecological adaptation to climatic conditions (Aguilar and Jacobsen, 2003). Consequently, germplasm from the NWA region has evolved to be highly diverse, demonstrating adaptation to different local climatic patterns throughout the four ecoregions where quinoa is grown in NWA (Curti *et al.*, 2012).

A study performed on 11 native accessions of NWA quinoa germplasm revealed that duration of development largely explains the phenotypic variation structure. Analysis of variation in time from planting to flowering revealed three phenological groups: early in the Altiplano, intermediate in the dry valleys, and late in the wet valleys, with strong genotype control (high G/G × A relationship) of that variation. An experiment involving six accessions belonging to those three phenological groups was carried out to evaluate the impact of photoperiod on flowering time (visible flower buds). The accessions were exposed to three photoperiods (natural [~12 h], 15 and 18 h) under field conditions at the Faculty of Agronomy of the University of Buenos Aires (UBA). The ranking of duration from emergence to visible flower buds was similar to that in evaluations at the region of origin, and all genotypes revealed photoperiod sensitivity with a quantitative short-day response. The experiment revealed major variation between genotypes in terms of sensitivity for time to flowering, from 4.7° to 30°Cd h-1 (Tb = 3°C). This sensitivity revealed a close negative association with altitude of origin (R2 = 0.98). The highest sensitivity value corresponded to a valley accession and is similar to that previously quantified for genotypes from the valleys of Peru and Ecuador, and the Peruvian Altiplano, but less than that of the 'Nariño' variety from Colombia (60°Cd h-1). The lowest value observed in an Altiplano accession was lower than that estimated in Coastal varieties (12°Cd h-1, 'Baer' variety) in central Chile (Bertero *et al.*, 1999, 2000). Given that genotypes responded to photoperiod in the entire range explored, the threshold photoperiod value and minimum duration of this phase could not be estimated (the latter is an estimate of temperature sensitivity). Nonetheless, the variation observed between genotypes under the natural photoperiod (200–543°Cd) also points to variation for that feature.

Genotype by environment interaction patterns within native germplasm

The quinoa-growing region in northwest Argentina (NWA) presents high environmental variability, both seasonal and spatial. As a result, the site–year combinations used to establish comparative yield studies may complicate the choice of genotypes via strong genotype by environment (G × A) interactions. In a study in which six comparative multi-environmental yield tests were performed on a set of 12 genotypes selected from the germplasm collection, there was strong variation between genotypes and environments for grain yield, its physiological determinants (biomass and harvest index) and numeric yield components (grain number and weight). The proportion of variance explained by G × A interactions for yield was higher than genotypic variance, and lesser – although still significant – for physiological determinants.

Cluster analysis on G × A bimodal matrices (standardized by environment) divided the genotypes into four groups with different response patterns. Environments were divided into two groups based on genotype discrimination. In both cases they comprised genotypes and/or environments of the Altiplano and Inter-Andean valleys, respectively. On the other hand, ordination analysis revealed a repeatable pattern of genotype discrimination, suggesting that the quinoa-growing region in NWA may be divided into two mega-environments. Phenological differences between genotypes, in conjunction with environmental differences in the incidence of mildew (*Peronospora farinose* f. sp. *chenopodii* Fr.) or risk of frost, generated changes in yield rankings between genotypes between environments, and determined specific adaptations to different agro-ecological conditions. On the basis of these observations, it is possible to avoid these interactions by selecting for specific adaptation in each agro-ecological zone. Genotypic variation in flowering time is the main source of genotype variation for grain yield, via its influence on the quantity of aerial biomass in valley environments. The harvest index was the main yield determiner in Altiplano environments. On the other hand, grain number was the numeric component that explained genotype yield variation in both mega-environments.

Quinoa production outlook

There is little background information on quinoa production in Argentina; the crop was not even registered in the National Food Code until 2013. Efforts (often isolated) to preserve and promote quinoa began over 20 years ago, with increased attention since 2001. This culminated in a large (~500 accessions) repository for the safekeeping, study and use of germplasm. The collection provides a broad genetic base to cover regional demand, develop the crop and obtain improved local varieties with resistance to biotic and abiotic factors – essential to face the challenge of production in this new climactic context.

There is potential to increase the area under cultivation and – given the ancient history of quinoa in the region – the crop also has strong cultural roots. The area stretches across the northwest of the country, on an environmentally heterogeneous stretch of land at 1 100–3 800 m asl. The production system is primarily manual, with little use of technology; however, soybean farmers and wine producers have recently been incorporating quinoa crops in industrial farming models (Figure 4). The total growing area for this region is estimated to measure 151 ha, including the provinces of Catamarca (74 ha), Salta (47 ha) and Jujuy (25 ha), with an average yield of 1.25 tonnes/ha, for 133 production units, with an average surface area of 1.14 ha/unit. The provinces of Buenos Aires and La Pampa in southern-central Argentina have a growing area of at least 26 ha with an average yield of 1.6 tonnes/ha (Alarcón, 2012).

Quinoa production in Argentina in 2009–2011 is estimated to be 97–150 tonnes, representing 0.2% of world production. These estimates indicate an average annual growth rate of 8%, with a peak of 30% in 2009. National production for 2013 was thus projected to be 886 tonnes (FAO, 2002–2011). During this year, however, high international prices (~USD3 200/tonne – Estrada Z, 2012), combined with the surge resulting from industrialized farming methods, have prompted farmers in NWA to produce at unprecedented levels. In June 2013, during a meeting on quinoa cultivation attended by 160 producers, a mere three had a combined planted area of 295 ha in Valle de Lerma and Quebrada del Toro, both in the province of Salta. This figure is close to the total 400 ha of quinoa growing area reported in the local press. In the coming production years, the figure is expected to easily reach the thousands.

Most local quinoa production is sold as grain without added value. Nevertheless, in Cusi-Cusi (Jujuy), where local production is aimed at the “functional foods” market, construction has begun of a grain processing plant. There has also been progress in added-value products, with the development of three variations on quinoa: i) popped quinoa, ii) quinoa flour and iii) an ingredient used in confectionery.

Furthermore, the provincial government of Catamarca, as part of a public–private partnership, has launched a project with farmers in the department of Tinogasta. This project includes industrialization of the production of pasta and milk enriched with quinoa, iron, zinc and vitamin C, to satisfy the dietary needs of people in the 4–14 age group. At present, however, the demand from food and confectionery companies for quinoa with added value exceeds by far current production, particularly in the case of companies focusing on *haute cuisine*. The immediate outlook is that of a steady increase in demand that will be difficult to satisfy in the short term.

Farmers in the department of Yavi (Jujuy) have also made significant production efforts. They are registering as local seed and grain producers in Seclantás and Luracatao (Salta), and are receiving guidance on how to obtain varieties using native germplasm.

Finally, it is important to highlight that action is required regarding factors that negatively impact yield. Within a general framework of quinoa cultivation and comprehensive development of the crop, in a highly competitive agricultural context, there is continued progress in terms of technological adjustments for planting, seed production, disease management, herbicide use, and harvest and postharvest operations. State institutions involved in agricultural processes have undertaken these tasks.

Given quinoa’s: i) enduring presence, ii) broad genetic base, iii) hardiness, iv) excellent nutritional value and v) great industrial potential, it seems destined to become one of the country’s most important crops, and the most important crop in northwest Argentina.

Figure 4: First mechanized quinoa harvest in Valles Calchaquíes, province of Salta, 10 June 2013 (photo: Santiago Arnaude).

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Section 6

Experimentation and current distribution

CHAPTER 6.1.1

Adaptation and scope for quinoa in northern latitudes of Europe

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a new crop, currently being tested in northern Europe, where its close relative, fat hen (*C. album*), is already a well-known weed species. During the Iron Age, European fat hen was a secondary crop, either collected or cultivated. Therefore, the present day introduction of quinoa to northern Europe is based on the utilization of a closely related species in ancient times. Quinoa is one of the oldest existing crops, and was first detected by Europeans when Columbus discovered South America at the beginning of the sixteenth century. Quinoa was not then brought to Europe, however, so the crop literally remained unknown outside the Andean countries until North Americans came to Bolivia and Peru in the late 1970s in order to import quinoa as a food product to the United States of America. Quinoa was at that time also introduced to the United Kingdom, Denmark and the Netherlands, where studies on the crop were initiated, to be later followed by trials elsewhere in Europe and the rest of the world. Nevertheless, there is at present very little commercial production of quinoa outside the Andes; but it is increasing, and there is good potential for further expansion of global production. According to FAO, quinoa is regarded as a new world staple and is predicted to spread fast across the globe (FAO, 2013). Due to the increasing global demand for quinoa, both as an Andean export commodity and for agricultural development purposes, there is huge interest in testing quinoa for cultivation under a range of environmental and geographical conditions. One of the environments most distanced from the crop's natural conditions is northern Europe. Research carried out in Europe, from south to north, has demonstrated the potential of quinoa for production under European conditions, with varieties adapted to longer days, increased humidity and intensive mechanization. Most recently, quinoa has successfully been grown commercially in Australia and France, and is on the verge of taking the same step in a number of other countries.

Introduction

The *Chenopodium* genus comprises around 250 species from all over the world. It is considered one of the most nutritious genera in existence, due to its protein and dietary fibre content, as well as healthy levels of fat, ash and minerals (Repo-Carrasco *et al*., 2003). Several species of *Chenopodium* have been independently domesticated. Most domestic forms of *Chenopodium* are grown as seed crops, for example, *C. pallidicaule*, although others, such as *C. nuttalliae* in Central America, are also used as a spinach-like vegetable. The oldest domesticated

Chenopodium species identified to date is the South American quinoa, developed in the Andes about 7 500 years ago (Pearsall, 1992). It reached North America in around 1200 A.D. Other species were independently domesticated, rather than being spread by trade.

In northern Europe, *C. album*, a global weed species, was a secondary crop in Denmark during the Iron Age (1200 B.C. – 400 A.D.) (Stokes and Rowley-Conwy, 2002). Various prehistoric finds from Denmark, such as a deposit of 1.5 litres of seeds, calculated to comprise 2.4 million seeds, from the first few centuries A.D., demonstrate the separate gathering or cultivation of this species for food (Helbaek, 1954). Seeds of *C. album* were also present in the stomach contents of the bog bodies from Tollund (Helbaek, 1950) and Grauballe (Helbaek, 1958). The plants for these meals were probably deliberately harvested by Iron Age farmers, who collected the whole plant for subsequent threshing and drying (Glob, 1969). *C. album* seeds are also known to have been used at the site of Voldtofte, Denmark, due to finds from as early as the Late Bronze Age (1570–1200 B.C.) (Rowley-Conwy, 1982, 2000).

C. album was also used as pasture for milking cows in Denmark during the Second World War (1940–45), as farmers discovered that it secured good milk production. The question is whether the Danish and European adaptation of *Chenopodium* should concentrate on *C. album* or *C. quinoa.* It was decided to focus on quinoa, as it is a long journey to transfer a wild species, such as *C. album*, to a crop (Risi and Galwey, 1984, 1989a; Jacobsen, 1997). Although quinoa is a tropical crop, it is also a highland crop and grows at relatively low temperatures.

Crop adaptation

It is necessary to adapt well-known crops to a range of stress factors, both abiotic and biotic, some of which are aggravated by actual and predicted climate changes. These stresses will necessitate the search for adaptation to photoperiods of new regions, especially in crops with good tolerance to stresses, such as quinoa. The standard approach for adaptation of crop species and cultivars to new day lengths and thermal environments has been to manipulate flowering to match phenology with specific climatic conditions, and to regulate the number of days of the plant growth cycle spent in vegetative and reproductive stages (Bertero *et al*., 1999; Lawn, 1989; Lawn *et al*., 1995).

Quinoa might be used for crop diversification in Europe and other parts of the world, outside its genetic origin, as an alternative for marginal agricultural land. For this reason, it has to be adapted to new regions of the world outside the Andes.

Historically, quinoa has been continuously selected for new environments in the Andean region, as it spread gradually from its centre of origin around the Titicaca Lake between Peru and Bolivia. The distribution from the lake went both northwards to Ecuador, Colombia and Venezuela, and southwards to Chile and Argentina, as well as down from the highlands to the valleys and coastal regions of the Andean countries. It was a slow process, however, due to the vast range of environments and the irregular climatic conditions in the Andean region (Bertero *et al*., 2004).

The present adaptation of quinoa to new environments has been relatively fast. No other crop has been introduced as rapidly. The introduction of potato, which was brought to Europe when the Spanish invaded South America in the early sixteenth century and was rapidly distributed throughout Europe, was not accepted commercially until 200 years later, around the start of the industrial revolution in late eighteenth century (Chapman, 2013). Soybean originated in China, and soon spread to Southeast Asia under the Ming Dynasty (Hancock, 2004). It arrived in Europe and America in the eighteenth century, but it did not become important outside Asia until the twentieth century (Hymowitz and Harlan, 1983).

Kiwi fruit is another story of a recent global success. It originated in China under the name “Chinese gooseberry”, and in the early twentieth century spread to New Zealand and was then exported to the United States of America just after the Second World War under its new name (Ferguson, 1999). For quinoa, just 50 years ago, no attention was paid to the species, not even in the Andean region, and no development, breeding or scientific research was being carried out. Quinoa was of value solely to the Andean farmers, while in urban areas it was considered a whole grain of inferior quality (Vietmayer, 1989).

In the past, introduced crops took as long as 200 years to attain acceptance and popularity on a broad scale (potato and soybean), while the kiwi fruit managed the same process in only 50 years. Quinoa has so far taken approximately 30 years, and it is close to a global success.

All stages of development in quinoa are sensitive to changes in photoperiod, but in particular the reproductive phase (Bertero *et al.*, 1999). Day lengths over 12 hours produce major detrimental effects on the development of quinoa (Christiansen *et al.*, 2010). The most important effects of an extended photoperiod are seen after flowering as the seed fill and maturation stages are disrupted, hindering continued vegetative growth and flowering (Bertero *et al.*, 1999, 2004; Christiansen *et al.*, 2010). This makes quinoa a facultative short-day plant (Bertero *et al.*, 1999; Christiansen *et al.*, 2010), which means that flowering occurs under any photoperiod, while reproductive development is inhibited by photoperiods longer than those found in its place of origin (Bertero *et al.*, 1999, 2004; Christiansen *et al.*, 2010). It is recommended to study the physiology, as well as the photoperiod effects (Christiansen *et al.*, 2010).

Quinoa has great potential for production in Europe (Galwey, 1993; Jacobsen, 1997; Jacobsen and Stølen, 1993). However, regions interested in introducing quinoa have longer days than those in its centre of origin, and it is, therefore, necessary to carry out studies on physiological mechanisms and photoperiod responses (Adolf *et al.*, 2012).

European quinoa history

Quinoa research breeding programmes were not initiated until the 1960s in the Andean countries (McElhinny *et al.*, 2007). There were some early attempts in Europe to introduce quinoa, but the genotypes of quinoa screened originated from Bolivia and Peru and, therefore, did not mature at high latitudes (Simmonds, 1965). Breeding programmes outside the Andes were initiated in the United States of America and Europe in the 1980s, with the objective of adapting quinoa in terms of early maturity under new climatic and agronomic conditions.

Quinoa's introduction to Europe began in the 1970s, when it was brought to the United Kingdom following recollection expeditions to South America. A breeding programme was initiated at Cambridge University (Fleming and Galwey, 1995; Galwey, 1989; Risi and Galwey, 1984, 1989a, b, 1991). In 1987, the programme was continued in Denmark after the establishment of collaboration between Galwey and Jacobsen (Jacobsen and Risi, 2001). Both countries worked on a broad range of genotypes obtained from earlier British recollections. Uniform lines were developed and given identification codes, but no varieties were registered. Quinoa breeding in the Netherlands began in 1986 based on accessions from gene banks, botanical gardens and universities. After evaluation, uniform lines adapted to the climate of Western Europe were selected (Mastebroek *et al.*, 2002). A stability analysis of the selection time for some quantitative traits of quinoa concluded that height, inflorescence, size and stage of development could be satisfactorily performed in the early stages of a breeding programme, and potential parental lines were identified in one population (i.e. from 14 lines grown during five seasons) for their use in the development of new varieties suitable for north European conditions (Jacobsen *et al.*, 1996). In 1993, a project was supported by the European Union, entitled "Quinoa - A multipurpose crop for EC's agricultural diversification", with field trials in the United Kingdom, Denmark, the Netherlands and Italy (Galwey, 1993). Other countries showing an interest in the crop at that time, in the light of the promising results of the EU project, were Sweden, Poland, Czech Republic, Austria and Greece, who all participated in the American and European Test of Quinoa, supported by FAO (Izquierdo *et al.*, 2003; Jacobsen, 2003; Iliadis *et al.*, 1997, 2001; Ohlsson, 1997). Finland also had trials ongoing (Keskitalo, 1997). Results from the American and European Test of Quinoa showed that the growth period in southern Europe was 100–116 days for the varieties which were able to mature, which is less than the growth period of 110–180 days in northern Europe (Mujica *et al.*, 2001).

In the United Kingdom, quinoa is sold in health food shops, but its main application is as a game-cover crop, alone or mixed with kale. A blend of early-, medium- and late-maturing types of quinoa is sown, mainly for pheasants and partridges, causing natural seed drop throughout the hunting season from October to January (Nicholls, 1996). Quinoa seed for game crops is grown successfully in south-east England. More recently, in Denmark, there has been attention on quinoa for people with coeliac disease as a potential alternative to the cereals, wheat, rye and barley, which all contain gluten (Jacobsen, 1997; Jacobsen and Bach, 1998; Jacobsen and Stølen, 1993; Jacobsen *et al*., 1994, 1996, 1997; Lomholt, 1996). In addition, projects on the production of green pellets from quinoa have been conducted. There is no commercial production of quinoa in Denmark, and Danish consumers currently pay approximately EUR10/kg for quinoa imported from Bolivia. In Denmark and Sweden, yields have been low (if harvested at all), with only European and Chilean varieties maturing (Izquierdo *et al*., 2003). As of a few years ago, improved cultivars of quinoa have been tested as far north as Norway and Iceland. The further north, the shorter the growing season, due to later spring and earlier autumn, both imply lower temperatures.

In the United States of America, during the early 1980s, Colorado State University introduced quinoa at northern latitudes in Colorado, and a commercial production on 500 ha was soon achieved. Today, varieties adapted to the conditions in the centre of the United States of America, in the foothills of the Rocky Mountains, are cultivated on around 50 ha (personal communication). Most of the production takes place in the highlands of the San Luis Valley at an altitude of approximately 2 000 masl.

In Canada, a region similar in size to northern Europe, quinoa has been grown since the early 1990s, mainly in Saskatchewan. The current production level is around 800 ha (personal communication). In the vicinity of Canada, field tests have been initiated in the state of Washington, with the aim of introducing quinoa to the northern United States of America as a staple crop.

In 2009, the first large-scale, commercial quinoa production trial in Europe was carried out. It took place with a French asparagus company AbbottAgra (www.abbottagra.com), now also a quinoa company, in northwest France, in the department of Maine-et-Loire. Production was 140 tonnes of quinoa on 100 ha in 2009, 210 tonnes on 150 ha in 2010, and 270 tonnes on 250 ha in 2011. In both 2010 and 2011, the crop suffered from lack of spring rain. Yields reached up to 3 tonnes/ha, by using Dutch, sweet, relatively late-maturing cultivars.

The distribution of quinoa for research purposes and initiating commercial production in northern Europe is seen in Figure 1.

Figure 1. Distribution of quinoa in northern Europe (marked in red)

Breeding

Breeding of quinoa in new regions should concentrate on uniformity, early maturity, high yield and quality, as well as industrial uses of the seed and of specific ingredients. The ideal variety of quinoa for seed production in northern Europe is one which matures uniformly and early. A growing period of less than 150 days would normally be regarded as beneficial. Quinoa should also have a consistently high seed yield and it should be short and non-branching to facilitate mechanical harvesting (Figure 2). Saponin is a bitter compound present in varying amounts in the seed hull of most cultivars. Their function is a general defence against biotic stresses and for this reason they may be desirable in organic production. However, the presence of saponins requires that seeds be dehulled and washed before consumption – traditionally a labour-demanding process. In the case of commercial production with industrial processing techniques, saponin removal means increased costs. Size, shape and compactness of the inflorescence may be important for the rate of maturation. A large open inflorescence will dry more quickly after rain and morning dew than a small, compact one, but it may also be prone to seed loss, as quinoa is not very domesticated, and there are few modern varieties available. Fodder types should be tall, leafy and late maturing, with a

Figure 2: Two types of quinoa, branching and non-branching

high dry matter yield and preferably a low saponin content. Quinoa should be considered for cultivation in temperate climates, as it offers good potential in organic farming systems. Quinoa has been selected as a potential new protein crop for organic feed in Denmark. Field trials in Denmark have demonstrated seed yields of 2 tonnes/ha, with 12–16% protein content and 6–8% fat. There is wide variation in seed yield depending on year and location; this may be due to crop establishment or to weed control measures and harvest and post-harvest techniques, which still need to be optimized (Jacobsen *et al*., 2010).

In the Netherlands, breeding programmes led to the first European variety, 'Carmen', characterized by low stature, compact panicle and early maturation. Further research aimed to increase yield and reduce the saponin level (Limburg and Mastebroek, 1996; Mastebroek and Limburg, 1996; Mastebroek and Marvin, 1997). A second variety, 'Atlas' – the first sweet, saponin-free variety outside the Andes – was launched. At present, there are both Dutch and Danish varieties of quinoa registered in Europe (Naturerhverv, 2013) (Table 1).

Quinoa crop management in northern Europe

Jacobsen *et al*. (1994) found that quinoa cultivars selected for north European growing conditions were well adapted to sandy soils in Denmark, although a significant yield increase was experienced when the amount of nitrogen fertilizer was increased from 40 to 160 kg N/ha. Yield increased by 16%, 11% and 3% when the nitrogen supply was increased from 40 to 80, from 80 to 120 and from 120 to 160 kg N/ha, respectively. In southern Germany, quinoa cv. 'Faro' and 'Cochabamba' responded well to N fertilization, with a 94% yield increase at 120 kg N/ha (Schulte auf'm Erley *et al*., 2005). N fertilization was effectively utilized for quinoa seed production within the studied range up to 120 kg fertilizer N/ha. Estimated yield potential often exceeds the observed yields, indicating that even higher rates of nitrogen application may increase yield (Ørum *et al*., 2013).
Hoeing increases yield more than harrowing due to better weed control, but overall yield increase can be achieved by adopting either method. Regression analysis showed that crop yield is related to weed dry matter and showed no indications of higher crop damage associated with weed harrowing. Protein content is low when weeds are not treated, and increases significantly when weeds are controlled. In conclusion, inter-row hoeing is more efficient than weed harrowing in terms of weed control. Nevertheless, weed harrowing should be optimized in future trials in narrow row spacing systems, as results indicate that weed harrowing is an effective supplement to inter-row hoeing (Jacobsen *et al*., 2010).

A model expressing yield as a function of plant density shows the optimal plant density for high yield to be 327 ± 220 plants/m^2. This plant density is the top point of the curve relating yield to plant density. However, the large standard deviation indicates that similar yields may be obtained from a wide range of densities (Jacobsen *et al*., 1994).

The inheritance of some qualitative characters is known, including genetic and cytoplasmic male

Table 1: Registered European quinoa cultivars

Cultivar	Origin	Registration	Expiration	Breeder	Note
Carmen	Netherlands	16/6 1997	1/5 2022	PRI	
Atlas		16/11 1999	18/10 2024	PRI	Sweet
Pasto		16/2 2005	30/1 2030	PRI	Sweet
Riobamba		16/2 2005	30/1 2030	PRI	Sweet
Carina		Com		CPRO-DLO	
Dorado		Com		CPRO-DLO	
Serena		Com		PRI	
Puno	Denmark	1/1 2010	13/12 2034	Quinoa Quality	
Titicaca		1/1 2010	13/12 2034	Quinoa Quality	

Table 2: Development stages of quinoa (after Jacobsen and Stølen, 1993)

Stage	*Description*	*Stage*	*Description*	*Stage*	*Description*
0	**Vegetative phase**	8	**Anthesis**	14	**Seed set** 1/3 seed set
1	**Bud formation** Bud covered by leaf	9	Half flowering	15	Half seed set
2	Bud visible	10	Full flowering	16	2/3 seed set
3	Bud distinct			17	Full seed set
4	Bud ca. 0.5 cm	11	**Floral dehiscence** Onset	18	**Maturity** Leaves: Green > yellow
5	Bud ca. 1 cm	12	Most flowers dehisced	19	Yellow > green
6	Onset of pyramid shape	13	Only wilted anthers	20	Mature
7	Distinct pyramid shape			21	Wilted

sterility, and this may be valuable for future breeding (Jacobsen and Stølen, 1993). A developmental stage scale has also been defined (Table 2).

The Danish quinoa accession, 'Olav', has been demonstrated to have a base temperature (temperature at which germination is initiated) of 3°C, an optimum temperature of 30°–35°C, and a maximum temperature of 50°C. The thermal time requirement to germination, defined as root protrusion, is 30°Cd (Jacobsen and Bach, 1998). The base temperature of 3°C is in the normal range for temperate crops, while the optimum temperature is similar to that for tropical crops (Garcia-Huidobro *et al.*, 1982), indication that the Danish quinoa could germinate and establish satisfactorily in both temperate and tropical regions. The low thermal time for root protrusion (30°Cd) shows a quick response to temperature, which is beneficial in northern regions where the growing season is short.

A description of cultivation instructions for quinoa production under north European conditions is given in Plate 1, based on experiences from Denmark and other north European countries during the last 20 years of research.

General discussion

There is an increasing interest in quinoa in the global market. This is in part due to the extraordinary nutritional characteristics of quinoa. Its high nutritional quality makes it beneficial not only for vegetarians and vegans, but also for health-conscious people. Furthermore, quinoa is gluten free, which makes it favourable for people suffering from coeliac disease. Imports of quinoa by the European Union have increased rapidly in recent years. Particularly in France, the Netherlands and Germany, and more recently in the United Kingdom and Scandinavia, quinoa consumption has increased. Bolivia provides 94% of EU quinoa imports (CBI Market Information Database).

Quinoa is an alternative to stable, global food products, such as rice and wheat flour, and it is superior in terms of nutritional value and abiotic stress tolerance. Therefore, it is believed that the current trend will continue, which is why 2013 was nominated the International Year of Quinoa by FAO. The potential for cultivation of quinoa in Europe was previously estimated at 2 million ha (Galwey, 1993), but to substitute a mere 10% of rice with quinoa will require over 30 million ha more worldwide. The potential is huge, also in northern Europe, where for several years companies have been working with Bolivian quinoa to develop new products. Northern Europe has also demonstrated its ability to develop new kinds of cuisine, looking towards, for example, Nordic food.

Quinoa is destined to play an important role in the future of Nordic food preparation, in both high level gastronomy and simple everyday dishes.

Plate 1: Instructions for mechanized cultivation of quinoa in northern Europe

Establishment
The most critical period in the cultivation of quinoa in northern Europe, and elsewhere, is the initial establishment, which has to be quick and efficient. Quinoa is sensitive to suboptimal conditions at the time of sowing – deep sowing, heterogeneous seed bed, low soil temperature and especially poor seed quality – which all lead to yield reduction.

Seed bed
The seed bed must be optimal, fine textured and with sufficient humidity for quick germination and establishment of the plants. The seed bed should be free from weeds at sowing time. Weed problems are most severe at a late sowing.

Sowing conditions
Optimal sowing conditions are created by using high quality seeds with a high germination percentage and vitality, sowing at 1–2 cm depth in a uniform, fine-structured, humid seed bed, with a soil temperature above 0°C.

Sowing date
Early sowing after the winter and as soon as the frost has left the soil has given good results, if the first month of spring (April) is relatively dry. If the period after sowing is humid and cold, seedlings or plants with 2–4 leaves can be attacked by soil-borne diseases and pests, such as Fusarium sp.
Quinoa should be grown as a spring crop, as it cannot overwinter in the field.

Row spacing
Quinoa can be sown on 50, 25 or 12.5 cm. If quinoa is sown with a row spacing of 25–50 cm, hoeing can be applied. If a sowing is done at cereal distance (12.5 cm), weeds can only be controlled by harrowing.

Sowing rate
There is no correlation between plant density and yield, which shows the compensatory capability of quinoa. If there are few plants, they will be large and each have high yield. However, a relatively high density is preferred in order to secure uniform plants and maturity, therefore, 100 plants/m2 is recommended, obtained with a sowing rate of approximately 10 kg/ha.

Sowing in an optimal seed bed

Emerged quinoa seedlings

Weeds
No herbicides controlling two-leaved weed species can be used in quinoa. For this reason, and given the interest in producing quinoa organically, the mechanical methods of hoeing, harrowing and flame treatment have been studied.

Flame treatment: As quinoa seems to emerge faster than any weed species, it is not possible to use flames to combat weeds before quinoa emergence.

Hoeing: For optimal weed control, it is important to sow in a clean seed bed, and allowing weeds

Harrowing

Hoeing

to germinate in a false seed bed can be very effective. Hoeing should take place as early as possible, but without covering the quinoa plants with soil between rows. In a subsequent control, it is possible to drive faster, creating a hilling which will have a positive effect on weed control in the row. Hoeing allows for accurate treatment between the rows, which makes it easier to control weeds, as it is possible to work deep in the soil and at high speed without damaging the quinoa. Crop soil cover should be avoided, although quinoa is relatively tolerant and may survive being covered.

Harrowing: This technique is easy to perform irrespective of how the crop was sown, and it can be done at high speed. The disadvantage is that the crop must be ahead of the weeds in order to avoid damage. It has, however, been demonstrated that quinoa can tolerate quite tough harrowing without damage to the crop.

Both weed control strategies result in loss of quinoa plants. Early hoeing may cause crop soil cover and result in loss of plants. Harrowing results in loss of the smallest plants as it is necessary to drive relatively fast for maximum weed control. Seed yield is highest with an efficient weed control strategy, and in general hoeing has given better results than harrowing. Prices for hoes and harrows are similar.

Manure

In an organic production system, nitrogen is normally applied to quinoa in the form of manure containing 80–120 kg N/ha. Quinoa may respond positively to higher levels.

Diseases and pests

Normally there are relatively few problems with diseases and pests in quinoa, although downy mildew (Peronospora variabilis) is seen every year everywhere quinoa is grown. This is especially the case under humid conditions with temperatures of 15°–20°C. The disease is less important if the summer is dry. Lack of disease control may result in significant yield decrease.

Harvest

Early harvest is essential in mountain regions and at high latitudes, requiring early establishment and growth in the spring. This enables the crop to avoid a cold, humid autumn climate in northern latitudes, which makes harvest more difficult, increases drying costs and reduces seed quality. At high altitudes, it is desirable to avoid drought and frost towards the end of the growing season. A late sowing or a cold growing season will delay development and harvest.

Harvest can be carried out with a combine harvester, putting the bridge close together, and reducing the air current. Yield is up to 2 tonnes/ha with properly adapted cultivars.


Good plant density in a clean crop


Quinoa close to maturity


Sowing dates, early and late


Commercial harvest


Seed yield


Harvested seed

Nutritional value

Quinoa has a high oil content (6% compared with 2% in cereals), and a high content of polyunsaturated fatty acids (omegas). It has a high protein content (14–18%), including a high lysine and methione content (double that of cereals). Quinoa has a high iron content (50% higher than in cereals), higher than any other crop.

Uses

Quinoa is attractive for food as well as animal feed. The main use of the primary product – the seed – is human consumption, and in South America the other plant parts are used for animals. Also in northern Europe, the main market is the food market. However, quinoa has a high feed value, as a result of the protein quality, starch content and high methionine composition, making it perfect for pig and chicken feed.

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CHAPTER 6.1.2

Quinoa d'Anjou: the beginning of a French quinoa sector

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Abstract[1]

Given the major increase in European quinoa imports between 2002 and 2007 (from 1 500 to 6 000 tonnes, with an annual growth rate of 20–30%) and the price per kg of grain imports (almost EUR2/kg), the Pays de La Loire Region decided to support the creation of a quinoa sector in France between 2009 and 2012. The project brought together different stakeholders: from seed production (Abottagra), agricultural cooperatives (CAPL) and research (UR LEVA, UR Grappe of group ESA Angers and Wageningen University). The main objective was to develop and operate the commercial distribution of "Quinoa d'Anjou", producing seeds of acceptable quality for customers, and the initial aim was to achieve a cultivated area of 500 ha and a mean yield of 3 000 kg/ha. Scientific support was concentrated in three main areas: i) Establishment of an experimental farm and initiation of a breeding programme of varieties adapted to European climatic conditions with help from breeders from Wageningen University (three adapted varieties without saponin were available to start the project, thanks to a previous breeding programme led by Wageningen University). ii) Adaptation of crop management to European agriculture (sowing date and density, nitrogen fertilization, harvesting etc.). iii) Organoleptic studies to identify differences between Andean and European quinoa and the consequences for agrifood industries. After just 3 years, the sector was becoming organized with a regular producers' group, efficient seed processing before commercialization, gradual integration of the production by agrifood industries, and introduction of a local food system. Experiment results demonstrate that the yield potential is very good and the seed quality differs from that of Andean quinoa.

The objective of this project is to develop production and commercial distribution of quinoa in France, and more precisely in the Loire Valley, by seeking technical solutions to produce seeds of acceptable quality for customers. Indeed, this highly nutritional product is not yet grown in Europe despite increasing customer demand: imports quadrupled between 2002 and 2007 and the value of the European market is estimated at EUR25 million.

Geographic localization and pedoclimatic conditions

The French quinoa sector is located in the Pays de la Loire Region and more specifically in Anjou (Figure

[1] The results of this project are confidential, which explains the lack of detail in some parts of the chapter.

Figure 1: Localization of production area in France

1). Plant production in this area is traditionally very diversified: crop cultivation, wine-making, horticulture, seed production, and the various actors in the sector are adapted to this heterogeneity.

The growth cycle of quinoa can be long (6–9 months), depending on the variety, and late varieties are unable to reach maturity at local latitudes. In addition, the pollen is very sensitive to high temperatures (Jacobsen and Stolen, 1993), therefore, cultivation is not possible in regions with early summer heat risk. On the other hand, high temperatures and sunlight are required at the end of the growth cycle to ensure seed filling.

The climate in Anjou seems appropriate to the culture of quinoa. It is characterized by mild temperatures between March and August (average 14.5°C), with a maximum temperature of 23°C during the grain filling period (July–August) and regular rainfall of 280 mm between March and August. However, an increased risk of water stress was observed between May and August during the last 10 years.

Soil types in quinoa production areas are varied and include loamy (the most common), sandy (found in the Loire Valley) and calcareous clay soils.

The economic context

When the project began, France was the biggest consumer in Europe, accounting for almost half of consumption. European imports increased from 1 500 tonnes in 2002 to 6 000 tonnes in 2007 (Figure 2), with an annual growth of 20–30%. Compared with wheat (125 million tonnes/year), the market is still small but it presents high growth potential.

In 2008, import prices varied between EUR1 and 1.50 /kg (Figure 2), with prices affected both by increase in demand and by reduced production in South America due to climatic conditions and production capacity limitations. In 2012, the market was even tighter: the import price rose to over EUR2 /kg, the price to consumers was EUR3–6 /kg and the value of the European market was around EUR25 million per year.

Can a quinoa sector in Europe be economically viable?

Production of quinoa in Europe is possible: trials in the last 20 years show that genotypes insensitive to day length achieve yields of 1–5 tonnes/ha. The best results (3–4 tonnes/ha) have been obtained using varieties selected by geneticists at the University of Wageningen in the Netherlands.

Two French agrifood industries – leaders in the distribution of conventional and organic quinoa in France – are very interested in quinoa and their requirements alone may exceed 1 000 tonnes per year as soon as the supply is guaranteed.

In Europe, quinoa is directed at a specific dietary market, and a gluten-free product, free from traces

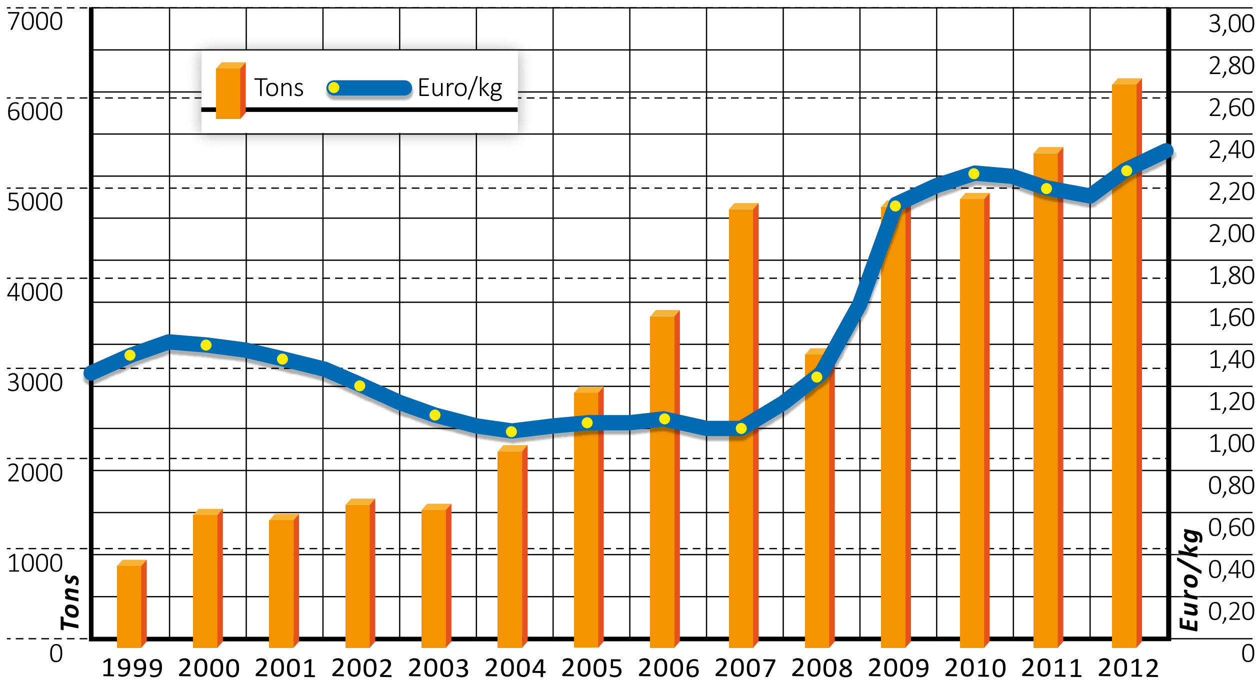

Figure 2: EU imports data (Eurostat, 2013)

of pesticide residues is required. In order to achieve this, the various operations carried out before commercialization (collecting, drying, cleaning and storage) must be specific to quinoa and a major investment is required.

Local farmer network

Several farmers, members of the CAPL cooperative (*Cooperative Agricole des Pays de la Loire*), agreed to produce quinoa. In 2009, there were 20 farmers producing Quinoa d'Anjou, 28 in 2010, 40 in 2011 and 38 in 2012. The sowing area also increased: from 123 ha in 2009 to 186 ha in 2010, reaching 400 ha in 2011.

The core of the quinoa network comprises 19 farmers who have produced quinoa for at least 3–4 years, actively participating in crop management development. They are convinced of the potential of quinoa.

Over a 4-year period, the total income of quinoa farmers exceeded EUR1 million, with an average income of EUR1 400/ha and almost EUR2 000/ha in 2012 as a direct result of improved crop management practices.

In conventional agriculture, the average yield is over 2 tonnes/ha, with the best performance exceeding 4.5 tonnes/ha. In organic agriculture, on the other hand, the average yield is around 800 kg/ha, with the best performance exceeding 2 tonnes.

In 2013, confident in conventional production capacity, but discouraged by high prices for conventional wheat and noting the market's clear preference for organic quinoa, the group decided to focus almost exclusively on cultivation of organic quinoa. However, conventional yields again exceeded 2 tonnes/ha (with the best field surpassing 4 tonnes/ha), while almost all the organic fields failed to even reach harvest. It would appear that the greatest constraint to organic quinoa production in France is natural nitrogen availability during cold, wet springs.

French-grown conventional quinoa has meanwhile become more attractive to buyers, and the group has therefore decided to focus on conventional production while continuing to study the challenge of organic production from new angles.

Genetic resources

The species has wide genetic variability and, therefore, genetic solutions can potentially be found to

a range of problems related to quality and performance: photoperiod, precocity, size and colour, and saponin-free seeds.
The Anjou project has been based on 'Pasto' and 'Atlas', two varieties developed by Wageningen University in the Netherlands. Wageningen began breeding quinoa in 1986 using two sources of genetic material: day-length insensitive, bitter material from the Chilean lowlands and short-day, low-saponin material from Ecuador and Peru. The varieties 'Atlas' and 'Pasto' are both adapted to Europe's long days and climate conditions (early maturing with a growth cycle of 6 months) and are characterized by a very low saponin content and, therefore, the grains do not have a bitter taste. 'Atlas' is a tall, leafy plant, characterized by late flowering but fast senescence, while 'Pasto' is a dwarf, compact plant with early flowering and slow senescence. 'Atlas' and 'Pasto' were protected by plant breeders' rights in the European Union in 1999 and 2007, respectively. Following the success of the Anjou project, Wageningen has significantly increased its quinoa breeding activities. For the foreseeable future, the varieties produced by Wageningen are likely to remain the most suited to large-scale commercial production in France.

Adaptation of crop management to European agriculture conditions

Sowing

Soil tillage is important, because the seeds need a fine soil – not a cloddy seed bed – to ensure soil–seed contact and achieve even and high emergence percentages. The appropriate technique is to first plough the field and then use a rotary harrow.
The optimal sowing period is between February and mid-March in order to avoid water stress during flowering and grain filling (sowing during April is not recommended, particularly in predominantly sandy soils) and to also avoid strong competition from weeds (especially *Chenopodium album*). The seed can germinate at very low temperatures (-1°C) and the seedling is resistant to freezing (up to -6°C for as long as 5 hours) (Bois *et al*., 2006).
Two years of trials have shown the optimum density to be 70–140 plants/m^2 with a row spacing of 12.5 cm, corresponding to a sowing rate of 8–10 kg/ha and a sowing depth of 1–2 cm.

Nitrogen fertilization

The plant response to nitrogen is a key factor in crop management. Two years of trials demonstrated that the nitrogen requirement is around 35 U of nitrogen/tonne. Nitrogen deficiency affects mainly the number of seeds per panicle, rather than the actual weight or seed size. The nitrogen balance method (considering the soil mineral nitrogen content and mineralization of soil organic nitrogen) is used to determine total nitrogen input.
Nitrogen inputs must be staggered to optimize the N uptake and minimize environmental impact (N leaching). Indeed, greenhouse trials have shown that nitrogen uptake increases significantly from the panicle stage. Producers are recommended to do a first N input at the 3–4 leaf stage (30–40 kg/ha) and a second input at the 8–10 leaf stage.
The relationship between yield and nitrogen requirement is linear up to a nitrogen availability of 170–230 kg/ha. There is a direct link between nitrogen intake and yield potential (in experimental conditions, the maximum yield is 10 tonnes/ha)

Pest control

Regular monitoring and surveillance of quinoa crops has allowed identification of the main pests (flea beetles, aphids, *Cassida nebulosa*), diseases (mildew) and weeds under pedoclimatic conditions. Early sowing at a density slightly higher than the optimum density can limit losses due to insects and weeds, although some species are still a problem (*Chenopodium album*).
In conventional farming, insecticide application se-

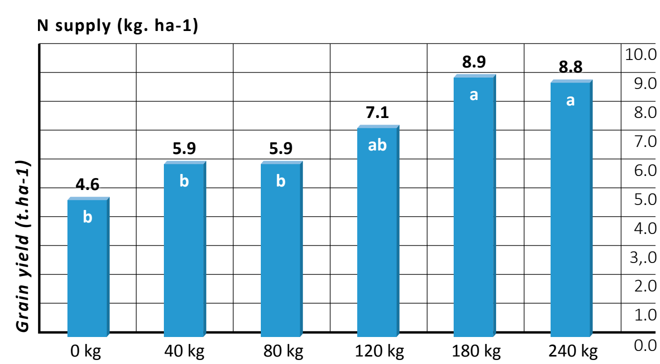

Figure 3: effect of nitrogen fertilizers on grain yield (Soil Mineral Nitrogen before sowing = 58 kg.ha-1), means denoted by different letters are significantly different at P<0,05 (ANOVA)

cures performance without leaving residues in the crop. In the quinoa trials, insecticides (pyrethroids) were applied according to the European regulations established for cereals.
Trials have shown that the various weeding techniques (harrow, hoe, plastic mulching and herbicides before sowing) give inadequate results.

Harvesting

Harvesting takes place between mid-August and mid-September. The main problem with mechanical harvesting are the climatic conditions during maturation, as they affect the seed humidity rate and leaf senescence. Harvesting a "green" crop causes the combine harvester to dysfunction, resulting in significant grain losses. An alternative technique is mowing at grain maturity and swathing, which reduces grain loss and does not cause grain quality to deteriorate. The optimal setting of the combine is very close to that used for cereals, but with slightly different parameters in order to limit shattering during mowing and avoid contamination by soil particles.

Almost all fields were harvested by two farming organizations specialized in quinoa harvesting and in possession of the specific equipment, cleaning machines and optimization settings.

Grain storing and processing before commercialization

CAPL, the agricultural cooperative which develops the farmer network, has established a quinoa-specific chain (silo, trucks, double-bottomed box etc.) to avoid contamination by other gluten-containing species, and no producer delivers his crop directly to the silo.

A workstation has also been specifically developed to clean quinoa, and includes a brush to whiten darker grains and a densimetric table to remove the sand brought by the mowing/swathing harvesting technique. The quinoa seeds then flow into an optical sorter which removes the grey and black seeds. Finally, the seeds flow into a series of sieves so that the product can be classified by seed size.

Quality studies of Quinoa d'Anjou compared to quinoa imports

A comparison (of the physical properties and taste) was made between experimental and commercial Anjou quinoas and the commercial brand, Quinua Real, originating from Bolivia. There are many differences between Anjou and Real quinoa grains, as the quality depends on the variety. Anjou grains are browner (Table 1) and smaller than the South American grains, and they have a stronger taste and specific aromas when cooked.

Variety	L*	C*	h*
Atlas	64.56[b]	24.52[b]	79.40[c]
Pasto	64.07[b]	26.00[c]	77.92[b]
QS	61.04[a]	26.93[d]	77.09[a]
Real	79.82[c]	20.33[a]	84.65[d]

Table 1: Color of the experimental quinoa varieties
*L = clarity note
*C = chroma (saturation)
*h = hue angle (color parameter)
Means in the same row followed by the same letter are not significantly ($P < 0.05$) different by LSD test.

Agronomic practices, such as nitrogen fertilization, have no impact on grain size, but grains tend to be darker as nitrogen supply increases.

Post-harvest treatments can also affect the grain quality of Quinoa d'Anjou. Brushing of the grain surface slightly reduces both the mean size of the grains and the heterogeneity of the size (Figure 4).

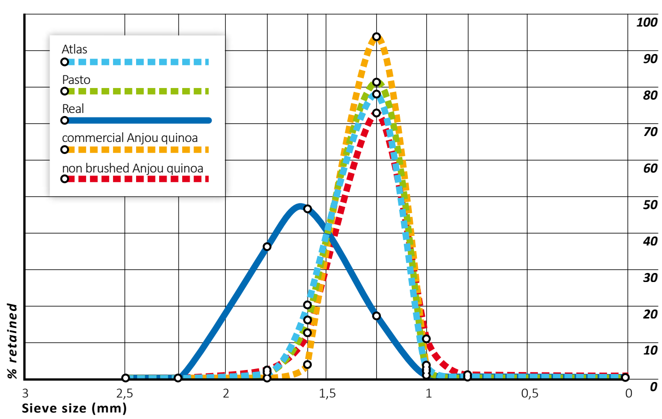

Figure 4: Particle size distribution of quinoa varieties obtained by sieving and influence of post-harvest treatment

Uses and markets

Differences in use between Anjou and South American quinoas have also been investigated. Several cooking methods can be adopted, and the cooking time and water requirement can vary, depending on the origin of the quinoa: Quinoa d'Anjou has a longer cooking time (Table 2) and needs more water than Quinua Real (Table 3).

Variety	Cooking time (min)
Atlas	18.85[b]
Pasto	19.56[b]
QS	19.79[b]
Real	14.50[a]

Table 2: Cooking time of the experimental quinoa varieties Means in the same row followed by the same letter are not significantly ($P< 0.05$) different by LSD test.

Conclusion

The French quinoa sector is becoming more secure, due to the stabilization of both the farmer network and the agrofood demand. In the light of the success of the quinoa supply chain in Anjou, a project to develop a sector in Belgium is now underway. Certain aspects of crop management require improvement, for example, reducing losses during harvesting or weed control (in particular in organic production). Studies have also begun to investigate the possibility of intercropping with legumes: while there is only limited scientific and technical knowledge available on nitrogen fertilization and weeding of quinoa in France, legumes as intercrops are known to provide ecosystem services for nitrogen fixation and for competition against weeds.

Acknowledgement

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Table 3: cooking time and water volume for cooking commercial quinoas

Cooking method	Anjou Quinoa		Real Quinoa	
	parts liquid to 1 part quinoa (vol)	cooking time (min)	parts liquid to 1 part quinoa (vol)	cooking time (min)
Boiling	excess water	17	excess water	14.5
Absorption	4	15	3	15
Pilaf/*'au gras'*	3.5	20	3	20
Oven steamed	2	60	1.6	60

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CHAPTER: 6.1.3

Quinoa in Italy: research and perspectives

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Abstract

An increasing number of studies have been performed in recent years in Italy on quinoa (*Chenopodium quinoa* Willd.). Interest in this Andean seed crop is mainly due to its resistance to the abiotic stresses affecting Mediterranean agro-ecosystems, in particular drought and salinity, and to the high nutritional value of its seeds. The principal research activities in Italy currently focus on the agronomic, biological and nutritional aspects of quinoa. Several field trials were carried out at CNR-ISAFoM in Ercolano (Napoli) to evaluate, in terms of growth, yield and physiological aspects, the adaptability of quinoa to Italian pedoclimatic conditions, and the crop's response to different agronomic management practices. Post-harvest chemical and product analyses were also performed to evaluate seed quality and aptitude for food processing. Quinoa's tolerance to salinity stress was investigated under controlled environmental conditions at the University of Bologna, where morphological and metabolic responses were analysed. All of these studies were conducted within national and international research projects with the collaboration of foreign research centres (CEAZA, Chile) and universities (University of Copenhagen), mainly using plant material selected in Denmark or sourced from the Andean region. This chapter describes the results of the main research activities carried out in the last decade by Italian institutions and discusses the potential for the introduction of quinoa cultivation in Italian cropping systems.

1. Introduction

The "boot-shaped" Italian peninsula forms a natural bridge in the Mediterranean Sea between the continents of Europe and Africa (Figure 1).

Italy is characterized by a range of different climates – alpine Mediterranean, peninsular and Po Valley – the result of its long shape incorporating high mountains (Alps and Apennines) and of its proximity to the Mediterranean Sea. There is, therefore, a transition between dry tropical and temperate climates, and as a consequence a vast range of agricultural crops are grown. Extensive crops, such as wheat and maize, are commonly found in the Po Valley, while vineyards, olive groves, citrus orchards and vegetable cultivation occupy the agricultural lands of central and southern Italy.

The majority of Italian farms (about 75%) have specialized production: olives (21.3%), cereals, oil seed

Figure 1. Italian peninsula highlighted in green

and protein crops (12.2%) and vineyards (9.9%), while 10.5% are engaged in mixed cropping and 10.4% in general field cropping (EUROSTAT, 2009).

According to data from the Sixth General Census of Agriculture of the Italian National Statistical Institute (ISTAT), in 2010 Italy's total UAA (utilized agricultural area) amounted to about 12.8 million ha, of which around 2.4 million ha (19% of UAA) are irrigated. Water is a strategic factor for agricultural development; it is estimated that around 40% of agricultural output depends on irrigated crops (INEA, 2011).

Since much of Italy's agricultural production depends on irrigation water availability, it is currently under threat from climate change.

It is predicted that the climate will change as a result of global warming, with drier and hotter summers in the Mediterranean region , and with hot dry spells all over Europe (IPCC, 2007).

Climate change scenarios for the Mediterranean Basin up to 2050 (Figure 2) show a clear trend towards decreased precipitation (10–15%) and increased length of the dry period, as well as increased temperature (1.25°–2.5°C) (Ragab and Prudhomme, 2002).

The Italian Ministry for Environment, Land and Sea confirmed in the Fifth National Communication under the UN Framework Convention on Climate Change that the mean temperature during recent decades in Italy increased more than the global mean. In particular, in 2006, the mean temperature increase compared with the reference 30-year period 1961–1990 was about 1°C in Italy, compared with a global mean increase of about 0.5°C.

Many studies on precipitation in Italy show significant annual negative trends in the southern regions; indeed, Campania, Basilicata, Calabria and Sicily recorded a decrease in rainfall of up to 20%, with an increase of the number of highly intensive precipitation events.

The reduction in rainfall and the increase in precipitation intensity obviously affect the total availability of water resources in the soil and the extension of the agricultural area, resulting in higher runoff, soil erosion, less accumulation of water in the reservoirs and reduced availability of water for irrigation purposes. Furthermore, a warmer climate and drought lead to an increase in evapotranspiration demand by crops.

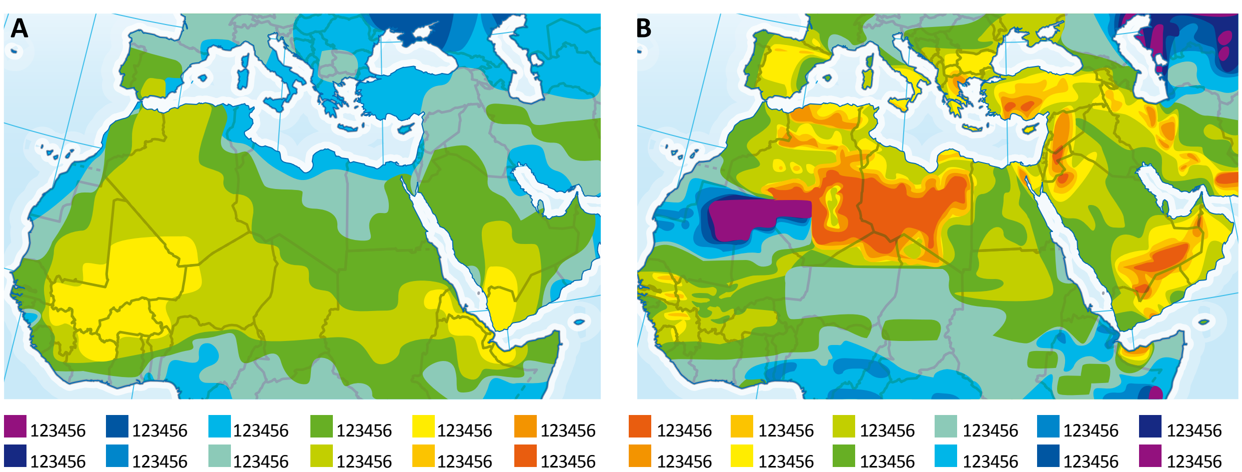

Figure 2. Predicted changes in annual air temperature (a) and precipitation (b) in the Mediterranean Basin until 2050 (Ragab and Prudhomme, 2002).

Under these conditions, water resources are an increasingly limiting factor, leading to greater competition between agriculture, urban and industrial uses.

Water availability is related not only to the climatic conditions of the different geographical areas, but also to socio-economic factors and the problems of water quality deterioration due to environmental pollution (Kirnak, 2006). The intensive use of limited water resources causes excessive extraction of underground water, resulting in intrusion of seawater in coastal areas and soil salinization (Pagliuca *et al*., 2009; Navarro *et al*., 2007). This phenomenon becomes increasingly evident in periods of peak water requirements for crops and it limits productivity (Maas and Hoffman, 1977). Water scarcity and soil salinization (Figure 3) are already a problem in many Italian agricultural areas; secondary soil salinization as a result of the use of saline waters for irrigation affects about 3.2 million ha and occurs in most Italian regions to varying degrees (Dazzi and Lo Papa, 2013).

It is estimated that within the next 25 years, salinization may result in the loss of 30% of current agricultural land, a figure set to rise by up to 50% by 2050 (Altman, 1999; Ashraf, 1994), due also to the rapid rate of population growth.

Various studies have been done to show how climate change will have mixed effects on crop phenology and yields.

- Moriondo and Bindi (2007) showed that the increasing temperature simulated by RCMs (regional climate models) and GCMs (global climate models) in the Mediterranean Basin is expected to induce earlier development of crops and a reduction in the length of the growing season of typical Mediterranean crops, such as durum wheat (*Triticum turgidum* L.), sunflower (*Helianthus annuus* L.), grape (*Vitis vinifera* L.) and olive (*Olea europea* L.). These responses may permit some crops to avoid summer drought stress, but at the same time, the changes in climate may imply an increased possibility of occurrence of extreme climate events (e.g. frost and heat waves) at sensitive phenological stages, with a negative effect on final yield quantity and quality.
- Wolf and Menne (2007) concluded that the expected lengthening of the growing period (by 10–15 days per 1°C rise in yearly average temperature) and consequent shortening of the cold winter period will make olive, citrus, vine and durum wheat cultivation possible in the north of Italy, while corn will suffer in the south. Such changes will directly affect both farming practices (e.g. the need to introduce new cultivars and species) and food and agriculture transformation industries (e.g. change in location or increased transportation costs).

One option available to Italian agriculture is the introduction of species capable of tolerating drought, frost and high soil salinity while ensuring acceptable yields. One such crop is quinoa (*Chenopodium quinoa* Willd.), which exhibits higher resistance to adverse abiotic factors compared with traditional crops (Jacobsen *et al*., 1994, 2009).

Quinoa is a facultative halophytic species, and some cultivars are able to grow under extreme saline conditions (up to soil electrical conductivity of 52 dS/m) (Adolf *et al*., 2012; Shabala *et al*., 2013).

Thanks to its resistance to abiotic stresses, quinoa could be successfully cultivated in those areas most

Figure 3. The areas highlighted in blue indicate those areas where it is possible to find salt-affected soils (Dazzi and Lo Papa, 2013).

affected by climate change (Jacobsen *et al*., 2012) or in marginal areas less suitable for common crops.

Quinoa is an answer to the increasing market demand for healthy food: its seeds are of high nutritional value and its protein content is superior to that of common cereals (12–20% of the seed's dry weight). The quality of the proteins is also a valuable characteristic of quinoa, as they contain essential amino acids in quantities close to human requirements (Schlick and Bubenheim,1996). Indeed, the balanced composition of the quinoa protein is comparable to that of milk proteins (casein).

Furthermore, the seeds of quinoa are commonly known to be gluten free. Quinoa thus provides an alternative to normal cereals in coeliac diets and, for this reason, the Italian Coeliac Association (AIC) has included quinoa in the coeliac food list.

Quinoa is also a potentially fascinating alternative to traditional crops which have been cultivated less in recent years for agricultural policy reasons. The tobacco sector in Italy was reformed by the EU's Common Agricultural Policy (CAP) in 2003 which stopped subsidies for tobacco production. Italy's tobacco production in 2011 was about 70 000 tonnes on over 8 900 ha. Compared with 2010, these data show a reduction of 20%, in terms of both volume and surface area, and the decrease is even higher when compared with the situation prior to application of the reform (i.e. before 2005).

The main reasons for the increasing interest in quinoa in Italy in recent years are the effects of climate change on agriculture, the increase in demand for healthy food and changes in agricultural policy.

A number of studies have been carried out in recent years in Italy to evaluate the adaptability of quinoa to Italian pedoclimatic conditions under different agronomic management practices and to assess its response to different abiotic stresses, in particular drought and salinity.

2. Research activity

Important research activities on quinoa are being carried out at the Institute for Agricultural and Forest Systems in the Mediterranean of the Italian National Research Council (CNR-ISAFoM) located in Ercolano-Napoli and at the University of Bologna.

2.1 Open field research works

Field trials began in 2006 at ISAFoM-CNR to test quinoa as a crop. These studies aimed to evaluate the quantitative and qualitative responses of quinoa under combined abiotic stresses (salt and drought stress) and assess its adaptability to the Mediterranean environment of southern Italy. Research has focused mainly on the agronomic, biological and nutritional aspects of quinoa in order to understand the potential impact of quinoa on Italian agro-ecosystems. Outputs from agronomic trials have been utilized in crop modelling to better manage and plan the cultivation of quinoa under different environmental conditions.

2.1.1 Projects and collaborations

ISAFoM's research activities on quinoa were carried out within the context of national and international research projects (Table 1) in cooperation with Italian and foreign universities and research centres. During 2006–07, quinoa was studied within the project "CO.Al.Ta. II" (Alternative Crops to Tobacco) founded by the European Community (EC). The aim of the project was to explore possibilities for diversification in traditional tobacco-growing areas of Italy, such as the province of Caserta, following the CAP reform for the tobacco sector (EG Nr. 864/2004 from 29 April 2004, effective on 1 January 2006). Quinoa was tested as a possible alternative crop with positive results: given its rusticity and the high nutritional value of its seeds, a quinoa crop can ensure a satisfactory income and employment, without requiring subsidization. During the Co.Al. Ta. II project, seed quality (in particular, saponin content) was researched in collaboration with the Department of Food Technology (DISTAAM) of the University of Molise.

From 2008 to 2013, ISAFoM-CNR participated as a partner in the EU project "Sustainable water use securing food production in dry areas of the Mediterranean region" (SWUP-MED). The tasks of ISAFoM within the project were: a) to test new crops, such as quinoa and amaranth, displaying the potential to cope with multiple stress factors in a Mediterranean environment of southern Italy; b) to apply sustainable agronomic interventions and identify suitable cultural practices to mitigate multiple abiotic stresses in order to stabilize and improve yield and quality of selected crops and species; and c) to use the models in order to find the best practices integrating water, crop and field management while saving freshwater, producing optimum yield and safeguarding the environment.

The research activity on quinoa within the SWUP-MED project was carried out in collaboration with the *Institut Agronomique et Veterinaire Hassan II* (IAV, Morocco), the Centre for Ecology and Hydrology of Wallingford (CEH, United Kingdom), the International Centre for Agricultural Research in the Dry Areas (ICARDA) of Aleppo (Syria), the Faculty of Agriculture of Cukurova (UWA, Turkey) and the Faculty of Life Sciences of the University of Copenhagen (UCPH, Denmark).

In 2010, modelling was carried out on quinoa using the SALTMED model, in collaboration with the CEH within the Short Term Mobility (STM) programme founded by the Italian CNR.

In 2011, testing on quinoa began within the "CISIA" project, funded by the Italian CNR, with the aim of improving the valorization and sustainability of agrofood products in southern Italy. As of 2013, the knowledge and results obtained have been disseminated to farmers and stakeholders in the region of Campania within the framework of the project "Quinoa Felix – Introduction of quinoa (*Chenopodium quinoa* Willd.) in the Campania Region for high nutritional and functional value food production", in collaboration with the University of Molise and the CNR-Institute of Food Science (ISA) of Avellino. The "Quinoa Felix" project also involves a private farm, located near Avellino, for the introduction of quinoa on an initial area of 1 ha, and a local industrial bakery, for the production of goods made with quinoa flour. The project is still underway and the possibility of spreading the cultivation and use of quinoa to other farmers and local entrepreneurs is being evaluated.

2.1.2 Genetic resources

Chenpodium quinoa Willd. is a seed crop originating in the Andes and presenting wide genetic variability. The origin of quinoa domestication appears to be the area around Lake Titicaca (Gandarillas, 1979; Pearsall, 1992), where the greatest genetic diversity and variation is found. Institutions in Bolivia hold the most important germplasm banks in

Table 1. Research activities carried out on different quinoa genotypes by ISAFoM-CNR within different projects, including activities in progress and those planned for 2014.

YEAR	Genotype	Origin	Project	Experimental site
2006-2007	Q52 (Titaca)	Denmark*	Co.Al.Ta. II	Vitulazio
2006-2007	Regalona Baer	Chile	Co.Al.Ta. II	Vitulazio
2009-2012	Titicaca	Denmark*	SWUP-MED	Vitulazio
2011-2012	Puno	Denmark*	SWUP-MED	Vitulazio
2011-2012	Kurmi	Bolivia	SWUP-MED	Vitulazio
2011-2012	Real	Bolivia	SWUP-MED	Vitulazio
2011-2012	Blanquita	Bolivia	SWUP-MED	Vitulazio
2011-2012	Janca Grano	Bolivia	SWUP-MED	Vitulazio
2011-2012	Jujuy Rosada	Argentina	SWUP-MED	Vitulazio
2012-2014	Titicaca	Denmark*	Quinoa Felix	Vitulazio-Avellino-Ercolano
2012-2014	Puno	Denmark*	Quinoa Felix	Vitulazio-Avellino
2012-2014	Kurmi	Bolivia	CISIA	Vitulazio
2012-2014	Real	Bolivia	CISIA	Vitulazio
2012-2014	Blanquita	Bolivia	CISIA	Vitulazio
2012-2014	Janca Grano	Bolivia	CISIA	Vitulazio
2012-2014	Jujuy Rosada	Argentina	CISIA	Vitulazio
2012-2014	Amarilla de Marangani	Peru	CISIA	Vitulazio

*selected from material originating from a cross between southern Chilean and Peruvian lines

Figure 4. Volturno River plain (Pagliuca *et al.*, 2009)

the world for this species, accounting for over 2 700 accessions (del Castillo *et al*., 2007). Since there is no quinoa variety domesticated in Italy, the studies at ISAFoM used seeds received from foreign institutions and of different origins, in particular: the Danish-bred cultivars 'Puno' and 'Titicaca' (Razzaghi *et al*., 2012), selected from material originating in southern Chile; a Chilean variety 'Regalona Baer'; four Bolivian cultivars 'Kurmi', 'Janca grano' 'Blanquita' and 'Real'; the Peruvian 'Amarilla de Marangani', and the cultivar 'Jujuy rosada' originating from Argentina.

2.1.3 Experiments and results

In 2006, field trials began at the ISAFoM research station in Vitulazio on the Volturno River plain (Figure 4) (14°50'E, 40°7'N, 25 m asl), an irrigated area in southern Italy. The soil of the trial site is characterized by a clay–loam texture.

The main agronomic practices applied during the field trials were seed bed preparation (carried out just before sowing by harrowing twice with a disc harrow and a rotary harrow) and inter-row manual weed control throughout the growing cycle (Jacobsen *et al*., 2010). Fertilization was 80 kg/ha N (NH_4NO_3) and 40 kg/ha P (P_2O_5). The nitrogen was divided into two equal parts and supplied at sowing and again during vegetative growth before flowering. Quinoa was harvested manually.

During 2006–07, a biannual field trial was performed within the Co.Al.Ta.II project to evaluate the effect of different sowing dates (5 April and 4 May) on yield and quality of seeds of two quinoa genotypes, 'Titicaca' (KVLQ52) (Figure 5) and 'Regalona Baer' (Figure 6), under rainfed conditions (Pulvento *et al*., 2010).

Figure 5. Quinoa cv. 'Titicaca' cultivated under rainfed conditions at the CNR-ISAFoM research station located in Vitulazio (CE) in the 2007 field trial.

Figure 6. Quinoa 'Regalona Baer' cultivated under rainfed conditions at the CNR-ISAFoM research station located inVitulazio (CE) in the 2007 field trial.

The results (Table 2) show that in the climatic conditions of southern Italy, early sowing (beginning of spring) gave higher yields (3.3 tonnes/ha) than later (May) sowing (1.5 tonnes/ha). The total yield ranged from 1.9 to 3.4 tonnes/ha, considering both genotypes, and was comparable with yields reported in the Andean region. The results suggest that both genotypes could be cultivated successfully in this climatic region. The different quinoa samples showed a protein content between 16.2% and 16.8% – i.e. higher than in cereals.

The saponin content of seeds, determined by chromatographic analysis, was significantly higher for cv. 'Regalona Baer' (329.0 mg/100 g dry weight) than in cv. 'Titicaca' (213.8–238.9 mg/100 g dry weight).

Harvested seeds were ground into flour and used to make different types of pasta and bread (Iafelice *et al*., 2009). A sensory evaluation was conducted testing pasta made with 50% and 20% of quinoa flour and product control (100% type "0" flour). Pasta made using 50% quinoa flour was not acceptable because there is a strong deterioration of taste and flavour (due to the presence of a strong smell of grass). On the other hand, pasta made with 20% quinoa flour had an acceptable sensory profile. The panel of judges highlighted the differences in tannin flavour and taste, but overall gave the quinoa-based product a positive evaluation. Bread made with 20% quinoa flour was also tested and the panel expressed a highly positive opinion with regard to the appearance and colour of both the crust and the inside of the loaf, but the taste and smell were considered unusual, negatively affecting the product value.

During 2009–10 cv. 'Titicaca' was tested in a biannual field trial in Vitulazio. The aim was to evaluate the quantitative and qualitative response of the crop (Figure 7) under combined salt and drought stress (Pulvento *et al*., 2011 2012).

Treatments irrigated with well water (Q100, Q50 and Q25) and corresponding treatments irrigated with saline water (Q100S, Q50S and Q25S) with an electrical conductivity (ECw) of 22 dS/m were compared. Q100 was the control receiving 100% of the water necessary to replenish the root zone (0.00–0.36 m) field capacity (FC). For Q50 and Q25, 50% and 25%, respectively, of the water volume used for the control treatment was applied.

The saline water was prepared by adding sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium chloride ($MgCl_2$) and magnesium sulphate ($MgSO_4$) to the well water. The water obtained had an ionic content similar to that of well water and seawater mixed in a 1:1 ratio. The aim was to simulate highly salinized groundwater due to seawater intrusion in the area surrounding the experimental site (Figure 8).

Figure 7. Quinoa cv. 'Titicaca' irrigated with saline water at CNR-ISAFoM research station located in Vitulazio (CE) in the 2010 field trial. Different phenological stages during the growth season are shown: (a) vegetative stage, (b) panicle formation and (c) maturity.

During the trial, yield, growth and physiological parameters of the crop were evaluated; ion accumulation in the various organs and qualitative aspects of quinoa seeds were also assessed.

In both years, seed yield, above-ground biomass and harvest index (HI) were not negatively affected by salt and drought stresses; the average annual seed yield was 2.3–2.7 tonnes/ha.

Differences were detected for the 1 000-seed weight, which was higher for saline than non-saline treatments.

Table 2. Yield parameters of two quinoa genotypes during the field trials conducted at CNR-ISAFoM research station in Vitulazio (CE) during 2006 - 10. "A" and "M" indicate sowing in April and May, respectively.

YEAR	Genotype	Seed Yield *t ha⁻¹*	1000 seed weight *(g)*	Harvest index
2006	Titicaca$_A$	3.3	3.6	0.6
2006	Titicaca$_M$	1.5	2.1	0.4
2006	Regalona Baer	3.4	2.3	0.3
2007	Titicaca	1.9	3.0	0.4
2007	Regalona Baer	3.0	1.8	0.3
2009	Titicaca	2.7	2.4	0.4
2010	Titicaca	2.3	2.7	0.4

Figure 8. Irrigation system with a) tanks for collecting irrigation water; b) sand filters; c) surface drip irrigation lines.

Quinoa plants responded differently to drought and salinity. Plant growth and water productivity (WP, kg/m^3) – defined as the ratio between total seed yield and total amount of water applied (rainfall and irrigation water) to the crop – were not influenced by saline irrigation, and the salinity tolerance of quinoa was thus confirmed. Salt tolerance is conferred by the plant's capacity to incorporate salt ions in the tissues (stems, roots, leaves) while preserving seed quality. Reduction of the irrigation water to 25% of the full irrigated treatment (Q25) caused an increase in WP and reduced dry matter accumulation in the leaves. Q25 plants were initially negatively affected by severe drought with a reduction of the relative growth rate (RGR) and net assimilation ratio (NAR), followed by adaptation. Quinoa may be considered a drought-tolerant crop that adapts its photosynthetic rate (NAR) to compensate for reduced growth (Riccardi *et al*., 2010 2013).

Cocozza *et al*. (2013) highlighted the main ecophysiological traits of quinoa during the trial. As water and salt stress developed and leaf water potential (Ψ_{leaf}) decreased, the leaf osmotic potential (Ψp) declined (below -2.05 MPa) to maintain turgor. Stomatal conductance *(gs)* decreased with the reduction in Ψ_{leaf} (with a steep drop at Ψ_{leaf} between -0.8 and 1.2 MPa) and Ψp (with a steep drop at Ψp between -1.2 and -1.4 MPa). In both years, salt and drought stress did not markedly affect the relationship between water potential components, relative water content (RWC) and *gs*. Ψ_{leaf} and *gs* were inversely related to water limitation and soil salinity experimentally imposed, showing exponential (Ψ_{leaf} and turgor pressure, Ψp, vs *gs*) or linear (Ψ_{leaf} and Ψp vs soil water content – SWC) functions. At the end of the experiment, salt-irrigated plants showed a severe drop in Ψ_{leaf} (below -2 MPa), resulting in stomatal closure through interactive effects of soil water availability and salt excess to control the loss of turgor in leaves. The effects of salinity and drought resulted in strict dependencies between RWC and water potential components, showing that regulating cellular water deficit and volume is a powerful mechanism for conserving cellular hydration under stress, resulting in osmotic adjustment at turgor loss. The extent of osmotic adjustment associated with drought was not reflected in Ψp at full turgor. As soil was drying, the association between Ψ_{leaf} and SWC reflected the ability of quinoa to explore soil volume to continue extracting available water from the soil. In 2009, there was no variance in leaf abscisic acid (ABA) content under concomitant salinity and drought stress conditions, while in 2010, there was a difference between Q100 and Q100S. Quinoa showed good resistance to water and salt stress through stomatal responses and osmotic adjustments that played a role in the maintenance of a leaf turgor favourable to plant growth and crop yield.

Neither drought nor salt stress affected the main qualitative aspects of the seed, and the protein content ranged from 14.7% to 16.6% on a dry weight basis.

HPLC-DAD-ESI-MS analysis of phenolics (Gómez-Caravaca *et al*., 2012) indicated that irrigation with 25% of full water restitution – with and without the addition of salt – caused an increase in free phenolic compounds of 23.16% (with salt) and 26.27% (without). In contrast, bound phenolic compounds were not affected by environmental stresses.

Saponins were evaluated in terms of sapogenins (Gómez-Caravaca *et al*., 2012; Pulvento *et al*., 2012; Lavini *et al*., 2011). Gas chromatographic (GC) analysis was carried out to evaluate saponin aglycones (sapogenins) derived from the acid hydrolysis of seed samples. Three major quinoa saponin aglycones were identified: oleanolic acid (36–50% of the total), hederagenin (27–28%) and phytolaccagenic acid (21–36%).

GC analysis showed that the samples grown under saline treatments had a higher level of sapogenins compared those grown under non-saline treatments, and that the sapogenins decreased under reduced irrigated (Q25 and Q50, compared with Q100). In situations of severe water deficit, saponin content decreased by 35%, 45% and 50%, respectively, when salt stress was added.

The experimental data collected during the 2009–10 biannual field trial were also used to calibrate and validate SALTMED (Ragab, 2010; Ragab *et al*., 2005a b) – an integrated, physically based model that simulates on a daily basis the main processes of the soil–water–plant continuum (Pulvento *et al*., 2013).

The results showed that the SALTMED model (See the specific chapter about this model in this book "*SALTMED : Modellization of crop development*") is an important tool for assessing the impact of water resource management for irrigation purposes and for predicting quinoa adaptation to different environments and types of agricultural management.

From 2011 to 2013, other field trials were performed in Vitulazio to compare dry matter, seed yield and quality of different quinoa genotypes (Table 1) grown under rainfed conditions. Preliminary results, not yet published, show that the Danish cultivars, 'Titicaca' and 'Puno', are the best grain producers under Mediterranean conditions, while others cultivars from south America either did not reach physiological maturity or produced very small quantities of seeds.

Research activities on quinoa are ongoing at ISAFoM within the CISIA and Quinoa Felix projects with the following objectives: *a)* test the response of quinoa to different agronomic practices; *b)* deliver typical food products made with quinoa flour; and *c)* disseminate knowledge about quinoa's characteristics to local farmers and stakeholders.

New field trials are also ongoing to test mechanization (Figure 9) of the main cultural practices (sowing, weeding and harvesting) that would allow the diffusion of quinoa cultivation in Italy.

Figure 9. Mechanization of quinoa sowing

2.2 Research under controlled environmental conditions

At Bologna University (Department of Biological, Geological and Environmental Sciences and Department of Agricultural Sciences), research is underway to study morphological and physiological elements of quinoa halophytism and variability in salt tolerance among different cultivars. Orsini *et al*. (2011) analysed several morphological and metabolic responses in parallel following exposure to salinity in one quinoa accession ('BO78', from Collipulli in the Araucanía region of southern Chile). *In vitro* seed germination was initially delayed by a 150 mM NaCl treatment, but it eventually reached the same level as the control (0 mM NaCl), while seedling root growth was enhanced; germination and seedling root growth were both moderately inhibited (~35–50%) by 300 mM NaCl. In pot-grown plants, plant size was reduced by increasing salinity (0–750 mM NaCl). Transpiration and stomatal conductance were decreased at the highest salinity levels tested. Changes in stomatal density and size were consistent in both adaxial and abaxial leaf surfaces as a response to salt, although reductions in density and index were perceivable already at low salinity levels, whereas stomatal size was only diminished at the highest salt concentrations. The density of epidermal bladder cells (EBCs) on the leaf surface remained unaffected up to 600 mM NaCl. Tissue content of Na^+ and Cl^- increased dramatically with salt treatment, but resulted in only a 50% increase in Na^+ from 150 to 750 mM NaCl. Internal K^+ was unaffected up to 450 mM NaCl, but increased at the highest salinity levels tested. Excretion through sequestration into EBCs was limited (generally 20%) for all ions, indicating that the role played by these salt glands on overall plant ion homeostasis may be limited. A modest dose-dependent proline accumulation and concomitant reduction in total polyamines and putrescine efflux occurred in NaCl-treated plants. The results confirm the importance of inorganic ions for osmotic adjustment, the plant's ability to maintain K^+ levels and the involvement of putrescine efflux in maintaining ionic balance under high salinity conditions. Conversely, ion excretion and proline appear to play a minor role in quinoa adaptation to salinity. The plant response involved several adaptive strategies at morphological (reduction of stomatal size and density), physiological

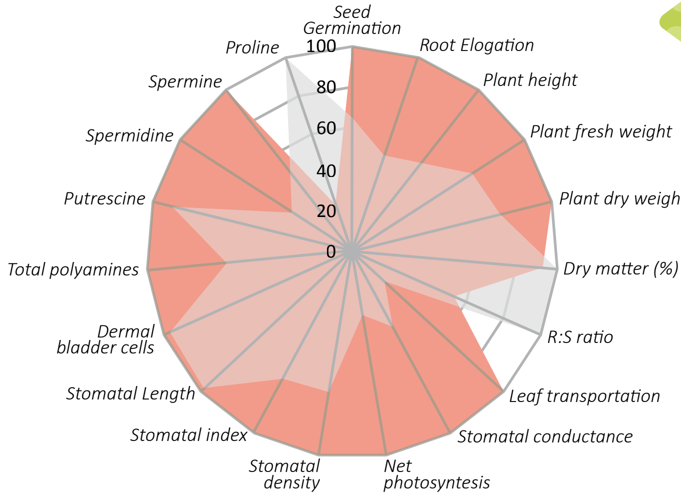

Figure 10. Response of quinoa accession 'BO78' to 0 (black area) and 300 (grey area) mM NaCl. Values expressed as percentage of their maximum value. Seed germination and root elongation from *in vitro* experiments were measured at 15 days from sowing and 7 days from germination, respectively. All other measurements from *in vivo* plants.

(down-regulation of water loss and stomatal conductance) and biochemical (ion homeostasis and depletion of polyamines) levels. These can, therefore, be considered useful indicators of adaptation to salinity in this quinoa accession. Some confirm previous knowledge of quinoa or halophytic dicots, while others, such as EBC density and ion sequestration capacity and polyamins extrusion, require further investigation to assess their role in this and other chenopods.

To investigate which tolerance mechanisms might account for varietal differences, Ruiz-Karrasco *et al*. (2011) compared four genotypes ('BO78', 'PRP', 'UDEC9', 'PRJ') from central and southern coastal regions of Chile for their growth, physiological and molecular responses to NaCl at the seedling stage. Seeds were sown on agar plates supplemented with 0, 150 or 300 mM NaCl. Germination was significantly reduced by NaCl only in 'BO78'. Shoot length was reduced by 150 mM NaCl in three out of four genotypes, and by over 60% at 300 mM (except 'BO78' which was closer to controls). Root length was hardly affected or was even enhanced at 150 mM in all four genotypes, but it was inhibited, especially in 'BO78', by 300 mM NaCl. Thus, the root/shoot ratio was differentially affected by salt, with the highest values in 'PRJ' and the lowest in 'BO78'. Biomass was also less affected in 'PRJ', the genotype with the highest increment in proline concentration upon salt treatment. Free putrescine declined dramatically in all genotypes under 300 mM NaCl; however, (spermidine + spermine)/putrescine ratios were higher in 'PRJ' than in 'BO78'. Quantitative RT-PCR analyses of two sodium transporter genes, *CqSOS1* and *CqNHX*, revealed that their expression was differentially induced at shoot and root level, and between genotypes, by 300 mM NaCl (see Chapter 2.7).

3. Perspectives

Future climate change will negatively affect Italian agro-ecosystems, reducing water availability, water quality and crop productivity.

Quinoa has a potential role in future diversification of agricultural systems in Italy, especially in areas most affected by abiotic stresses, such as drought and salinity. The studies conducted have shown that the crop can be successfully cultivated under southern Italian environmental conditions, giving considerable seed yield also under unfavourable environmental conditions. Some quinoa varieties are much more able than traditional commercial crops to withstand drought or salinity.

Since quinoa has relatively good resistance to pests and diseases, it is also a suitable crop for organic production.

The high nutritional value of its seed makes quinoa an interesting ingredient for typical Italian food products, and could represent an added value in the high quality food market. Quinoa is also a valid alternative for preparation of gluten-free foods and drinks for coeliacs. According to the Italian Ministry of Health's annual report to parliament on coeliac disease (2011), the number of coeliacs in Italy is around 600 000. The trend is an annual increase of 19%.

Research programmes and further studies on quinoa should support the development of this valuable but underutilized crop; concerted efforts, such as improving agricultural techniques and mechanization of quinoa farming, need to be taken into consideration. This can be done through dissemination and demonstrations of best farming methods. Italian farmers will then be in a position to learn more about how to improve the production of this crop.

Basic research conducted in Italian universities in collaboration with other research institutes should be encouraged in order to contribute towards breeding improved varieties better adapted to national agro-environmental conditions.

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CHAPTER: 6.1.4

Quinoa Experimentation and Production in Turkey

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Abstract

The purpose of this chapter is to provide information on improving food crop production in arid and semi-arid regions, especially in the semi-arid Mediterranean region of Turkey, which is influenced by multiple abiotic stresses. In particular, the authors focus on the diversification of crop production and introduction of new climate-proof crops and cultivars with improved stress tolerance, such as quinoa. The stresses are becoming even more pronounced under the changing climate, which is predicted to bring drier conditions, increasing temperatures and greater variability, resulting in desertification. As quinoa is drought resistant, it is traditionally cultivated under rainfed conditions, even in semi-arid locations. However, when researchers started to study the impact of additional water on quinoa production, they found that deficit irrigation (DI) was highly beneficial in various experimental locations. On the other hand, quinoa is rarely cultivated under full irrigation, as in tests it performed only slightly better than quinoa cultivated under DI (in addition to the fact that sufficient water for full irrigation is mostly unavailable). Studies carried out in the Mediterranean region of Turkey indicated a positive response of quinoa to full irrigation – both with saline water and with freshwater – compared with DI. Quinoa is a facultative halophyte and can grow in non-saline to extremely saline conditions, depending on the cultivar. Seed production is enhanced by moderate salinity (EC in the 5–15 dS/m range) and some cultivars can still produce relatively good yields at an EC of 40–50 dS/m. This chapter also deals with problems of introducing quinoa in this part of the world, genetic resources used, the current state and perspectives of cultural dissemination in Turkey, uses and markets, and questions and problems about its dissemination.

Introduction

Food production and water use are inextricably linked. Water has always been the main factor limiting crop production in much of the world where rainfall is insufficient to meet crop demand. With the ever-increasing competition for finite water resources worldwide and the steadily rising demand for agricultural commodities, the call to improve the efficiency and productivity of water use for crop production, to ensure future food security and to address the uncertainties associated with climate change, has never been more urgent (Steduto *et al.*, 2012).

In order to meet global food, feed and biofuel demand and to alleviate hunger and poverty, there is no alternative but to increase agricultural productivity (i.e. crop yield per unit area) and the associated total and individual factor productivities (i.e. biological output per unit of total production input, and output per unit of individual factors of production such as energy, nutrients, water, labour, land and capital). Thus, to feed the world in 2050 and beyond, further crop production intensification and optimization are required. However, until now, agricultural intensification has tended to have a negative effect on the quality of many essential resources (e.g. soil, water, land and biodiversity) and on ecosystem services, causing yield and factor productivity growth rates to decline (Derpsch and Friedrich, 2010). Another challenge for agriculture is its environmental footprint and climate change. Agriculture is responsible for about 30% of total greenhouse gas emissions of CO_2, N_2O and CH_4, and it is also directly affected by the consequences of a changing climate (IPCC, 2007).

Drought and salinity are common adverse environmental factors affecting plant growth and they determine the global geographic distribution of vegetation and restriction of crop yields in agriculture (Gregory, 2006; Lin *et al*., 2006; Schulze *et al.*, 2005). In the southern Mediterranean region, food crop production is restricted by limited water resources, drought and salinity. Under semi-arid and arid conditions in Mediterranean countries affected by multiple abiotic stress factors further influenced by climate change, the typical crop cultivation is cereals in low-yielding monoculture or combined with fallow. Food crop production in the arid and semi-arid regions (especially the semi-arid Mediterranean region of Turkey) influenced by multiple abiotic stresses, can be improved by further diversifying crop production and introducing new climate-proof crops and cultivars with improved stress tolerance, such as quinoa. Quinoa (*Chenopodium quinoa* Willd.) is a halophyte with the potential to become an important crop in arid regions and saline habitats and satisfy a growing world market (Jacobsen and Shabala, 2013). The abiotic stresses in the Mediterranean are becoming even more pronounced due to the changing climate which is predicted to bring drier conditions, increasing temperatures and greater variability, resulting in desertification.

Salinity and drought are the major problems in agriculture in arid and semi-arid regions, and considerable areas are lost due to salinization each year (Munns and Tester, 2008). New approaches are required to cope with this situation, and one option is the use of crop species with tolerance to soil salinity (Koyro *et al*., 2008). One of those, and perhaps the most promising, is quinoa (*Chenopodium quinoa* Willd.). The wide genetic variability in salinity tolerance in quinoa provides an excellent source for selection and breeding for higher tolerance (Gomez-Pando *et al*., 2010; Ruiz-Carrasco *et al.*, 2011; Adolf *et al*., 2012a). Quinoa combines a high natural tolerance to salinity (Hariadi *et al*., 2011; Razzaghi *et al*., 2011a; Pulvento *et al*., 2012; Yazar *et al*., 2013a) and a number of other environmental stress factors (Jacobsen *et al*., 2003; Razzaghi *et al*., 2011b), with high nutritional quality (Repo-Carrasco *et al*., 2003; Stikic *et al*., 2012). Quinoa belongs to the Amaranthaceae family and is a halophyte with over 3 000 accessions presenting a wide range of diversity in terms of salinity tolerance and other characters. It has been shown that varieties from the Bolivian Altiplano are less affected by salinity than varieties from non-saline areas (Adolf *et al*., 2012b).

Chenopodium quinoa Willd. is an Andean pseudocereal cultivated since 5 000 B.C. in its native area. During the European colonization of South America, quinoa was scorned by the Spanish *conquistadores*, and even actively suppressed because of its status within indigenous non-Christian ceremonies (Mujica *et al*., 2001). Recently, it has been introduced in the United States of America and Canada and also in Europe, where it is a candidate crop for agricultural diversification. The species is an annual Amaranthacea with good adaptability to different environmental conditions. It is drought resistant, and tolerant to frost, saline soils, diseases and pests (Mujica *et al*., 2001; Jacobsen *et al*., 2003; Jacobsen *et al*., 2005).

This traditional Andean seed crop has been cultivated in the Peruvian and Bolivian Andes for more than 5 000 years (Pearsall, 1992). It grows under unfavourable soil and climatic conditions (Garcia, 2003) and is rapidly gaining interest throughout the world (Jacobsen, 2003) because of its robust character and its high nutritional value. The seeds have a high protein content and a balanced presence of essential amino acids (e.g. lysine), as well as being rich in vitamins and minerals (Comai *et al*., 2007). It is robust with good tolerance to frost (Jacobsen *et al.*, 2005), drought (Geerts *et al*., 2008a) and soil salinity (Sanchez *et al.*, 2003; Jacobsen *et al.*, 2003; Jacobsen and Shabala, 2013). Quinoa is an annual crop species belonging to the C3 group of plants (Jacobsen *et al.*, 2003). It grows to a height of 0.5–2 m, terminating in a panicle that consists of small flowers producing 1 seed per flower. The 1 000-grain mass is generally low (3–6 g) due to the small seed size (Geerts *et al.*, 2008b). The seed are of high nutritional value, but they also contain the anti-nutritious component, saponin, in varying concentrations, depending mainly on the variety (Ward, 2000), and these saponins need to be removed before consumption. Different agronomic characteristics of a large number of quinoa varieties are listed by Bhargava *et al*. (2006).

Map 1. Location of Turkey

Agricultural Situation in Turkey

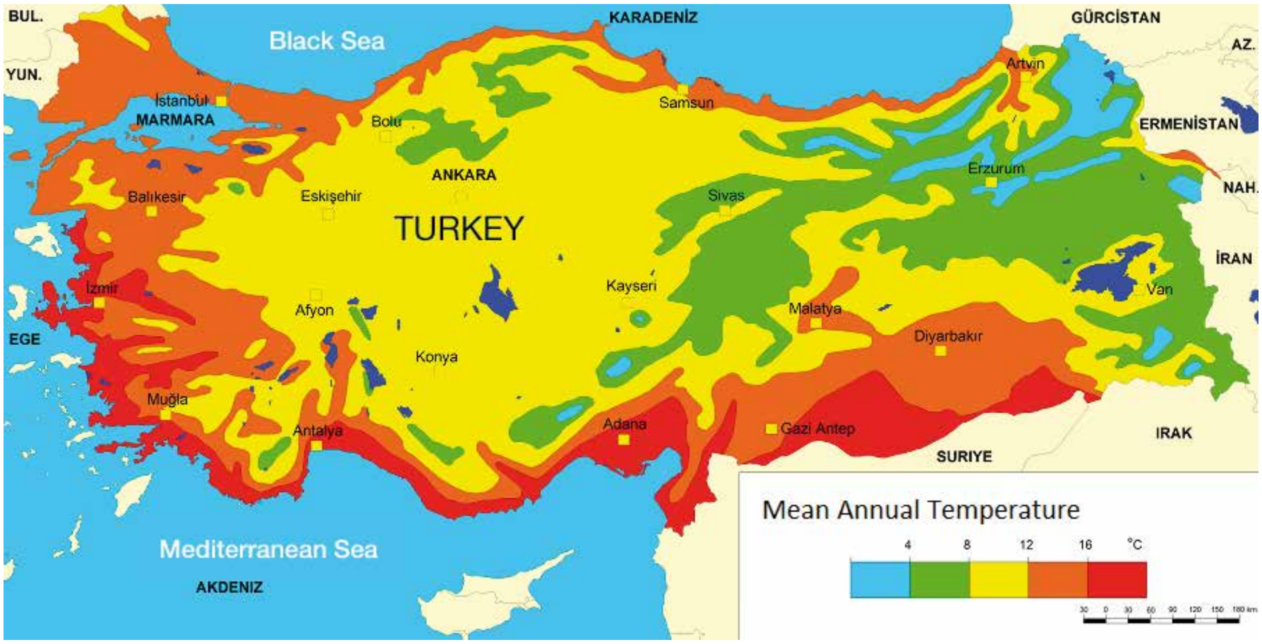
Turkey is located in a unique geographical position at the junction of three continents: Asia, Europe and Africa (Map 1). This “crossroads” location, combined with diverse geomorphological and climatic conditions, means that Turkey is a key country for global biodiversity conservation with species originating from the north (Europe), the east (western Asia) and the south (Africa). Turkey is one of the most important countries in the world in terms of agricultural genetic diversity and resources. Many annual and perennial, herbaceous and woody plants used in Mediterranean and temperate agricultural systems originate from Turkey and the country is recognized as a “centre of domestication” where ancient agriculture started several thousand years ago (Tan, 2003). Important crops originating from Turkey include wheat, barley, oats, peas and lentils, as well as many cultivated fruit species, such as cherries, apricots, almonds and figs. Turkey is also home to a number of ornamental flowers, the most notable being the tulip. There are two aspects to Turkey’s significance as a centre of crop genetic diversity. First, it is still home to many wild relatives of cultivated crops. Second, there are high levels of genetic diversity among local cultivated crop varieties. This is the case in particular in marginal mountain areas, where traditional farming methods have been maintained, rather than in the intensively cultivated coastal regions or the Anatolian Plateau.

Turkey has a total area of 778 997 km^2 with a population of 76 million. It is a high altitude country, averaging 1 132 m asl. The European part of the country (Thrace) is a fertile hilly land, while the Asian part (Anatolia) consists of an inner plateau with mountain ranges along the north and south coasts. This plateau rises from sea level in western Anatolia to 800–1 000 m asl in the centre and > 1 700 m asl in the east. Soils are generally poor and productivity is limited by their depth, combined with altitude, low rainfall and steepness. Only 15.2% of soils have a depth of > 90 cm, and the majority (72.1%) are shallow (20–50 cm) or very shallow (0–20 cm).

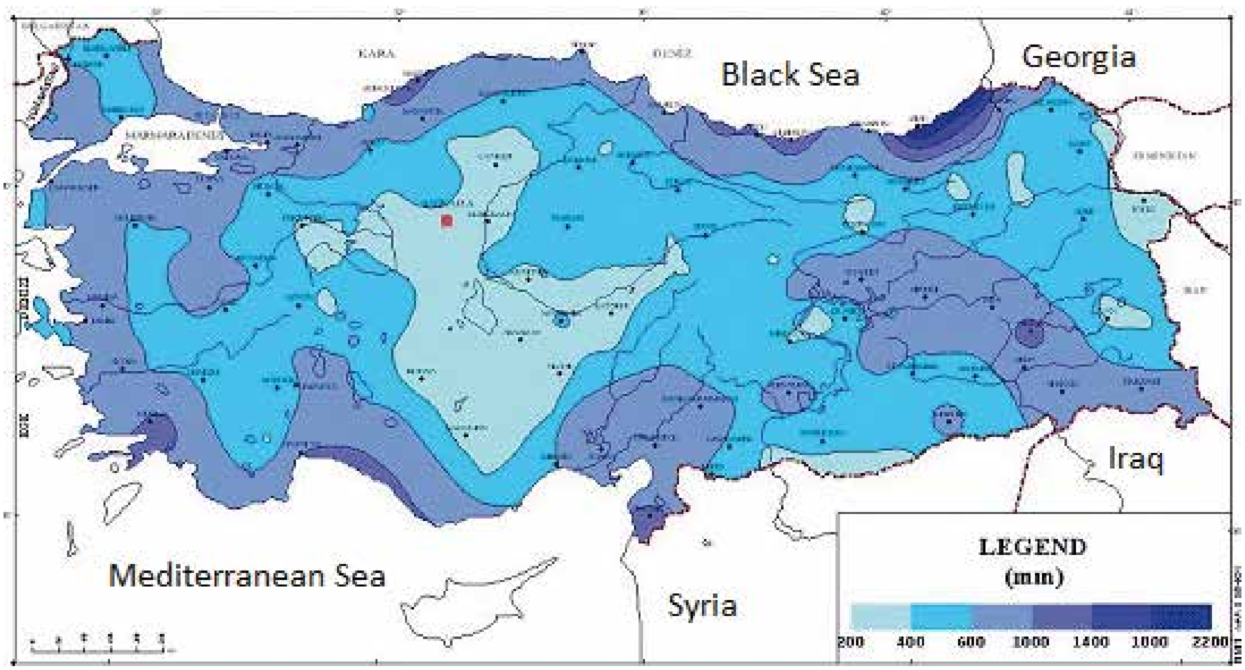
Turkey has a semi-arid climate, but the diverse topography (in particular, the existence of mountains parallel to the coast) produces marked differences in the climatic conditions of the various regions. The southern coastal areas of the Aegean and Mediterranean regions enjoy a Mediterranean climate with hot, dry summers and mild, rainy winters. In contrast, the Black Sea climate of the northern coastal areas is much wetter and cooler throughout the year, while the high plateau of Central Anatolia is characterized by a steppe climate with relatively little annual precipitation and much greater differences in temperature between the cold winters and hot summers (Map 2). Average precipitation in Turkey is 646 mm per year, but there are huge variations between regions: from almost 2 500 mm in the high mountains of the eastern Black Sea region to 250–300 mm in some parts of central Anatolia (Map 3).

The favourable climate and a strong farming tradition continue to make agriculture an important sector of the Turkish economy. Although agriculture’s contribution to the total GDP fell from 26.1% in 1980 to 8.2% in 2012, almost one-third of the Turkish population is involved in agriculture and 11% of total exports are agricultural products (Çakmak, 2004).

Approximately half (53%) of Turkey’s total area of 77.9 million ha is currently used for crop and livestock



Map 2. Mean Annual Temperature distribution in Turkey (www.meteor.gov.tr)



Map 3. Average annual precipitation distribution in Turkey (www.meteor.gov.tr)

production, including an estimated 26.6 million ha of cultivated land used for arable crops (cereals, pulses and industrial crops), forage crops for animal feed, fruit and vegetables, vineyards and fallow land. The majority of this cultivated land is privately owned. Approximately 5.2 million ha of land are currently under irrigation, since without irrigation much of the land can only support low-yielding dryland crops. In addition, there are 14.2 million ha of grasslands and rangelands (dry grasslands); they are predominantly state-owned and used for common grazing, while some privately-owned meadows are used for hay-making. The remaining land area includes an estimated 20.8 million ha of forest (99% state-owned) and approximately 16 million ha of non-cultivated land, including built-up areas. The majority of farms are typically small-scale and fragmented, except in the more prosperous and fertile coastal regions. Data from the 2012 agricultural census reveal the following: average farm size is 6.1 ha; over 83% of farmers own < 10 ha of land (occupying approximately 42% of total cultivated land); less than 1% of farms have > 50 hectares (occupying approximately 17% of total cultivated area) and these include the large-scale, specialized horticultural producers located in the Aegean and Mediterranean regions (TUIK, 2012).

Subsistence and semi-subsistence farming is an important element of Turkish agriculture and provides income security and a livelihood to the majority of the rural population. However, these farms are also characterized by low productivity, high hidden unemployment and lack of competitiveness. The majority of the sector is also “informal” with only a small minority of farmers paying income tax or participating in the national self-employed social security scheme (OECD, 2008). It is also difficult to apply traditional market and price policies, because they only market a small part of their production. Crops are the most important agricultural product and contribute approximately 55% to the total value of agricultural production. Given the immense range of environmental conditions, farmers produce a wide variety of fruits, vegetables, cereals and industrial crops, such as cotton, sugar beet and tobacco. In terms of land use, the most widely grown crops are cereals, especially wheat, while in terms of economic output (including exports), the most valuable crops are fresh and dried fruits and nuts, including citrus fruits, grapes, olives and hazelnuts. Livestock farming is also an important part of the agricultural economy (Yesilada *et al*., 2010).

As a result, it is vital to develop alternative sources of income and new employment opportunities in rural areas. This is especially important since the number of farms is decreasing rapidly as rural people migrate to the urban areas. Between 1991 and 2011, the total number of farmers in Turkey declined by 25% from 4.1 to 3.0 million, and this trend is likely to continue.

Quinoa experimentations and production in Turkey

Quinoa was introduced in Turkey for the first time in 2008 as part of a European Union project within the seventh framework programme titled “Sustainable water use securing food production in dry areas of the Mediterranean region” (SWUP-MED). The strategic objective of the project was to improve food crop production in the Mediterranean region under multiple abiotic stresses. These stresses are becoming more pronounced under a changing climate, predicted to produce drier conditions, increasing temperatures and greater variability, resulting in desertification. One of the specific objectives of the project was to introduce and test new climate-proof crops, such as quinoa and amaranth, using cultivars with improved stress tolerance and selecting promising varieties of new crops. Several field experiments were carried out in the Mediterranean region of Turkey, testing climate-proof crops (quinoa and amaranth) in different locations presenting different challenges.

Field experiments were set up in order to evaluate the yield response of quinoa (*Chenopodium quinoa* Willd. L ‘Titicaca’) to saline and freshwater under Mediterranean climatic conditions in 2009, 2010 and 2011 (Yazar *et al*., 2013a). This research was conducted in the experimental field of the Irrigation and Agricultural Structures Department of the Cukurova University in Adana, Turkey (Maps 1 and 2). The station is located at 36º59’N, 35º18’E, 50 m asl. The soil of the experimental site is classified as the Mutlu soil series (Palexerollic Chromoxeret) and has a clay texture throughout the soil profile. The available water-holding capacity of the soil is 198 mm in the 120 cm soil profile. The 2009 experiment compared four different irrigation treatments: full irrigation using freshwater (FIF); full irrigation using saline water (FIS); deficit irrigation (DI); and partial root-zone drying (PRD). DI and PRD treatments were irrigated using freshwater. Under PRD, half the root zone is wetted while the other half is kept partially dry. The 2010 experiment included a total of nine different irrigation treatments: full irrigation using freshwater (FIF); full irrigation using saline water at different salt concentrations (FIS-40 dS/m; FIS-30 dS/m; FIS-20 dS/m; FIS-10 dS/m); deficit irrigation (DIF-50; DIF-75; DIS-40); and dry treatment. The 2011 experiment considered eight different irrigation treatments: full irrigation using freshwater (FIF); full irrigation using saline water at different salt concentrations (FIS-

30 dS/m; FIS-20 dS/m; FIS-10 dS/m); deficit irrigation (DIF-50; DIF-75); PRD; and dry treatment. Saline water was prepared by diluting seawater with canal water. A day-length-neutral variety, 'Titicaca', the seeds of which were selected at the University of Copenhagen from material originating from southern Chile was used (Christiansen *et al.*, 2010). Quinoa seeds were sown by hand (3–4 cm apart with 50-cm row spacing) on 10 April 2009, 26 March 2010 and 28 March 2011, respectively. At planting, a composite fertilizer (15-15-15) was applied by broadcasting at a rate of 75 kg/ha N, P_2O_5 and K_2O, and incorporated into the soil. Quinoa plants were thinned to approximately 15 cm apart between the plants in the row. Drip irrigation systems were laid out in the plots, and irrigation treatments were started. Salinization was induced at the beginning of floral bud formation. In 2009, quinoa received a total of 302 mm under FIF, and 151 mm under DI and PRD. In 2010, a total of 320 mm of irrigation water was applied to full irrigation treatments, both fresh and saline; DIF-50 and DIS-50 received 160 mm and DIF-75 received 240 mm. In 2011, only single irrigation was applied to treatments due to above average rainfall during the growing season.

Tables 1–3 show the grain yield, irrigation water applied, dry matter yield (DM), seasonal crop water use (ET), water productivity (WP) and irrigation water use efficiency (IWUE) for quinoa under the different irrigation treatments in the Mediterranean region of Turkey.

Irrigating quinoa with saline water under Mediterranean climatic conditions showed that plant growth is not negatively impacted by irrigation water salinity of 40 dS/m compared with freshwater irrigation. However, the water stress to which plants were subjected under DI treatments resulted in a considerable reduction of plant biomass yield. Soil salinity was highest in the top 10 cm soil layer of FIS-40 treatment plots, followed by DIS-50 and FIS-30 treatments. Salt accumulation decreased with soil depth. The higher the irrigation water salinity, the greater the soil salinity. Deficit irrigation with salinity of 40 dS/m gave the highest salt accumulation in the top layer. Yield parameters (e.g. above ground biomass, seed yield and HI) all indicated good adaptation of 'Titicaca' in the Mediterranean environment. Saline and water stress did not interfere with crop yield, and quinoa may, therefore, be defined as a crop tolerant to salinity and drought.

Re-use is an important and natural method of managing drainage water. In order to obtain the maximum benefit from a water supply and to help dispose of drainage water, strategies for water re-use have evolved. Water re-use must take into account both short- and long-term needs, considering both local and off-site effects. In regions of limited irrigation water supply, drainage water can be used as a supplement. However, exactly which crops can be irrigated depends on the quality of the drainage water. Field experiments were carried out in 2012 and 2013 in the experimental fields of the Soil and Water Resources Research Institute in Tarsus, Turkey. The objective was to evaluate the effect of planting dates and of supplemental irrigation using drainage canal water (Yazar *et al.*, 2013b). The station is located at 37°01'N, 35°01'E, 10 m aasl. The soil is classified as Arikli: silty–clay–loam, with a relatively high water-holding capacity. The experiment was laid out using two line-source irrigation systems to

Table 1. Grain yield, irrigation water applied, dry matter yield (DM), seasonal crop water use (ET), water productivity (WP), and irrigation water use efficiency (IWUE) data of Quinoa under different treatments in the Mediterrranean region of Turkey in 2009

Treatments	Irrigation (mm)	ET mm	Grain Yield (kg/ha)	1000 Seed Weight (g)	DM (g/m^2)	Plant Height (cm)	HI %	IWUE kg m^3	WP kg m^3	LAI
FIF	383	450a	2120	2.6	1932.5	130	52.3	0.55b	0.47b	4.5a
DIF	202	343b	1691	2.2	1812.5	113	48.2	0.84a	0.49b	3.0c
PRD	202	321b	1873	2.1	1649.2	116	53.1	0.93a	0.58a	2.8c
FIS	383	462a	1784	2.4	1917.9	127	48.1	0.46b	0.39c	3.9b
LSD		58	ns	ns	ns		ns	0.073	0.087	0.94

Table 2. Grain yield, irrigation water applied, seasonal crop water use (ET), water productivity, and irrigation water use efficiency data of Quinoa under different treatments in the Mediterrranean region of Turkey in 2010.

Treatments	Irrigation (mm)	ET mm	Grain Yield g/plant	Grain Yield (kg/ha)	1000 Seed Weight (g)	WP kg/m³	IWUE kg/m³	HI %	Relative Yield Reduction
FIF	320	576	29,86ab	2986	2,52	0,52	0,88	44	5,8
FIS-40	320	466	31,70a	3169	3,14	0,68	0,94	46	0,0
DIS%50	160	348	17,78c	1778	3,20	0,51	1,00	45	43,9
FIS-30	320	481	21,64bc	2164	2,72	0,45	0,62	51	31,7
FIS-20	320	524	23,62abc	2362	2,96	0,45	0,68	48	25,5
FIS-10	320	516	27,35abc	2735	2,50	0,53	0,80	48	13,7
DIF%50	160	322	18,89c	1889	3,12	0,59	1,07	48	40,4
DIF%75	240	483	23,16abc	2316	2,92	0,48	0,89	44	26,9
DRY	0	247	17,13c	1714	2,51	1,39	0,00	45	46,0
LSD			8.773		0.04074				

Table 3. Grain yield data of Quinoa under different treatments in the Mediterrranean region of Turkey in 2011.

Treatment	Plant Height cm	Panicle weight g/plant	Stem Weight g/plant	Biomass g/plant	Grain Yield per plant g	1000 Seed weight, g	HI %
DRY	80	31.18	12.40	43.58	23.19b	2.56c	53
FI-100	84	41.09	14.38	55.47	31.80a	2.83ab	57
DI-75	74	37.98	11.35	49.34	26.35ab	2.82ab	53
PRD	70	36.41	10.91	47.32	26.47ab	2.78ab	56
DI-25	82	33.94	11.35	45.29	24.37ab	2.98a	54
FS-10	70	36.97	11.98	48.96	28.58a	2.98a	58
FS-20	69	41.88	10.29	52.18	30,63a	2.90a	59
FS-30	73	36.45	9.95	46.40	27.00ab	2.72ab	58
LDS			1.		7. 837	0.04211	

allow gradual variation of the irrigation, placed at right angles to the source. One line-source sprinkler system was set for each water resource. Four irrigation levels – one full (I1) and three deficit (I2–I4) – were envisaged. I2, I3 and I4 treatments represent deficit irrigation of approximately 20, 50 and 80%, respectively. The quinoa variety, 'Titicaca', was planted on 11 April 2012 (normal planting) and on 30 April 2012 (late planting). Quinoa seeds ('Q52') were provided to a seedlet-producing company in Mersin, and the seedlets were then transplanted to experimental plots (20 cm between plants, 50-cm row spacing). The quality of the drainage water varied: from 0.573 dS/m in June to 1.684 dS/m in April. Seedlets were transplanted (20 cm between plants, 50-cm row spacing) on 11 April and 30 April 2012 for normal and late planting, respectively. At both planting times, 70 kg/ha composite fertilizer (20-20-20) was applied and incorporated into the soil. On 15 May 2012, 50 kg/ha urea (46% N) was applied. Quinoa was harvested on 10 July and 20 July 2012, respectively.

The amount of irrigation water applied in the treatment plots varied between 71–311 mm (normal planting) and 95–395 mm (late planting). Seasonal water use varied from 222 mm in rainfed to 456 mm in I1 level (normal planting); the corresponding values for late planting were 208 and 473 mm. Irrigation levels significantly ($P \leq 0.05$) affected quinoa grain yield. Quinoa grain yields in the normal planting plots were generally higher than in the late planting plots. Thus, the differences in yield between the normal and late planting treatments are statistically significant. The highest grain yield of 6.38 tonnes/ha was obtained from treatment plots adjacent to the line-source in I1 treatment under normal planting conditions. For late planting, the highest grain yield (2.61 tonnes/ha) was obtained in I1 treatment plots. The lowest yields were in the I5 treatment plot (2.21 and 1.10 tonnes/ha for normal and late planting, respectively). Grain yields fell significantly as the amount of irrigation water decreased. Water use efficiency (WUE) was 1.00–1.57 kg/m^3 under normal planting and 0.53–0.75 kg/m^3 under late planting. Plant height at harvest varied between 50.8–75.3 cm for normal planting and 47.0–75.3 cm for late planting. I1 treatment produced the highest plant height in both normal and late planting. Mean plant height was significantly affected by both irrigation time and irrigation level. As the amount of irrigation water applied decreased, plant height also decreased significantly. Other yield attributes also varied with irrigation, for example, 1 000-grain weight values went from a low of 3.03 g to a high of 3.29 g in normal planting, and from 2.62 to 2.79 g in late planting. The 1 000-grain weight increased as the distance from the lateral increased, and as the amount of irrigation water decreased, the 1 000-grain weight increased. The results show that grain yield, seasonal water use, water use efficiency (WUE) and irrigation water use efficiency (IWUE) all depend on the controlled ranges of soil water content. The grain yield response to irrigation varied considerably depending on the soil water content and rainfall distribution during the growing season. Significant linear relationships were found between the seed yield and ET under normal and late planting (Figure1a). The relationship between irrigation water and yield of quinoa is best described by a strong polynomial function for each planting time (Figure1b). Quinoa seed yield increased with increasing ET in both normal and late planting times, while water use decreased significantly in those treatment plots receiving limited irrigation.

Economics of quinoa production and markets

The current market for quinoa in Turkey is very limited. In order for the market to expand, an effort must be made to educate people in Turkey about what quinoa is and how to cook it. An international market exists for quinoa, and quinoa is available for sale in most health food stores and hypermarkets in Turkey. Quinoa consumption is limited to those with knowledge of health foods who value quinoa for specific health benefits, including its gluten-free status. Quinoa is not a product "consumed by the masses", but rather one "discovered" by educated, health-conscious consumers.

In Turkey, this crop has been available in health food stores, and more recently in grocery stores in big

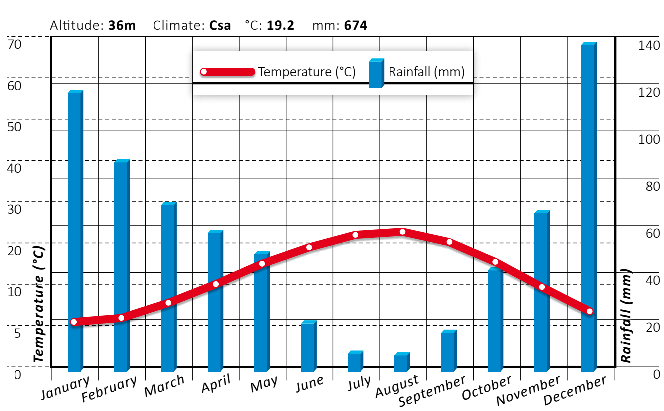


Figure 1a. Average monthly temperature and precipitation of Turkey

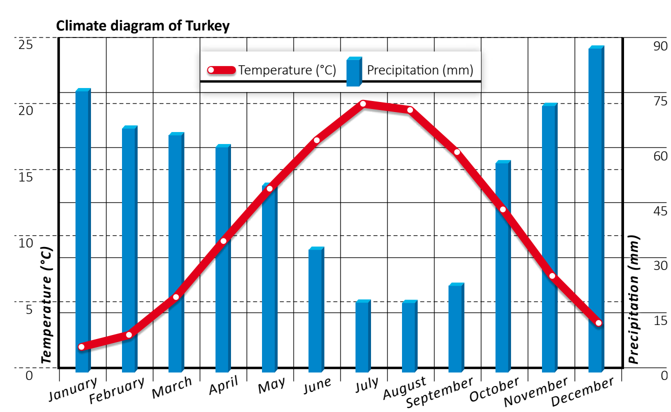


Figure 1b. Average monthly temperature and precipitation in Adana, Turkey

cities, at prices ranging from EUR5.0 to 8.0 per kg. The high nutritional quality, good flavour and versatility of quinoa mean that there is a good potential market. Prospective growers in areas with suitable environmental conditions are advised to make contact with a marketing group to discuss quinoa seed contracts before going ahead and raising the crop. The market prospects and growth potential of quinoa are incentives for the food industry, which is ready to make quinoa products. However, there are also disincentives – notably cultural problems and suboptimal yields – and companies are therefore holding back until there is a larger, more reliable supply available to processors.

Since quinoa production is not practised commercially in Turkey, production costs for quinoa under rainfed conditions have been estimated using the data obtained from the experimental work carried out in Adana. The cost of rainfed quinoa production is estimated at TRY1 324/ha. For irrigated quinoa, the total production cost is approximately TRY3 000/ha for pressurized irrigation system. In comparison, total production costs of rainfed wheat and chickpea are TRY1 653/ha and 1 463/ha, respectively (Yazar *et al.*, 2013c). The cost of production for growers in the Mediterranean is expected to decrease as they become more familiar with the crop and obtain higher yields. The production cost per kg of quinoa is currently TRY 0.60–0.80 for rainfed and TRY0.50–0.90 for irrigated conditions (in 2013, 1 TRY (Turkish lira) was equal to USD0.55 and EUR0.42).

Evaluating farmers' perceptions and obtaining feedback about the adoption of new crops (such as quinoa and amaranth) is necessary in order to improve the efficiency of research, technology exchange and information flow to policy-makers. It is important to discern and understand what influences farmers' behaviour and attitudes towards adoption of a new crop. Farmers must analyse the financial and social costs and benefits of new crops, farming practices and economic activities. Therefore, in order to develop and implement guidelines for recruiting quinoa growers and promoting long-term producer participation in Turkey, it is essential to understand the factors farmers consider when evaluating land use change, production activities on the farm and resource allocation. Quinoa represents an emerging market, currently in the research and demonstration project stage. Most quinoa research has been centred in the Mediterranean region. Quinoa is a valuable food crop: its production can benefit farmers and taxpayers. Since the market for quinoa is not well developed, it is necessary to gather information about producers' attitudes toward quinoa markets, the net returns required to produce quinoa and the acreage that can potentially be converted to quinoa. The purpose of this study is to assess producers' views on quinoa markets, their willingness to produce quinoa, and the area and type of agricultural production that could be converted. A survey was conducted to obtain information about Adana farmers' views on quinoa production (Xhoxhi, 2012; Pedersen *et al.*, 2013). A logit model was then used to show which farm and farmer characteristics most affect adoption rates of quinoa. Using the estimated logit model, an analysis was done to predict the likelihood of quinoa adoption by survey respondents who did not know if they would be interested.

Structured interviews were used for data collection among farmers. The results reveal poor farmer knowledge of quinoa, with differences in knowledge among the farmer categories studied. Overall, farmers have a good perception of new technologies and are willing to adopt the crop. Economic factors are the most important issues for farmers considering its adoption. The crop's sustainability in Turkey depends on this factor, and it should be considered very carefully, especially in view of current complexities in the world quinoa market. Trial projects with educational programmes incorporating farmers' knowledge and perception are a good way of introducing the crop to farmers in Turkey (Lind *et al.*, 2013).

There appears to be significant market potential for quinoa, since it is a gluten-free product. Strategies aimed at introducing this crop in the Mediterranean region should attempt to establish links between food companies or farmers' cooperatives operating in these regions and the farmers. A strategy is therefore required to increase quinoa awareness among such companies. Moreover, the potential benefits of gluten-free food in industrialized countries and in cities in Turkey and Morocco (e.g. Istanbul, Ankara, Adana) should be addressed. By raising awareness of these benefits, it is more likely that the introduction of quinoa in the farmers' production system will be a success.

The creation of a market for quinoa in Turkey has several beneficial effects for the two regions. One such benefit is with regard to production on lands with elevated levels of salinity and not used for other crop production. Quinoa also presents drawbacks, however: low yields and high labour requirements compared with corn and wheat. Low yield is particularly negative in terms of crops grown for local consumption. On the other hand, quinoa reaches higher prices than either corn or wheat in the international market, potentially compensating for low yields and high labour costs.

Wossink and Boonsaeng (2003) observed that farmers' perception and knowledge is crucial for successful research and development strategies. They stated that many promising agricultural policies have failed because they did not match farmers' needs and perception. Perception generally refers to how people select, organize and interpret information gained through the senses or experience. Sustainability of agricultural production is largely dependent on farmers' actions and their ability to make decisions on the basis of the knowledge and information available to them (Rahman, 2003). However, the role of perception has received very limited attention in studies regarding farmers' adoption of a new technology. Programmes tend to fail to address situations where farmers' knowledge is lacking and inadequate. In order to prevent failure with quinoa and ensure sustainable adoption of this new technology, a good understanding of the knowledge, needs and perception of farmers is essential before a systems approach can be devised to introduce the crop. This study complements the limited scientific studies available to date on the sustainability of quinoa. Furthermore, an assessment of farmers' perception and potential problems is necessary to eliminate any pro-innovation bias that may unknowingly characterize the research (Rogers, 1995).

The study aims to provide scientific information on the social and psychological factors influencing acceptability of the new crop and large-scale quinoa production in Turkey. It will be instrumental in bridging the current social and psychological knowledge gap on quinoa. It aims to gain an understanding of the psychological and social factors underlying the adoption of a new crop by farmers. Psychological factors account for uncertainties in the minds of adopters of this innovation, and if the farmers' perceived problems can be understood and solved, then uncertainty can be reduced (Rogers, 1995). Social factors, on the other hand, are related to the extent to which other farmers are dependent on the subjective evaluation of the innovation by individuals they consider to be more like themselves (i.e. farmers who have previously adopted the innovation) (Rogers, 1995).

In addition, the study will help promote sustainable large-scale quinoa production to ensure a reliable income for farmers and reduce poverty levels in Turkey. If the potential ability of Turkish farmers to sustainably produce quinoa on a large scale is identified, policy-makers and investors can then be provided with evidence of the viability of the project.

Finally, the study sets out to design appropriate educational programmes to fill in the gap in farmers' knowledge and help them gain an objective perception of the crop before and during its introduction. This is of paramount importance, because quinoa is a new crop and farmers must have sufficient knowledge to ensure sustainable cultivation.

Farmers listed the desirable conditions for adoption of a new crop: market availability, financial aid, education on cultivation, factory availability, good prices, and availability of high-yielding and early-maturing varieties. It is economic factors that dominate their needs. These findings agree with those of Negatu and Parikh (1999): marketability and grain yield are the two most important ingredients affecting the decision to adopt. Fernandez-Cornejo and McBride (2002) pointed out that an innovation's profitability (yield, input cost and cost of adoption relative to current management practices) is the main motivation behind adoption.

This study offers insight into farmers' knowledge and perception of quinoa and why this is important for sustainability of the crop in Turkey. The principal conclusions and recommendations of this research are as follows:

- Economic factors dominate farmers' concerns and the major factors are low crop prices and high input costs. Social problems include administrative bottlenecks, political interference and absence of transparency in pricing, while pests and diseases, as well as drought, rank highest among the environmental problems.
- Economic advantages are key to influencing whether farmers adopt quinoa. Market availability, financial aid, training in cultivation, high yield, good prices and early maturity were important factors conditioning commitment to the innovation-decision process. These economic factors also influenced previous post-adoption behaviour.

- Farmers' *awareness* and *how-to knowledge* of sugar beet is generally low. There is, therefore, a knowledge gap for quinoa among the Turkish farmers studied.

- Overall, there is no difference in the perception of new technologies between quinoa trial and non-trial farmers. They all have a positive perception towards innovation and are willing to adopt quinoa. An analysis of their post-adoption behaviour, however, shows that they will stop cultivating quinoa if they find it is not meeting their needs.

- For quinoa to be sustainable in Turkey, the findings on knowledge and perception should be incorporated into an educational programme for farmers before the crop is introduced on a large scale. Since the domains of sustainability are interlinked rather than distinctive, the social/psychological sustainability analysed by this research can only work if the economic and biological aspects complement it.

- Introduction of new crops on a trial basis is very important, as it affords farmers, extension officers and interested parties the opportunity to monitor project progress and carry out effective educational programmes. Further trials should be established in coordination with the agricultural ministry to build and improve farmers' *awareness* and *how-to knowledge*. An educational programme should be established to consider the local knowledge of the farmers and the extent to which they can depend on previous knowledge. Good knowledge will help farmers have the right perception of the crop and this is crucial in influencing the decision of those farmers who have yet to adopt the crop.

- Immediate steps should be taken to establish a market in order to be able to proceed with large-scale trials. The current small-scale trials cannot highlight all the difficulties potentially arising in large-scale production. Market availability is fundamental for trial farmers, as it will determine whether they continue cultivating it, it will influence future adoption decisions and will help farmers form a conclusive perception of the crop.

- Future research should look at the economic sustainability of quinoa in Turkey and how the project can be carried out in order to bring benefit to all groups.

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CHAPTER 6.1.5

Status of quinoa production and research in Morocco

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Abstract

Climate change, population increase and overgrazing have created an urgent need in Morocco to develop a new approach for preserving native crop genetic resources and protecting food security – especially for the rural poor. The preliminary diagnosis, carried out in 2000 in the Khenifra region in the Middle Atlas Mountains, aimed to identify social, economic and technical constraints. Crop diversification and the introduction of adapted cultivars were identified as major priorities. Quinoa is a potentially new crop with high nutritional value, of special interest for areas that experience frequent drought conditions.

The specific aim of the IAV-BYU project, initiated in 1999, was to address food security issues for Moroccan subsistence farmers by selecting new crops with suitable genotypes to improve dietary needs. Important scientific outcomes, related to quinoa cultivation, adaptation and productivity, were achieved.

In 2008, the area under quinoa cultivation was extended to the semi-arid region of Rhamna through the "Sustainable water use securing food production in dry areas of the Mediterranean region" project (SWUP-MED) sponsored by the European Union. Quinoa was selected by SWUP-MED to specifically diversify and improve the sustainability of the Mediterranean cropping systems.

The 24 quinoa accessions introduced into the area were derived from the FAO-CIP international nursery (Izquierdo *et al*., 2003). During the initial 3-year period, the material was planted and assessed at three experimental sites. The best-performing genotypes were selected and distributed to local farmers for *in situ* evaluation and farmer selection. Several lines were developed by the IAV programme, and in 2005 suitable lines were given to farmers in the Middle Atlas (Khenifra) and High Atlas (Marrakech) regions. Advanced lines were also introduced to the Bouchane and Agadir regions in 2009 as part of the SWUP-MED project.

Demand for quinoa is quite high at national level, but can vary dramatically at local level. For example, quinoa production in 2012 in Khenifra was almost non-existent due to the lack of a local market to sell quinoa products. Similarly, in Bouchane, total production did not exceed 0.5 tonnes in 2012 because of the lack of a local market. In various regions across the country, many private farmers have taken the initiative to grow quinoa. Seeds – and perhaps more importantly, knowledge of a suitable crop management system – are not currently available to assist such initiatives.

Figure 1. Morocco in the Mediterranean map

Context for introducing quinoa in Morocco

Climate change and world population increase have created a threatening situation at international, regional and local level. In Morocco, overgrazing throughout the temperate zones means that it is now crucial to design strategies to preserve native crop genetic resources and maintain food security. IAV Hassan II and BYU initiated a joint project in 1999 to find concrete ways to improve the conditions of the mountain population in three villages in Middle Atlas, Morocco (Figure 1). The preliminary diagnosis aimed to identify the social, economic and technical constraints, in order to develop a strategic plan and appropriate tasks. The priority actions that emerged were crop diversification and introduction of adapted cultivars. A list of potential species was established.

Quinoa has good potential under drought conditions; it has high nutritional value and is rich in proteins and amino acids. Compared with wheat, quinoa has 40% more iron, 400% more calcium and 20% more protein, and a substantially higher lysine content. Quinoa can make an important contribution to the local diet. It could also contribute to improving the crop management system, given that the traditional cereal-legumes rotation has been neglected since the early 1980s when drought became a persistent occurrence. A quinoa-cereal rotation represents a suitable alternative to cereal monocropping or cereal-fallow cropping , both of which were widely practised in the Middle Atlas region at the time the project began.

The IAV/BYU quinoa crop diversification subproject had four overarching components/goals. The first goal was to improve the food security of Moroccan subsistence farmers by introducing new crops with superior nutritional value and abiotic stress tolerance. The second goal was to teach local farmers how to improve heterogeneous crop genetic resources through selection of superior types and how to conserve *in situ* these adapted plant materials. The third goal was to educate local families in food preparation and marketing of the introduced crops. Finally, the fourth goal was to provide collaborative international educational opportunities for IAV and BYU students. Important scientific outcomes of this project included the publication of peer-reviewed data related to quinoa production in hot, arid regions of Morocco, as well as the conservation, characterization and domestication of Chenopodiaceae germplasm native to Morocco.

Testing of quinoa began again in 2008 in the semi-arid region of Rhamna under the EU project SWUP-MED (Sustainable water use securing food production in dry areas of the Mediterranean region). The project's principle objective concerns the implementation of new sustainable crop management technologies able to improve crop productivity and Mediterranean cropping systems in the current situation of climatic change and population increase. Quinoa was selected a second time as the elite crop to be introduced in rotation with cereals and other crops as grain legumes.

History of the research areas

Quinoa in the Middle Atlas region

During the first phase of quinoa introduction and adaptation in Khenifra in 2000–07 (Figure 2), the programme had two main phases. The first was implemented over 3 years and involved the assessment of the adaptation of the introduced germplasm. The second phase involved selection of the best genotypes based on productivity (yield), tolerance to pathogens and pests, and seed quality.

Adaptation phase

Throughout the first project phase, quinoa was grown on small plots (≤ 0.25 ha) for experimental evaluation and selection of suitable genotypes or propagation of seeds. With the exception of tillage, which was mechanized, all the cropping tasks – planting, weeding and harvesting – were done manually. Agronomic experiments entailed comparisons between sowing modes (broadcasting and row-hand seeding) and dates (winter, Nov.–Dec. and early spring, Feb.–Mar.).

The first quinoa germplasm entries were planted in 2000 and consisted of 14 accessions believed to be tolerant to drought, obtained from the CIP-FAO international quinoa nursery. The experiment evaluated the accessions for their adaptation to the Moroccan environment in order to identify high-yielding genotypes with drought tolerance. The introduced germplasm was evaluated at the *Institute Agronomique et Vétérinaire Hassan II* (latitude 33.9814294, longitude -6.364133, 46 m asl), Rabat; at the *Institut Technique d'Agriculture* (ITA) of Ben Khlil (latitude 32.75043, longitude -5.684133, 833 m asl), Khenifra; and on three subsistence farms in the village of Agoudim, Khenifra (latitude 32.8585064, longitude -5.623453, 845 m asl).

Figure 2: Quinoa introduction sites

Photo 1: Quinoa field at Omar Ghanem farm, May 2004

Selection and evaluation phase

The quinoa selection phase was initiated after four evaluation seasons. In June 2004, BYU geneticists (Jellen and Maughan) and three farmers from Agoudim (Omar Ghanem, Oumessaoud Bouhouida and Mustapha Talaghram) selected the best genotypes in their fields (Photo 1). From the quinoa adaptation trials on the ITA experimental farm in Ben Khlil and farmers' plots, 300 genotypes were selected on the basis of their phenotypic traits (Table 1). They were then harvested separately to establish the first genetic pool for the quinoa selection programme. Expansion of the panicle, sensitivity to pathogens and pests, seed size and colour, and grain yield were the main selection criteria adopted (Photo 2).

Table1. Selected accessions from farmers' plots

Farmer plot	Accession	Panicle type	Precocity group
Mustapha Talaghram	113	2	2
	114	2	4
	119- 121	2	5
	109	3	2
	200	3	3
	108	4	5
	202	4	3
	105	6	5
Omar Ghanem	137	1	5
	11	2	1
	130	2	2
	208	2	3
	210	3	3
	123	6	2
	214	7	3
Oumessaoud Bouhouida	150	2	5
	151	2	5
	222	4	3
	230	5	3
	142	6	2
	143	6	5
	138	7	2
	224- 226	8	3

Photos 2. Diversity in the panicle shape and colour

The following growing season, the 300 established lines were again screened at the Rabat and Khenifra sites in replicated two-row plots. Both BYU partners visited in May 2005 to participate in the second selection cycle. This time, the number of accessions was reduced to 30, selected from the original 300 genotypes.

In the 2006–07 cropping season, the 30 selected lines were tested with two sowing dates (November and February) and in two localities (IAV Rabat and Tnine Ait Boukhayou at My Bouazza – latitude 33.293804, longitude -6.286926, 912 m asl). In the same season, a new introduction was made of 25 USDA-NPGS accessions received from Dr Jellen; they were sown in Rabat for seed increase and to study their behaviour under local environmental conditions.

In 2008–09, the remaining germplasm consisted of 20 accessions. A total of 13 selection lines, plus 7 maintained from the 25 introduced USDA-NPGS accessions, were evaluated for a second season at the IAV Rabat site.

Quinoa in Bouchane, Rhamna

Evaluation for drought tolerance

During the second phase of the quinoa introduction programme in Morocco (2008–2012), quinoa cultivation was extended to the semi-arid regions of Bouchane in the region of Rhamna (latitude 32.2732, longitude -8.390808, 310 m asl) and Ait Melloul Agadir (latitude 30.35.076, longitude -9.475965, 19 m asl) within the framework of the EU SWUP-MED project (Photo 3). Germplasm developed over 8 years of quinoa evaluation and selection in Khenifra, Rabat and Moulay Bouazza was used. The main objective of this second phase was to identify cropping systems for improved sustainable water use efficiency. Quinoa, newly introduced, was tested as a rotational crop with cereals under dryland and irrigated conditions. Crop stress physiologists, agronomists and graduate students from the IAV Hassan II Rabat and Agadir campus and from the University of Marrakech were involved in the EU project programme. Following the experiments at Rabat, Bouchane and Ait Melloul, five advanced lines from the IAV selection programme were evaluated for their yield potential and their response to supplementary and deficit irrigation. The experiments also in-

Photo 3: Quinoa farmers' workshop in Bouchane, June 2009

vestigated the use of fertilization and of wastewater irrigation in quinoa productivity.

Mechanization of quinoa cropping

In the second phase of SWUP-MED, agronomic experiments were held to investigate the effects of nitrogen fertilization and crop automation (tillage, seeding and harvesting). This information (in the form of technical data sheets) is critical if the cultivation of quinoa is to be increased in Morocco. IAV agronomists reported the initial results at the SWUP-MED conference in Agadir, Morocco in March 2013.

Genetic resources used and origin

The first quinoa introduction to Morocco in 2000 consisted of 14 accessions from the FAO-CIP nursery, selected for their resistance to drought and tolerance to other abiotic factors. They originated from: Peru – Puno (15S, 70W, 3 825 m asl, 450 mm), Arequipa (16S, 72W, 2 350 m asl, 0 mm) and Lima (12S, 77W, 200 m asl, 10 mm); Bolivia – Choquenaira (16S, 68W, 3 822 m asl, 450 mm); and Kenya – Naivacha (0S, 36E, 1 829 m asl, 729 mm), and comprise 14 cultivars and entries ('Masal 389', 'Ecu-420', 'G205DK', 'Pandela', 'Utusaya', 'Huariponcho', 'Ayara' [wild], 'Sayana', 1 [80] 1, 03-08-51, 03-08-907, 03-21-79BB, 03-21-72 R M, 24 [80] 3).

In 2006, a second quinoa collection was introduced from the USDA seed bank, comprising 25 accessions from the United Kingdom ('RU-2'), IBTA-Bolivia ('Kamiri', 'Sayana', 'Ratuqui'), the Netherlands ('NL-6'), Denmark ('G-205-95', 'E-DK-4'), Chile ('Baer II'), Brazil ('02-EMBRAPA'), Peru Puno ('03-21-07988', '03-21-072RM', 'ILLPA-INIA', 'Kancolla', 'Salcedo'), Pjino ('Huariponcho'), Cusco ('CICA-17', 'CICA-127', 'CICA-17'), Colombia ('Narino'), UNA-Argentina ('Jujuy') and INIAP-Ecuador ('Ecu-420', 'Ingapirca').

International collaborations

Main international collaboration

Eric N. Jellen Ph.D., Professor and Chair, Department of Plant and Wildlife Sciences, Brigham Young University, 275 WIDB, Provo, Utah, 84602, United States of America. The collaboration with Dr Jellen was established following an initial visit to Morocco in July 2000, when it was determined that crop diversification should take priority, and quinoa was suggested as a potential new alternative crop for introduction to the project site. Jeff Maughan Ph.D., a professor in the same department and an expert in molecular genetics and genomics, became involved in 2004. He contributed during field selections and helped develop DNA-based markers (SSRs, SNPs) that have been used to evaluate genetic diversity in quinoa, including field selections from Morocco.

Sven-Erik Jacobsen, Assistant Professor, Department of Agriculture and Ecology, Crop Science, Hojbakkegard Alle 3, DK-2630 Taastrup, University of Copenhagen, Faculty of Life Science, Denmark. The collaboration with Dr Sven Jacobsen began when he shared genetic material for experiments in Khenifra as part of the CIP-FAO programme aimed at extending quinoa cultivation to arid and semi-arid regions of the world. The results were presented and published at the European Crop Science Conference held in Copenhagen in July 2004 (Benlhabib *et al.*, 2004). In 2007, Dr Jacobsen, together with a team of Mediterranean scientists, was awarded a PF7 project which partly financed the second phase of quinoa introduction and evaluations in Morocco (2008–2012).

Germplasm evaluation under different environments

In 2000, 14 accessions with drought tolerance were introduced from CIP-FAO. The main objective of the introduction was to select specific genotypes with improved drought tolerance, high yield and adaption to the agroclimatic conditions of Morocco.

Khenifra and Rabat region

The specific objective in the first season was to test the adaptation of the 14 accessions to the Moroccan agroclimate. Preliminary experiments were conducted on three experiment sites: IAV Hassan II; ITA Ben Khlil; and the Omar Anwar farm, Agoudim, branch Ait Ali o' Kassou. The results highlighted the varieties' wide diversity in several agronomic characteristics: plant size, root depth, panicle form and colour, stem colour, seed colour and diameter, sensitivity to diseases and pests, precocity etc. Variety 'G205-95DK' was the earliest, flowering after 55 days. The seeds of 'G205-95DK' (and of 'Pandela' and 'Utusaya') are large and light in colour (Benlhabib *et al.*, 2004). High temperature (47°C) had a profound impact on plant fertility, and only early-flowering varieties escaped pollen kill due to the heat. Initial research demonstrated that 'G205-95DK', 'Pandela', 'Sayana' and 'Utusaya' were semi-adapted to the Moroccan environment. Most characteristics showed significant variation between sites, and the majority of genotypes performed better at higher elevations in Khenifra.

The first quinoa trial also suggested that biotic disease sensitivity was variable among the 14 accessions. No variety was unaffected. The wild 'Ayara' strain was the most sensitive to insect damage. At IAV, insecticide treatment (Paraban) successfully limited insect damage. Aphid injury was observed but it did not seriously affect the health of the plant.

Seed size differed from variety to variety. Grain diameter was 0.5–2 mm, with improved varieties having the largest seeds. Seed colour varied from black, brown and yellow to creamy. As previously suspected, correlation between seed size and colour was confirmed with larger seeds having a lighter seed colour. Both characters were used as indirect selection criteria.

In Rabat, the accessions had a wide range of yields: from 0.255 to 1.512 tonnes/ha. Cultivars 'G205-95DK', 'Pandela' and 'Utusaya' were the most productive, with better adaptation under the coastal conditions that characterize the Rabat site (Benlhabib *et al.*, 2004). The other accessions were discarded, not only because of their lack of adaptation, but also because of their susceptibility to disease and, in particular, the sterility caused by high temperatures at the flowering stage (Table 2).

Table 2: Quinoa grain yields (tonnes/ha) at the three experimental localities in 2000–01

Accessions	ITA Khenifra grain	IAV Rabat grain yield	Agoudim Khenifra
03-21-72 RM	0.00	0.924	0.0144
03/08/51	0.00	1.188	0.902
03-08-907	0.00	0.255	0.521
03-21-79BB	0.00	1.155	0.070
Sayana	2.383	0.354	0.150
1 (80) 1	0.00	0.274	0.360
24 (80) 3	0.006	0.410	0.455
Masal 389	0.00	0.228	0.00
Ecu-420	0.00	1.025	0.221
G205-95DK	4.644	1.512	3.463
Pandela	0.900	0.840	0.164
Utusaya	0.508	1.267	0.506
Huariponcho	0.00	1.10	0.038
Ayara (wild)	0.00	0.855	0.267

At ITA Ben Khlil, quinoa yield showed positive correlation with earliness (precocity) and negative correlation with sensitivity to high temperatures during the flowering stage (Benlhabib *et al.*, 2004). 'Sayana', 'G205-95DK' and 'Pandela' were the earliest accessions and also the most productive. The other accessions exhibited only productivity. High temperatures (> 47°C) and dry wind (Chergui) at flowering (June–July) negatively impacted plant fertility.

In addition to high temperatures and precocity, soil texture and soil fertility also had a significant impact on yield. Yields in Khenifra were relatively higher than in Rabat ('G205-95DK' yielded 1.64 vs 1.114 tonnes/ha), suggesting that under suitable conditions (early sowing and mild temperatures), some accessions have high productivity potential in Khenifra.

In 2001–02, seed of the four best varieties ('G205-95DK', 'Pandela', 'Utusaya' and 'Sayana') was increased and evaluated in two additional cropping seasons at IAV Rabat and ITA Ben Khlil, and on two farms in Agoudim.

In 2004, the best three varieties of quinoa ('G-205-95DK', 'Sayana' and 'Pandela') were planted on yield plots at the ITA in Khenifra. All fields were planted in December, but the crop did not emerge until February due to insufficient midwinter rains. The 'G-205-95DK' cultivar was heterogeneous for several traits, notably colour and leaf shape. Minor viral infection was noted on a small number of plants, and several plants had the dwarfing/thick leaf phenotype that could be indicative of a chromosomal abnormality or aneuploidy.

At the Agoudim site (planted by Omar Ghanem), plants were highly heterogeneous, and many exhibited a red stem (similar to Achachino-type 'Real' variety). Plant development closely resembled that observed in the Altiplano, with low saponin seeded plants being selectively stripped of their seeds by ants. The field was used to select desirable genotypes, specific compact head types and early maturity characteristics. The 2004 trials show December to be a suitable planting time for large-scale production of quinoa, especially in the foothills of the Middle Atlas (Khenifra).

In the 2004/05 season, a 3-month spring drought and extreme cold in January (as low as -8°C) at the ITA Khénifra site, had little or no effect on quinoa plants which exhibited limited freeze damage (under a weekly irrigation scheme). About 30 genotypes of the more than 300 lines planted in a replicated trial were selected. The main selection criteria were large white seeds, stand uniformity, early-intermediate maturity and yield. Almost all the quinoa lines had good seed set, despite the exceedingly warm (> 30°C) temperatures in late April.

Moulay Bouazza region

In 2006, 30 quinoa genotypes, selected for drought, cold and heat tolerance, were planted in a multilocation and multi-sowing-date replicated yield trial at Tnine Ait Boukhayou (Moulay Bouazza) and IAV Hassan II (Rabat). The 30 genotypes exhibited good adaptation and stable yield in the Middle Atlas region. The collected data clearly showed February planting to be more suitable than November planting at all experimental sites. The tested genotypes were well adapted to the altitude and in general performed better in Moulay Bouazza. The accessions revealed different pheno-agromorphological traits. The sowing date and location affected plant size, number of nodes and susceptibility to pathogens: plant size and number of nodes were higher in Moulay Bouazza, February sowing date: injuries from disease were frequent in Rabat (due to the coastal location's humid climate) and practically absent in Moulay Bouazza. All 30 accessions were susceptible to insects, especially aphids, in both locations.

Maturity was especially influenced by the sowing date and was more synchronized in the February sowing. Growth cycles were slightly shorter in Moulay Bouazza. Yields were variable and low in Rabat, due to diseases, pests and avian predation, and more consistent in Moulay Bouazza, with the highest yields coming from February planting. The location and sowing date did not affect seed diameter, weight and colour, or saponin content. All genotypes performed better in Moulay Bouazza. Accessions 112 and 143 were ranked first with the best performance in the primary selection criteria: earliness, short stature, high yield, tolerance to mildew and green bug, and seed quality.

Experiments conducted

Wild Chenopodium in Morocco

Morocco is a centre of diversity for the *Chenopodium* species. During visits to quinoa fields in the High Atlas region, three wild weedy species of *Chenopodium* (*C. album*, *C. murale* and *C. vulvaria*) were identified growing in abundance as weeds. In 2006, a wild *Chenopodium* collection expedition covered several regions in the country and more than eight species were identified and collected.

In the vascular flora of Morocco, 26 genera and 89 species of the Chenopodeaceae are reported; of these, 45 are rare or very rare (Fennane and Ibn Tattou, 1998, 2005), and several have an agronomic or therapeutic relevance. In North Africa Flora, 14 *Chenopodium* species are described: *C. multifidum*, *C. ambrosiodes*, *C. botrys*, *C. suffriticosum*, *C. capitatum*, *C. vulvaria*, *C. hybridum*, *C. wall*, *C. urbicum*, *C. glaucum*, *C. giganteum*, *C. serotinum*, *C. chenopodioides* and *C. album* (Mayor, 1962). The Morocco catalogue lists nine species in the genus: *C. ambrosiodes*, *C. vulvaria*, *C. wall*, *C. rubrum* (sp. *crassifolium*), *C. album*, *C. opulifolium*, *C. giganteum*, *C. multifidum* and *C. virgatum* (Jahandiez, 1932). The Hands-on Morocco Flora references ten species: *C. album*, *C. ambrosioides*, *C. bonus henricus*, *C. chenopodoides*, *C. giganteum*, *C. multifidum*, *C. wall*, *C.*

opulifolium, *C. suffritucosum* and *C. vulvaria* (Fennane *et al.*, 1999).

In parallel to quinoa adaptation and selection work, a research project was conducted on the collected *Chenopodium* species at the IAV University of Rabat. The study concerned the morphological, palynological aspects of wild Moroccan *Chenopodium* (El Rhzaoui, 2006). A total of 35 populations were studied and they included *C. album*, *C. ambrosiodes*, *C. opulifolium*, *C. multifidium*, *C. vulvaria*, *C. chenopodioides* and *C. giganteum*. Morphological trait analysis was carried out on more than 200 samples; data analysis highlighted significant variability among and within species due to genetic and environmental effects. Pollen analysis of 15 collected *Chenopodium* populations revealed similarities in pollen shape (oblate spheroid or breviary), but differences in the number of pores. Pollen pore density was found to be an important taxonomic criterion when sorting species (El Rhzaoui, 2006). The preliminary cytogenetic study revealed variation in the ploïdy among species; however, further research is required for genome analysis to understand *Chenopodium* karyotype evolution. The *Chenopodium* species predictably differed for several traits, and to better appreciate the diversity of wild *Chenopodium*, molecular techniques are recommended.

Agronomic experiments

In 2008–09, the best 20 quinoa selections (13 selected lines and 7 newly introduced accessions) were growing under Rabat conditions (Azouz, 2009). Yield varied from 1.2 tonnes/ha in the Coastal cultivar 'BAER II' from Chile to 3.2 tonnes/ha in the Altiplano variety 'ILLPA-INIA' from Puno Peru. The average yield was 2.346 tonnes/ha – significantly more than the average yield of 1.4 tonnes/ha previously measured in Khenifra (Benlhabib *et al.*, 2004). The difference between the two yield trials was principally attributed to better crop management. Important variability was displayed among accessions for a series of morphological and agronomical traits: plant size, number of nodes, internode length, inflorescence shape, disease injury, total biomass and grain yield. Accessions 'S119B' and 'S135' yielded more than 2.8 tonnes/ha.

In the 2010–11 seasons, five advanced quinoa lines selected for their adaptation to the local environment were evaluated under farmers' field conditions in semi-arid Bouchane. Yield evaluations in three separate farm trials showed that line 142 had the highest yield: 1.52 tonnes/ha under irrigation and 0.93 tonnes/ha under rainfed cropping (Filali, 2011). Quinoa line 123 presented the lowest yield. The Danish variety 'Titicaca' had the highest harvest index (0.46) and largest grain yield per plant. Adaptation trials showed that under the rainfed conditions of Bouchane, quinoa lines performed better than in the Rabat environment. The morphophenological characterization experiment showed that flowering stage is reached after 40–50 days and physiological maturity after 70–105 days.

Tillage investigation

During the second period (2008–2012) of quinoa introduction to the semi-arid and arid regions of Bouchane and CHA Agadir, most investigations were related to stress physiology, agronomy and genetic evaluation and characterization. Despite persistent efforts to select quinoa lines adapted to the soil and climate conditions of Morocco, a major problem remains stand establishment. Farmers currently adopt a range of tillage, using on-farm available tillage implements, such as the disc harrow with cover cropping. Seeding is generally done by hand, and there are no specific recommendations for the seeding rate. In 2011, an experiment at the INRA Experiment Station in Koudia (southeast of Rabat) was done on sandy–loam soil to compare three tillage systems: one-pass using a tine cultivator followed by a roller (CD-R); one-pass using a disk harrow (CC); and no-till seeding or direct seeding (DS) (Oussible *et al.*, 2012). The primary objective was to develop an automated strategy for quinoa crop management. Growth- and yield-related parameters, such as the soil's physical properties and root growth, were measured and they revealed the advantages of a direct-seeding system: DS produced the highest plant population at harvest (55 208 plants/ha), largest grain yield (0.64 tonnes/ha) and highest harvest index (0.46).

Physiological and anatomic studies conducted

Another study at the University of Cadi Ayad, Marrakech, examined antioxidant enzyme activities in the quinoa leaves, since variations in water availability and fertilization had been previously reported to generate biochemical reactions in plant cells. Stress induced by these factors had been associated with enhanced reactive oxygen species (ROS) generation, which caused oxidative damage. To study the biochemical reactions to water stress and organic fertilization, four water supply regimes (100, 50, 33% evapotranspiration [ETc] and rainfed [RF]) and manure supply were tested. At the flowering stage, superoxide dismutase (SOD), polyphenoloxydase (PPO), peroxidase (POD) and catalase (CAT) were measured in quinoa leaves. The results suggested that antioxidant enzymes play an important role in reducing the oxidative effect in quinoa exposed to water stress (Fghire *et al.*, 2012). SOD activities increased significantly (322.42%) in rainfed treatments. CAT, POD and PPO activities also increased significantly (87.4%, 72.8% and 520.35%, respectively) in rainfed treatments in comparison with 100% ETc. Manure almost neutralized the effect of drought except in rainfed treatments. PPO activity showed a significant augmentation in the non-fertilized and fertilized assays: 190.16% and 253.28%, respectively, in 50% ETc; 462.67% and 229.14% in 33% ETc; and 520.35% and 106.16% in rainfed treatment (Fghire *et al.*, 2012).

In 2012, another study conducted at IAV Rabat assessed stomata density variation among accessions and its correlation to drought tolerance. A total of 52 quinoa accessions selected for their adaptation were used in the analysis and compared with two native, widely distributed wild species (*C. album* and *C. murale*) and with two Danish varieties adapted to the Moroccan environment ('Puno' and 'Titicaca'). The results revealed variation in the stomata density in relation to genotype and to leaf position on the principal stem. The stomata density varied from 140 to 544 stomata per mm². The selection 'W142/2' had the highest stomatal density. Stomatal density decreased from the apical leaf to the basal leaf. The stomatal density in the Danish variety 'Puno' decreased markedly from the apical leaf basipetally, from 529 to 272 stomata per mm². The cultivar 'Sayana' and selection 'Wafa6' presented the lowest stomatal density at the apical leaves: 310 and 246 stomata/mm², respectively. In selection 'W142/2', stomatal density decreased steadily from the apical to the basal leaf, from 627 to 176 stomata per mm² (Thabit, 2012).

Irrigation, salinity and treated water experiments

Between 2010 and 2012, several experiments were conducted under field and pot-sown conditions at IAV-CHA, Agadir, in order to evaluate the effects of salt, of drought priming and deficit irrigation using treated wastewater, and of organic amendments on quinoa growth and productivity. The results indicated that quinoa seedling growth decreased as salinity increased. At salinity levels of up to 10 dS/m, grain yield remained stable, but yields decreased by 34% at 30 dS/m EC compared with the control (Hirich *et al.*, 2013a). In the deficit irrigation experiment, maximum grain yield and water productivity were obtained when 50% deficit irrigation was applied at the vegetative growth stage (Hirich *et al.*, 2012a). There was significant variation among accessions in terms of grain yield. Deficit irrigation during vegetative growth conserved 20% of the water supply (690 m3/ha) compared with the control (Hirich *et al.*, 2012c). Hirich *et al.* (2013b) also demonstrated that the organic amendment significantly improved grain yield and total biomass under deficit irrigation conditions. The IAV-CHA experiments at Agadir also proved that quinoa helps in leaching salt from the soil, as salt accumulation in the soil was significantly reduced when quinoa was irrigated with treated wastewater.

In farmers' trials carried out in Bouchane, yield varied depending on the mode of irrigation and the skill level of the farm's crop manager (Table 3). Wa-

Table 3: Yield fluctuation in relation to farm manager's skill and irrigation method

Name	Management skill	Irrigation method	Yield tonnes/ha
Abbadi Driss	Efficient	Surface irrigation	3.450
Wafi Mohamed	Quite efficient	Surface irrigation	2.670
Khilani Mohamed	Efficient	Surface irrigation	3.000
Jabrane Ahmed	Conventional	Rainfed	0.890

ter was the main limiting factor, and yield increased three- to fourfold under irrigation. Nitrogen fertilization had a minor effect on yield under rainfed cropping (Benlhabib *et al*., 2013).

Genetic and molecular characterization

From the germplasm developed through recurrent and pedigree selection, 78 accessions selected on the basis of drought tolerance and adaptation to the coastal environment, were evaluated at IAV Hassan II, Rabat. A total of 23 quantitative and qualitative characters were used to evaluate the genetic diversity of and relationship among accessions. Positive correlations were observed between yield and plant height and fresh and dry weight; on the other hand, days to flowering was negatively correlated with grain yield. The first four Principal Components (PC) contributed 74.76% of the total variation: plant size, days to grain filling and days to maturity were correlated to PC1, while seed size, inflorescence density and mildew resistance were correlated to PC2. Hierarchical cluster analysis sorted the 78 quinoa accessions into four main groups (Manal *et al.*, 2013a).

A total of 94 quinoa accessions, including the 78 selected accessions evaluated above, were subjected to the single nucleotide polymorphism (SNP) technique analysis at the molecular lab in the Department of Plant and Wildlife Sciences, BYU, Provo, Utah. A set of 96 SNPs were used to analyse patterns of genetic diversity. The 94 accessions were split into two main groups based on maturity with structure analysis, and 88.01% of the genetic variation was explained by variation within the subgroups. The highest heterozygosity group was found within the medium maturing subgroup (He = 0.456). The fixation index value (IF = 0.15) highlighted important diversification within the two principal populations. Cluster analysis and PCA divided all quinoa accessions into four discrete clusters. Molecular analysis provided a new set of easy to use and highly informative genetic markers in quinoa and helped identify a diversified germplasm set to enhance the breeding programme (Manal *et al.*, 2013b).

To date, 100 quinoa lines have been characterized and evaluated. Of these, 45 proved to be adapted,

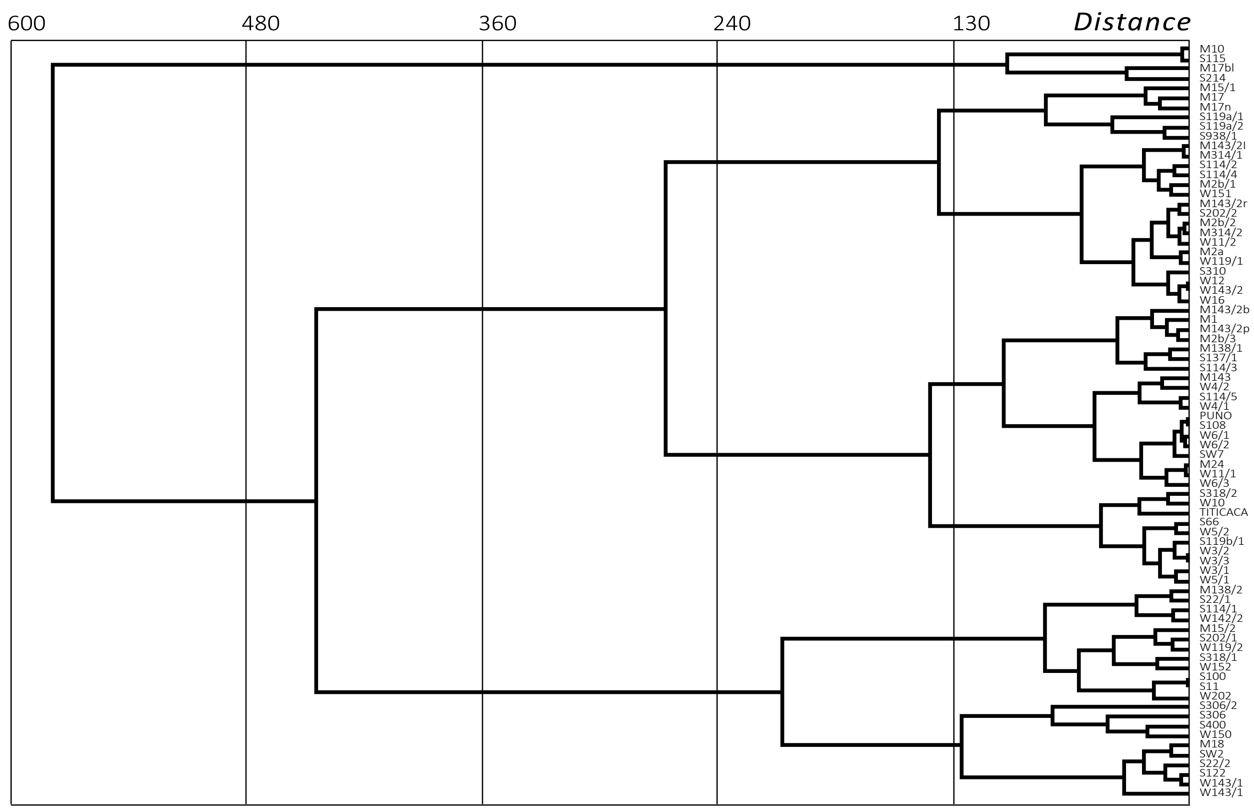

Figure 3. Dendrogram based on agronomic and morphological traits using UPGMA

productive, genetically stable and homogenous, and will be subjected to seed increase and yield assays in two or three localities. In 2014 also, a crossing activity between selected lines will be initiated to develop superior genotypes. The quinoa programme has for 2 years been developing a technical data sheet to promote quinoa cultivation on a large scale and to better manage all the cropping tasks.

Current state and perspectives of crop dissemination

Crop dissemination

In Morocco, quinoa production and consumer demand are still only emerging. A consistent effort is required to promote the product and educate consumers about quinoa's nutritional value and commercial benefit. Quinoa cultivation has been introduced in several regions through the IAV-Hassan II programme and collaborative projects. Quinoa seeds have been distributed to farmers and rural associations in different regions. Farmers have also received information about crop management.

During the IAV-BYU project (2000–06), quinoa seeds were distributed in several localities in the Khenifra region: Ait Ishak, Ouaoumana, Kerouchen and Boumia. Quinoa trials were also conducted in the High Atlas region: in2002–04, in Aït Lekkak village, Oukaimeden (2 016 m asl); and in 2002–07, in Tizi Oushen village, Siti Fatma. Local farmers reacted positively to quinoa, because it showed good growth and yield. In 2006, quinoa was introduced in the Moulay Bouazza region, where one genotype's adaptation and evaluation trial took place on a private farm in Tnine Ait Boukhayou.

In 2007, a quinoa workshop was organized in Rabat involving 15 young farmers from the Shoul and Sidi Slimane organic farming associations. The meeting aimed to raise awareness about quinoa's nutritional value and crop management. Seeds were distributed to participants in order to start quinoa cultivation. Subsequent visits were organized to follow up with the crop during its cultivation on several farms.

Under the SWUP-MED project, quinoa seeds were widely disseminated in Rhamna, Marrakech and Agadir, involving farmers from Bouchane, Sidi Bouathmane, Itlil village (Jbilat), Skoura, Jemât Shim (Safi), Berchid and Dar Bouazza (Terre Humanisme Maroc, Casablanca) and Massa (Agadir). In 2011, quinoa was tested under irrigation at Itlil village, Jbilat, north of Marrakech (31.894548N, -8.030889W), and plant development and yield were very attractive to local farmers because the soil and climate are generally very restrictive to crop production. At Skoura village, at the *Carrefour des Initiatives et des Pratiques Agroécologiques* (CIPA) centre, quinoa was cultivated to identify the optimal sowing date (Oct. 2011 – Mar. 2012) and to promote production of the crop among local farmers and visitors.

Quinoa cropping was expanded in 2012 to the southern (Saharan) side of the High Atlas Mountains through an informal seed exchange with a Peace Corps volunteer who introduced quinoa seeds in Ikniouen (latitude 31.1736495, longitude -5.6734057, 1 912 m asl), a rural community in the Ouarzazate region, and in Baknou (latitude 32.23333, longitude -3.93333, 1 196 m asl) in the Errachidia region. A growth chart with instructions in the local Arabic and Tamazight (Berber) languages was developed.

Several farmers began to cultivate quinoa as a commercial crop in the Casablanca, Marrakech, and Settat regions. During the 2013 cropping season, a nationwide restorer group, "Rahal", began to grow quinoa on a 4-ha area in Bouskoura near Casablanca. Depending on the production and financial return, the group may seek to increase the size of the project and extend the crop to other regions.

Through collaboration between the IAV programme and the Association of Moullablad, two quinoa trials were installed recently under rainfed conditions in Ain Sbit, 80 km from Rabat. The experiments aimed to investigate crop mechanization, the effects of nitrogen fertilization on yield and seed production. The main objective was to produce the quinoa data sheet and promote large-scale cultivation. The initial results were encouraging, showing significant improvements in crop installation, grain stretching and seed production (Photos 4, 5, 6).

Crop promotion initiatives

Since its introduction to Morocco in 2000, quinoa has been the focus of several promotion initiatives at annual farmers' workshops, women's gastronomic workshops, and in national, regional and local exhibitions.

Quinoa extension workshops

Since its introduction to Morocco, quinoa has been presented in several workshops organized by the IAV quinoa team, with the participation of mainly agriculture extension agents, technicians, associations members and farmers.

In May 2006, a workshop was organized on quinoa cropping practices at ITA Ben Khlil for the benefit of farmers, DPA (*Direction Provincial d'Agriculture*) and CT (*Centre de travaux*) technicians and ITA Ben Khlil staff. Videos and PowerPoint presentations illustrating quinoa cropping, conditioning, marketing and consumption were shown. Leaflets, information sheets and quinoa recipe booklets were provided to participants. The quinoa experimental trial was visited and selection criteria were discussed.

Gastronomic workshops

During the 13 years since quinoa was first introduced to Morocco, a number of other activities have been organized to promote the crop and its management, nutritional awareness and financial benefits. In the autumn of 2004, a 3-day workshop on cooking quinoa and its adaptation to local recipes was held at the women's association in Tighassline, Khenifra. On the last day, there was a reception for visitors where they could taste different foods made from quinoa.

Quinoa exhibitions

Quinoa was exhibited for the first time at the International Agriculture Forum of Meknes in April 2006 as part of the collaborative effort between IAV-Hassan II, ITA Ben Khlil and producers of the Khenifra region. In 2011–12, it was exhibited by the local Association of Bouchane, a SWUP-MED project partner, in the Rhamna regional pavilion.

In 2011, quinoa began to appear in organic stores and in hypermarkets at a price MAD50–80/kg (MAD = Moroccan Dirham). Coeliac associations in Rabat and Marrakech have been importing quinoa for several years and are potential customers. Quinoa is a high-quality food, with a prized taste and multiple uses, and will, therefore, quickly win over the national market. The quinoa selection programme

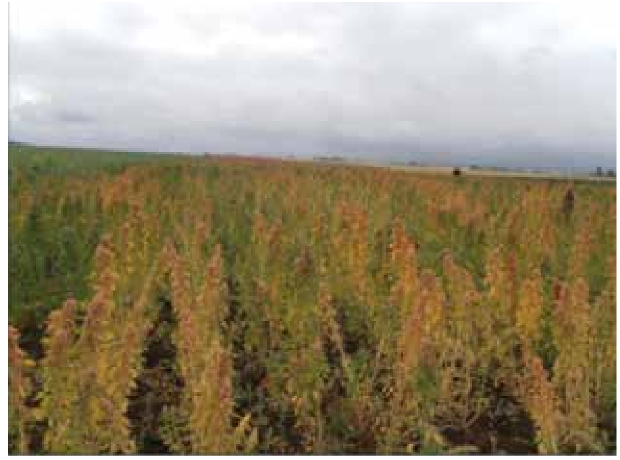

Photo 4: Quinoa field at Karim Moullablad farm, May 2013

Photo 5: Quinoa production at Karim Moullablad farm, June 2013

Photo 6: Quinoa grains stretching machine, June 2013

with international collaboration is working on specific constraints, such as biotic and abiotic stresses, as well as the improvement of seed quality, productivity, yield and adaptation to the local environment.

Uses and Markets

The most desirable outcome of the Khenifra project was the adoption of quinoa as a high protein food by the indigenous people. To date, however, its consumption at local and national level is still minimum and continued awareness-raising efforts are required. Production as an export-cash crop is an additional favourable outcome that is more attractive to producers.

In 2004, a farmer from Agoudim village, Khenifra, produced over 150 kg of quinoa grain, and sold about 80 kg to a relative living in Canada. The first official community sale was completed in 2004 with the Maghreb-Bio Association in Marrakech, an organization that specializes in organic products and has imported quinoa in the past. Since 2011, the local association in Bouchane has been producing quinoa, selling the harvested crop at MAD60–80/kg and at an export price of EUR12 /kg. Local production is still limited and haphazard, and further effort is needed to raise awareness of the crop among both farmers and consumers.

An organic store based in Casablanca sells quinoa and reported that customers were responding enthusiastically with an increase in demand during the past 3 years. The product is currently imported from Peru and Bolivia in 400-g packs and retails at MAD35 per pack.

The Rahal Group hosted the CREMAI International Hotel, Restaurant and Bakery Professionals Exhibition involving 185 participants in Casablanca in March 2013. The exhibition saw a 23% increase in participation compared with the year before. Four days of professional meetings were dedicated to hotel and catering businesses, with one topic focused on the culinary aspects of quinoa. The chair of the Rahal Group also presented a talk entitled “Quinoa: A New Trend in the Culinary Business”.

Quinoa is now a star product on practically all continents. Within the national strategy for agricultural development, “*Plan Maroc Vert*”, initiated in 2008, the Marrakech-Tensift-Al Haouz region defined its main goal to be the advancement of local agriculture by supporting the development of special products, through cooperative assistance, and their production and marketing. The regional office of the Agriculture Ministry recently defined specific target crops for the region, including fennel, cumin, ornamental iris, the Chiadma grape, *Alouidane gumbo* (okra), and new quinoa – introduced in 2009 through SWUP-MED and adapted to the region’s climate and soils.

Future perspectives

A range of factors affect quinoa production, including optimal sowing date and nutrient requirements, and constraints still need to be overcome, including crop stand establishment, sensitivity to high temperatures, weed control and saponin removal. Furthermore, it is essential to design a product marketing strategy and raise awareness of farmers, and of agriculture ministry and other government officials.

In Bouchane, the local association that was the main SWUP-MED local partner, elected quinoa as a special crop for development in the region. However, quinoa yield and production costs remain unpredictable, and crop management techniques and certified seed for cultivation need to be developed.

At the “Sustainable Use of Water and Food Safety in the Mediterranean Region Under the Influence of Climate Change” conference held on 10–15 March 2013 in Agadir, participants emphatically recommended the establishment of an international quinoa network to promote its cultivation in the Mediterranean Basin, as it is a valuable crop in terms of both food security and nutritional quality.

With the impending effects of global climate change, quinoa represents a highly valuable crop for food security due to its adaptation to a variety of different environmental stresses (drought, salinity, high temperature) and its resistance to diseases. It is strongly recommended for marginal soils and can be cultivated under a range of environmental conditions which most other crops cannot tolerate. For example, quinoa is potentially productive under seawater irrigation and can even be used as a remediative crop to extract salt from contaminated soils.

The establishment of an international quinoa network will promote the crop in the Mediterranean Basin through the exchange of scientific information among collaborative research organizations, the organization of training courses on crop management for the benefit of farmers and crop technicians, and the awareness-raising among consumers of quinoa's nutritional virtues.

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CHAPTER 6.1.6

Greece

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Abstract

In southern Europe, Greece was the first country to become involved (in1995) in the American and European Test of Quinoa, organized by the Food and Agriculture Organization of the United Nations (FAO) and the CIP-DANIDA Quinoa Project. Greece comprises a wide variety of ecological zones, from hilly and mountainous areas with humid and low mean temperatures, to coastal and low plain areas with dry and hot temperatures. Quinoa seems to be well adapted to altitudes of 500–1 300 m asl. Preliminary results from field trials in central Greece – the largest agricultural plain in Greece – show that quinoa is agronomically viable. With the introduction of proper technology, it will be possible to increase yields and expand the cultivation of quinoa to lands otherwise unsuitable for agriculture. Quinoa may be well adapted to most soil orders (Entisols, Inceptisols, Alfisols) and to soils from sandy–loam to loamy–sand texture. Early experiments showed that drought, low relative humidity, temperatures above 32°C and long days (during anthesis) were unfavourable for quinoa seed production. Crusting and drying up of the soil surface had a negative effect on the proportion of emerged seedlings. The optimal sowing density is considered to be 25 plants/m^2. Early sowing in March gave good results, while late sowing resulted in poor germination. Subsequent trials showed that under dry conditions only 8 out of the 25 varieties originating from Europe and Latin America produced seeds. These varieties were evaluated for their yield potential and seed composition under contrasting soil properties (neutral vs saline-sodic). Seed yield in the saline-sodic soil decreased by up to 45%. Mineral and protein content (15–18.5%) in quinoa seeds was higher in the cultivars from Latin America. Breeding efforts using mass selection procedures for the creation of new varieties from plants surviving in saline–sodic soils were, however, discontinued due to lack of financial support. Trials were later conducted in central, northern, and central-southern Greece to evaluate weed control, yielding potential, organic fertilization, cultivation practices and the allelopathic potential of quinoa. It was concluded that the active ingredients in the tested herbicides were not selective for broad-leaved weeds and cannot be safely used for quinoa. No herbicides have as yet been approved for use on quinoa in Greece. The application of various organic fertilizers (seaweed compost and humus) produced no significant differences in quinoa plant morphological properties and yield. On the other hand, minimum tillage (MT)

was more advantageous than conventional tillage (CT). No significant differences were found in the saponin content between MT and CT. The quantification of the allelopathic activity of quinoa plant extracts revealed that all the tested species (oat, common bean, duckweed) showed greater phytotoxic response than the inflorescences of quinoa. In order to expand quinoa in Greece, it is necessary to consider a range of issues: the shortage of processing plants and absence of manufacturing potential; scarce farmer knowledge; the small average size of land parcels; the distance from European markets; and the absence of incentives. The agricultural research authorities must promote applied research on quinoa (irrigation management, fertilization plans etc.), organizing germplasm evaluation in different agro-ecological zones and establishing genetic improvement programmes in order to expand the crop.

Crop production trends in Greece within the European context – New alternative and promising crops

Greek agriculture is directly influenced by the European Common Agricultural Policy (CAP) and the main objectives are to: (i) safeguard and guarantee long-term European food security; (ii) contribute to growing world food demand, expected by FAO to increase by 70% by 2050; (iii) support farming communities that provide food which is sustainably produced; and (iv) maintain viable rural communities (EC, 2010). In recent years, Greek agriculture (an integral part of the European agricultural sector) has suffered the consequences of the economic crisis, expressed primarily through the decline of trade, reduced demand and lack of resources for support and development. Furthermore, Greece has to deal with the impact of climate change, which increases the frequency and severity of extreme climatic conditions affecting potential crop yields (EC, 2009). Soil quality degradation and loss of plant biodiversity directly affect intensive and conventional farming practices (FAO, 1998; Lal, 2009).

Interest in cultivation of new crops has increased in Europe in recent decades. Some are cultivated only to a limited extent, while others are grown as part of demonstration projects. There are some fertile plains in Greece, but most of the land area is hilly or mountainous. In general, the plains are characterized by dry climatic conditions (especially during irrigation periods), insufficient water resources and small agricultural holdings. Prior to the recession, Greece's agricultural sector recorded a very marked decline, with a significant decrease in farm income (particularly pronounced in 2008, at approximately 11%). Nevertheless, the agricultural sector provides an important range of products and services to the food industry. Greece is an agricultural country, with 11.6% of the employed civilian working population engaged in employment in the agriculture, forestry, hunting and fishing sector (EC, 2012). The importance of agriculture in the Greek economy, as in almost all member states of the European Union, is in continuous decline. This is clearly shown by the negative trend of the contribution made by agriculture (agriculture, hunting and forestry) to the total gross domestic product of the Greek economy during the period 2001–2011 (from 5.9% in 2001 to 2.5% in 2011) (EC, 2012).

The edaphoclimatic conditions favour the production of a wide range of high-quality products. Greek agriculture in recent decades has traditionally been based on products such as cotton, cereals, olives and olive oil, fresh fruits and grapes. Greece also has a competitive advantage in the production of fruits (rather than vegetables), although certain fruits and vegetables (grapes, asparagus, canned peaches and processing tomatoes) are dynamic and are exported mainly to European countries. In the light of the introduction of many potential new crops in Europe, there has been much debate in Greece about the need to explore new ways to achieve more effective land use planning. Greek agriculture needs to explore the possibility of cultivating neglected crops (e.g. some legume species) and introducing new crops following experimentation for adaptability. New crops include stevia (*Stevia rebaudiana*) to replace tobacco (*Nicotiana tabacum* L.), traditionally cultivated in several parts in Greece, blueberry (*Vaccinium corymbosum* L.), common sea-buckthorn (*Hippophaes rhamnoides*), black chokeberry (*Aronia melanocarpa*) and Goji berry (*Lycium barbarum*). Another crop of specific interest is quinoa and it has been tested since 1995, primarily in central Greece.

Quinoa (*Chenopodium quinoa* Willd.) is a very interesting crop due to the nutritional characteristics of its seed and its potential industrial uses in various sectors (e.g. paper production). In 1996, quinoa was classified one of the most promising crops for humanity and the United Nations (UN) declared 2013 the "International Year of Quinoa" (IYQ), as well as the "International Year of Water Cooperation". IYQ aimed to focus world attention on quinoa's nutritional, economic, environmental and cultural value. The main objective of designating 2013 the International Year of Quinoa was to raise awareness of how quinoa can provide nutrition, increase food security and help eradicate poverty around the world (FAO, 2013).

In the Mediterranean Basin, Greece was the first European country to introduce quinoa in the mid-1990s, and experiments were performed to assess its adaptation (Karyotis *et al.*, 1996; Taviani *et al.*, 2008). Greek research institutes showed interest in quinoa as early as 1995 and initiatives were taken by researchers from the National Agricultural Research Foundation (presently General Directorate of Agricultural Research, Hellenic Ministry of Rural Development and Food). Scientists participated in the respective European research networks and were involved in the American and European Test of Quinoa organized by FAO and coordinated by the National Agrarian University La Molina in Lima-Peru.

Pedobioclimatic conditions for the cultivation of quinoa in Greece

Quinoa thrives under adverse pedoclimatic conditions and tolerates abiotic stresses. It is grown in drained, easily worked, medium-deep soils, with an adequate supply of nutrients, and requires a relatively long growing period. The crop can be adapted in soils with sandy to loamy texture and also under a wide range of pH (4.8–8.5), depending on the ecotype. It is reported to tolerate saline soils or irrigation with a high salt content (Garcia, 1991; Jacobsen *et al.*, 1999).

Greece's terrain and pedological considerations

Greece is located in southern Europe, in the Mediterranean Basin (Figure 1). It covers a total area of 131 957 km^2 and – with the exception of central and eastern Macedonia, Thessaly and Kopaida, where there are flat plains – the terrain is characterized by hilly and mountainous areas with various shapes of relief. According to FAO estimates, around 45% of the Greek territory (60 000 km^2) is characterized by soils without severe constraints. Around two-thirds of the country (86 000 km^2) is vulnerable to erosion and desertification risk, and shallow soils cover 27% (36 000 km^2).

In the lowlands, especially the soil depressions of Thessaly (central Greece), there is an area of approximately 6 500 ha with saline and/or alkaline soils. Proximity to the sea, combined with overexploitation of the groundwater for irrigation, result in seawater intrusion, which affects soil salinization and alkalization. Salinity and alkalinity influence the productivity of many agricultural crops and little is known about quinoa adaptation under specific Mediterranean soil environments. Preliminary results from field experiments conducted in central Greece show that quinoa exhibits yield potential even on degraded soils (Karyotis *et al.*, 2003) (Tables 1 and 2). The results show that under saline-sodic soil conditions, seed yield of quinoa was reduced on average by 45% compared with seed yield of plants grown under neutral soil pH (1.630 tonnes/ha) (Table 5).

Figure 1: Location map of Greece in (A) Europe and (B) in the Mediterranean basin

Table 1: Properties of two soils used for quinoa experiments in Thessaly (Central Greece)

Soil parameters	Soils orders	
	Vertisol	*Inceptisol*
Clay	50	29
CEC (cmol kg^{-1})	29.5	15.3
Soil texture	Clay	Clay loamy
pH (1:1)	7.0	8.9
Soil organic matter %	1.32	0.89
N_{tot} (g kg^{-1})	1.65	0.49
$CaCO_3$ (%)	0.44	2.20
EC (dS m^{-1})	< 3.0	6.5

Table 2: Nutrients and micronutrients of the soils used for quinoa's experiments in Thessaly (Central Greece)

Nutrients (cmol kg^{-1})	*Vertisol*	*Inceptisol*
K^+	1.73	0.22
Na^+	0.35	4.69
Mg^{++}	6.20	1.66
Trace elements extracted by DTPA (mg kg^{-1})		
Fe	8.30	10.90
Zn	3.17	9.55
Mn	12.90	5.90
Cu	2.19	2.57

Agricultural land in central Greece where quinoa was initially tested is located mainly on alluvial deposits characterized by high variability in soil texture, acidity, amount of available nutrients and hydromorphy. Under the influence of soil genesis factors (parent material, time, climate, organisms and topography), soils in Thessaly have been classified as Entisols, Inceptisols, Alfisols, Vertisols and Mollisols (Soil Taxonomy, 1999). Current floodplains comprise mainly recently formed Entisols characterized by the continual deposition of alluvial material.

The Entisols of central Greece comprise the suborders, Fluvent and Orthent. Fluvents are stratified soils originating from alluvial deposits and characterized by a xeric soil moisture regime and thermic soil temperature regime. They are deep and their texture varies significantly from sandy or sandy–loam, to loam and clay. They are rich in calcium carbonate and the risk of erosion is low due to the gradual slope. In areas with strong gradients and high risk of erosion, soils belong to the Orthent suborder. Soil productivity is low to medium, and erosion often causes the parent material to appear on the soil surface. Quinoa's adaptation to Entisols is limited by factors related to climate (temperature, uneven rainfall) and by scarcity of irrigation water. High temperature in the blooming stage restricts pollination and seed yield is extremely low. Another problem is the shallow soil depth in certain soils located on steep slopes vulnerable to erosion.

The Inceptisols in Thessaly have a moderately fine to fine texture and are usually rich in calcareous material, without severe drainage problems. The soil organic matter is less than 2%. Heavy textured soils are characterized by cracks during dry periods, reaching a depth of > 50 cm, and their clay content is usually more than 40%. Quinoa performs better in Inceptisols than in Entisols due to the better soil conditions (soil texture, soil nutrient availability) and increased water-holding capacity. High temperature during anthesis (> 32°C) and water scarcity is a problem for soils located in the lowlands, while at altitudes of 500–1 000 m asl, quinoa can be better adapted.

Most Alfisols (well developed and slightly acidic) in Thessaly are classified as Xeralfs because of the xeric soil moisture regime. The main soil characteristic is the presence of a well-developed argillic horizon. Soils are slightly acidic, the surface horizons have been partially eroded due to leaching of exchangeable calcium, potassium and magnesium. These clay horizons have a strong, angular blocky structure and, in hydrological terms, belong to moderately drained or well-drained classes. Quinoa adapts rather well, although there are potential problems at altitudes of > 1 000 m asl due to leaching of exchangeable cations and the presence of exchangeable aluminium in strongly acidic soils.

Vertisols are characterized by high clay content and deep surface cracks during the dry period. They are generally fertile soils with a neutral or slightly alkaline soil pH, and in central Greece they sustain crops such as cotton, corn, wheat or sugar beet. They have strong structure and drainage differs significantly between soil units. All Vertisols have a clay soil texture and require careful handling and appropriate farming practices. Most are located in the lowlands and the main problems are related to water scarcity and increased temperature, especially in June and July. If the water table is very shallow, water drainage is insufficient and quinoa may not adapt well.

Mollisols are developed on calcareous parent materials, usually derived from the decomposition of tertiary marly substrates, and they occupy only a small percentage of the cultivated land in Thessaly. The topsoil is rich in organic matter content, degree of base saturation and strong soil structure. The soil texture is clay–loamy or clay, while the pH is slightly alkaline or alkaline. No particular problems with draining are observed. The surface horizons have a slight, moderate or fine texture, while the subsurface horizons have a clay texture. These soils occupy a small percentage of the cultivated land, and are located in hilly or mountainous areas without severe fertility problems. Soil water conditions are favourable and temperature is lower in the blooming stage. Given the lower temperatures in early spring, late sowing of quinoa (mid-April) is suggested. At these altitudes, quinoa can be cultivated as a rainfed crop. Quinoa is not suggested for shallow soils (< 40 cm).

A map showing the altitudinal zones was compiled on the basis of the variability of soil properties in the arable land of Greece and the climatic conditions (Figure 2). It is suggested to cultivate quinoa at altitudes of 500–1 300 m asl, given the favourable moisture and temperature conditions. Moreover, no irrigation is necessary to obtain profitable yield. However, field trials must be established in order to assess the adaptability and profitability of quinoa cultivation. Another advantage of cultivating quinoa in the highlands is that it protects the slopes from erosion.

For reasons related to the application of proper agricultural practices, soil units were grouped into a limited number of so-called "soil class units" (Figure 3). The grouping was based on soil texture, slope and hydromorphy (recorded on the soil maps). With regard to soil texture, three main groups were recognized: coarse, medium and heavy texture. The soils were then grouped into lowland and hilly, using a threshold of 6% of the mean soil slope. In terms of hydromorphic characteristics, two categories were established: those with good to fair and those with insufficient hydromorphy. To create a manageable number of "soil classes", the coarse and medium

Figure 2: Map of altitudial classes in Greece

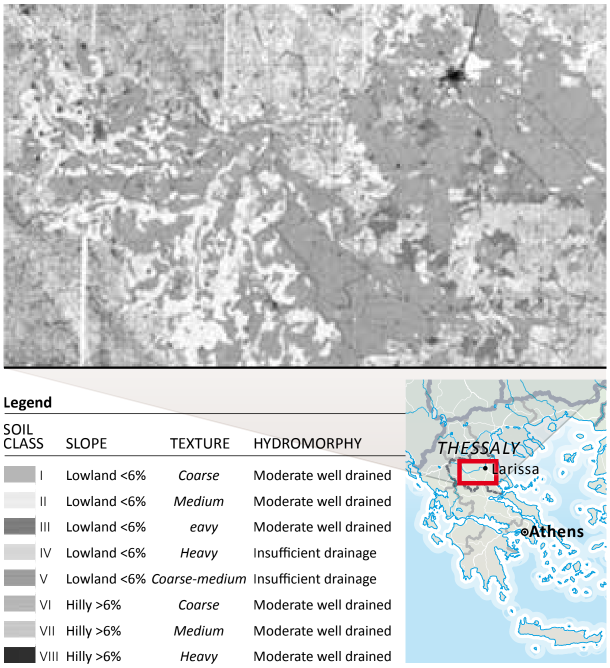

Figure 3: Map of soil groups in part of Thessaly, Central Greece (Compiled by the Institute for Soil Mapping and Classification; Tsitouras, Karyotis & Toulios 2012)

Figure 4: Distribution of annual precipitation (mm) in Greece

texture soils with insufficient hydromorphy were grouped together. Quinoa adapts well on soils with sandy–loam to loamy–sand texture. According to the Thessaly soil map (Figure 3), quinoa adapts well to most soil categories.

2.2. Climatic conditions and suitability for quinoa cultivation in Greece

Greece is a typical Mediterranean country with climatic conditions favourable for the cultivation of most crops. The climate is usually wetter in the west and drier in the east **(**Figure 4). It is generally temperate and mild, with wet, cool winters and hot, dry summers. The climate varies with altitude: hilly and mountainous areas are more humid with a lower mean temperature than the lowlands. Greece's climate can be divided into four different types:

- Dry Mediterranean
- Humid Mediterranean
- Continental Mediterranean
- Alpine Mediterranean

In regions with a dry Mediterranean climate, the summers are dry and precipitation is in the form of showers or thunderstorms; winters are wet and any snow does not last long. The humid Mediterranean climate is characterized by mild winters with little and sparse snowfall, although frost can occur; precipitation is abundant throughout the year and annual rainfall can reach 1 000 mm in some parts of western Greece. Summers, on the other hand, are hot reaching extremely high temperatures in some districts. In areas with a continental Mediterranean climate, winters are cold, with locally abundant snowfall, and summers are very hot. There are significant differences between summer and winter precipitation, but most rainfall is in late autumn. The Alpine Mediterranean climate is characterized by harsh winters with abundant snowfalls and relatively cool summers.

Quinoa is sensitive both to day length (classified as a short-day plant) and to temperature, requiring relatively cool temperatures for optimum growth, – its life cycle is the result of these two factors (Bertero, 2001). Research in the United States of America reported that temperatures over 35°C tend to cause plant dormancy or pollen sterility (AAFRD, 2005); indeed, in trials over several years, quinoa failed to set seed in the lowlands of central Greece where temperatures are very high. In the evaluation of 25 quinoa varieties from Europe and Latin America, only six European and two Latin American varieties produced seeds while the remaining 17 (from Latin America) produced only panicles and flowers (Iliadis and Karyotis, 2000). The sowing date was the beginning of March and the tested varieties grown under irrigation conditions developed well and matured for harvest about 100–120 days (end of July) after germination (Table 4). It is assumed that seed production did not depend on photoperiod sensitivity, because all varieties with small differences flowered at approximately the same time (about 60 days after germination, Table 4). However, extremely high temperatures (> 32°C) and long days (during anthesis) may explain why only eight varieties produced seeds. High temperatures in Greece were considered to be unfavourable for seed production of quinoa. If the temperature rises above 35°C during the flowering season, the pollen dries up and fertilization and seed production decrease. Drought is a common phenomenon in central Greece, and low relative humidity during anthesis restricts pollen viability.

Quinoa plants are tolerant to light frosts (from -1° to -3°C). However, plants are not affected by temperatures below -6°C once the grain has reached the soft dough stage. Quinoa flowers earlier when grown in areas with shorter day length, and in general it is not widely adapted due to temperature sensitivity. Experiments under central Greece conditions showed that high temperatures (> 30°–32°C) and long days (during flowering) are unfavourable for seed production, and the low relative humidity during anthesis restricts pollen viability. High temperatures and long days were found unsuitable for quinoa growth and production (Bois *et al*., 2006; Iliadis *et al*., 1997; Jacobsen *et al*., 1994).

According to data (1974–2004) received from the Meteorological Station of Larissa, average annual precipitation in the area is about 423 mm and the average annual temperature is 15.7°C. The mean temperature of the coldest month (January) is 5.2°C and of the hottest (July) 27.2°C (Table 3). Taking into account the absolute minimum temperature reached during March, farmers are recommended to avoid early sowing of quinoa in the first half of March, especially in areas where frost is probable.

Rainfall is distributed unevenly during the year. Most precipitation occurs in October, November and December, and the least in June, July and August. The ombrothermic diagram (Figure 5) shows clearly that the mean monthly temperature varies greatly between 5.2° and 27.2°C, while monthly

Table 3: Climate data of the period 1974-2004 from the meteorological station of Larissa, Central Greece (Longitude 22º, 25´; Latitude 39º, 36´; Altitude 73 m asl)

Month	Mean Temp. (ºC)	Abs. Max. Temp. (ºC)	Abs. Min. Temp. (ºC)	Relative Humidity (%)	Rainfall (mm)
January	5.2	22.8	-21.6	79.6	32.5
February	6.8	25.2	-10.5	75.1	31.7
March	9.4	27.5	-7.0	73.4	36.7
April	13.8	32.4	-4.4	68.7	33.0
May	19.7	40.0	1.4	61.6	38.2
June	25.0	42.2	7.0	49.2	25.6
July	27.2	45.2	11.0	46.6	19.0
August	26.2	45.0	10.0	50.0	16.4
September	21.8	39.2	0	58.9	30.2
October	16.2	36.8	-2.0	70.0	52.2
Novembee	10.8	29.6	-7.0	79.5	56.9
December	6.6	23.2	-17.5	82.2	50.8

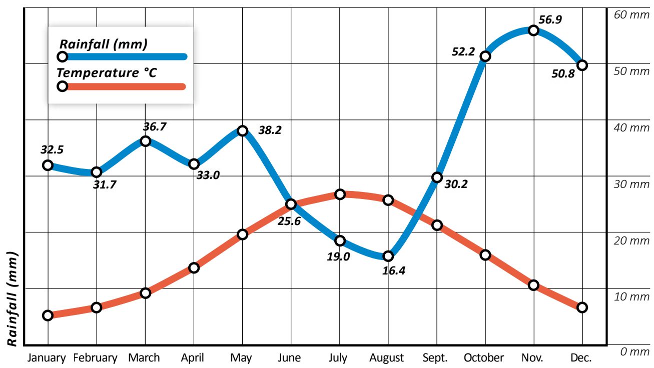

Figure 5: Ombrothermic diagramm in the location of the experiments (Larissa, Thessaly, Central Greece, Altitude 73m a.s.l. Latitude 39º 36´, Longitude 22º 25´)

Photo 1: Plantation of early sowing (5th of March, 1998) Quinoa (cvs: "FARO" and "407") in experiments conducted in Larissa, Thessaly, Central Greece (Longitude 22º, 25´; Latitude 39º, 36´; Altitude 73 m a.s.l.) (Iliadis *et al* 1999).

Photo 2: Poor Quinoa (cvs: "FARO" and "407") crop establishment in a late sowing date (2d of May, 1998) in the Region of Larissa (Thessaly, Central Greece) (Longitude 22º, 25´; Latitude 39º, 36´; Altitude 73 m a.s.l.) (Iliadis *et al* 1999).

rainfall ranges from 16.4 to 56.9 mm. From May to the end of September, a water deficit is recorded, and during this period quinoa must be irrigated to ensure acceptable yield potential. The extreme maximum temperatures reached on certain days in the lowlands (> 40°C) can produce a significant drop in seed yield of quinoa (normally harvested at the end of July); it is, therefore, thought that this crop (depending on the ecotype) may be better adapted to the hilly and/or mountainous areas of Greece.

Experiments carried out in 1998 in the lowlands of central Greece (Iliadis *et al.*, 1999) showed that seed yields of the cultivars 'FARO' and '407' were higher (1.99 tonnes/ha and 2.23 tonnes/ha, respectively) following early sowing in March; in contrast, late sowing in May was unsuitable (0 tonnes/ha and 0.09 tonnes/ha, respectively) due to poor seed germination (Photos 1 and 2). Climate data for 1998 show that the minimum mean monthly air temperature in March was low (-5.2°C), due to frost in the first week of the month. However, the minimum mean monthly soil temperature for the same period was 6.7°C.

Of the three altitudinal classes shown in Figure 2, 500–1 000 m asl and 500–1 300 m asl are considered suitable for quinoa cultivation because of:

- ample soil moisture and favourable temperature conditions; and
- exploitation of marginal soils cropped to cereals.

In these mountainous areas, the constraint of low temperatures in March could be overcome by late sowing (approximately second week of April). However, further field experimentation is required in order to define and validate the appropriate sowing dates.

International collaborations and experiments in Greece

Introduction of quinoa in Greece

Greece was among the European countries (Sweden, Poland, Czech Republic, Austria and Finland) involved since 1995 in the American and European Test of Quinoa, financed by FAO (Iliadis *et al.*, 1999, 2001). The quinoa project was undertaken by the International Potato Center (CIP) in Peru and aimed to improve quinoa cultivation, introduce new uses of the crop and enhance market demands. Quinoa seed material was provided for field experiments in the participating European countries in order to test its adaptation and the application of various farming practices under different climatic and soil conditions.

Research activities with quinoa in Greece

Introduction of quinoa in the plain of central Greece

The first experiments for the adaptation of quinoa in the Mediterranean Basin were conducted in Greece. There is wide variability among varieties, and cultivation potential is great, with positive results even in the warmer climates of the Andes (Karyotis *et al.*, 2003; Taviani *et al.*, 2008). Early research in Greece took place in Larissa, central Greece (22º25'N, 39º36'E, 73 m asl) (Figure 6) between 1995 to 2004. Experiments were carried out within the framework of COST ACTION 814 (1995–2000, "Crop development for the cool and wet regions of Europe") and continued with COST ACTION 852 (2001–06, "Quality legume based forage systems for contrasting environments"). The quinoa trials were conducted at the research stations of the Fodder Crops and Pastures Institute in cooperation with the Institute for Soil Mapping and Classification of Larissa (National Agricultural Research Foundation, N.AG.RE.F., Hellenic Ministry of Rural Development and Food).

The aim of the preliminary experiments in 1995 was to assess the adaptation of two quinoa varieties under Greek pedoclimatic conditions (Karyotis *et al.*, 1996). Seed material of the quinoa varieties 'Olav40' and 'KVL68' was provided by the Royal Veterinary and Agricultural University (Department of Agricultural Sciences) of Copenhagen. The results indicated that both varieties have production potential in Greece, with 'Olav40' yielding considerably higher (1.4 tonnes/ha) than 'KVL68' (0.44 tonnes/ha) under similar soil and climatic conditions.

Research was extended until 1996, and an experiment took place at the Fodder Crops and Pastures Institute to investigate how sowing density (50 and 100 plants/m^2) could affect seed yield and other quality characters of two quinoa and two amaranth (*Amaranthus caudatus*) accessions (Iliadis *et al.*, 1997). The quinoa accessions were 'Faro' and 'No 407'; the latter originated from Chile and was selected and adapted in Greece. Amaranth outyielded quinoa, and seed yield increased with plant density in all accessions of the two species. The protein content of the seeds of both species was on average high (15%) and was not affected by plant density.

The effect of sowing date on seed yield and quality was also investigated in a slightly alkaline clay soil (Iliadis *et al.*, 1999). The quinoa accessions 'Faro' and 'No 407' were tested for three different sowing dates (beginning of March, April and May). The results showed that for both cultivars, seed yield was higher at the earliest sowing (5 March 1998), while the yield obtained from the second sowing (1 April 1998) decreased by 30–50% in both cultivars. The latest sowing (2 May 1998) was unsuitable and resulted in poor germination (Photo 2). Therefore, the most suitable sowing date is the beginning of March for both cultivars, while the May sowing gives poor plant density due to poor seed emergence. The cultivar 'No 407' was more productive than 'Faro' for all sowing dates. The best seed quality (protein and minerals) was achieved with the second sowing date. However, precise results depend on the quinoa ecotypes tested.

Another 25 promising cultivars from different agro-ecological regions (Europe and Latin America) were evaluated in 1999 under Greek soil and climatic conditions to evaluate seed yield potential and other agronomic characteristics. The varieties originated from Europe and Latin America and some of them proved suitable for seed production (Iliadis and Karyotis, 2000), with their origin playing a dominant role. Only eight (six European and two Latin American) of the 25 examined varieties produced seeds, while the other 17 produced only panicles and flowers (Table 4). All the European varieties were well adapted, while most of the Latin American varieties failed to produce seed because of the extremely elevated temperatures (> 30ºC) during flowering.

The seeds of the eight most promising varieties were subsequently analysed in the laboratory for protein and mineral content. Their yielding potential was further explored (Karyotis *et al.*, 2003) in experiments conducted in two locations with contrasting soil properties (Tables 1 and 2) and (Photos 3 and 4).

Table 4: Evaluation of 25 quinoa varieties for seed yield and other characteristics (Iliadis & Karyotis 2000) in Central Greece *(average of four replications)*

		Varieties	Origin[1]	SY	F	H	DSW	M
1	European varieties	E-DK-4-PQCIP-DANIDA-UNA	Denmark	1496	59	113	3940	110
2		RU-2-PQCIP-DANIDA-UNA	England	794	60	100	4810	100
3		RU-5-PQCIP-DANIDA-UNA	England	1018	60	109	3920	106
4		NL6-PQCIP-DANIDA-UNA	Holland	1100	60	90	2880	101
5		G-205-95-PQCIP-DANIDA-UNA	Denmark	1106	60	118	3320	113
6		Control 'N° 407'	Greece[2]	812	60	168	4500	116
7	Latin American varieties	02-EMBRAPA	Brazil	459	64	101	1630	102
8		BAER-II-U-CONCEPTION	Chile	302	60	157	3670	110
9		Cica-127-Cusco	Peru	0	68	156	6660	120
10		Cica-17-Cusco	Peru	0	66	141	7400	120
11		Huariponco-CRIDER-Puno	Peru	0	60	115	3320	118
12		Kancolla-UNA	Peru	0	58	123	4730	122
13		Narino-INIA-Pasto	Colombia	0	77	144	2250	132
14		Salcedo-INIA-Puno	Peru	0	60	113	4830	118
15		Ratuqui-IBTA	Bolivia	0	59	125	2600	118
16		Kamiri-IBTA	Bolivia	0	60	152	2880	121
17		Real-IBTA	Bolivia	0	60	108	2530	120
18		Juiuy-UNA	Argentina	0	60	119	4250	120
19		Sayana-IBTA	Bolivia	0	62	127	2290	124
20		Ingapirca-INIAR	Ecuador	0	68	113	4060	124
21		03-21-079BB-Una-Puno	Peru	0	68	117	4740	124
22		03-21-072RM-Una-Puno	Peru	0	63	109	3830	110
23		Ecu-420-INIAP	Ecuador	0	66	118	4820	119
24		Canchones-Uap-Iquique	Chile	0	0	106	180	122
25		Illpa-UNIA-Puno	Peru	0	61	107	2180	120
			$LSD_{0.05}$	117	1.64		1150	1.44
			CV	15.98	12.40		12.46	9.32

SY=Seed Yield (Kg ha^{-1}), F =Beginning of flowering (days after germination), H=Plant's height (cm) at harvest, DSW= Dry Stems Weight (Kg ha-1), M= Plant's maturing for harvest (days after germination).

[1] Sanchez et al (1998); [2] Originated from Chile and selected and adapted in Greece

Photo 3: Quinoa grown on a *Vertisol* with neutral soil conditions (pH=7) in experiments in Larissa, Central Greece (Longitude 22º, 25´; Latitude 39º, 36´; Altitude 73 m a.s.l.) (Karyotis *et al* 2003)

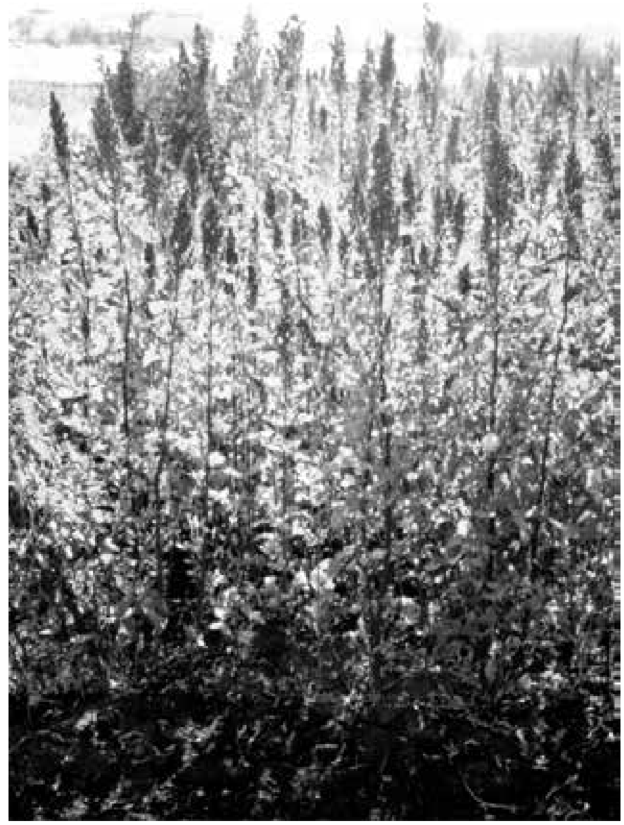

Photo 4: Quinoa grown on an *Inceptisol* with saline-sodic soil conditions (pH=8.9) in experiments in Larissa, Central Greece (Longitude 22º, 25´; Latitude 39º, 36´; Altitude 73 m a.s.l.) (Karyotis *et al* 2003)

The majority of the quinoa varieties tested were not selected to tolerate unfavourable physical and chemical soil conditions, and yield needs to be improved by plant breeding and sustainable soil management. It was observed that seed yield in marginal (saline–sodic) soil decreased by 45%. In both locations, the varieties originating from South America accumulated more protein in the seeds, which also had superior mineral composition (Tables 5 and 6, adapted from Karyotis *et al*., 2003).

In a breeding programme started in 2002, mass selection procedures were used for the creation (from plants surviving in saline-sodic soil) of new varieties suitable to marginal soils, with 23 families created (Iliadis *et al*., 2004).

The main observations and conclusions derived from the experiments conducted in central Greece during the period 1995–2004 can be summarized as follows:

– A wide range of soils are suitable for quinoa production, but crusting and drying up of the top soil can restrict germination potential. Heavy soils may be used for quinoa cultivation under appropriate fertilization and irrigation regimes (Iliadis *et al*., 1999; Iliadis *et al*., 2001).

– Early sowing (March) is suitable for quinoa production (in areas where frost avoidance is ensured), while late sowing (May) is unsuitable and results in poor germination.

– High temperatures and long days (during anthesis) are unfavourable for seed production. Drought is a common phenomenon, especially in central Greece, and low relative humidity during anthesis restricts pollen viability.

– Optimal sowing density is 25 plants/m^2 or 10 kg/ha.

– Variety, cultivation practices and soil-climatic conditions are among the main factors affecting quinoa's yield potential. Seed yields under central Greece conditions for some varieties exceed 1–1.5 tonnes/ha and biomass production of the dry stems var-

Table 5: Seed yield (dt ha^{-1}), whole seed protein concentration (%) and content of P, Ca, K, Na, (g kg $^{-1}$) in the seeds at harvest for eight quinoa varieties at two experimental locations in Central Greece *(Adapted from Karyotis et al 2003)*

Varieties	Origin	Seed yield				Proteins				Phosphorous (P)		
		L1	L2	L1–L2		L1	L2	L1–L2		L1	L2	L1–L2
E-DK-4-PQCIP-DANIDA-UNA	Denmark	22.1	6.2	15.9	**	14.89	17.97	– 3.08	**	3.30	3.36	– 0.06
Baer-II-U-Conception	Chile	13.1	6.0	7.1		16.59	18.81	– 2.22	*	3.46	3.72	– 0.26
RU-2-PQCIP-DANIDA-UNA	England	18.7	5.3	13.4	*	15.50	18.72	– 3.22	**	3.31	3.59	– 0.28
RU-5-PQCIP-DANIDA-UNA	England	23.0	10.7	12.3	*	14.30	19.03	– 4.73	**	2.97	3.26	– 0.29
NL6-PQCIP-DANIDA-UNA	Holland	10.1	7.5	2.6		16.17	18.33	– 2.16	**	3.68	3.92	– 0.24
G-205-95-PQCIP-DANIDA-UNA	Denmark	12.5	5.2	7.3	**	15.09	18.28	– 3.19	**	3.48	3.37	0.11
02-EMBRAPA	Brazil	15.1	5.9	9.2		15.33	19.03	– 3.70	**	3.57	3.90	– 0.33
'N° 407'	Chile	15.6	12.7	2.9		14.91	17.41	– 2.50		3.18	3.39	– 0.21
Avg.(locat.)		16.3	7.4	8.9	**	15.35	18.45	– 3.10	*	3.37	3.56	– 0.19
LSD[†]		8.10	6.70			0.86	1.62			0.36	0.34	

Varieties	Origin	Calcium (Ca)				Potassium (K)				Sodium (Na)			
		L1	L2			L1	L2	L1–L2		L1	L2	L1–L2	
E-DK-4–PQCIP-DANIDA-UNA	Denmark	3.31	2.34	0.97		18.89	15.71	3.18		0.40	1.49	– 1.09	**
Baer-II-U-Conception	Chile	5.38	2.39	2.99	**	22.50	14.42	8.08	**	0.37	1.01	– 0.64	
RU-2-PQCIP-DANIDA-UNA	England	4.53	2.30	2.23	**	17.25	15.82	1.43		0.37	0.97	– 0.60	*
RU-5-PQCIP-DANIDA-UNA	England	3.68	2.04	1.64	*	18.62	14.61	4.01	*	0.34	1.02	– 0.68	*
NL6-PQCIP-DANIDA-UNA	Holland	4.74	2.60	2.14	*	17·69	16.21	1.48		0.38	1.08	– 0.71	
G-205-95-PQCIP-DANIDA-UNA	Denmark	4.68	2.46	2.22	**	16.10	15.33	0.77		0.36	1.20	– 0.84	
02-EMBRAPA	Brazil	5.48	3.76	1.72		22.63	20.03	2.60		0.39	2.13	– 1.74	
'N° 407'	Chile	4.40	1.94	2.46	*	21.67	13.77	7.90	*	0.34	0.84	– 0.50	
Avg.(locat.)		4.53	2.48	2.05	**	19.42	15.74	3.68	*	0.37	1.22	– 0.85	**
LSD[†]		1.67	0.75			5.16	1.92			0.09	1.08		

L1 is the location with the *Vertisol* soil order (neutral soil conditions) and L2 is the location with the *Inceptisol* soil order (saline-sodic soil conditions). See also Tables 1 & 2.
*, **, denotes significant differences between the locations at 5 % and 1 % probability level respectively, according to Student's pair–wise t test
[†] LSD: for comparing the varieties within the locations at 5 % probability level

ies between 6 and 8 tonnes/ha. Moreover, quinoa seeds are rich in protein and mineral content.

– Dry stems of the plants left after seed harvest contain a high percentage of fibres (45%). Dry plant material sent to the *Istituto Poligrafico e Zecca Dello Stato Roma* in Italy where it was used to produce paper.

– Quinoa is tolerant to the drought conditions which prevail in the plains of Greece. However, irrigation is required, especially in the early stages of growth (4th to 6th leaf stage).

– Quinoa tolerates marginal, saline-sodic soils. Certain quinoa varieties can adapt under marginal environments, producing seeds with high protein and mineral content (Karyotis *et al*., 2003).

– Results from the American and European Test of Quinoa show that the growth period in Greece is 100–116 days for varieties that mature, while the growth period in northern Europe is 110–180 days (Jackobsen, 2003).

Other locations in Greece where quinoa was tested or grown

Table 6: CWhole seed content of Mg (g kg $^{-1}$) and Fe, Zn, Mn, Cu, B (mg kg $^{-1}$) for eight quinoa varieties at two experimental locations n Central Greece *(Adapted from Karyotis et al 2003)*

Varieties	Origin	Magnesium (Mg)				Iron (Fe)				Zinc (Zn)			
		L1	L2	L1–L2		L1	L2	L1–L2		L1	L2	L1–L2	
E-DK-4-PQCIP-DANIDA-UNA	Denmark	4.73	3.70	1.03	*	178	146	32		56	38	18	**
Baer-II-U-Conception	Chile	6.43	3.59	2.84	*	205	106	99	*	67	44	23	**
RU-2-PQCIP-DANIDA-UNA	England	5.15	3.76	1.39	*	136	128	8		62	37	25	**
RU-5-PQCIP-DANIDA-UNA	England	5.12	3.50	1.62		94	111	– 17		53	34	19	**
NL6-PQCIP-DANIDA-UNA	Holland	5.28	4.03	1.25	**	155	120	35		63	41	22	**
E-DK-95-PQCIP-DANIDA-UNA	Denmark	4.85	3.80	1.05	*	186	191	– 5		61	39	22	**
02-EMBRAPA	Brazil	6.49	4.57	1.92		127	257	– 130		63	42	21	**
'N° 407'	Chile	5.11	3.46	1.65	**	129	96	33		60	38	22	**
Avg. (locat.)		5.40	3.80	1.60	**	151	129	22		61	39	22	**
LSD†		1.40	0.49			58	66			9	4		

Varieties	Origin	Manganese (Mn)				Copper (Cu)			Boron (B)			
E-DK-4-PQCIP-DANIDA-UNA	Denmark	76	48	28	**	13.9	17.0	– 3.1	43	44	– 1	
Baer-II-U-Conception	Chile	83	49	34	**	17.2	18.6	– 1.4	44	34	10	
RU-2-PQCIP-DANIDA-UNA	England	74	43	31	**	15.1	16.4	– 1.3	35	39	– 4	
RU-5-PQCIP-DANIDA-UNA	England	70	43	27	**	11.8	12.4	– 0.6	38	36	2	
NL6-PQCIP-DANIDA-UNA	Holland	73	47	26	**	13.8	15.3	– 1.5	41	42	– 1	
E-DK-95-PQCIP-DANIDA-UNA	Denmark	81	49	32	**	15.4	16.3	– 0.9	35	38	– 3	
02-EMBRAPA	Brazil	100	66	34	**	16.7	18.5	– 1.8	43	45	– 2	
'N° 407'	Chile	91	54	37	**	15.6	16,.	– 0.7	47	34	13	*
Avg. (locat.)		81	50	31	**	14.9	16.4	– 1.5	40	39	1	
LSD†		16	10			2.0	3.3		12	6		

L1 is the location with the *Vertisol* soil order (neutral soil conditions) and L2 is the location with the *Inceptisol* soil order (saline-sodic soil conditions). See also Tables 1 & 2.
*, **, denotes significant differences between the locations at 5 % and 1 % probability level respectively, according to Student's pair–wise t test
† LSD: for comparing the varieties within the locations at 5 % probability level

In Velestino (eastern Thessaly, central Greece, 39º22΄N, 22º45΄E) and in Grevena (western Macedonia, northern Greece, 40º05΄N, 21º25΄E) (Figure 6), weed control, yield and fertilization trials on quinoa were conducted by the University of Thessaly from 2008 to 2010 (Lolas, 2012). In the Velestino trials, the active ingredients of the tested herbicides were not sufficiently selective for broad-leaved weeds and cannot be safely used with the quinoa crop. Weed control is, therefore, performed with carvings 20 and 40 days after quinoa seed germination, and in cases of high infestation of broad-leaved weeds, additional carvings are required within 20–60 days after germination. Weeds impact grain yield and care should be taken to set up the appropriate sowing dates for quinoa. Early sowing (beginning of March) successfully competes with rapidly growing weeds, because quinoa exhibits slow growth during the first 2 weeks after emergence, when competition from rapidly growing weeds is greater. Early sowing also gives higher seed yields (Iliadis *et al.*, 1999). However, further studies are needed to explore the effectiveness of selective herbicides in quinoa. It should be noted that there are as yet no approved herbicides for use in quinoa in Greece. Considering that there are no recommended herbicides for quinoa cultivation, and taking into account the market demand for quality organic products, it is imperative to explore efficient cultural techniques to suppress weeds. In Greece, organic farming offers a future and presents a new challenge. In Greece, organic agricultural production is

Figure 6: Map of Greece showing the approximate locations where quinoa has been tested or grown

favoured by the prevailing pedoclimatic conditions (mild climate), the limited agrochemical pollution, the presence of small family farms and consumer demand for quality products. Moreover, organic farming favours products with good organoleptic properties and it enjoys higher prices than conventional farming. Organic farming should therefore be taken very seriously by all stakeholders and requires the support of the European Union for efficient development.

Experiments by the University of Thessaly over 3 years (2008–10) in Velestino and Grevena in central Greece tested the varieties 'Faro' and 'CO 407' (seed material supplied by Dr D. Gimplinger from Vienna Agricultural University, BOKU), and yields ranged from 0.78 to 4.25 tonnes/ha (Lolas, 2012), with the highest yields obtained with early sowing in April. Iliadis *et al.* (1999) performed trials in a slightly alkaline soil and found that yield increased by 30–50% with early sowing compared with late May sowing. Variety 'CO 407' is a short, early-maturing variety (approximately 100 days from sowing), with dense and reddish inflorescence and good resistance to seed fall. It has a pleasant taste and a protein content of 16–18% (compared with 12.5–14% in other varieties). 'Faro' is a high-yielding variety, with height of 1.2 m, light green leaf colour, yellow seeds and green-yellow inflorescence, maturing in about 100 days from seed emergence (sea level quinoas). In the district of Mesolakos (western Macedonia) in 2009, quinoa was tested in the villages of Agapi and Trikokia (northern Greece) with the financial support of the prefecture of Grevena and with farmer's participation.

The crop was cultivated at experimental level in relatively small areas in Greece and, therefore, no severe crop diseases were observed – just limited damage in the Grevena trials in northern Greece from *Chaetocnema* sp. and *Haltica* sp. at the 7th to 10th leaf stage. Quinoa fields in southern Europe (Italy, Greece) report the presence of *Epitrix subcrinita* Le Conte (Chrysomelidae, Coleoptera), and plants at emerging stage are attacked by leafhoppers (Cicadellidae, Homoptera) (P. Casini and C. Iliadis, personal communication, cited in Rasmussen *et al*., 2003). It is expected that as the crop expands and is more widely cultivated, serious disease problems may arise.

On the farm of the Agricultural University of Athens (Attica, central-southern Greece, 37º59´N, 23º42´E, Figure 6), during the growth period March–August 2011, field trials were conducted to study the effects of three kinds of fertilizer (seaweed compost, humus and control) on the growth and production of organic quinoa (Katsenios, 2012). The Bolivian organic cultivar 'Royal' (Davert), certified by the organization "Bio-Latina", was used to study plant height, fresh and dry matter, leaf area index (LAI) and seed yield. No significant differences between seaweed compost and humus were revealed, while the control treatment had a greater impact than the other two on all the plant properties. Application of humus gave the highest dry matter (10.857 tonnes/ha), followed by seaweed compost (10.148 tonnes/ha) and the control (8.7 tonnes/ha). Humus gave a seed yield of 2.2 tonnes/ha – significantly higher than the control, but not than seaweed compost (Katsenios, 2012).

In the area of Agrinio (Aetolia-Acarnania, western Greece, 38º35'N, 21º25'E, Figure 6) between May and September 2011, organic field trials were carried out to study two different tillage systems (conventional and reduced tillage), combined with three different fertilization regimes (control, cow manure and seaweed compost), and their effects on total soil nitrogen %, density of quinoa root system, dry and fresh weight, LAI and seed yield (Katsenios, 2012). Reduced tillage had a positive impact compared with conventional tillage, and significantly affected soil total N and root density, resulting in higher aboveground biomass. Of the fertilization regimes, cow manure had a positive effect on all properties and produced higher yields than seaweed compost (not statistically different); the control gave consistently lower values. Reduced tillage resulted in significantly higher seed yields (2.532 tonnes/ha) compared with conventional tillage (2.418 tonnes/ha). Cow manure gave significantly higher quinoa seed yields (2.584 tonnes/ha) compared with the control (2.365 tonnes/ha), but only slightly higher than compost (2.475 tonnes/ha).

The effect of organic fertilization on weeds and on the allelopathy of quinoa was also investigated at the experimental field of the Agricultural University of Athens (Attica, central-southern Greece, 37º59´N, 23º42´E, 170 m asl, Figure 6) (Gournaki, 2012). Studies were carried out on the allelopathic effects of four tissue types of quinoa (roots, shoots, inflorescence and leaves) on the growth of oat (*Avena sativa)* and the weeds, *Avena sterilis* and *Echinochloa* spp., and on the effect of weed density taking into consideration the type of fertilization (compost, humus and control). It was found that only the growth of quinoa in the field influenced the appearance of weeds – not the type of fertilization. In similar experiments, the allelopathic activity of four plant tissues (leaves and stems, roots and inflorescences) of *Chenopodium quinoa* on above-ground (seedlings) and below-ground (roots) growth of oat in pot experiments was evaluated by Bilalis *et al.* (2013). The different plant tissues of quinoa exhibited different allelopathic activity. Oat growth (fresh and dry weight of above- and below-ground parts) was significantly inhibited by the phytotoxic activity of inflorescence tissues, leaves and roots of *Chenopodium quinoa.* The quantification of phytotoxicity of quinoa plant extracts by means of three bioassay methods (seed germination and radicle growth of oat, fresh and dry weight of common bean, and fresh weight of duckweed) revealed that all the tested species (oat, common bean, duckweed) showed greater phytotoxic response from the inflorescences than the other tissue parts (leaves, roots) of quinoa (Bilalis *et al.*, 2013).

Fertilization trials (control, cow manure, compost) on quinoa were established in 2010–11 in Agrinio (Aetolia-Acarnania, western Greece, Figure 6). In addition, these experiments were conducted to determine the effect of cultural practices (minimum tillage [MT] and conventional tillage [CT]) on yield and quality characteristics of quinoa (Bilalis *et al.*, 2012). The highest leaf area index (4.47–5.03), quinoa dry weight (8.65–9.29 tonnes/ha) and quinoa root density (1.03–1.21 cm/cm^3) were found in MT. Quinoa saponin content is very important for the industry. No significant differences were found in saponin content between MT and CT. The highest seed yield (2.485–2.643 tonnes/ha) and highest saponin content (0.42–0.45%) were found in cow manure and compost treatments. The highest saponin yield (7.70–12.05 kg/ha) was produced in the MT system. The results indicate that minimum tillage (MT) and organic fertilization increase saponin content and yield of quinoa.

Current situation and perspectives of quinoa in Greece

The crop is not yet commercially cultivated in Greece, with the exception of a very small area (around 1 ha) in the region of Lamia (central-southern Greece, Figure 6) in 2012 in collaboration with the University of Thessaly (Lolas, 2012). The income expectations of the farmers may be high with the introduction of quinoa – a new promising and unknown crop. Nevertheless, it will be several years before a real breakthrough, and commercialization cannot seriously begin for another 5–10 years. Quinoa needs to be further explored and domesticated, and new varieties must be selected and adapted to Greek climatic conditions in several agro-ecological zones. In central Greece, some cultivars (in particular, those originating from Europe) show promise and acceptable yield potential. Plant breeding for crop adaptation and increased yield stability must be given high priority, and research must also focus on product development and marketing.

In Greece, breeding efforts began in 2001 to produce new high-yielding quinoa varieties suitable for both neutral and saline-sodic soils. Field observations indicated that varieties were not morphologically uniform, with variability mainly in the type of inflorescence and the grain colour. In 2001, single plant selections were made and the most productive plants from each variety (mostly unbranched, with compact inflorescences and large white grains) were chosen in both soil types used for experimentation. This genetic material was used in a breeding project which started in 2002 to create high-yielding varieties suitable for neutral and saline-sodic soils with compact inflorescence and large white grains. The selection trials were established in Larissa (central Greece) under irrigation. Emphasis was given to plants cultivated in saline-sodic soil, and five varieties were selected (Iliadis *et al*., 2004). The pure line selection breeding method was used. A total of 23 selections, already pure lines, originating from both soil types, are now available and have shown promising grain yield at single plant level (Table 7). These selections were to undergo evaluation in dense sowing in typical seed yield experiments, but experimentation was discontinued in the absence of financial support.

Uses and by-products of quinoa

Quinoa may be considered a "neglected" crop, and only recently is it being used as a novel functional food ("superfood") (FAO, 2013). Quinoa is an alternative crop exhibiting high nutritional values, such as protein seed flour (14–18%) and excellent amino acid balance (Oelke *et al*., 1990; Aluko and Monu, 2003; Abugoch *et al*., 2009). It is an important source of minerals (calcium, magnesium, iron, copper, zinc, manganese) and vitamins (A, B2, E) and is gluten-free. As a gluten-free product, it is recommended for coeliac people. The only treatment currently available for coeliac disease is a lifelong avoidance of gluten ingestion. Patients have to follow a very strict diet and avoid any products that contain wheat, rye or barley (some authors include oats). Patients with coeliac disease cannot eat some common foods such as bread, pizzas and biscuits or drink beer. Given the unique properties of gluten, it is a big challenge for food scientists to produce good quality gluten-free products (Shoenlechner *et*

Table 7: Single plant selections made from the higher seed yielding plants of the varieties in the two soil types (Iliadis *et al* 2004)

N° of single plants	Source of selection	Soil type[1]	Plant growth type[2]	Plant height (m)
1	'Nº 407'	N	UN-B	1.57
2		S–S	UN-B	1.57
3		N	UN-B	1.30
4		S–S	UN-B	1.51
5		S–S	B	1.57
6	RU–5–PQCIP–DANIDA–UNA	S–S	UN-B	1.15
7		S–S	UN-B	1.28
8		S–S	UN-B	1.30
9		N	UN-B	1.25
10		S–S	B	1.30
11		N	B	1.50
12	BAER–II–U–CONCEPTION	N	B	1.50
13		S–S	UN-B	1.36
14		S–S	UN-B	1.00
15		N	UN-B	1.45
16		S–S	UN-B	1.17
17	02–EM BRAPA	N	B	1.17
18		N	UN-B	1.01
19		N	B	1.15
20		S–S	B	1.21
21	G-205-95-PQCIP–DANIDA–UNA	S–S	B	-
22		S–S	B	-
23		N	B	1.21

[1] Type of soil from which plants were selected: N= neutral, S-S=saline sodic,

[2] B=branched plants, UN-B= un-branched plants

al., 2008). Quinoa has been used on a small scale to make bread, cookies, muffins, pasta, snacks, drinks, flakes, breakfast cereals, baby foods, beer and diet supplements (FAO, 2013).

In recent years, Greece has witnessed an increasing interest in gluten-free products. The Greek Coeliac Association (http://www.coeliac.gr/), a member of the Association of European Coeliac Societies (http://www.aoecs.org/), actively participates in activities in order to disseminate knowledge of coeliac disease and facilitate the daily lives of sufferers. Lack of information, combined with relatively limited experience of quinoa cultivation, results in a knowledge gap for Greek and Mediterranean agriculture and the agrofood industry.

The Hellenic Food Authority (EFET) announced in May 2013 a series of events within the framework of the project "Know In Target" (available at http://www.knowintarget.eu/final-and-international-conference-in-athens-30-and-31-may-2013/) addressing important issues in the agrofood sector with regard to both sectorial competitiveness and consumer welfare. Among the topics discussed was "Innovation and Entrepreneurship in Food Production" with special attention to gluten-free products. The workshop's objectives included: inform the productive sector of the concerns of people with coeliac disease; raise public awareness; change the attitudes and behaviour of consumers with regard to coeliac disease; and face the challenge of the design of gluten-free foods. The meeting was attended by, among others, business executives, representatives of industry bodies, managers of public institutions and scientists from the fields of medicine, food and nutrition.

In recent years, numerous agricultural, biomarket, health, fitness-oriented and social media internet sites have appeared with an abundance of information on quinoa. It must be underlined that the consumption of quinoa is still limited and is preferred by consumers who have specific interests in health care or the environment.

Potential for research, development and dissemination of results

Quinoa production requires conditions found only at high altitudes (cool night time temperatures, hot days). These requirements restrict quinoa production to a few climatic regions and successful production can be expected mainly in the highlands and in the cool regions of northern Greece.

The main potential problems for the expansion of quinoa in Greece are related to:

– limited processing plans and absence of manufacturing potential;
– farmer awareness and knowledge of quinoa production and post-harvest handling, and of the quinoa varieties available, well adapted to Greek conditions;
– distance from European Union markets;
– absence of incentives by the European Commission for quinoa and support payments in relation to other competitive crops (i.e. cotton); and
– limited consumption of quinoa in Greece, concentrated among consumers with knowledge of health foods or who value it for its health benefits including its gluten-free status.

To promote consumption, funding must be obtained to support the participation of farmers in training courses and internal consumption of quinoa should be promoted (through school breakfast programmes, maternity subsidies and the army food programme). At present, quinoa consumption is very low throughout the country.

Agricultural Research Institutes in Greece should coordinate the work of the various demonstration projects related to applied research on quinoa (adaptation, water scarcity, fertilization plans, proper agricultural practices etc). A regional programme is required for soil and irrigation management, the development of new sowing technologies, fertilization and proper irrigation practices, and the development of new harvest and post-harvest technologies.

The creation of financing mechanisms can provide better capital access conditions to farmers and processing companies. However, taking into account the economic conditions of Greece in recent years, funding cannot easily respond to the needs of companies and farmers. It is necessary to introduce and establish a network of farmers oriented to international markets.

Agricultural production in Greece is labour-intensive with relatively limited use of technologies. Another constraint is the small average size of parcels of land, leading to increased production costs and restricting the adoption of soil and water management systems – as a result, sustainable cultivation is not always possible.

Research by the National Agricultural Research Foundation in Greece (N.AG.RE.F.) shows that in the region of Thessaly (the largest agricultural plain in Greece), quinoa is agronomically viable. Trials conducted in the field suggest that, with the introduction of the appropriate technologies, crop yield can be increased significantly and quinoa cultivation can be expanded, using lands previously considered unsuitable for agriculture (i.e. alkaline soils).

The development of agricultural technology entails the provision of farming machinery and equipment, such as planter, harvester, dryer and thresher. The Ministry of Rural Development and Food must also invest in infrastructure and build roads, provide education to farmers and give access to credit. It is also necessary to invest in germplasm collection and evaluation, in genetic and agronomic programmes, and in post-harvest handling and industrialization. Marketing campaigns are required to provide information on prices and markets and to stimulate demand and mass consumption.

Universities and Agricultural Research Institutes should continue research and hold trials in a greater variety of soils in most agro-ecological zones of Greece. Ecoregions could be identified for quinoa cultivation, taking into account the crop's resistance to drought and its yielding capacity even on marginal soils (Iliadis *et al*., 2001; Jacobsen *et al*., 2003) and in areas with low temperatures. Quinoa is a promising and profitable crop. Quinoa yield, as with many other crops, depends on many factors, such as variety, cultivation practices and agroclimatic conditions. Yields vary considerably from year to year and from region to region and may depend on successful crop establishment, the presence of weeds, harvest and cultivation techniques and other factors. One factor affecting quinoa's growth and development and resulting in reduced yields is the combination of high temperatures and long days (long photoperiod) during the growing period. Rotation schemes for quinoa are similar to those for potato. The effects of monocropping and crop rotation on yield, above-ground biomass and weed populations of four Andean crops: potato (*Solanum tuberosum*), melloco (*Ulucus tuberosus*), lupine (*Lupinus mutabilis*) and quinoa (*Chenopodium quinoa)*, planted both with and without fertilizers, were studied by Nieto-Cabrera *et al*., (1997), and the most recommended sequences are fertilized potato followed by non-fertilized quinoa, and melloco followed by quinoa with or without fertilization. This practice improves the quinoa yield and thus the fertility of the soil; furthermore, the life cycle of various pathogens is destroyed and any unused residual fertilizers applied to previous crops are efficiently utilized by quinoa, decreasing its dependency on N fertilizers.

Quinoa is imported from Bolivia and Peru, the main suppliers of this product. According to IICA (*Instituto Interamericano de Cooperacion para la Agricultura*, 2010), the European Union (EU) is the world's largest consumer of quinoa. There are no data available on quinoa consumption in the EU, but the market size can be estimated based on trade data, since the EU does not produce significant quantities of quinoa. For 2009, the EU market is estimated at EUR14 million/6 500 tonnes. The estimated market value for 2005 was EUR3 million, indicating rapid market growth. Of the EUR14 million of imported quinoa, 94% was sourced in Bolivia and 6% in Peru. Most export opportunities for quinoa can be found in Western Europe, with France, the Netherlands and Germany currently the largest importers. However, these countries also re-export the imported quinoa to other EU countries, especially the Netherlands. Other potentially interesting importers are the United Kingdom, Spain, Italy, Denmark and Sweden. Western Europe is also the largest market for organic food products, with Germany being the front runner, accounting for about one-third of the total EU organic food market.

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CHAPTER 6.2

Quinoa in the Indian subcontinent

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Abstract

The Indian subcontinent is a large land mass covering India, Pakistan, Nepal, Sri Lanka and Bangladesh and it sustains 20% of the world' population. The area is prone to degradation of its natural resources due to intensive cultivation leading to declining soil fertility, changes in water table depth, deterioration in the quality of irrigation water, and rising salinity in the region. Much of the population has little access to a protein-rich diet, since wheat and rice are the principal food grains grown and consumed in the area. The growing population necessitates increased food production combined with a shift towards environmentally sound sustainable agriculture. It is therefore important to select crops requiring fewer inputs while able to respond to the nutritional deficiency prevalent in the region. Quinoa is still an "underutilized" crop, given its nutritional superiority over traditional crops and its wide adaptability to diverse agronomic conditions, and its commercial potential in South Asia has remained untapped. Quinoa grain has a high protein content and good amino acid spectrum, and has an important role in combating the "silent hunger" of poor populations with little access to a nutritious diet. Quinoa's ability to produce high protein grains under stressful conditions makes it important for the diversification of future agricultural systems, especially in the Indian subcontinent. The worldwide popularity of quinoa and initial promising reports from Asia make it an important candidate as an alternative crop in this region.

Introduction

The Indian subcontinent is the southern portion of Asia, mostly situated on the Indian Plate and projecting southwards into the Indian Ocean. It is surrounded by the Himalayas in the north, the Arakanese in the east, the Hindu Kush in the west, and extends southwards into the Indian Ocean with the Arabian Sea to the southwest and the Bay of Bengal to the southeast (Chapman and Baker, 2002). The region comprises five major states, namely India, Nepal, Pakistan, Bangladesh and Sri Lanka (Table 1), and two small countries, Bhutan and the Maldives. The total area is approximately 4.4 million km^2, and is home to about 22% of the world population. The Indian subcontinent exhibits enormous diversity in terms of agroclimatic regions and edaphoclimatic conditions and includes lofty mountain ranges, highlands and plateaus, deserts, large fertile river valley plains, and coastal areas (Balfour, 1976; Shukla *et al.*, 2005a; Saini, 2008).

The Indian subcontinent

The Indian subcontinent is in the second stage of demographic transition, i.e. high birth rates and low death rates, with a consequently high rate of population growth. India, with 1.27 billion people and a population density of 382 persons per km^2, is the second most populous country in the world with a population predicted to rise to > 1.53 billion people by the end of 2030. Table 1 depicts the population size, population density and growth rate for all the countries in the Indian subcontinent. The region is home to a large number of the developing world's poor. According to the World Bank's recent poverty estimates, about 571 million people in the region survive on less than USD1.25 a day, and constitute more than 44% of the developing world's poor. The region also has the largest number of malnourished children in the world, with malnutrition rates in some areas higher than in Africa.

The increasing population in this part of the world demands not only an increase in food grain production but also a shift towards environmentally sound and sustainable agriculture. During the last 50 years, agriculture has transformed significantly from subsistence to intensive, requiring farm mechanization and increased labour, as well as greater inputs of high-yielding varieties, chemical fertilizers and pesticides (Bhargava *et al*., 2008a). While yields have increased significantly, farmers have run up increasing debts (due to input requirements), undue pressure has been placed on the fragile agro-ecosystems, and increased homogeneity and monocropping has resulted in loss of agrobiodiversity as well as frequent crop losses due to pathogen infestations. The situation is compounded by the overdependency on a few plant species, with just 12 species providing 75% of the world's food supplies, and the three major crops (rice, wheat and maize) providing 50% of the world's food (Bermejo and Leon, 1994; FAO, 1996; Heywood, 1999; Thies, 2000). This condition prevails in spite of the fact that about 7 000 plant species have been cultivated for hundreds of years and are still in use in various parts of the world today (IPGRI, 2002). The emphasis on a handful of major crops has narrowed the number of species upon which global food security depends and many species are no longer a priority. The consequences of crop failure resulting from unforeseen stresses, pests and diseases are potentially catastrophic (Prescott-Allen and Prescott-Allen, 1990). There has been a recent impetus in different aspects of research on underutilized crops, and several important programmes have been undertaken to promote such crops for agricultural systems, as an alternative source of nutrition.

Underutilized minor, orphan or neglected crops are those which were once widely grown and consumed, but have now fallen or are falling into disuse (Hammer *et al*., 2001). This is often the case for indigenous plant species (rather than non-native or adapted introductions), which often form a complex part of the culture and diets of the people who grow them (Mayes *et al*., 2012). Underutilized species are traditionally appreciated by communities for their role in income generation, adaptability to marginal farming conditions, relevance to local food culture and diverse nutritional and nutraceutical value (DEFRA, 2005; Mwangi and Kimathi, 2006; Hawtin, 2007; Bhargava *et al*., 2008a; Hughes 2009; Mahyao *et al*., 2009; Bala Ravi *et al*., 2010; Shukla

Table 1. Demographic profile of countries of the Indian subcontinent.

Country	Population	Growth rate	Fertility rate (Children born/woman)	Population density (Persons/km^2)*
India	1 220 800 359	1.41	2.5	411
Pakistan	187 343 000	1.60	3.58	229
Bangladesh	142 316 000	1.57	2.6	1 174
Sri Lanka	20 263 723	0.91	2.17	323
Nepal	26 494 504	1.59	2.95	189
Bhutan	708 427	1.20	2.13	19
Maldives	394 999	1.30	1.90	1 107

*FAO and World Bank population estimates.

et al., 2010; Padulosi *et al*., 2011). The exceptional hardiness of many of these species and their ability to cope with adverse growing and climatic conditions offer great promise in the face of climate change (Bala Ravi *et al*., 2006). Use of these species can make an important contribution to the food security and well-being of the poor. Underutilized crops have enormous potential to alleviate hunger directly, by increasing food production in limiting environments where the yield of traditional major crops is severely affected. They can raise nutritional levels and increase incomes and, therefore, also the purchasing power of the poor (Mayes *et al*., 2012).

Chenopods in the Indian subcontinent

Chenopodium is the principal genus in the Chenopodiaceae family, which includes plants such as sugar beet, beetroot and spinach (Bhargava *et al*., 2005a). Chenopods are cosmopolitan in distribution and occur in every part of the world (Hickey and King, 1988). The genus *Chenopodium* includes herbaceous (sect. *Agathophyton*), suffrutescent (sect. *Ambrina*) and arborescent (sect. *Skottsbergia*) perennial species, most of which occur as colonizing annuals (Wilson, 1990). Ethnic communities in the subcontinent have always used chenopod leaves to treat urinary troubles (Bakshi and Sensarma, 1999) and to remove intestinal worms (Singh *et al*., 2003). Ancient Indian medicinal texts describe the plant as having oleaginous, diuretic and aphrodisiac properties, effective in the treatment of eye diseases, piles and heart and spleen ailments (Kirtikar and Basu, 2001). The first record of chenopod farming in Asia, specifically in the Himalayan region, dates back over 150 years (Roxburgh, 1832; Thomson, 1852). Chenopods are currently cultivated in the watersheds of the Chenab, Ravi, Beas, Satluj and Yamuna rivers in the western Himalayas, in the hilly areas of northern Bengal, watershed of the Teesta River, and in several states in northeast India (Joshi, 1991; Partap *et al*., 1998). *C. album*, ranked among the top ten weeds of the world (Holm *et al*., 1977), is grown in the northwest Himalayan region as a subsidiary food crop in mixed farming systems, particularly multiple cropping systems (Partap and Kapoor, 1985, 1987). The plant is cultivated in this region for its nutritionally rich grain, as a fodder crop and as pot herb (Partap, 1990). Over 90% of families in the region cultivate chenopods and utilize almost every plant part for various purposes. In addition to being used for food, the plant is also used as fuel and for the preparation of alcoholic drinks (Partap *et al*., 1998). However, in the Indo-Gangetic Plains, *C. album* is not cultivated but is weeded out from other crops and sold in local markets for consumption as a pot-herb.

Quinoa and its relevance in the Indian subcontinent

Quinoa (*Chenopodium quinoa* Willd.), an underutilized Andean crop, has gained worldwide attention because of its ability to grow in various stress conditions, such as soil salinity, acidity, drought and frost, exhibiting a high level of resistance to these environmental stress factors (Jacobsen *et al*., 2003; Gómez-Pando *et al*., 2010). Environmental stresses, such as water stress, temperature stress and salt stress, also happen to be among the major productivity constraints in the Indian subcontinent often causing extensive crop losses. The situation is compounded by the fact that agriculture is the mainstay of the economy in most of the countries in the region. Quinoa is an important food source for human consumption in the Andean region and has immense industrial value (Bhargava *et al*., 2006a; Fuentes and Bhargava, 2011). The crop grows in different ecological zones, from sea level to 2 000–4 000 m asl (Bazile *et al.*, 2013; Fuentes and Bhargava 2011). Quinoa may be classified as “underutilized” in the Indian subcontinent, because, despite its wide adaptability, rusticity and nutritional superiority, its commercial potential remains untapped. Much of the population has little access to a protein-rich diet, since rice and wheat are the principal food crops. Quinoa has a very protein-rich grain with a good amino acid spectrum, and can, therefore, contribute to a balanced diet and can play an important role in combating the “silent hunger” of poor populations with little access to proteins (Bhargava *et al*., 2006a). Furthermore, improved technologies and links with other sectors, such as product development and marketing, can help the industry tap quinoa’s potential for diverse applications.

Genetic resources and field results

The evaluation of quinoa in the Indian subcontinent has produced impressive results with the crop showing good adaptation and abundant yield.

India, located between 8° and 38°N and 68° and 93.5°E, has a very wide range of agroclimatic regions and edaphoclimatic conditions (Bhargava *et al.*, 2006a). Research on quinoa has been underway at the National Botanical Research Institute (NBRI), Lucknow, since the early 1990s. The NBRI is located at the heart of the Indo-Gangetic Plains (IGP), a region of land covering much of India, Pakistan, Nepal and Bangladesh (Table 1). IGP is characterized by fertile soils and an abundant water supply (Aggarwal *et al.*, 2004). Research intensified in 2000, when extensive field trials were performed as part of a coordinated effort by different departments, namely genetics and plant breeding, lipid chemistry, plant pathology, experimental taxonomy and biomass biology (Bhargava *et al*., 2005b, 2006a, 2007, 2008b, c; Kumar *et al*., 2006). Trials in the Indo-Gangetic Plains have shown that the crop can be successfully cultivated in this region, with many cultivars giving high yield (Bhargava *et al.*, 2007). The quinoa experiments in the Indian subcontinent are primarily based on germplasm obtained from United States Department of Agriculture (USDA) and IPK Gatersleben, Germany. The most comprehensive report from India (Bhargava *et al*., 2007) lists germplasm primarily from the South American countries of Bolivia, Chile, Peru and Argentina (Table 2). A total of 27 germplasm lines of quinoa and 2 lines of *C. berlandieri* subsp. *nuttalliae* were evaluated for 12 morphological and 4 quality traits in Lucknow (26.5°N, 80.5°E, 120 m asl), Uttar Pradesh, in the crop years 2002/03 and 2003/04. The general weather conditions for both crop years are presented in Table 3. The experimental site had sandy–loam soil and no chemical fertilizer was applied either before or during the experiment. No fungicides or insecticides were used during the experiment. In the IGP, quinoa is usually sown at the onset of winter, from mid- to late November, and harvested in February or March, depending on the maturity period of the variety. The 29 germplasm lines evaluated had an average pre-flowering growth period of about 82 days and took around 48 days for grain maturity (Table 4). Thus, the total growth period in north Indian conditions was less than that reported in South America (110–190 days) (Jacobsen and Stolen, 1993) and similar to northern Europe (Jacobsen, 1998). The harvest index presented tremendous variability and ranged from 0.26 to 1.43, indicating high efficiency of the reproductive partitioning (Table 4) (Bhargava *et al*., 2007). Seed protein among the lines ranged from 12.55 to 21.02% with an average of 16.22$\pm$0.47%; seed carotenoid ranged from 1.69 to 5.52 mg/kg with an average of 2.83$\pm$0.16 mg/kg (Table 5). The carotenoid content in the leaves was 230.23–669.56 mg/kg, and was comparatively higher than in the seeds. The leaf carotenoid content was higher than that reported for spinach, amaranth and *Chenopodium album* (Gupta and Wagle, 1988; Prakash and Pal, 1991; Shukla *et al*., 2003; Bhargava *et al*., 2006b). Of the lines with high leaf carotenoid, 70% also had high seed carotenoid. Quinoa had a higher protein content than commonly used cereals and compared favourably with other underutilized crops like *Amaranthus* (Bressani *et al*., 1987; Shukla *et al*., 2004, 2005b) and *Fagopyrum* (Steadman *et al*., 2001), and even some underutilized legumes like *Cassia floribunda* (Vadivel and Janardhanan, 2001). The seeds' high protein content is indication of the crop's potential as a low-cost source of protein to eliminate protein malnutrition in developing countries like India, where low incomes restrict consumption of meat and pulses for much of the population. Quinoa could be immensely useful for obtaining high-quality protein concentrates to solve the problem of chronic malnutrition affecting urban and rural populations in developing countries. An assessment of the crop's seed yield potential showed that 41% of the accessions were high-yielding. Accessions of Chilean and United States origin showed greater adaptability to north Indian conditions (Bhargava *et al.*, 2007). It was suggested that quinoa might serve as an alternative winter crop for the North Indian Plains and other subtropical regions with similar agroclimatic and edaphic conditions (Bhargava *et al.*, 2007). Quinoa has the potential to play a pivotal role in the future diversification of agricultural systems in India, not only at the high altitudes of the Himalayan region as a summer crop, but also in the North Indian Plains.

Pakistan is located between 24.53°N, 67.00°E and 35.44°N, 74.37°E, and has less than 240 mm of rainfall and 1 066 m^3 per caput water availability per annum. It is classed among the high water stress countries of the world (FAOSTAT, 2008; Munir, 2011). The country has a high proportion of salt-affected soils, and almost one-third of the total cultivated land has saline, saline–sodic or sodic soils (Khan, 1998). Pa-

Table 2. Germplasm lines, their source, origin and seed colour
(Reprinted from Bhargava *et al.* 2007, with kind permission from Elsevier)

Germplam line	Source	Status*	Origin*	Altitude* (m)	Seed colour
C. quinoa Willd. CHEN 58/77	IPK, Germany	-	-	4000	Light
C. quinoa Willd. CHEN 67/78	IPK, Germany	-	Puno, Peru	-	Dark
C. quinoa Willd. CHEN 71/78	IPK, Germany	-	Bolivia	-	Light
C. quinoa Willd. CHEN 33/84	IPK, Germany	-	-	-	Light
C. quinoa Willd. CHEN 84/79	IPK, Germany	-	Cuzco, Peru	3200	Light
C. quinoa Willd. CHEN 92/91	IPK, Germany	-	Columbia	-	Light
C. quinoa Willd. CHEN 7/81	IPK, Germany	-	-	-	Light
C. quinoa Willd. PI 614938	USDA	-	Oruro, Bolivia	-	Light
C. quinoa Willd. PI 478408	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 478414	USDA	Cultivar	La Paz, Bolivia	3800	Dark
C. quinoa Willd. PI 596498	USDA	Landrace	Cuzco, Peru	3030	Light
C. quinoa Willd. Ames 13219	USDA	-	La Paz, Bolivia	3700	Light
C. quinoa Willd. Ames 13719	USDA	-	New Mexico, USA	-	Light
C. quinoa Willd. PI 587173	USDA	Cultivated	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 510532	USDA	Cultivated	Peru	3000	Light
C. quinoa Willd. PI 614883	USDA	-	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 584524	USDA	Cultivated	Chile	-	Light
C. quinoa Willd. Ames 22156	USDA	Cultivar	Chile	-	Light
C. quinoa Willd. Ames 13762	USDA	-	Nueva Mexico, USA	-	Light
C. quinoa Willd. PI 614881	USDA	-	Jujuy, Argentina	-	Light
C. quinoa Willd. PI 510537	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. PI 510547	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. Ames 22158	USDA	Landrace	Chile	-	Light
C. quinoa Willd. PI 510536	USDA	Cultivated	Peru	-	Dark
C. quinoa Willd. PI 478410	USDA	Cultivar	La Paz, Bolivia	3800	Light
C. quinoa Willd. PI 433232	USDA	-	Chile	-	Light
C. quinoa Willd. Ames 21909	USDA	Landrace	Oruro, Bolivia	3870	Light
C. berlandieri subsp. *nuttalliae* PI 568155 (Saff.) Wilson and Heiser	USDA	Landrace	Mexico	1680	Dark
C. berlandieri subsp. *nuttalliae* PI 568156 (Saff.) Wilson and Heiser	USDA	Landrace	Mexico	2700	Dark

*From germplasm database

kistan has seen significant reductions in crop yields as a result of: large tracts of salt-affected soils; significant areas of cultivable wastelands with marginal or brackish irrigation water; uncertain climate-dependent irrigation sources; poor fertile tracks; and adverse climatic phenomena (Government of Pakistan, 2009; Munir, 2011). Climatically resilient and highly adaptable crops and climate-proof cropping systems are emerging (Munir 2011). As with other parts of southern Asia, crops such as quinoa are needed, not only to avoid failure but also to produce sufficient grain to meet dietary needs under unfavourable conditions (Munir, 2011).

Quinoa was introduced in Pakistan in 2007 in central Punjab to minimize the dependency of the masses on conventional food (Munir *et al.*, 2012). In Pakistan, the crop has been successfully culti-

Table 3. Weather conditions during the first and second experiments
(Reprinted from Bhargava *et al.* 2007, with kind permission from Elsevier)

Experiment I (2002-2003)	Temperature (°C) Max.	Min.	Mean	Dew point (°C)	Wind (km/hra)
November	24	16	20	14	3
December	18	11	15	10	4
January	15	7	11	8	3
February	24	11	18	12	6
March	30	16	23	13	7
April	38	23	30	15	9
Experiment II (2003-2004)					
November	28	14	21	12	2
December	21	11	16	11	4
January	10	8	9	7	3
February	25	12	19	11	4
March	34	17	26	13	5
April	37	23	30	16	7

vated on experimental farms in Faisalabad, Chakwal and Bahawalpur. The University of Agriculture, Faisalabad, has taken giant steps towards making this crop a reality in Pakistan (Figure 1). The university trial sites were situated at 184 m asl, in a subtropical region with a rich sandy–loam and loamy soil texture. Grain yields of up to 2.7 tonnes/ha are likely to increase further as farmers improve their understanding of its production and appropriate technologies (personal communication).

Further quinoa trials in Pakistan have demonstrated that the seed yield of the different accessions varies depending on the growing environment, with some accessions exhibiting good stability in the new environment. The short-statured accessions of Danish origin set seed in the shortest time, while the Chilean accessions originating from near sea level produced viable seeds with a medium-duration life cycle. The fiscal balance sheet showing the coefficient of profitability indicates that quinoa has the potential to be introduced as a new cash crop in the region and is a potentially sound choice for farmers with smallholdings (Munir *et al.*, 2012). Quinoa shows promise as an important new crop for Pakistan agriculture, providing highly nutritive and versatile food products for the population and new raw material for industry. Cultivation is feasible, particularly in marginal environments afflicted by drought or salinity stress, currently suffering from very low productivity (Jacobsen *et al.*, 2002). The crop offers hope in northern Pakistan where conventional agriculture is difficult due to loss of fertile soil and the shortage of suitable crops to improve the agricultural economy; quinoa has adaptability to severe winter conditions and could help alleviate poverty in such areas. It can also help improve food production in the western dry mountains of Balochistan, where the degraded land and declining groundwater resources severely hamper production of many crops. In summary, the assessment of quinoa in Pakistan shows that it is a potential drought- and salinity-tolerant crop with a wide range of adaptability under the varying climatic conditions of the Punjab Province of Pakistan, and it can be recommended for general cultivation once the production technologies are fully developed (Munir, 2011).

Current state in the Indian subcontinent

Cultivation of quinoa is becoming more widespread in the Indian subcontinent. The crop has been successfully cultivated in the drought-prone Anantapur district of Andhra Pradesh within the framework of “Project Ananta” (Deccan Chronicle, 2013; The Times of India, 2013). Quinoa was considered suitable for the weather conditions in Anantapur: it was cultured in the laboratory in February 2013 and

Table 4. Mean performance of 29 lines for 12 morphological traits in Chenopodium (Reprinted from Bhargava *et al*. 2007, with kind permission from Elsevier)

Germplasm lines	Origin	Days to flowering	Days to maturity	Plant height (cm)	Leaf area (cm^2)	Primary branches /plant
C. quinoa CHEN 58/77	-	73.55	117.67	45.41	15.71	16.56
C. quinoa CHEN 67/78	Puno, Peru	74.55	119.44	59.63	6.12	16.70
C. quinoa CHEN 71/78	Bolivia	79.33	131.67	46.33	26.94	15.44
C. quinoa CHEN 33/84	-	101.55	144.00	42.33	9.46	16.96
C. quinoa CHEN 84/79	Cuzco, Peru	86.00	121.67	86.97	17.47	22.11
C. quinoa CHEN 92/91	Columbia	81.89	123.22	77.49	24.69	14.06
C. quinoa CHEN 7/81	-	85.11	133.78	123.56	22.14	28.00
C. quinoa PI 614938	Oruro, Bolivia	71.00	109.33	11.27	5.67	10.00
C. quinoa PI 478408	La Paz, Bolivia	71.33	109.33	17.67	8.93	8.55
C. quinoa PI 478414	La Paz, Bolivia	83.66	134.11	78.98	21.53	20.55
C. quinoa PI 596498	Cuzco, Peru	83.77	129.00	65.87	20.82	17.33
C. quinoa Ames 13219	La Paz, Bolivia	81.99	129.98	53.96	11.75	19.21
C. quinoa Ames 13719	Neuva Mexico, USA	82.21	120.28	115.52	25.03	27.74
C. quinoa PI 587173	Jujuy, Argentina	85.33	125.78	101.03	30.91	16.74
C. quinoa PI 510532	Peru	86.67	157.11	144.03	22.02	25.55
C. quinoa PI 614883	Jujuy, Argentina	70.78	109.89	54.89	12.33	21.89
C. quinoa PI 584524	Chile	81.33	127.00	115.89	29.64	25.00
C. quinoa Ames 22156	Chile	80.55	126.00	106.44	26.16	20.44
C. quinoa Ames 13762	Nueva Mexico, USA	79.33	132.44	123.72	5.00	23.00
C. quinoa PI 614881	Jujuy, Argentina	87.11	127.22	113.00	25.00	24.56
C. quinoa PI 510537	Peru	84.33	124.00	100.00	14.39	25.44
C. quinoa PI 510547	Peru	82.11	131.78	66.67	16.02	14.11
C. quinoa Ames 22158	Chile	80.89	131.11	80.27	23.25	21.24
C. quinoa PI 510536	Peru	73.78	115.22	31.05	4.42	17.53
C. quinoa PI 478410	La Paz, Bolivia	82.77	126.78	101.10	17.29	22.61
C. quinoa PI 433232	Chile	81.00	130.00	108.66	23.01	20.89
C. quinoa Ames 21909	Oruro, Bolivia	82.55	152.44	82.44	25.87	21.00
C. berlandieri subsp. *nuttalliae* PI 568155	Mexico	91.33	163.33	139.44	21.44	35.74
C. berlandieri subsp. *nuttalliae* PI 568156	Mexico	85.33	152.33	135.44	13.53	29.11
Medio ±S.E.		81.76 ±1.18	129.51 ±2.51	83.76 ±6.79	18.15 ±1.44	20.62 ±1.08
CD (5%)		2.41	5.14	13.90	2.94	2.21
CD (1%)		3.26	6.93	18.76	3.97	2.98
CV		7.82	10.44	43.67	42.75	28.32

Germplasm lines	Origin	Inflorescence length (cm)	Ilnflore-cence/ plant	Seed size (mm)	1000 seed weight (g)	Dry weight/ plant (g)	Harvest index	Seed yield (t/ha)
C. quinoa CHEN 58/77	-	2.93	41.19	1.58	1.81	6.31	1.07	2.11
C. quinoa CHEN 67/78	Puno, Peru	1.71	91.63	1.34	0.78	5.75	0.74	3.75
C. quinoa CHEN 71/78	Bolivia	3.39	127.73	1.97	2.85	7.21	1.43	3.27
C. quinoa CHEN 33/84	-	2.42	13.85	1.57	2.07	3.84	1.40	1.33
C. quinoa CHEN 84/79	Cuzco, Peru	1.00	117.78	2.21	3.57	10.47	1.32	3.44
C. quinoa CHEN 92/91	Columbia	2.25	64.11	2.01	3.70	10.21	0.88	2.25
C. quinoa CHEN 7/81	-	4.09	141.55	2.09	3.65	28.00	1.41	9.83
C. quinoa PI 614938	Oruro, Bolivia	1.07	11.67	1.73	1.87	1.11	1.06	0.32
C. quinoa PI 478408	La Paz, Bolivia	0.84	14.65	2.17	2.87	1.26	1.19	0.47
C. quinoa PI 478414	La Paz, Bolivia	1.60	106.48	1.81	3.03	14.00	1.25	6.07
C. quinoa PI 596498	Cuzco, Peru	2.47	90.33	2.03	3.08	19.89	0.79	3.93
C. quinoa Ames 13219	La Paz, Bolivia	2.64	114.66	2.06	3.54	15.08	0.73	2.80
C. quinoa Ames 13719	Neuva Mexico, USA	2.67	98.00	2.15	3.65	32.03	0.99	9.33
C. quinoa PI 587173	Jujuy, Argentina	2.25	68.50	2.01	4.09	15.47	0.81	3.17
C. quinoa PI 510532	Peru	2.24	138.22	1.51	1.25	52.89	0.29	1.68
C. quinoa PI 614883	Jujuy, Argentina	3.61	45.89	1.73	1.77	3.03	0.97	1.00
C. quinoa PI 584524	Chile	2.51	137.55	1.58	3.02	29.86	0.90	6.60
C. quinoa Ames 22156	Chile	1.60	85.55	1.93	3.51	17.21	1.21	5.03
C. quinoa Ames 13762	Nueva Mexico, USA	4.31	136.44	1.83	2.75	35.21	0.94	8.50
C. quinoa PI 614881	Jujuy, Argentina	3.01	114.22	2.05	2.94	24.16	1.34	8.25
C. quinoa PI 510537	Peru	1.44	136.00	1.78	2.71	13.02	1.32	4.39
C. quinoa PI 510547	Peru	2.08	68.92	1.82	3.13	12.67	1.33	4.70
C. quinoa Ames 22158	Chile	3.85	40.29	1.95	3.17	12.70	1.18	4.85
C. quinoa PI 510536	Peru	1.79	21.03	1.93	2.34	1.38	1.28	0.67
C. quinoa PI 478410	La Paz, Bolivia	0.90	118.33	1.80	2.63	29.00	0.43	3.13
C. quinoa PI 433232	Chile	4.54	74.22	1.77	2.28	13.11	1.09	3.56
C. quinoa Ames 21909	Oruro, Bolivia	2.12	132.22	1.83	3.31	15.97	1.15	9.08
C. berlandieri subsp. *nuttalliae* PI 568155	Mexico	6.47	114.78	1.58	1.28	28.94	0.26	2.01
C. berlandieri subsp. *nuttalliae* PI 568156	Mexico	4.77	103.39	1.65	1.37	15.05	0.65	2.32
Mean ±S.E.		2.64 ±0.24	88.59 ±7.81	1.84 ±0.03	2.69 ±0.15	16.37 ±2.24	1.01 ±0.06	4.06 ±0.52
CD (5%)		0.49	15.99	0.06	0.30	4.58	0.12	1.06
CD (1%)		0.66	21.57	0.08	0.41	6.18	0.17	1.43
CV		49.62	47.48	11.41	31.97	73.85	32.16	68.34

Table 5. Mean performance of 29 lines for 4 quality traits in *Chenopodium*
(Reprinted from Bhargava *et al.* 2007, with kind permission from Elsevier)

Germplasm lines	Origin	Total chlorophyll (mg/g)	Leaf carotenoid (mg/kg)	Seed carotenoid (mg/kg)	Seed protein (%)
C. quinoa CHEN 58/77	-	1.03	389.83	1.73	13.22
C. quinoa CHEN 67/78	Puno, Peru	1.70	531.03	3.12	21.02
C. quinoa CHEN 71/78	Bolivia	1.82	534.80	3.15	19.37
C. quinoa CHEN 33/84	-	0.55	230.23	1.69	16.92
C. quinoa CHEN 84/79	Cuzco, Peru	1.12	414.73	2.30	18.84
C. quinoa CHEN 92/91	Columbia	1.68	521.83	2.00	13.93
C. quinoa CHEN 7/81	-	1.92	632.40	3.30	17.31
C. quinoa PI 614938	Oruro, Bolivia	1.16	338.23	2.84	17.83
C. quinoa PI 478408	La Paz, Bolivia	1.19	330.03	2.74	15.23
C. quinoa PI 478414	La Paz, Bolivia	1.86	588.23	3.88	17.86
C. quinoa PI 596498	Cuzco, Peru	1.65	551.07	2.68	15.09
C. quinoa Ames 13219	La Paz, Bolivia	1.32	421.03	2.02	12.55
C. quinoa Ames 13719	Nueva Mexico, USA	1.36	466.13	1.75	17.71
C. quinoa PI 587173	Jujuy, Argentina	1.85	580.43	3.86	14.66
C. quinoa PI 510532	Peru	1.34	483.13	2.06	14.51
C. quinoa PI 614883	Jujuy, Argentina	1.25	434.67	3.15	19.48
C. quinoa PI 584524	Chile	2.04	669.56	2.87	13.01
C. quinoa Ames 22156	Chile	1.86	611.83	2.81	14.24
C. quinoa Ames 13762	Nueva Mexico, USA	1.60	519.90	2.08	15.47
C. quinoa PI 614881	Jujuy, Argentina	1.42	481.23	3.33	13.89
C. quinoa PI 510537	Peru	1.59	511.77	3.82	19.78
C. quinoa PI 510547	Peru	1.22	416.30	2.35	20.43
C. quinoa Ames 22158	Chile	1.06	414.63	2.40	16.09
C. quinoa PI 510536	Peru	1.09	371.80	2.84	20.39
C. quinoa PI 478410	La Paz, Bolivia	1.43	480.07	1.97	13.08
C. quinoa PI 433232	Chile	1.51	479.47	2.13	14.23
C. quinoa Ames 21909	Oruro, Bolivia	1.55	504.07	3.15	16.20
C. berlandieri subsp. *nuttalliae* PI 568155	Mexico	1.17	601.90	5.52	13.28
C. berlandieri subsp. *nuttalliae* PI 568156	Mexico	1.20	528.50	4.73	14.82
Mean ±S.E.		1.43 ±0.06	484.09 ±18.37	2.83 ±0.16	16.22 ±0.47
CD (5%)		0.12	37.62	0.32	0.96
CD (1%)		0.16	50.75	0.44	1.29
CV		23.07	20.42	31.80	15.90

Figure 1. Map showing countries of the Indian subcontinent and sites of field trials of quinoa.

small offshoots planted in March. Its growth was phenomenal and, despite the severe summer, the crop yielded well. The time-period from saplings to maturity was about 150 days (Deccan Chronicle, 2013). The seed, supplied by the Union Ministry of Agriculture to the AMR-APARD (AMR-Andhra Pradesh Academy of Rural Development), was grown in demonstration plots and was revealed to be a valid alternative to groundnut – a crop with deteriorating cultivation in the district due to the progressive decrease in rainfall. The AMR- APARD, located in Hyderabad, Andhra Pradesh, India, has focused for over 54 years on building capacity for sustainable development of the rural poor. The prospects of quinoa in southern India are being explored in other areas as well. A number of private companies are planning to extensively cultivate quinoa in Tamil Nadu, Gujarat and Rajasthan in farmers' fields (personal communication). The Humana People to People India is planning to introduce quinoa in central Uttar Pradesh to benefit marginal farmers and the crop's performance will be assessed.

Uses and Markets

The demand for quinoa is increasing in many parts of India and it is being imported at high prices. In Andhra Pradesh, quinoa is sold at a price of nearly INR1 500/kg. "Organic Quinoa", based in Bangalore, is marketing quinoa at INR595 per 500 g (INR = Indian rupee). Experimental trials have been successful, with good yields which could give great returns for the local farmers. Also in Pakistan, demand for this "magic" crop is growing, but availability is less due to high cost. If cultivation becomes more widespread, the cost of quinoa can be massively reduced, making it available for the common man. Moreover, marginal farmers can also export quinoa to other countries where demand for the grain is high.

Dissemination of quinoa in southern Asia

The availability of information is a major constraint in the promotion of underutilized species (Padulosi *et al*., 2002). Factors hampering the development of underutilized crops include lack of knowledge (both of quality traits genetics and of agronomy), lack of interest of farmers afraid of the risks of cultivation, absence of a market, lack of experience and inadequate financial resources (Polok *et al*., 2008). In southern Asia, farmers tend to be less enthusiastic about new crops and show interest only when high returns are guaranteed. Many farmers practise subsistence agriculture, growing cereal crops for personal use only. To increase the popularity of quinoa in the region, priority must be given to the following:

(i) Initiation of participatory research in all aspects of the crop, most importantly crop stability and selection of genotypes suited to different agroclimatic conditions.

(ii) Invoking the interest of farmers by disseminating information to producers regarding the benefits of the crop in terms of income generation and nutritional security.

(iii) Dissemination of detailed information to farmers regarding cultivation practices, agronomy and pathology of the crop.

(iv) Sharing information about quinoa cultivation, agronomic requirements, local uses and values, and its potential contribution to local food security and environmental sustainability.

(v) Providing free or subsidized high-quality seeds to farmers in the early years to relieve them of the burden of arranging germplasm best suited to local conditions.

(vi) Providing a marketing infrastructure where the produce is collected directly from the farmers' fields, especially in the initial period until a proper mechanism is in place. Government agencies can play a major role, setting up strategic alliances with agencies or organizations with experience in quinoa marketing, processing and product development. Improved commercialization creates better opportunities for income generation by marginal farmers who can hugely benefit from cultivating this crop.

(vii) Inclusion of quinoa in crop insurance schemes which exist in India for selected crops. This would instil confidence in producers and make them consider quinoa cultivation as less risky.

(viii) Improving public awareness and raising interest in quinoa to create a favourable environment for its sustained production and use. This entails a coordinated effort by governments, research institutions, the private sector and consumers, as both the public and the producers should be aware of the benefits that arise from wider use of this crop.

Conclusion

Quinoa is highly adaptive under marginal agro-ecological and edaphic situations, and can thus enhance the food and nutritional security of local communities and improve income in southern Asia. The crop has great potential to alleviate hunger and malnutrition in the Indian subcontinent by increasing food production in challenging environments where major crops are severely limited. However, this could be achieved by an integrated effort at all levels: information, awareness, popularization, research and marketing.

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CHAPTER 6.3.1

Assessment and adaptation of quinoa (*Chenopodium quinoa* Willd) to the agroclimatic conditions in Mali, West Africa: an example of South-North-South cooperation

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Abstract

Quinoa's adaptation was tested in Mali, West Africa, where the difficult agroclimatic conditions are similar to those in central northern Chile. The traditional varieties used were predominantly from Chile ('A64', 'BO25', 'BO78', 'PRP', 'PRJ', 'UDeC9', 'R49', 'VI-1', 'Regalona', 'Mix'), plus two crop cultivars from Argentina ('Roja Tastina' and 'Sajama') and one varietyfrom Bolivia. Trials began in 2007 and continue today. They tested sowing in the rainy season (June–Oct.) and in the dry season (Nov.–Mar.). Pests, diseases and yields were assessed, taking into account also the grain storage conditions and more sustainable soil management (compost). Some Altiplano cultivars were recalcitrant ('A64', 'R49' and 'MIX'), while the traditional varieties from central southern Chile gave satisfactory yields (1–2 tonnes/ha). Ideally, seeds should be sown each season to avoid a reduction in germination vigour which is caused by the ambient humidity and high temperatures characteristic of *in situ* storage in tropical zones. The crop cycle is 90–100 days for the accessions from Chile and up to 108–119 days for the accessions from Argentina. The panicles can be attacked by fungal diseases that reduce productivity in the rainy season. The presence of phytophagous insects (*Bemisia*, *Aphis* and *Aspavia* genera) was observed, as well as Coccinellidae, which are their natural predators in biological control. Quinoa has the potential to improve the supply of high quality protein in Africa. Pests in the rainy season and insect infestation can be controlled by adopting ecological management practices, using saponins from the same quinoa varieties. The limiting factor is the energy requirement for using water (not readily available in the dry season) and for mechanized threshing. The population's use and acceptance of quinoa can be expected to be high, on the basis of past experience introducing other crops from America (potato, maize and tomato) to this continent and given the culinary similarity with millet and rice.

Context and problems of introducing quinoa to this part of the world

Africa is a region characterized by serious nutritional problems. In most African countries, over 20% of the population is malnourished and infant mortality exceeds 75% for children under five. In 2012, across the Sahel, an estimated 1.1 million children under the age of five were at risk from severe acute malnutrition. Therefore, in April, UNICEF launched SahelNOW, a campaign to raise global awareness of the imminent crisis. For the first time in history, UNICEF's offices and national committees came together to join social networks that were used as the principal means of communication for advocacy and fundraising. The campaign mobilized Goodwill Ambassadors from UNICEF at national and global level to alert the world about the convergence of a series of conditions threatening the nutritional status of children in nine countries: Burkina Faso, Cameroon, Chad, the Gambia, Mali, Mauritania, the Niger, Nigeria and Senegal. SahelNOW boosted the conventional media coverage and was described as innovative by CNN. In 2012, UNICEF's national committees raised USD29.8 million to help provide treatment to save the lives of over 920 000 severely malnourished children under five (UNICEF, 2013). In addition, the chronic lack of rain in the entire sub-Saharan region has worsened according to studies conducted by the Intergovernmental Panel on Climate Change (Figure 1).

In this region, agriculture's main objective is food security for the population. Thus, subsistence farming is the principal activity in the region, although cotton, maize, peanut and other crops are sold on a regular basis to generate family income. In the case of Mali (West Africa) around 90% of the population depend on cereal production (predominantly sorghum, millet, maize and rice), yielding about 1 tonne/ha (Soumaré *et al.*, 2008). In addition to drought, the duration of the rainy season is very varied. Soils are very poor and some elements in the soil (Al, Fe) constitute a limiting factor (Gigou, 1987; Gigou *et al.*, 1998; Traoré *et al.*, 2004).

These difficult conditions generate tremendous fluctuations in annual production, while there is a need to secure production for the fast-growing population. In this context, agricultural diversification and soil improvement are useful tools for facing these challenges. Agro-ecological farming (Altieri, 1995) offers

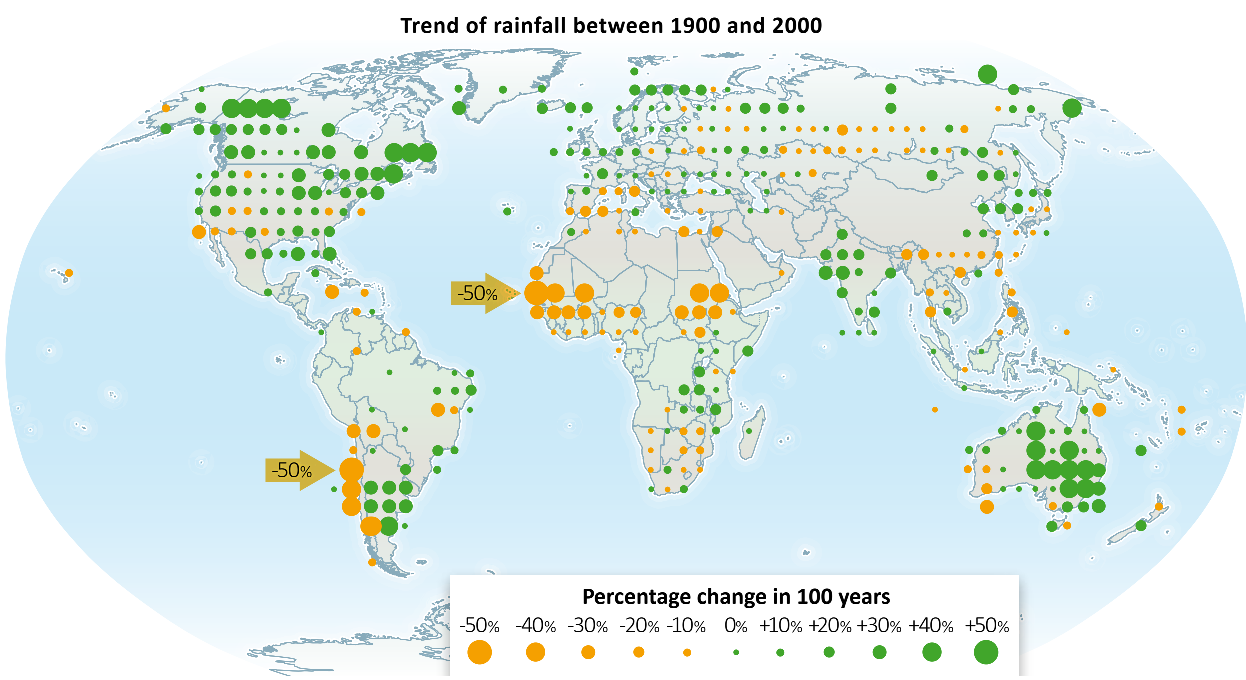

Figure 1. Map of the world's rainfall balance (deficit/excess in 100 years). The deficit of over 50% (arrows) in some parts of sub-Saharan Africa is comparable to central northern Chile (Coquimbo region). Source IPCC 2001.

a solution for populations whose limited financial resources are insufficient for other more technological alternatives, such as chemical fertilizers or genetic modification. Furthermore, technological solutions increase the effect of greenhouse gases and are unsustainable or beyond the economic reach of developing countries (Anon., 2010).

It is in this context that the potential of quinoa emerges. It is a highly nutritious plant, tolerant to various types of abiotic stress, capable of diversifying the crop production systems in many countries (Glass and Johnson, 1974; Jacobsen, 2003; Jacobsen *et al*., 2003) with problems of drought, food insecurity and poverty, such as Mali. For this reason, FAO declared 2013 the International Year of Quinoa (IYQ), just one year before 2014, the International Year of Family Farming.

Historically, farmers in Africa have always been open to experimenting with new varieties or crops in order to improve their living conditions (Chevassus-au-Louis and Bazile, 2008; Louafi *et al*., 2013). **Rural seed production systems** strengthen exchanges between farmers and support the capacity to introduce and test new agrobiodiversity (Bazile *et al.*, 2008; Coulibaly *et al.*, 2008). There is always the risk of losing some of the local biodiversity, and this should be carefully assessed before increasing production (Bazile, 2006).

Historical account of the areas of research and the disciplines involved during the period 2006–2013

In 2006, teams from Italy, Argentina, Chile and Mali studied the tolerance of Chilean quinoas to salt stress. While the main objective was to investigate the tolerance mechanisms to saline stress in *Chenopodium quinua* Willd., the same genes are also tolerant to water-related stress factors, such as drought and frost (see chapter 2.2). The Malian team conducted field assessments on the adaptability of registered and traditional varieties of quinoa and studied their tolerance to the soil and climatic conditions in Mali. The assignment began in 2007 after a researcher from the *Institut Polytechnique Rural* (IPR) in Mali spent 6 months in Chile (CEAZA) and Argentina (University of Buenos Aires and INTA) learning the basics about this crop from the southern Andes.

The trials in Mali involved behavioural tests on quinoa seeds from Chile and Argentina. Drawing on the huge geographic distance between the north (18°S) and the far south (40°S) of Chile, traditional crop varieties were used, adapted over thousands of years to different combinations of photoperiod, temperature and rainfall, generating tremendous crop genetic diversity (Fuentes *et al*., 2012). Agronomic trials were conducted on crop cycles in dry and rainy seasons, and seed storage tests were carried out. Both activities followed agro-ecological protocols in line with ecologically and economically sustainable farming. Parallel studies in Chile, Italy and Argentina were conducted to assess the genetic mechanisms and responses that high tolerance to different types of stress confers to some of the traditional varieties tested in Mali (Orsini *et al*., 2012; Ruiz-Carrasco *et al*., 2012).

Genetic resources used and their origin

By the end of 2007, at the end of Dr Coulibay's stay in South America, 12 accessions of quinoa, including 10 from Chile and 2 from Argentina (Table 1), were provided for the adaptation trials in Mali's Sudano-Sahelian zone in the IPR/IFRA's experimental plots in Katibougou (75 km northeast of Bamako). The trials were conducted in successive years, each year using the results and seeds obtained. For the rainy season trials, an unspecified commercial variety of Bolivian origin was included (purchased on the European market).

International collaboration

International collaboration began thanks to the Third World Academy of Sciences, which together with the International Centre for Genetic Engineering and Biotechnology launched an international call for projects. The participation of developed countries depended on active collaboration with developing countries. For this reason, the call for tender was answered by an institution from a developed country (University of Bologna, Italy) and two research centres from developing countries (Centro de Estudios Avanzados en Zonas Áridas, CEAZA, Chile, and the University of Buenos Aires, Argentina). In addition, Mali, West Africa, was invited to participate, thanks to a former collaboration between the University of Bologna and a professor and researcher from the *Institut Polytechnique Ru-*

Table 1. Passport data for the quinoa accessions being assessed in Mali.

Accession	Origin	Grade of selection	Seed bank
A64	North Alteplano (District of Colchane)	Seed color (Yellow)	University of Arturo Prat, Iquique
R49	North Alteplano (District of Colchane)	Seed color (Red)	University of Arturo Prat, Iquique
Mix	North Alteplano (District of Colchane)	No selection	University of Arturo Prat, Iquique
PRP	Central coast of Chile (Palmilla locality, District of Pichilemu)	No selection	CEAZA collections for INIA seed bank
PRJ	Central coast of Chile (District of Pichilemu) Central Chile (District of Pichilemu)	No selection	CEAZA collections for INIA seed bank
VI-1	Central coast of Chile (District of Chanco) Southern Chile (District of Cunco)	No selection	CEAZA collections for INIA seed bank Collection of Univ. of Concepción, Chillán
UdeC 9	Southern Chile (District of Collipulli)	No selection	AGROGEN bank, donated to INIA
BO25	Hybrid variety Altiplano variety (Bolivia/Argentina)	No selection	AGROGEN bank, donated to INIA BAER seeds
BO78	Altiplano variety (Bolivia/Argentina)	No selection	Univ. of Buenos Aires bank, Argentina
Regalona		Selection for best yield and grain size	Univ. of Buenos Aires bank, Argentina
Sajama		Low saponin content	
Roja Tastina		No selection	
Boliviana	Unknown	Unknown	European market

ral for Agricultural Research and Training (IPR/IFRA) in Katibougou.

Another project involving France (CIRAD, IRD, INRA), Mali (IER, ICRISAT) and Chile (CEAZA), funded by the French National Research Agency (ANR, 2008–2012), studied quinoa seed systems in Chile and compared them with sorghum and millet systems in Mali (Bazile *et al.*, 2011 and 2012).

Experiments conducted and the results

The experiments concentrated on four areas: (1) sowing in the dry season (Nov.-–Mar.); (2) sowing in the rainy season (June–Aug.), the period preferred by farmers because rain is more abundant; (3) seed germination and storage; and (4) use of different types of compost to improve soil quality. In all the studies, special attention was given to assessing the presence of diseases and insects that are potential crop pests or predators (biological control).

1. Assessments in the dry season

The Chilean and Argentinian varieties of quinoa (Table 1) were sown in experimental plots of 18 m2 on 15 November 2007, which corresponds to the dry season. Five or six seeds were sown in **pockets** spaced at two different densities (every 10 and 20 cm) with 50 cm between the rows, except for the Argentinian varieties, for which less material was available. These varieties were sown at the higher density only (every 10 cm). Irrigation was spaced at 15-day intervals (3/4 of field capacity) and every 10 days after flowering. The only fertilizer used was compost from cattle manure (at a rate of 8 tonnes/ha). In the second period of assessment in the dry season (2008/09), seeds from plants that produced panicles and grains in 2008 were used (Figure 2). This time, seeds were sown slightly later (5 and 15 December 2008) in the same conditions as for the first period, using seeds obtained from the harvest in the first assessment. The germination capacity was assessed for the seeds from the first harvest. Not all results were viable (see results section).

2. Assessments in the rainy season.

In the 2009 rainy season (June–Aug.), trials were conducted to verify the findings of preliminary trials carried out in the 2008/09 rainy season which saw the emergence of fungal diseases that **decimated** the panicles (Figure 3). The Chilean Altiplano ecotypes ('A64', 'R49', 'MIX') were not used because there were recalcitrant seeds (no germination) following harvest in the dry season in Mali. In addition to direct sowing in the field (a little over 0.3 ha), the seed germination capacity was tested in the laboratory (evaluated at 5 days, n = 50 seeds), using cotton and soil from the same experimental plots.

3. Studies of seed germination and storage.

The quinoa seeds that arrived in Mali for the first time were assessed for their capacity to germinate at successive stages, involving quality assessment in storage conditions at ambient temperature. Temperatures ranged between 21°C and 26°C, with extremes (outside ambient temperature) of 45°C in the dry season and maximum inside ambient temperatures around 10°C less. Maximum ambient humidity was quite high in all periods (> 50%), therefore the seed weight was assessed before and after 3 months of storage. Once the first seeds were produced in Mali, germination was assessed following 12 hours in damp cotton under laboratory

Figure 2. Quinoa plants (traditional variety 'Roja Tastina') near the time of harvest at the IPR/IFRA experimental station in Katibougou, Mali, in February 2008 (dry season). The water tanks that can be seen in the background provide gravity-fed irrigation.

Figure 3. Plant from the variety 'BO78' during the rainy season in 2008, with fungal damage (unidentified) on the panicle that caused the entire apical panicle to abort and the plant to become bushy (many branches).

conditions at 5, 11 and 12 months post-harvest and after 3 years.

Insect control involved an assessment of the entomofauna that developed in 100 g packs of quinoa seeds. Controls (n = 8 for each variety) were compared with treatments in which seeds of each variety were juxtaposed and combined with material bags containing 10 g of dry residue (whole) or 6 g of ground residue (meal) from a plant species that is a potential insect repellent. Two plant species were tested for this biopesticide potential: *Cassia nigricans* and *Hyptis spigicera.* Seeds from 8 of the 12 varieties ('Boliviana', 'PRP', 'PRJ', 'VI-1', 'UDEC9', 'Regalona', 'BO25' and 'BO78') were assessed (for germination and associated entomofauna) after 3 months.

4. Study of the responses to compost use.

The yields of the same eight quinoa varieties tested for grain resistance to insect attacks were tested for sowing in the dry season (2 December 2010 to March 2011). There were three soil fertilizer treatments: manure from cattle/sheep (composted) applied at 8 tonnes/ha and at 4 tonnes/ha (control), and compost from the same manure but modified by earthworms, applied at 8 tonnes/ha. The harvest took place on 12 March 2011. Yields were recorded and the presence of insects was determined for each traditional variety.

Results

1. Assessments in the dry season.

The 12 traditional varieties from central southern Chile and the seeds harvested in Argentina showed germination rates of between 73% ('PRP') and 97% ('Sajama'). The north Altiplano varieties from Chile ('A64', 'R49', 'MIX') were recalcitrant (no germination). Ambient temperatures in the first period ranged from a minimum of 8.7°C (January 2008) to a maximum of 36.6°C (February), while in the second period they ranged from 14°C (December 2008) to 39°C (March 2009). Relative humidity ranged from 21% (February 2009) to 82% (November 2008).

Grain yields for the 2007 sowings varied from less than 0.5 tonnes/ha ('Sajama' at the highest sowing density) to just over 2.5 tonnes/ha ('BO25' and 'UdC9' at the lowest density) (Figure 4). In general, the best yields were obtained with the lowest sowing densities. Six of the ten traditional Chilean varieties achieved yields of around 2 tonnes/ha or more. The best yields were observed for 'UdeC9', 'BO78', 'BO25', 'PRJ' and 'PRP', all of which are from central and southern Chile.

For yields in the second campaign (sown on 5 and 15 December 2008), no grains were produced by seeds from Argentinian harvests ('Sajama' and 'Roja Tastina'). In general, yields were higher at lower seed densities and varied between 0.5 and 1.5 tonnes/ha. For 15 December sowing, yields were 0 tonnes/ha for the Argentinian varieties and 0.5–1 tonne/ha for the other varieties. There were no significant differences between the two sowing densities. For 5 December sowing, temperatures were lower during flowering than for 15 December sowing, and yields were at least 0.5 tonnes/ha higher for early sowing.

2. Assessments in the rainy season.

Seed germination rates in these trials were slightly higher for the traditional varieties germinating in cotton (80–97% on day 2) than in soil from the same plot (60–80% on day 2). 'Sajama' and 'Roja Tastina' varieties failed to germinate and 'Boliviana' germinated at a rate of less than 5%. 'BO25' was the slowest to germinate, both in cotton (60% on day 5) and in soil (just over 50% on day 5). Fungal disease was observed on seeds on day 5: the highest level of contamination (> 70%) in 'UdeC9', 'Roja Tastina' and 'Sajama' from Argentina and the lowest (< 20%) in 'BO25' and 'BO78' from Chile's humid south. All the other varieties showed intermediate results. The type of fungal disease and whether or

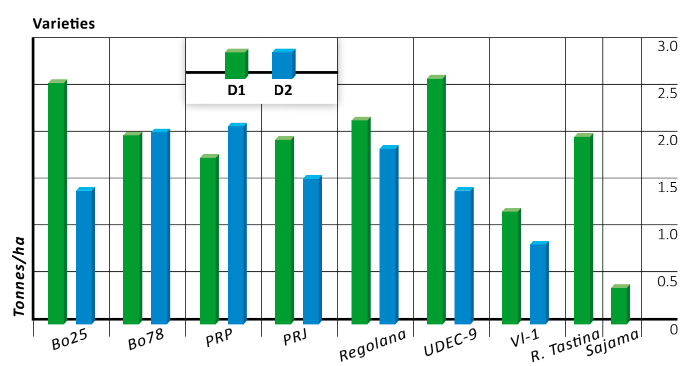

Figure 4. Quinoa grain yields (tonnes/ha) for sowing in 2007 at low (D1) and high (D2) density.

not it was already present on the seeds at the time of harvest is not known. Fungal diseases were recorded on day 5 of incubation.

In the field, emergence varied between 23.7% for 'BO25' and 51.27% for 'VI-1'. Panicles were also attacked by fungal disease (Figure 3). The variety 'BO25' had over 40 standing plants on 25 July 2009 and was least affected (16.7%). 'PRP' was the most affected, with fewer than 40 standing plants on the same date (54.5% of contamination). The other varieties ('BO25', 'PRJ', 'UdeC9') all had some degree of fungal contamination, and fewer than 15 plants survived the rainy season. The variety 'Boliviana' had just two surviving plants, while 'Roja Tastina' and 'Sajama' did not produce any plants.

The phtyophagous insects observed belonged to 30 species (13 unknown), 22 families and 7 different orders (Orthoptera, Homoptera, Heteroptera, Dermaptera, Diptera, Coleoptera and Hymenoptera), while the beneficial organisms (entomophagous organisms, predators) belonged to 10 families from 5 orders (Orthoptera, Heteroptera, Diptera, Coleoptera and Hymenoptera).

There are no available data on yields, given the high level of fungal infection observed in the two rainy seasons.

3. Studies on seed germination and storage.

In Mali, seed germination (assessed on day 5, observed at 5 months on 6 August 2008) was between 25% ('Roja Tastina') and 98% ('Sajama'). The majority had germination rates of about 65%. **In month 11 (25 January 2009), germination dropped below 70% for the varieties 'VI-1', 'Roja Tastina', 'Sajama', 'R49' (<5%) and 'Boliviana'. All the other varieties had values of over 70%, almost 100%** ('BO25', 'PRJ'). In month 12 (4 February 2009), 'Roja Tastina' had the lowest germination rate (30%). In the other varieties, 'BO78', 'PRP', 'PRJ', 'VI-1' and 'UdeC9s', germination ranged from 80% ('BO25') to 98% ('Regalona'). Seeds stored for 3 years had much lower germination rates – between 2% ('R49') and 12% ('PRJ', 'UdeC9' and 'BO25').

In the assessment of grain storage and the presence of insects, observations from the first grain inspection detected (using 10 g dry matter of *Cassia nigricans*) only two Coleoptera on eight varieties, and on the seeds only of 'Regalona'. With 10 g dry matter of *Hyptis spigicera,* Lepidoptera also appeared, although only on seeds of 'Regalona'. With 6 g of ground *Cassia nigricans,* one Coleoptera appeared again on 'Regalona' seeds and 15 Lepidoptera on 'Boliviana' seeds. With 6 g of ground *Hyptis spigicera,* only three Coleoptera and a single Lepidoptera appeared on 'Regalona'. The results were the same for the dried residues (with or without grinding) of both plant species.

Germination after 120 hours is approximately 90% for all traditional varieties. Fungal infections appeared on all varieties, with the lowest incidence (20% of seeds) on 'BO25' and the highest on 'VI-1' and 'UdeC9' (> 50% of seeds). After 3 months, the emergence of rootlets and cotyledons (indicators of seed germination vigour) decreased by at least 50% compared with initial observations (for the treatments with both dried plants). This could be related to the biochemical reactions resulting from the absorption of ambient humidity, since the weight of seeds increased by up to 3% after 3 months of storage at ambient temperature, particularly for the Altiplano varieties.

4. Study of the responses to compost use.

The best yields were obtained with half the dose of compost made from sheep/cattle manure. Application of worm compost to the eight varieties produced yields of between 0.8 tonnes/ha ('UdeC9') and 4.5 tonnes/ha ('BO25'), and an average yield of 2.6 tonnes/ha. For compost applied at a rate of 8 tonnes/ha, yields varied between 1.8 tonnes/ha ('Regalona') and 4.9 tonnes/ha ('PRJ'), with an average of approximately 3 tonnes/ha. When the dose was halved, yields were even higher, ranging from 2.2 tonnes/ha ('BO78') to 5.7 tonnes/ha ('BO25' and 'PRP') with an average of slightly over 3 tonnes/ha.

Compost applications also affected the insect burden on plants. For example, with vermicompost, thrip infestation did not exceed 150 insects/plant. In contrast, an 8 tonne/ha dose of normal compost resulted in over 600 thrips per plant, while halving the dose resulted in only 25 thrips/plant in the same period. Similar patterns were observed for other insects. For some varieties, insect abundance increased fourfold at certain times ('PRP' at the outset).

Current situation and outlook for the dissemination of the crop in the country in 5–10–20 years

At the end of the first campaign (2007–08), nine varieties seemed able to adapt to the dry season with an average production of 1–5 tonnes/ha, i.e. greater than farmers' yields for the traditional cereals (millet and sorghum) widely used in Africa. However, the trials were conducted on a small scale and at an experimental station with controlled parameters. Now, after 4 years of trials, there are enough frozen seeds to sow over 200 ha. A possible objective is to test quinoa in salinized soils where it is difficult to grow rice (the rice-growing territories of the Niger River, Lake Sélingué, the perimeter of Baguinéda, Dioro and Diré). During the next 5 years, the Malian people need to be better informed about quinoa. In 2008, trials took place to understand how well quinoa was accepted as a prepared food. Reception was good, grains were washed by hand to remove saponins and new recipes were created (Figure 5). In the next 10–20 years, quinoa could spread to the Kidal region.

It is important to remember that the strength of the organization in Mali does not depend only on the national authorities. The village chiefs and councils of elders are in a position to make decisions that the farmers are quick to follow. In addition, the national association of professional farmers' organizations (*Association des Organisations Professionnelles Paysannes*) is an important platform for diffusing innovations and an effective interface between research and farmers. For example, between 2005 and 2008, the NGO Helvetas persuaded village chiefs that organic cotton had a profitable future on the international market. In the 3-year period, Mali went from having just over 100 small-scale cotton producers (1 ha) to 6 000, with only a score of professional extension agents for the crop. Unfortunately, the international organic cotton market dropped its prices (for Turkey's entry into the market) and production was not very profitable. However, the experience demonstrated the Malian producers' versatility and capacity for rapid change and, moreover, it encouraged them to go back to their traditional agro-ecological practices, somewhat forgotten by the advent of the green revolution and agrochemicals. Thus, quinoa represents a potential solution for improving the prospects of maintaining ecological farming. From the outset, dissemination policies should raise public

Figure 5. Ms Salimata shows us a dish of quinoa prepared with vegetables and cheese, steamed in a cloth hung from a tree. The seeds come from the first experimental harvests in Katibougou (Mali, February 2008). The saponins were washed by hand. This demonstrates how well quinoa has been accepted for culinary use in Mali.

awareness about the product because it is highly nutritious, is tolerant to diverse abiotic stresses and provides an alternative for rotation (no risk of losing crop diversity).

When contemplating potential areas for quinoa cultivation in Mali, the fields near the Niger River should be taken into consideration: the groundwater is close to the surface and could be used to irrigate deep-rooting plants. Quinoa's poor performance during the rainy season confirms its potential for sowing out of season, allowing for further agricultural production in the cereal-growing zone. Good soil fertility is not a limiting factor when considering two annual crops in plots that would produce more food without overlapping with local species and varieties. To date, all the trials have been restricted to the IPR/IFRA experimental plots.

Nevertheless, cutting-edge research is required in, for example, phytopathogenic fungal attacks and insect attacks during seed storage, so that sowing times can be adjusted accordingly.

Access to water in the dry season is likely to be a limiting factor for research on quinoa. It is important to consider the energy required to obtain water, for example, by pumping from wells or from the Niger River. Energy is also needed to install threshing machines for dry grains, which would also allow use of saponins for pest and disease control in quinoa and other crops. Once there is access to water, it is important to avoid erosion through good soil management, for example by using compost and less tillage. This study clearly shows that yields improve considerably with the use of compost. All these considerations are more urgent and effective than, for example, improvement through genetic engineering, which is still very expensive. Lastly, it is important to monitor what is happening in India, where the 700 million small subsistence farmers, predominantly vegetarians, who desperately need to increase the sources of high quality protein, have already started to adapt varieties of quinoa (Bhargava, 2006).

Uses and Markets

The small quantities of quinoa harvested have already been used as an innovative food product in Mali (Figure 5). The organic cotton experience shows that it is not advisable to launch an agricultural product for export, with the sole objective of making money, in part because prices are frequently volatile, which can cause local tragedies. In Mali, millet and sorghum are produced as staple foods. Long before quinoa is a market item, it should be a high quality complementary food, as widely available as possible for malnourished people and children who do not have regular access to sources of animal protein.

Conclusion

In conclusion, quinoa is a crop that could be adapted to sub-Sahelian countries like Mali or other countries from both hemispheres, situated between 15°N and 15°S, with a climate characterized by contrasting dry and rainy seasons. Nevertheless, during torrential rain there is a high risk of losing seeds during the early stages of germination if the rainfall causes considerable soil erosion. Other losses can be due to fungal or insect attack (at both seed and plant stages). Traditional varieties should be selected in order to address these risks, and to have adaptation to the photoperiod and high temperatures during flowering. It is interesting to note that the varieties from southern Chile produced good yields during the dry season, particularly when organic compost was added to the soil. The seeds from these varieties demonstrated a high tolerance to ambient humidity when stored between sowing dates.

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CHAPTER 6.3.2

Production and utilization of quinoa (*Chenopodium quinoa* Willd.) outside its traditional growing areas: a case of Kenya

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Abstract

Quinoa (*Chenopdium quinoa* Willd.) has been cultivated for thousands of years in the Andean region. It has earned itself the title "super food" because of its high content of minerals, protein (16%) and vitamins. It is capable of growing in diverse environmental conditions, including saline, frost-prone, low altitude and arid zones. Demand for the crop is, therefore, increasing as more people learn about it. Quinoa production needs to spread to other areas, especially in Africa where it can act as a food security crop, increase crop diversity in agro-ecosystems and be part of the strategy to deal with climate change, and where its production labour demands can be met. Records are not clear as to when the crop was introduced in Kenya and other parts of Africa. As early as 1942, Elmer makes reference to the crop being grown at 2 000–2 400 m asl, but the source cannot be verified. The Food and Agriculture Organization of the United Nations (FAO) mentions its introduction in 1985, while Jacobsen (2003) cites 2001. The crop was not introduced systematically in Kenya, so detailed follow-up trials have not been possible. However, research was done on quinoa in Kenya in 1999–2000 by Oyoo, University of Nairobi, who evaluated some 24 quinoa genotypes sourced from Peru, Bolivia, the Netherlands, Denmark, Ecuador, Chile, Colombia, Argentina and Brazil through the International Potato Center (CIP), Nairobi. Bojanic (2011) mentions quinoa trials and quotes Jacobsen. Oyoo's work remains perhaps the most comprehensive on yield and yield-related traits, following performance trials of quinoa in Kenya – over a period of two crop seasons he identified five superior genotypes for direct released to farmers or for use in future breeding work. More recently, in 2006, he conducted further research to evaluate the incorporation of quinoa as green manure into bean cropping systems to control root-knot nematode. The results showed that quinoa has an intermediate effect in suppressing root-knot nematodes when used as an interplant. While the results are promising, little is known about quinoa outside the research environment. There is, therefore, a need for awareness-raising on the benefits of quinoa and its potential outside its production areas, and for coordinated research in various fields of quinoa physiology, breeding and agronomy to stimulate its spread and adoption.

Key words: Quinoa (*Chenopdium quinoa* Willd.), introduced in Kenya, research, superior genotypes, control, root-knot nematodes.

Introduction

On 2 July 2011, the 37th FAO Conference adopted Resolution 15/2011 and declared 2013 the "International Year of Quinoa", in support of the Government of Bolivia's proposal. The Resolution was adopted on the basis of the crop's exceptional nutritional qualities, its adaptability to various growing conditions and its potentially significant contribution to the fight against hunger and malnutrition. The Resolution was then forwarded to the Secretary-General of the United Nations for adoption (Bojanic, 2011).

The earliest records of quinoa (*Chenopodium quinoa* Willd.) cultivation date back to 5000 B.C. in Ayacucho, Peru, and to 3000 B.C. in Chinchorro, Chile, where it was grown by the Incas and Araucanian Indians in Argentina and Chile, and by the Chibcha Indians of Colombia. The crop is thought to have been domesticated around Lake Tititcaca (Fleming and Galwey, 1995; Jacobsen and Mujica, 2002), and is, therefore, of Andean origin. The crop was initially distributed during the expansion of the Inca Empire, reaching Bolivia, Ecuador, Peru, Argentina, Chile and Colombia (Jacobsen and Mujica, 2002). The Incas called it the "Mother grain" and it was second in importance only to potato in terms of cultivation. However, following the Spanish conquest, quinoa declined in importance and was replaced by other grains (Fleming and Galwey, 1995). Today, the crop is widely grown in many countries in the world, such as the United Kingdom, Canada, Denmark and the Netherlands (Bertero *et al*., 2004).

Interest in quinoa has grown significantly because of the many advantages it exhibits compared with other crops. It has excellent adaptability and can produce in unfavourable soil and climatic conditions and it has high nutritional value, including high protein content and low gluten content, and is rich in vitamins and minerals (it has even been said to be the most nutritious grain in the world) (Fleming and Galwey, 1995; Jacobsen and Mujica, 2002; Jacobsen, 2003; Bertero *et al*., 2004; El Hafid *et al*., 2005). Despite its good performance from sea level to over 4 000 m asl and in drought prone regions, quinoa's drought tolerance is not absolute. The crop needs some water applications during water-sensitive stages of growth and irrigation can significantly increase yields (Bosque Sanchez *et al.*, 2003; Garcia, 2003). It is also known to perform well under saline conditions (Eisa *et al*., 2012).

Many people and organizations feel that with the increasing world population, more attention should be paid to major world cereals in order to feed people. This may be the case for productive areas, but not for marginal and degraded ones where minor cereals and pseudocereals are more adaptive and higher-yielding (Williams, 1995). Quinoa is suited to such areas, especially in consideration of the situation of climate change faced by the world. The effects of climate change are well known and include: increased temperatures, high altitude and latitude areas more prone to warming, prolonged drought in arid and semi-arid regions, increased flooding, extreme weather events and rising sea level. Overall, the climate is changing faster than species are able to adapt. Conscious efforts are therefore necessary for life on earth to be sustainable.

Spread of Quinoa outside South America

While maize and potato spread from South America and achieved cosmopolitan distribution, quinoa did not spread beyond its centre of diversity. After the Spanish conquest, there was a marked decline in the cultivation and use of quinoa in Latin America following the introduction of barley and wheat; indeed, the crop became little known in large cities and was regarded as a low status native food crop of little interest. The Spanish conquerors also discouraged its cultivation because of its religious status in the Inca society (Cusack, 1984). Nevertheless, the crop continued to be grown by the indigenous populations in the mountain regions, where it has remained the most important grain crop because of its tolerance to drought, cold and poor soils (Rae *et al*., 1979 cited by Jacobsen and Stølen, 1993).

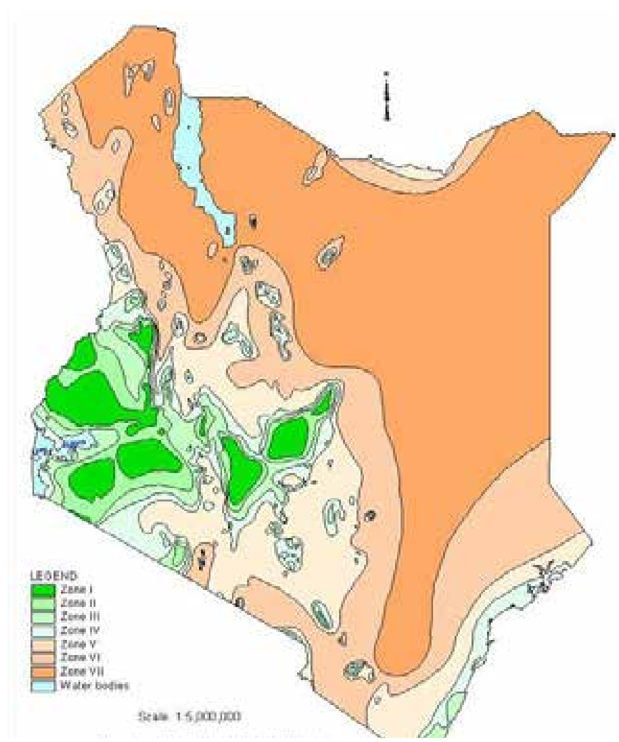
The crop entered the European Union in the 1970s when it was introduced in the United Kingdom before spreading to other countries. There are also records documenting that its cultivation in the region dates back further. Quinoa is used for health food products and as a game-cover crop, alone or mixed with kales (Jacobsen, 2003). The crop has spread to other continents – Asia, North America and Africa – where it has shown that it can perform well (Williams, 1995; Bojanic, 2011; Jacobsen, 2003).

Quinoa in the Context of Food Security and Climate Change in Kenya

Maps 1–3 show the position of Kenya in Africa, the agro-ecological zones the country is divided into, and the major towns. Taxonomic characteristics of the vegetation cover have been used to classify the country into ecological land units – called eco-climatic vegeto-ecological or agro-ecological zones – where climate, soil and topography have been isolated, combined and equated with their vegetation types to produce six ecoclimatic zones (Map 2): Zone I, Afro-Alpine moorland and grassland, found at high altitudes above the forest line; Zone II, humid to dry sub-humid climate; Zone III, dry sub-humid to semi-arid climate; Zone IV, semi-arid climate; Zone V, arid climate; and Zone VI, very arid climate. Zones IV to VI account for 72% of Kenya's total land area and are usually referred to as the arid and semi-arid lands (ASALs) of Kenya (Ojany and Ogendo, 1988). Rainfall in the ASALs is sparse and highly variable and therefore crop production is not assured. The major economic activities in these areas are linked to livestock production, but as lifestyles change in the face of climatic variability, livestock production is not safe and the local communities have recently been taking up cropping activities, sometimes supplementing rainfall with irrigation. These initiatives are the result of their inability to re-stock following prolonged droughts that wiped out entire herds. To ensure food security, cereal production by formally pastoral communities is slowly being adopted.

Agriculture is done mainly on a small scale on farms averaging 0.2–3 ha, in areas of high potential and mostly on a commercial basis. This small-scale production accounts for 75% of the total agricultural output and 70% of marketed agricultural produce. Over 70% of maize, 65% of coffee, 50% of tea, 80% of milk, 85% of fish and 70% of beef and related products are produced by small-scale farmers. In the rangelands, the small-scale livestock production system features mainly pastoralists (GoK, 2010). Smallholder farmers face great challenges as most

Map 1. Countries of Africa



Map 2. Kenya, agro-ecological zones

Map 3. Major towns of Kenya

of them are resource poor.

It is estimated that half of Kenya's population of 38.5 million is poor, with about 7.5 million people living in extreme poverty and more than 10 million people suffering from chronic food insecurity and poor nutrition (GoK, 2011). The causes of poverty are beyond the scope of this paper. Food security is one of the greatest problems in the country and its causes are numerous: lack of coherent food policy or strategy; low crop productivity; climate change and dependency on rainfed agriculture; uncoordinated marketing; need for expensive, inaccessible, low-quality yield-enhancing inputs (fertilizers and seed); land degradation; and limited value addition opportunities (Nyoro, 2011). Statistics in recent years show that at any one time, food assistance is required by about 2 million people, and this number may even double during droughts, heavy rains and/or floods (GoK, 2011).

The effect of climate change on low-resource farmers is immense. For these farmers, adaptation is the only way out, and can be achieved by developing mechanisms that enable them to cope with the changes, since they are not in a position to stop the causes of climate change (Khanal, 2009). For a country like Kenya, with a large population with limited coping strategies, crop choices that enable them to survive the harsh conditions are desirable. Plant genetic diversity can be crucial for breeding food crops and is one of the central preconditions for food security in resource-constrained smallholder systems where soils are poor.

To ensure quality food, a variety of crops is needed to guarantee provision of all nutrients. Therefore, in the search for crop diversity, a crop like quinoa – that not only does well in dry areas but also supplies quality nutrients – is needed. Quinoa has the ability to shield people from the vagaries of climate change.

History of Quinoa Research in Kenya

The National Gene Bank of Kenya (NGBK) conserves about 49 000 plant accessions, and only 4 000 samples have been distributed over a period of 15 years. They comprise at least 290 plant species distributed to at least 150 users within and outside the country (Mutegi *et al*., 2005). While some research was done on quinoa in 1935–39 and 1999–2000, no seeds were sent for conservation to the NGBK by the participating institutions (the colonial research institution and the University of Nairobi).

The early history of quinoa research in Kenya is documented by Elmer (1942) and is summarized herein. In 1935, cream-coloured seeds were obtained from the Royal Botanic Gardens and were planted in Kitale (Zone II) and Kapenguria (Zone IV), about 40 km north of Kitale (Map 3), in northwest Kenya at altitudes of 1 828 and 2 134 m asl, respectively. The seeds failed in Kitale but gave some yield in Kapenguria (2.3 kg from 100 seeds). It was recommended that planting be done at the end of the rainy season (July to Aug.) for harvest during the dry season (Nov. to Dec.). In 1939, seeds from Kapenguria were planted at the Scott Agricultural Laboratories (now National Agricultural Research Laboratories) outside Nairobi (Zone II) at 1 737 m asl. Rainfall was low (279 mm), but quinoa was the only crop in the area that gave some yield. In Kiambu (Zone II) in the uplands of central Kenya close to Nairobi, at 2 438 m asl, a yield of 0.85 tonnes/ha was obtained from poor soils. The results of the three experiments indicate that quinoa does not need fertile soils (which only make it grow tall and unproductive), grows in areas > 1 829 m asl, needs 381–635mm of rainfall during the growing season and requires a dry harvesting season 4.5 to 6 months after sowing. The agronomic practices adopted were appropriate to the Kenyan context at the time and it was recommended to use the crop as green manure – a practice adopted by Kimenju *et al*. (2008). The trials were designed to test the adaptability of the crop to the local environment, and for this reason, different sites were used: Kitale (0°6S, 34°45'E), Kapenguria (1°14S, 35°7'E), Kiambu (1°10S, 36°49'E) and Scott Agricultural Laboratories (1°14S, 36°43'E).

Following Elmer's report, there is no trace of quinoa research in the country until 1999 when the crop was re-introduced as part of a world multilocation trial. The research aimed to determine the adaptability and yield of quinoa cultivars under Kenyan conditions, and was conducted by Maurice Oyoo as part of his MSc thesis and several publications were made from the research. A total of 24 accessions were used, all sourced through the International Potato Center (CIP) office in Nairobi (Table 1).

Table 1: Genotypes used in the 1999 - 2000 trial by Oyoo et al. (2010)

GENOTYPE	COUNTRY OF ORIGIN
1. CICA-127	PERU
2. CICA-17	PERU
3. Huariponcho	PERU
4. Kancolla	PERU
5. 03-21-079BB	PERU
6. 03-21-072RM	PERU
7. IIlpa	PERU
8. Salcedo	PERU
9. Ratuqui	BOLIVIA
10. Kamiri	BOLIVIA
11. Real	BOLIVIA
12. Sayana	BOLIVIA
13. RU-2	ENGLAND
14. RU-5	ENGLAND
15. NL-6	HOLLAND
16. E-DK-4	DENMARK
17. G-205-95	DENMARK
18. Ingapirca	ECUADOR
19. ECU-420	ECUADOR
20. Canchones	CHILE
21. Baer	CHILE
22. Narino	COLOMBIA
23. Jujuy	ARGENTINA
24. Embrapa	BRAZIL

The highlights of this research are presented below:

Variation and Mean Performance Analysis of Quinoa in Kenya

The variance analysis revealed variability among the quinoa cultivars for all the morphological, agronomic and phenological traits studied (Tables 2 and 3), except for the number of days to branching in short rainy season (Table 3). This level of variation suggests genetic diversity among the quinoa cultivars evaluated.

Morphological, phenological and Agronomic performance of quinoa in Kenya

The results of crop performance for the year 1999 are presented in Table 4 below.

In 1999, cultivar 'RU 2' recorded the lowest number of days from sowing to emergence (7.0 days) in the long rainy season (Table 4). Other cultivars, '03-21-079BB', 'ECU-420' and 'Salcedo', emerged 7.3 days after sowing and 'E-DK-4', 'G-205-95' and 'Real' 7.7 days after sowing. 'Canchones' took the most to emerge (11.0 days). Other late-emerging cultivars were 'Sayana' (10.0 days), 'Baer' and 'CICA-127' (10.3 days) and 'Kamiri' (10.7 days). In 1999/2000, cultivar 'NL-6' recorded the shortest period, emerging 9.0 days after sowing (Table 4). Others were 'RU-2' and 'Ingapirca' (9.3 days), '03-21-079BB', '03-21-072RM Kacolla', 'CICA-17', '02-Embrapa', 'Canchones' and 'Narino' (9.7 days). The late-emerging cultivar in the short rainy season was 'Real' (12.3 days). It was followed by 'Sayana' (11.0 days) and then 'Ratuqui', 'Kamiri', 'Illpa', 'Baer' and 'CICA-127' (10.3 days). 'Salcedo', 'Huariponcho', 'ECU-420' and 'Jujuy' all took 10.0 days to emerge.

'Kancolla', 'CICA-127', 'ECU-420', 'E-DK-4 Narino' and 'Real' all took 20 days after sowing to register six true leaves (Table 4). Others were 'RU-2' and 'Illpa' (20.3days), 'CICA-17', 'RU-5', 'Sayana', '03-21-072 RM' and 'Ingapirca' (20.7 days) and '03-21-079BB' (21.0 days). 'NL-6' took the least number of days to reach the six true leaf stage in the second season (Table 5). Others were 'Ratuqui' (23.7 days), 'Salcedo', '03-21-79BB', 'Ingapirca', '02-Embrapa' and 'Kancolla' (24.0 days). 'Real' (25.7 days) took the longest period to reach the six true leaf stage. 'Canchones' and 'Kamiri' registered the longest number of days to six true leaves (22.7 days), close-

Table 2. Analysis of variance mean squares for 24 quinoa cultivars grown in Kabete during long rainy season (1999)

Source of variation	d.f.	Days to emergence	Days to 6 true leaves	Days to branching	Days to flower bud formation	Days to anthesis	Days to milky grain	Days to pastry grain	Days to physiological maturity	Panicle length (cm)	Biomass (g)
Blocks	2	0.79ns	0.22ns	0.06ns	3.50ns	1.54ns	14.43ns	8.79ns	7.63*	5.46ns	47.80ns
Cultivar	23	4.69***	2.05***	1.94***	6.68***	12.33***	294.14***	236.10***	302.55***	17.02***	795.04***
Error	46	0.40	0.38	0.66	1.49	2.11	8.69	6.79	1.56	2.48	37.79

Source of variation	d.f.	Plant height (cm)	No. of branches/plant	Leaf blade width (mm)	Leaf blade length (mm)	Leaf petiole length (mm)	Seed size (µm)	Harvest index	Seed yield/plant
Bloques	2	35.91ns	9.72ns	694.59***	178.01ns	66.84ns	0.0001ns	0.01ns	1.31ns
Cultivar	23	1810.68***	22.81***	98.98**	104.19ns	109.01***	0.07***	0.001*	46.71***
Error	46	62.43	1.34	34.60	60.63	43.41	0.01	0.01	2.77

P=0.05 df. Degrees of freedom
Ns: non significant *:-significant **:-very significant ***:-highly significant

Table 3. Analysis of variance mean squares for 24 quinoa cultivars grown in Kabete during short rainy (1999/2000) season

Source of variation	d.f.	Days to emergence	Days to 6 true leaves	Days to branching	Days to flower bud formation	Days to anthesis	Days to milky grain	Days to pastry grain	Days to physiological maturity	Panicle length (cm)	Biomass (g)
Blocks	2	0.04ns	1.35ns	0.51ns	0.26ns	0.06ns	0.79ns	0.39ns	2.06ns	2.56ns	4.62ns
Cultivar	23	1.34**	1.56*	0.74ns	2.89***	7.39***	290.38***	446.29***	507.74***	26.72***	521.8***
Error	46	0.35	0.72	0.60	0.31	0.43	1.13	1.52	1.82	1.60	12.62

Source of variation	d.f.	Plant height (cm)	No. of branches/plant	Leaf blade width (mm)	Leaf blade length (mm)	Leaf petiole length (mm)	Seed size (µm)	Harvest index	Seed yield/plant
Bloques	2	816.78***	1.98ns	38.78ns	37.79ns	25.17ns	0.01ns	0.003ns	0.10ns
Cultivar	23	1479.03***	16.77***	164.8***	161.57***	199.42***	0.071***	.0029	8.20**
Error	46	62.32	1.34	22.11	19.52	29.37	0.004	0.002	0.58

P=0.05 df. Degrees of freedom
Ns: non significant *:-significant **:-very significant ***:-highly significant

Table 4. Mean performance of 24 quinoa cultivars during long rainy season of 1999 at Kabete

Source of variation	Days to emergence	Days to 6 true leaves	Days to branching	Days to flower bud formation	Days to anthesis	Days to milky grain	Days to pastry grain	Days to physiological maturity	Panicle length (cm)	Biomass (g)
1	10.3ab	21.7abc	27.7ab	33.7b-f	41.3a-c	56.7f-h	64.7e-g	70.7 g	13.8bc	48.3c-g
2	7.3gh	21.0b-d	28.0a	34.7a-e	39.3c-f	69.7bc	74.7cd	84.3e	12.6b-d	45.1c-i
3	8.0e-h	20.0d	28.0a	35.7a-c	40c-e	58ef	75cd	84.3e	10.6de	52.6c-e
4	11.0a	22.7ª	27.0a-c	32.7ef	38.3d-g	53.7f-h	65.7ef	72g	9.7e	45.9c-i
5	10.3ab	20.0d	26.3b-d	36.7a	43ab	72.7b	79.7b	90.3b	15.2b	55.1c
6	9.3b-d	20.7cd	27.0a-c	36.7a	43.7a	62.3de	71.0d	79F	21.8a	87.1b
7	7.3gh	20.0d	27.7ab	36.7a	43ab	84.3a	90.7a	97.7a	13.9bc	54.7cd
8	7.7f-h	20.	25.3d	32.7ef	39.7c-f	52.0gh	61.0fg	65.0 h	13.4b-d	42.6d-i
9	10.3ab	22.0ab	28.0a	35a-e	40.3b-e	55.7f-h	61.0fg	65.0 h	12.9b-d	45.5c-i
10	7.7f-h	20.0d	26.7a-d	31.7f	36.3g	52gh	60.3g	65.7h	11.6c-e	37.8f-i
11	8.0e-h	21.0b-d	28.0a	33.3c-f	38.7c-g	67cd	77.7bc	84.7de	13.2b-d	37.4g-i
12	8.7def	20.3d	27.0a-c	33.7b-f	39c-g	56.7f-h	65.7ef	73.0g	12.1c-e	35.5hi
13	8.0e-h	20.7cd	27.0a-c	35.3a-d	39.0c-g	71bc	80.3b	87.7c	13.2b-d	47.2c-h
14	8.3d-fg	20.7cd	28.0a	33.3c-f	38.0e-g	54.7f-h	64.7e-g	73.0g	12.9b-d	34.0i
15	10.7a	22.7ª	27.7ab	35.3a-d	41a-d	57.7e-g	73.0cd	81.0f	11.1cde	49.9c-f
16	9.0 c-e	20.0d	26.7a-d	36.3a	43.7a	84.3a	91.0a	97.0a	15.4b	107.3a
17	7.3gh	20.7cd	25.7cd	32.7ef	37fg	52gh	61.0fg	65.0 h	11.1c-e	36.8g-i
18	10.3ab	22.0ab	27.7ab	33.7b-f	37.7e-g	51.7h	66.3e	73.0g	10.6de	45.1c-i
19	7.7f-h	20.0d	26.3b-d	33.0d-f	39.3c-f	52.7f-h	65.0e-g	71.7g	13.2b-d	40.7e-i
20	8.0e-h	20.7cd	27.7ab	36ab	39.3c-f	71bc	77.3bc	86.7cd	11.7cde	40.8e-i
21	7.0h	20.3d	26.0cd	33.0d-f	38.3d-g	52.0gh	61.0fg	65.0 h	12.5b-e	40.8e-i
22	8.0e-h	20.7cd	27.7ab	34.7a-e	40.3b-e	56.7f-g	66.3e	73g	12.8b-d	44.8c-i
23	7.3gh	21.0b-d	28.0a	35.7a-c	40c-e	57.7e-g	71.3d	83.3e	11.6c-e	44c-i
24	10.0a-c	20.7cd	27.7ab	33.3c-f	37.7e-g	55.7f-h	66.7e	72g	10.8c-e	46.3c-h
(%)CV	7.4	3.0	3.0	3.3	3.3	2.9	1.6	1.3	12.3	12.7
Medio	8.54	20.81	27.20	34.33	39.76	60.79	70.79	77.65	12.81	48.56
SE	0.633	0.618	0.815	1.148	1.307	1.741	1.126	1.034	1.575	6.147

1.Baer 2.03-21-079BB 3.Kancolla 4.Canchones 5.CICA-127 6.CICA-17. 7.ECU-420. 8.E-DK-4. 9.02-EMBRAPA. 10.G-205-95. 11.Huariponcho. 12.Illpa. 13Ingapirca. 14.Jujuy. 15.Kamiri. 16.Narino. 17.NL-6. 8.Ratuqui. 19.Real. 20.03-21-072RM. 21.RU-2. 22. RU-5. 23.Salcedo. 24.Sayana.
S.E Standard error
Source: Oyoo, 2002

ly followed by 'Baer' (21.7 days) and '02-EMBRAPA' and 'Ratuqui' (22.0 days) (Table 5).

Cultivar 'E-DK-4' took the shortest period to branch (25.3 days) in the first season (Table 4), compared with 'NL-6' (25.7 days) and 'RU-2' (26.0 days). Cultivars '03-21-079BB', '02-Embrapa', 'Jujuy' and 'Salcedo' took the most number of days to branch (28.0 days).

The shortest period after sowing to flower bud formation in the first season was 31.7 days, registered in cultivar 'G-205-95' (Table 4), while the highest number of days to bud formation was recorded in 'CICA-127' (36.7 days), 'CICA-17' (36.7 days), 'ECU-420' (36.7 days) and '03-21-072RM' (36.0 days). In the second season, the earliest cultivar to form flower buds was 'Canchones' (44.3 days), while 'CICA-127' took the longest reaching flower buds in 46.7 days (Table 5).

During the 1999 season, 'G-205-95' was the first cultivar to attain 50% flowering (36.3 days after sowing) (Table 4). This was not significantly different ($p \leq 0.05$) from the number of days observed for 'NL-6' (37.0 days), 'Sayana' (37.3 days), 'Jujuy' (38.0 days) and 'Canchones' (38.4 days). Cultivars 'CICA-17' and 'Narino' took the longest period to reach anthesis (43.7 days). Other late genotypes were 'CICA-127' (43.0 days), 'RU-5' (40.3 days) and 'Baer' (41.0 days). In the short rainy season, the shortest time to 50% flowering was 52.0 days after sowing, recorded in cultivar 'NL-6' (Table 5). Early flowering during this time was also observed in '02-Embrapa' (52.3 days), 'Kancolla' (52.7 days), 'Canchones' (52.7 days), 'RU-5' (52.7 days), 'Sayana' (52.7 days) and 'Illpa' (52.7 days). The highest number of days to 50% flowering in this season was observed in 'CICA-127' (56.7 days). The other cultivars that flowered late included 'ECU-420' (56.0 days), 'Narino' (55.0 days) and 'CICA-17' (53.3 days). These genotypes also had the best grain yields.

The earliest cultivar to attain the milky grain stage in 1999 was 'Ratuqui', which took 51.7 days (Table 4). Cultivars 'E-DK-4', 'G-205-95', 'NL-6' and 'RU-2' (52 days after sowing), 'Real' (52.7 days) and 'Canchones' (53.7 days) were also early for this trait. The longest periods to milky grain formation during the long rainy season were recorded in 'ECU-420' (84.3 days) and 'Narino' (84.3 days). They were followed by 'CICA-127' (72.7 days), '03-21-072RM' (71.0 days) and 'Ingapirca' (71.0 days). Results obtained in 1999/2000 show that 'ED-K-4', 'Canchones', '02-Embrapa' and 'NL-6' took the shortest time (60.0 days) after sowing to reach the milky grain stage, while 'ECU-420' (93.0 days) was the latest (Table 5).

Cultivar 'G-205-95' was the earliest to form pasty grains (60.3 days after sowing) in 1999 (Table 4). Other cultivars took a similar length of time to reach the pasty grain stage ($p \leq 0.05$): 'E-DK-4' (61.0 days), '02-Embrapa' (61.0 days), 'NL-6' (61.0 days) and 'RU-2' (61.0 days). The longest periods to pasty grains were recorded in 'Narino' (91.0 days) and 'ECU-420' (90.7 days), followed by 'Ingapirca' (80.3 days) and 'CICA-127' (79.7 days). In the short rainy season, 'RU-2' (66.3 days), 'G-202-95' (66.7days), 'ED-K-4' (67.0 days), 'Canchones' (66.7 days) and 'Baer' (66.7 days) reached the pasty grain stage in the shortest time (Table 5). Cultivars that took the longest time to reach pasty grain stage were 'ECU-420' (112.67 days), followed by 'Narino' (104.0 days) and 'CICA-127' (92.0 days).

The earliest-maturing cultivars during the 1999 long rainy season in Kenya were 'RU-2', 'NL-6', 'ED-K-4' and '02-Embrapa' (65.0 days) and 'G-205-95' (65.7 days) (Table 4). Others were 'Baer' (70.7 days), 'Real' (71.7 days), 'Sayana' (72.0 days), 'Canchones' (72.0 days) and 'RU-5', 'Illpa' and 'Jujuy' (73.0 days). The late-maturing cultivars were 'ECU-420' (97.7 days), 'Narino' (97.0 days) and 'CICA-127' (90.3 days). Cultivar 'ECU-420' (122.7 days) was the last cultivar to attain seed physiological maturity in 1999/2000 (Table 5). Cultivars 'Narino' (113.3 days) and 'CICA-127' (100.33 days) were second and third, respectively. The earliest-maturing cultivars in the second season were 'G-205-95' and 'RU-2', both maturing in 72.33 days after sowing (Table 5). Days to physiological maturity in these two cultivars were not significantly different ($p \leq 0.05$) from those recorded in 'ED-K-4' (73.0 days), '02-Embrapa' (73.0 days), 'Canchones' (74.0 days), 'RU-5' (74.33 days) and 'NL-6' (74.67 days).

The longest panicle length during the long rainy season was recorded for 'CICA-17' (21.8 cm) (Table 4). This was significantly different ($p \leq 0.05$) from that of 'Narino' (15.4 cm) and 'CICA-127' (15.2 cm). The cultivar with the shortest panicle was 'Canchones'

Table 5. Mean performance of 24 quinoa cultivars during short rainy season 1999/2000 at Kabete.

Cultivar	Days to emergence	Days to 6 true leaves	Days to bud formation	Days to anthesis	Days to milky grain	Days to pastry grain	Days to physiological maturity	Panicle length (cm)	Biomass yeld (g)
1	10.33bc	25.0a-c	46.0cd	53.0e-g	60.33h	67.0i	75.0j	15.9cd	16.25h-j
2	9.67cd	25.33ab	45.33b-f	53.0e-g	71.0e	80.33e	89.33f	15.7cd	14.69ij
3	9.67cd	24.0a-d	44.67ef	52.7e-g	70.33ef	77.0f	88.67f	14.3d-h	17.88g-j
4	9.67cd	24.67a-c	44.33f	52.3e-g	60.0h	66.67i	74.0jk	15.27c-e	16.92h-j
5	10.33bc	23.33cd	47.67a	59.67a	81.67c	92.0c	100.3c	21.7a	40.25b
6	9.67cd	24.0a-d	47.33ab	53.33cd	78.33d	86.33d	92.0e	18.23b	34.08c
7	10.0b-d	24.67a-c	48.0a	56.0b	93.0a	112.6a	122.7a	22.53a	41.26b
8	9.33cd	24.67a-c	44.67ef	53.0e-g	60.0h	67.0i	73.jk	14.7d-g	13.86ij
9	9.67cd	24.0a-d	45.0d-f	52.33fg	60.0h	66.67i	73.0jk	13.5d-h	12.97ij
10	9.67cd	24.33a-c	45.0d-f	53.0e-g	60.0h	66.33i	72.33k	12.27g-I	12.53ij
11	10.0b-d	24.67a-c	45.67c-e	53.0e-g	68.67fg	76.67f	88.67f	13.7d-h	19.22f-I
12	10.33bc	24.67a-c	45.33d-f	52.7e-g	61.0h	71.3gh	80.0gh	12.4g-I	25.35ef
13	9.33cd	24.0a-d	46.0cd	53.0e-g	77.0d	85.0d	95.0d	17.27bc	19.31f-I
14	10.0b-d	25.0a-c	45.0d-f	53.67de	61.33h	73.33g	81.33g	15.57c-e	26.46de
15	10.33bc	25.0a-c	45.67c-e	53.67de	62.0h	700h	81.33g	12.1hi	36.45bc
16	9.67cd	24.33a-c	46.67bc	55.0bc	89.0b	104.0b	113.3b	19.1b	70.33a
17	9.0d	22.67d	44.33f	52.0g	60.0h	66.67i	74.67jk	12.47f-I	11.94j
18	1033bc	23.7d-b	45.33d-f	53.0e-g	61.0h	70.33h	77.33i	13.13e-I	22.6e-h
19	12.33a	25.67a	45.67c-e	53.0e-g	60.67h	70.0h	78.0hi	12.77f-I	32.25cd
20	9.67cd	24.0a-d	46.0cd	53.0e-g	67.67g	78.67ef	88.0f	14.87d-f	24.91ef
21	9.33cd	24.67a-c	44.67ef	53.33d-f	60.33h	66.33i	72.33k	12.3g-I	14.22ij
22	9.67cd	25.33ab	45.0d-f	52.7e-g	61.33h	69.67h	74.33jk	13.5d-h	14.52ij
23	10.0b-d	24.0a-d	45.67c-e	53.33d-f	62.0h	73.33g	82.33g	13.8d-h	23.2e-h
24	11.0b	25.67a	45.67c-e	52.7e-g	61.33h	70.33h	80.33g	11.0i	24.5e-g
medio	9.96	24.47	45.60	53.49	67.0	76.11	84.49	14.96	24.43
%cv	5.9	3.5	1.2	1.2	1.6	1.3	1.1	8.6	14.5
S.E.	0.588	0.851	0.550	0.658	1.061	0.989	0.961	1.293	3.552

1.Baer 2.03-21-079BB 3.Kancolla 4.Canchones 5.CICA-127 6.CICA-17. 7.ECU-420. 8.E-DK-4. 9.02-EMBRAPA. 10.G-205-95. 11.Huariponcho. 12.Illpa. 13Ingapirca. 14.Jujuy. 15.Kamiri. 16.Narino. 17.NL-6. 8.Ratuqui. 19.Real. 20.03-21-072RM. 21.RU-2. 22. RU-5. 23.Salcedo. 24.Sayana.
S.E Standard error
Source: Oyoo, 2002

(9.7 cm). The other cultivars with short panicles during this time were '03-21-079BB' (12.6 cm), 'Kancolla' (10.6 cm), 'G-205-95' (11.6 cm), 'Illpa' (12.1 cm), 'Kamiri' (11.1 cm), 'NL-6' (11.1 cm), 'Ratuqui' (10.6 cm), '03-21-072RM' (11.7 cm), 'RU-2' (12.5 cm), 'Salcedo' (16.6 cm) and 'Sayana' (10.8 cm). 'ECU-420' and 'CICA-127' had the longest panicles (22.5 and 21.7 cm, respectively) in the short rainy season of 1999/2000 (Table 3). Others in this season were 'Narino' and 'CICA-17' (19.1 and 18.33 cm, respectively). The cultivar with the shortest panicle was Sayana (11.0 cm). The other cultivars with mean panicle lengths that were not significantly different ($p \leq 0.05$) from 'Sayana' included: 'RU-2' (12.3 cm), 'Real' (12.77 cm), 'Kamiri' (12.1 cm), 'Ratuqui' (13.13 cm), 'NL-6' (12.47 cm), 'Illpa' (12.4 cm) and 'G-205-95' (12.27 cm).

'Narino' had the highest biomass yield of 107.3 g per plant in the first rainy season (Table 4). This biomass yield was significantly different ($p \leq 0.05$) from that of 'CICA-17' (87.1 g) and 'CICA-127' (55.1 g). The lowest biomass yield was recorded in 'Jujuy' (34.0 g) followed by 'Ingapirca' (35.5 g), 'Illpa' (35.5 g), 'Huariponcho' (37.4 g) and 'G-205-95' (37.8 g). 'Narino' (70.3 g) also had the highest biomass yield in the second season (Table 5). In the second season, low biomass yields were recorded in 'NL-6' (11.94 g), 'G-205-95' (12.53 g), '02-Embrapa' (12.97 g), 'ED-K-4' (13.86 g), '03-21-079BB' (14.69 g), 'RU-2' (14.22 g) and 'RU-5' (15.52 g).

For plant height, in 1999, the shortest were 'Canchones' (74.0 cm) and 'NL-6' (84.8 cm) (Table 6). The other short genotypes were 'G-205-95' (92.3 cm), 'Jujuy' (97.3 cm) and 'Real' (92.5 cm). The tallest cultivars in this season were 'Narino' (167.9 cm), 'CICA-127' (164.6 cm), 'ECU-420' (160.9 cm) and 'CICA-17' (146.4 cm.). In 1999/2000, 'NL-6' recorded the shortest height (39.8 cm) at maturity (Table 5), followed by 'RU-5' (53.2 cm), 'G-205-95' (53.87 cm), '02-Embrapa' (53.4 cm) and 'Canchones' (54.13 cm). The tallest genotype was 'CICA-127' with a height of 132.66 cm. Other tall cultivars were 'ECU-420' (117.87 cm) and 'Narino' (113.63 cm) (Table7). Taller plants also exhibited superior grain and biomass yields in these trials.

'Canchones' had the least number of branches (14.3) in the long rainy season (Table 6), and others with few branches were 'NL-6' (15.7) and 'Sayana' (18.0). The highly branched cultivars were 'Narino' (27.0) and 'ECU-420' (24.7), '03-21-079BB' (23.7) and 'Kancolla' (23.3). The cultivars were not as branched in the 1999/2000 season as they were in 1999 (long rains). During the short rains, 'Baer' (2.7), '02-Embrapa' (2.2) and 'G-205-95' (2.0) had the least number of branches, while 'CICA-127' (11.3) was the most branched cultivar (Table 7). Other highly branched cultivars in the second season were 'Narino' (9.7) and 'ECU-420' (9.3).

Results from the 1999 trial indicated that 'Real' and 'CICA-17' had the largest seed sizes (2.40 and 2.34 µm, respectively) (Table 6). Others were 'Kamiri' (2.22 µm) and 'Illpa' (2.27 µm). 'Huriponcho' (1.88 µm), 'RU-5' (1.89 µm), 'Ingapirca' (1.89 µm), '02-Emprapa' (1.90 µm) and 'ECU-420' (1.90 µm) had small seeds. In the 1999/2000 season, 'Ingapirca' and 'Kancolla' had the smallest seed size of 1.86 µm (Table 7), followed by 'ECU-420' with a seed size of '1.87 µm'. Others were 'Ingapirca', 'Kancolla', 'ECU-420' and 'Baer' (1.96 µm), '03-21-079BB' (1.89 µm), '02-Embarapa' (1.88 µm), 'Huaripocho' (1.88 µm), 'Narino' (1.97 µm), '03-21-072RM' (1.89 µm), 'RU-2' (1.93 µm) and 'RU-5' (1.88 µm). During this season, 'Sayana' (2.32 µm) recorded the largest seed size, followed by 'Ratuqui' (2.28 µm), 'Kamiri' (2.23 µm), 'Illpa' (2.23 µm) and 'CICA-17' (2.22 µm).

'Illpa' (0.47) had the highest harvest index in the 1999 season (Table 4). Other cultivars with high harvest indices were 'Kancolla' (0.34), 'CICA-125' (0.34), 'ED-K-4' (0.38), 'Huriponcho' (0.41), 'NL-6' (0.40), 'Kamiri' (0.33), '03-21-072RM' (0.41), 'RU-5' (0.34) and 'Salcedo' (0.33). The lowest harvest index was recorded in 'Baer' (0.21). Others with low harvest index were 'Canchones' (0.24) and 'Narino' (0.25). In the 1999/2000 season, the highest harvest index was reached by '03-21-079BB' (0.48), 'ED-K-4' (0.49) and 'NL-6' (0.43) (Table 7). The cultivar with the lowest harvest index was 'Real' (0.15). Other cultivars with low harvest index were 'CICA-127' (0.18), 'ECU-420' (0.20), 'Narino' (0.17) and 'Kamiri' (0.17).

'Narino' and 'CICA-17' had the highest seed yields (26.5 and 25.7 g, respectively) during the first rainy season (Table 6). 'CICA-127' (22.2 g) and 'Kancolla' (17.5 g) also had high grain yields in this season. The lowest seed yields were obtained in 'Canchones' (10.6 g) and 'Jujuy' (10.7 g). In the 1999/2000 sea-

Table 6. Mean performance of 24 quinoa cultivars, long rainy season, 1999 at Kabete.

Cultivar	Plant height (cm)	No. of branches per plant	Leaf blade width (cm)	Leaf petiole length (cm)	Seed size (µm)	Harvest index	Seed yield/plant (g)
1	127.7cd	22.7b-d	50.7bc	51.3c-e	1.98f-h	0.21d	13.4e-g
2	122.3cd	23.7a-c	45.3c	51.3c-e	1.97f-h	0.29b-d	12.8fg
3	123.9cd	23.3a-c	44.3c	53c-e	1.96f-h	0.34a-d	17.5c
4	74.0i	14.3g	42.7c	48de	2.17b-d	0.24cd	10.6g
5	164.6a	20.7b-e	59.7ab	65.3ab	2.03d-h	0.34a-d	22.2b
6	146.4b	20.7b-e	51.3bc	63ac	2.34a	0.30b-d	25.7a
7	160.9a	24.7ab	49.0bc	60ad	1.90h	0.31b-d	16.7cd
8	115.4de	21.3b-e	48.0c	51.3c-d	2.01e-h	0.38a-c	16.2c-e
9	104.3e-g	21.7b-e	50.3bc	54.7b-e	1.90h	0.31b-d	13.9d-f
10	92.3gh	19.3c-f	51.3bc	51.7c-e	2.06d-g	0.35a-c	13.4e-g
11	115.6de	22.3b-d	45.3c	49.3de	1.88h	0.41ab	15.3c-f
12	109.9def	19.3c-f	43.3c	48.3de	2.27a-c	0.47a	16.2c-e
13	134.9bc	21.3b-e	43.0c	49.7de	1.87h	0.30b-d	14.1d-f
14	97.3f-h	21.0b-e	44.3c	49de	2.16c-e	0.32b-d	10.7g
15	111.8d-f	21.7b-e	44.7c	51.7c-e	2.27ab	0.33a-d	16.4c-e
16	167.9a	27.0a	65.7a	68.3a	2.08d-g	0.25cd	26.5a
17	84.8hi	15.7fg	41.7c	47.3de	1.97f-h	0.4ab	14.2d-f
18	99.2f-h	17.3e-g	47.3c	51.3c-e	2.07d-g	0.31b-d	14.0d-f
19	92.5gh	20.3b-e	45.7c	49.7de	2.40a	0.34a-d	13.6d-g
20	132.8bc	22.0b-d	48.7b	50de	1.96f-h	0.41ab	16.2c-e
21	104e-g	21.3b-e	41.0c	45.3e	1.92gh	0.37a-c	14.7c-f
22	114.2de	22.0b-d	48.7bc	55b-e	1.89h	0.34a-d	14.9c-f
23	122.2cd	19.0c-f	44.0c	50.7c-e	2.11d-f	0.33a-d	14.3d-f
24	98.9f-h	18de-g	41.3c	44e	2.07d-g	0.31b-d	14.2d-f
%cv	6.7	11.1	12.4	12.6	4.1	20.4	10.6
Mean	117.12	20.90	47.39	52.51	2.05	0.33	15.73
S.E.	7.902	2.319	5.882	6.630	0.084	1.666	0.068

1.Baer 2.03-21-079BB 3.Kancolla 4.Canchones 5.CICA-127 6.CICA-17. 7.ECU-420. 8.E-DK-4. 9.02-EMBRAPA. 10.G-205-95. 11.Huariponcho. 12.Illpa. 13Ingapirca. 14.Jujuy. 15.Kamiri. 16.Narino. 17.NL-6. 8.Ratuqui. 19.Real. 20.03-21-072RM. 21.RU-2. 22. RU-5. 23.Salcedo. 24.Sayana.
S.E Standard error
Source: Oyoo, 2002

son, seed yields were very low compared with the first rainy season, with 'Narino' recording the highest grain yield of 11.8 g (Table 7). 'CICA-17' recorded 9.30 g and 'ECU-420' 8.1 g. Cultivar 'G-205-95' had the lowest grain yield in the short rainy season (4.0 g).

Correlation analysis

In both seasons, days to flower bud formation, days to pasty grain stage, days to milky grain, days to physiological maturity, panicle length, biomass yield and plant height all had high and positive correlation with seed yield. The respective correlation coefficient between seed yield per plant and the above-mentioned characters was 0.664, 0.774, 0.530, 0.518, 0.721, 0.854 and 0.773 for first season and 0.580, 0.789, 0.760, 0.741, 0.646, 0.780 and 0.692 for the second season.

The correlations between seed yield per plant and 50% flowering (0.768), leaf petiole length (0.821), leaf blade length (0.717) and leaf blade width (0.595) were high and positive in the long rainy season. The correlations between seed yield per plant and days to branching (0.583) and number of branches (0.686) were also high and positive in the second season. Other characters exhibiting positive and significant correlation with seed yield per plant were the number of branches per plant (0.479) in the long rainy season and leaf petiole length (0.428) in the short rainy season. Analysis of the correlation of yield components clearly brought out the importance of biomass yield on seed yield for all the characters studied.

Path Coefficient Analysis

Oyoo (2002) also performed path coefficient analysis to estimate the direct and indirect effects of the various yield components on yield -. It was reported that the residual effect (R^2) was 0.3729 in the first rainy season and 0.1901 in the second rainy season, suggesting that the characters measured could account for 37.29 and 19.0% of the total variability of yield for the first and second seasons, respectively

Path coefficient analysis in 1999 revealed that biomass yield per plant had the highest direct effect (0.7438), followed by days to physiological maturity (0.6294) and harvest index (0.4257). Days to milky grain stage, days to pasty grain formation, number of branches per plant and petiole length – which all showed a positive and significant correlation to grain yield – exhibited negative direct effects of -0.3848, -0.2047, -0.0702 and -0.0722, respectively. The direct contributions of days to flower bud formation, days to 50% flowering, plant height, leaf blade width and leaf length were low (0.1741, 0.0024, 0.0483, -0.0722 and 0.1336, respectively), although they were highly associated with grain yield in the first season.

In the second season, plant biomass yield had the highest positive direct effects (0.7907) on seed yield. This was followed by harvest index (0.5501) and leaf blade length (0.5056). Days to milky grain formation and days to flower bud formation also had a high and direct effect on yield (0.3710 and 0.3093, respectively). The path coefficients indicate that biomass yield and harvest index have a high direct effect on seed yield in the long and short rainy seasons. This suggests that biomass yield and harvest index could be good yield predictors and yield could indirectly be increased by selecting for these characters.

The study concluded that 'Narino', 'CICA-127', 'CICA-17' and 'ECU-420' were superior cultivars in terms of biomass production and seed yield, and that adaptability and yield stability studies across several Kenyan environments could be useful in order to recommend different quinoa genotypes for cultivation in different agro-ecological regions. However, no further work has been done and no seeds were preserved for future trials in Kenya. However, to promote production and utilization of quinoa in Kenya and other regions of the world in a similar climatic zone, efforts need to focus on morphological and genetic characterization and evaluation of quinoa germplasm.

Quinoa can adapt to the degraded agro-ecosystems of Kenya, where productivity is low but human labour does not limit production. The crop can be used for purposes of climate change adaptation and as a food security stock, since it yields well in low fertility and low rainfall areas and farmers need to spend very little on agricultural inputs, such as fertilizers and chemicals to control diseases and pests. This is in line with the recommendations of Khanal (2009) on adaptation to climate change. Furthermore, it will increase species variety in agro-ecosystems which have undergone genetic erosion due to the unsuitability of some crops as a result of

Table 7. Mean performance of 24 quinoa cultivars, short rainy 1999/2000

Cultivar	Plant height (cm)	No. of branches per plant	Leaf blade width (cm)	Leaf petiole length (cm)	Seed size (µm)	Harvest index	Seed yield/plant (g)	Seed yield/plant (g)
1	62.23fg	2.7g	48.67b	65.67bc	38.67b-f	1.96e-h	0.34b-g	5.61e-h
2	81.47c-e	6.0c-e	37.33d-g	52.0f-j	38.33b-f	1.89gh	0.48a	6.99c-e
3	83.17cd	5.73c-f	40.33b-f	54.67e-j	39.0b-f	1.86h	0.38b-c	6.71c-f
4	54.13gh	5.03c-f	30.33g	46.33j	37.33d-f	2.2bc	0.35b-e	5.78d-h
5	132.66a	11.27a	58.33a	71.67ab	63.76a	1.99e-g	0.18j-l	7.21cd
6	90.17c	7.2c	38.0d-g	52.33f-j	42.0b-e	2.22a-c	0.27d-j	9.23b
7	117.87b	9.27b	64.67a	74.0a	64.67a	1.87h	0.2 i-l	8.08bc
8	66.43fg	3.8e-g	45.0b-e	64.33c-e	39.0b-f	2.07de	0.49a	6.74c-f
9	53.4gh	2.2g	42.0b-f	61.33c-e	36.0ef	1.88gh	0.37b-d	4.75hi
10	53.87gh	2.0g	36.0e-g	54.33e-j	30.33f	2.06de	0.32c-h	3.95i
11	76.0c-f	5.0c-f	34.33fg	48.0h-j	33.33ef	1.88gh	0.32c-h	6.22d-h
12	66.17fg	5.7c-f	42.33b-f	50.0g-j	39.67b-f	2.23a-c	0.26e-k	6.41d-g
13	83.07cd	6.4c	44.0b-e	60.0c-f	49.0b	1.86h	0.28c-i	5.44f-h
14	67.57e-g	4.1d-g	40.33b-f	49.67g-j	38.33b-f	2.02ef	0.22i-l	5.65e-h
15	61.63fg	5.23c-f	37.33d-g	49.0h-j	38.0c-f	2.23a-c	0.17kl	5.97d-h
16	113.63b	9.7ab	48.0bc	57.67c-g	48.33bc	1.97e-h	0.17kl	11.79a
17	39.77h	1.97g	46.33b-d	62.33c-e	37.33d-f	2.04d-f	0.43ab	5.17g-i
18	67.7e-g	5.33c-f	40.67b-f	51.67f-j	39.33b-f	2.28ab	0.24g-l	5.13g-i
19	75.93c-f	7.17c	41.0b-f	55.33e-i	47.33b-d	2.22a-c	0.15l	4.77hi
20	85.9cd	6.03c-e	42.0b-f	56.33d-h	42.0b-e	1.89gh	0.21i-l	5.11g-i
21	54.5g	3.53fg	39.0c-g	56.33d-h	39.67b-f	1.93f-h	0.39bc	5.46f-h
22	53.2gh	3.67fg	40.0b-f	59.0c-f	40.33b-f	1.88gh	0.34b-f	4.96g-i
23	72.8d-f	5.37c-f	47.33bc	58.67c-f	47.67b-d	2.14cd	0.25f-l	5.72e-h
24	65.67fg	6.07c-e	36.0e-g.	42.33i-j	38.67c-f	2.32a	0.24h-l	5.61e-h
Mean	74.57	5.44	42.00	42.33	56.63	2.04	6.19	0.29
%CV	9.4	21.3	12.9	10.9	7.6	3.1	12.3	18.2
S.E.	7.042	1.158	5.419	4.631	4.323	0.063	0.759	0.053

1.Baer 2.03-21-079BB 3.Kancolla 4.Canchones 5.CICA-127 6.CICA-17. 7.ECU-420. 8.E-DK-4. 9.02-EMBRAPA. 10.G-205-95. 11.Huariponcho. 12.Illpa. 13Ingapirca. 14.Jujuy. 15.Kamiri. 16.Narino. 17.NL-6. 8.Ratuqui. 19.Real. 20.03-21-072RM. 21.RU-2. 22. RU-5. 23.Salcedo. 24.Sayana.
Source: Oyoo, 2002

climate change. The particular advantage of quinoa in Kenya is that it has shown the ability to perform under low rainfall conditions (Elmer, 1942); Kenya is classified as about 80% arid and semi-arid, and quinoa can be used in those areas.

Potential use of Quinoa in the Incorporation of Green manure for Root-Knot Nematode Suppression

In research by the University of Nairobi, Kimenju *et al*. (2008) carried out an experiment to determine the suitability of green manure plants, including quinoa, as rotational crops with common bean to suppress root-knot nematode (*Meloidogyne* spp.). The plants were also evaluated as soil amendments in nematode control. The results (Tables 8 and 9) suggest that quinoa does not decrease the egg mass index of root-knot nematode in common bean and may not be suitable for the control of this pest when used as a rotation crop in Kenya. However, when used as green manure, quinoa recorded one of the highest reductions of egg mass index for the control of root-knot nematode.

The other mention of work on quinoa in Kenya is made by Participatory Ecological Land Use Management-Kenya (PELUM) in their Grow Bio Intensive Agriculture Program but they do not provide any details (PELUM, www.pelum.net).

Uses and Markets

Quinoa is not currently grown in the country, but some of its products are found in the major supermarket chains in large towns and cities. Their origin is mainly Europe and South America. For the local populations, the crop remains undiscovered; therefore, if the crop is produced locally, there is potential in terms of consumption. A legal framework exists in the country regulating plant genetic material for sustainable management and utilization of genetic resources for the benefit of the people. In order to manage this, various organs are in existence to facilitate this purpose. These include Kenya Plant Health Inspection Service (KEPHIS), National Environmental Monitoring Authority (NEMA) through Environmental Management and Co-ordination Act (EMCA) Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing and the National Council for Science and Technology (NCS&T).

Table 8: Galling indices, eggmass indices and reproductive factors of *Meloidogyne javanica* on common beans interplanted with green manure plants in a glasshouse

Green manure plant	GI	EMI	Rf
Calliandra calothyrsus	4.1	3.5	1.6
Canavalia ensiformis	4.1	3.0	2.0
Chenopodium quinoa	4.8	6.2	3.0
Crotalaria juncea	3.7	3.8	0.8
Desmodium uncinatum	2.8	3.2	1.0
Gliricidia sepium	3.4	2.7	1.2
Leucaenia leucocephala	3.8	3.3	1.2
Mucuna pruriensis	5.5	2.2	0.5
Sesbania sesban	4.7	3.8	4.5
Tagetes minuta	2.2	2.5	0.9
Tephrosia purprea	6.7	5.8	5.2
Tithonia diversifolia	2.7	2.5	0.8
Vicia villosa	8.2	6.7	5.7
Phaseolus vulgaris monocrop	4.8	5.7	3.0
LSD (p≤5)	1.4	1.5	0.2
CV(%)	29.9	36.2	9.7

Source: Kimenju *et al*. 2008

Table 9: Galling indices, eggmass indices and reproductive factors of *Meloidogyne javanica* on common beans interplanted with green manure plants in a glasshouse

	GI Test		EMI Test		
Green manure plant	**I**	**II**	**I**	**II**	**Rf**
Calliandra calothyrsus	4.50	3.90	4.70	4.10	1.60
Canavalia ensiformis	2.50	2.20	2.70	2.90	3.40
Chenopodium quinoa	3.70	4.40	5.30	5.70	3.50
Crotalaria juncea	1.00	1.20	1.70	1.20	0.70
Desmodium uncinatum	1.20	1.10	1.50	1.10	0.40
Gliricidia sepium	1.20	1.00	1.20	1.00	1.00
Leucaenia leucocephala	1.20	1.00	1.30	1.00	0.30
Mucuna pruriensis	2.20	2.10	2.70	2.40	2.30
Sesbania sesban	6.20	5.70	8.50	7.60	5.40
Tagetes minuta	1.30	1.60	1.50	1.70	0.20
Tephrosia purprea	7.70	7.70	9.00	7.60	5.70
Tithonia diversifolia	1.00	1.40	1.20	1.50	0.60
Vicia villosa	6.20	7.00	9.00	7.90	6.00
Phaseolus vulgaris monocrop	4.50	5.60	9.00	7.40	4.40
LSD (p≤5)	0.25	0.23	0.23	0.21	0.34
CV(%)	8.1	7.40	5.40	5.80	13.50

Source: Kimenju et al., 2008

Conclusion and perspectives

It is unfortunate that a crop like quinoa, with such a long history of introduction, failed to capture the interest of researchers and the farming community after showing its ability to perform as early as 1935. Reasons for this are a matter of conjecture. However, even after its reintroduction in 1999, the crop still failed to capture the interest of farmers and researchers. The Kenya Gene Bank holds very little information on the crop and to date, there are no signs that the crop has ever been grown on the University of Nairobi experimental plots. Given the situation of climate change and the poor nutritional status of rural Kenya, quinoa has the ability to change both the cropping landscape and the nutritional status of rural populations, in particular those from marginal areas who can hardly feed themselves. Domestication of the crop is necessary, not only for nutritional purposes but also as a means of diversifying cropping systems to make farms resilient to climate change and increase farm income.

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CHAPTER 6.4.1

Quinoa in the United States of America and Canada

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Abstract

Quinoa as a crop and food has a relatively recent history in North America. Significant research into quinoa as a potential crop began in the 1980s led by efforts at Colorado State University. Agronomic research and breeding efforts established quinoa as a crop in high-altitude locations of the Rocky Mountains. Multiple private efforts also occurred around this time, leading to the commercial cultivation of quinoa in the Canadian Prairies and in Washington State. Chilean Coastal varieties were found to be the most adapted to temperate latitudes and Chilean germplasm has formed the basis of quinoa variety development. Major challenges for quinoa in North America include heat susceptibility, downy mildew, saponin removal, and weed and insect pressure. As quinoa expands into new environments in North America, other challenges may appear such as pre-harvest sprouting and novel pests and diseases. Quinoa is increasingly popular, and demand in North America far exceeds supply. Currently, research on quinoa is being conducted by Brigham Young University, which is investigating salt-tolerance physiology and quinoa genetics and genomics in collaboration with PROINPA. Washington State University has recently initiated a breeding programme and has conducted agronomic research, investigating quinoa's potential as a crop in the Pacific Northwest. Additionally, researchers at various institutions have conducted research on quinoa pathogens, archaeology of quinoa domestication and phylogenetics of *Chenopodium*. For future dissemination of quinoa in North America, the development of early-maturing saponin-free quinoa varieties with greater heat tolerance will be crucial for the crop's success. Additionally, the development of lines with greater downy mildew resistance and pre-harvest sprouting tolerance will facilitate the crop's expansion in North America.

Introduction

Quinoa was introduced relatively recently to North America. While efforts to grow the crop were reported as early as the 1900s (Caldwell, 2013), interest in the crop began to increase in the 1970s and 1980s. Efforts by seed companies and private individuals resulted in cultivation of the first varieties in the late 1970s and early 1980s (Wood, 1989).

Quinoa was first grown commercially in the early 1980s, when efforts by a joint partnership between Sierra Blanca Associates and Colorado State University led to its introduction and cultivation within the United States of America (Johnson, 1990). Research continued at Colorado State University until 2003, and has been conducted at Brigham Young University since 1988 (Research History, 2012; S. Ward, personal communication, 31 May 2013).

In North America, quinoa remains a niche crop limited to specific geographic areas. Currently, the greatest area of quinoa cultivation in North America is in the Prairie Provinces of Canada, where cultivation became established in the early 1990s through the Northern Quinoa Association and later the Northern Quinoa Corporation (M. Dutcheshen, personal communication, 28 May 2013). Quinoa also continues to be grown on 20–40 ha in the San Luis Valley of Colorado (J. McCamant, personal communication, 19 May 2013), with cultivated area peaking in 1992 (Tobin, 1995). Quinoa was also grown successfully in northern Washington State in the late 1980s and early 1990s (J. Marcille, personal communication, 19 May 2013). Ward (1994) records quinoa cultivation over 500 ha in southern Colorado, Wyoming, and in northern New Mexico.

In recent years, interest in quinoa as a food has grown rapidly and there has been renewed interest in quinoa as a crop for North America. Starting in 2010, Washington State University began quinoa research, conducting agronomic studies and initiating a breeding programme. A partnership was formed between Washington State University (WSU), Brigham Young University (BYU), Oregon State University and Utah State University to collaborate on quinoa variety development for the western United States of America.

Challenges and Research Opportunities

Heat Susceptibility

The largest barrier to quinoa in North America is excessively high temperatures during the growing season. Quinoa has high heat susceptibility which can cause pollen sterility and dormancy at temperatures exceeding 35°C (Johnson and Croissant, 1985). Seed fill was shown to be affected by high temperatures, particularly when combined with the long photoperiod common to temperate latitudes (Bertero *et al*., 1999). Long photoperiods also have an effect on pollen viability (D. Bertero, personal communication, 16 Oct. 2013). Varieties originating from southern and central Chile were found to have the greatest heat tolerance, and were the best performing in trials in Washington State and Colorado (Johnson, 1990, unpublished data). This limits the range of quinoa germplasm available to North American growers. Given that many desirable traits, such as larger seed size and lack of saponins, are found in non-Chilean ecotypes of quinoa, breeding will be necessary to introgress these valuable traits into a more heat-tolerant Chilean background.

While Chilean quinoa has a high level of heat tolerance, it is still inadequate for areas experiencing temperatures above 35°C. Furthermore, the projected increase in heat waves due to global climate change threatens to shrink the area suitable for quinoa cultivation and increases the importance of introducing superior heat tolerance into quinoa (Meehl *et al*., 2007). Quinoa varieties identified with superior heat tolerance are being crossed in the WSU quinoa breeding programme with the goal of generating more heat-tolerant varieties. If genetic variation for heat tolerance within existing quinoa germplasm is insufficient, then *Chenopodium* spp. native to North America may provide an alternative source of heat tolerance. In the WSU quinoa breeding programme, crosses have been made between quinoa and *C. berlandieri* with the goal of introducing superior heat tolerance while selecting against problematic traits from the weedy parent, such as shattering and seed dormancy.

Little is known about the mechanisms of heat tolerance and susceptibility in quinoa, and there are, therefore, many opportunities for future research in this area. Pollen sterility, plant stunting and reabsorption of seed content have all been reported as detrimental response to high temperature (Johnson and Croissant, 1985; Bonifacio, 1995; Bertero *et al*., 1999). It is important to determine the relative effects on yield of pollen sterility and high temperatures at the different growth stages. If one particular life stage proves to be the limiting factor for yield, then that particular stage can be targeted for improvement.

Saponin Removal

Another great challenge is saponin removal. For quinoa to be marketable, the saponin coating found on many varieties of seed must first be removed. Saponins can be removed by abrading the seed using a grain dehuller or polisher, through washing, or by brushing (Darwinkel and Stølen, 1997; Johnson and Ward, 1993). The cost of establishing this infrastructure can be prohibitive for some farmers and it was a limiting factor for commercial quinoa production in Colorado (Johnson, 1990). Additionally, saponin dust created during removal from the seed can create health problems (Sweet, 1994). The development of saponin-free lines via introgression of a saponin-free allele (Ward, 2001) could facilitate quinoa's dissemination to new environments. However, saponin-free varieties may prove to be more susceptible to predation by birds.

Diseases

Given the wide range of environments across North America, many of the challenges faced by quinoa are specific to particular growing environments. Downy mildew (*Peronospora variabilis*), the most serious pathogen of quinoa, causes significant losses, reaching 99% under cultivation in South America (Danielsen *et al*., 2001). This pathogen has been reported in outdoor plantings in Pennsylvania and Washington State (Testen *et al*., 2012, unpublished data). Downy mildew may pose a significant problem for quinoa yields in locations with humid summers, such as the central and eastern United States of America and southeastern Canada. However, it has not posed a significant problem for growers in the San Luis Valley (J. McCamant, personal communication, 19 May 2013), and is unlikely to in the western part of the continent characterized by dry conditions throughout the growing season.

A recent report from Pennsylvania documented infection of quinoa by the pathogens *Passaloria dubia* (Riess) U. Braun and *Asochyta* sp. in field plantings (Testen *et al*., 2013a, b). The relative impact of these new pathogens remains to be determined.

Pre-harvesting Sprouting

Pre-harvest sprouting is a potential problem for areas with rainfall late in the growing season when seeds have begun to mature. This was a problem in variety evaluation trials in 2010 and 2013 held in western Washington State. Early rains in late summer resulted in pre-harvest sprouting in many varieties (unpublished data). Pre-harvest sprouting is a potential problem for locations on the west coast of North America, where the beginning of the seasonal rains may coincide with seed maturity. Additionally, locations with year-round precipitation could also face this problem. Such a problem has been seen in the Netherlands, where precipitation occurs year-round. Work in the Dutch quinoa breeding programme identified variability in sprouting susceptibility relative to the date of harvest, and this factor was successfully selected (Mastebroek and Limburg, 1997). Two varieties, 'QQ065' and '2WANT', were identified with significant dormancy to provide pre-harvest sprouting tolerance (Ceccato *et al*., 2011). Additionally, efforts by private quinoa breeder Frank Morton, in the Willamette Valley of Oregon, developed varieties with greater sprouting resistance. This indicates that pre-harvest sprouting in quinoa can be overcome and that resistance could be developed through the identification and improvement of existing tolerance within quinoa varieties. Alternatively, tolerance could be introgressed from the identified sources in accessions 'QQ065' and '2WANT'.

Weeds and Insects

Weeds and pest pressure are major challenges for quinoa cultivation. Little research has been done in North America on these issues. Closely related *Chenopodium* spp. are an important constraint when they are the main weed problem, given their similar growing habit and appearance to quinoa, particularly when young.

Reports from Colorado indicate a wide range of insect pests in farmers' fields shortly after the crop's introduction (Cranshaw *et al*., 1990)Chenopodium quinoa Willd. (Chenopodiaceae. Experience in Washington State indicates that *Lygus* spp. and *Hayhustria atriplicus* are the main two quinoa pests. Further research is needed in this area, particularly for *H. atriplicus*. Small honeydew particles produced by this pest are difficult to remove from harvested seed and are a significant post-harvest processing challenge (unpublished data).

Additionally, given quinoa's status as a relatively new crop, quinoa is likely to attract novel pests, particularly those that prey on related *Chenopodium* spp. – as has been seen in Europe, with the example of *Cassida nebulosa* and *Scrobipalpa atriplicella* (Sigsgaard *et al*., 2008).

Outcrossing

Crossing between quinoa and related species, such as *C. berlandieri*, may prove to be a challenge where maintaining varietal purity is important. Crosses between these species can be fertile, and crosses have been observed in Colorado, Oregon and Washington State (Wilson and Manhart, 1993; J. McCamant, personal communication, 19 May 2013; F. Morton, personal communication, 28 May 2013). Undesirable traits, such as black seed colour in a white-seeded variety, may pose a challenge to farmers looking to replant their seed. However, such crossings may also provide an opportunity, as native *Chenopodium* spp. may contain traits conferring greater environmental adaptation, such as increased heat tolerance or disease resistance. Such a spontaneous hybrid was developed into a variety by John McCamant at White Mountain Farm in Colorado (J. McCamant, personal communication, 19 May 2013).

Collaboration and Outreach

Farmer outreach constitutes a significant factor governing the success of the expansion of quinoa in North America. While farmers from many growing environments have expressed great interest in quinoa, successful cultivation is difficult due to the lack of infrastructure and bulk sources of proper seed. Often, seed sold as food is planted instead of the more adapted Chilean varieties, resulting in little or no yield.

The production and dissemination of more detailed and regionally specific growing guidelines will help foster successful expansion of quinoa. Seed of properly adapted varieties must be made available in bulk to growers. Currently, superior adapted Chilean varieties are only available in small quantities. Making this seed more readily available should be given high priority, as planting imported quinoa sold as food may introduce new strains of seed-borne quinoa diseases, such as downy mildew (*Peronospora variabilis*), which is present as oospores in the seed pericarp (Danielsen *et al*., 2004). *Peronospora variabilis* is heterothallic and has the potential for sexual reproduction if compatible mating types are present (Danielsen, 2001). The inadvertent introduction of new downy mildew strains could result in the establishment of mating populations in North America, if such populations are not already present. Risks also exist for the introduction of seed-associated pests such as *Eurysacca quinoae* (D. Bertero, personal communication, 16 Oct. 2013).

Once adapted cultivars are more readily available, farmers will generate valuable information as they become familiar with the challenges and agronomic practices that provide the best results for their particular environment. Much of the current knowledge of quinoa in North America has come from farmers and farmers working in collaboration with researchers. Such collaboration was vital to quinoa's successful establishment on the continent and will be key to the crop's further expansion.

Research and History

Early History

Sporadic accounts of quinoa trials and experiments date back to before research began in earnest in the 1980s in Colorado. Reports from Alaska indicate that the plant was investigated as early as 1900 (Caldwell, 2013). In 1948, quinoa's response to deficiencies of various nutrients was measured at the University of Arizona (Larrabure, 1948). Later, Torres (1955) investigated the tolerance of quinoa to salinity and sodicity. References in the 1950s suggest that the crop had been grown under field conditions with little success (Eiselen, 1956). In 1968, a thesis was written examining quinoa's growth response to temperature and oxygen tension (Aguilar, 1968). Around this time, work was carried out on the relationship between quinoa and huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) based on morphology and their ability to form hybrids (Nelson, 1968; Heiser Jr and Nelson, 1974).

Reports from the late 1970s and early 1980s suggest successful field plantings of quinoa. As early as 1978, a variety collected by Gabriel Howearth was found to set seed in southern Oregon. By 1980, quinoa varieties were being grown and offered by a seed company in western Washington State (Wood, 1989).

Colorado State University

The largest and most significant study on quinoa agronomy began in Colorado in the early 1980s. A partnership was formed between Colorado State University and the no-profit Sierra Blanca Associates (Johnson, 1990). Germplasm collection and variety testing were undertaken by Stephen Gorad and Dave Cusack of Sierra Blanca Associates, and quinoa was grown successfully in 1982 and 1983 (J. McCamant, personal communication, 19 May 2013). Variety testing was undertaken in several locations in 1984, and southern Bolivian and Chilean varieties were identified as the best adapted. Experiments were undertaken on nitrogen requirements, irrigation and plant density. The details of these experiments are not forthcoming, but recommendations based on the results are available (Johnson, 1990). One variety, 'Colorado 407', was released at this time. It represented a selection from a Chilean variety collected in Linares in central Chile (J. McCamant, personal communication, 19 May 2013).

Commercial production of quinoa began in 1987, when saponin removal equipment was received from the Pillsbury Company (Johnson, 1990). Removal of bitter saponins allowed quinoa to be marketed on a large scale. A short-lived North American Quinoa Producers Association was formed in 1988. However, after a boom in the late 1980s and early 1990s, quinoa production declined due to lack of profitability and has since remained relatively limited (J. McCamant, personal communication, 19 May 2013).

A study investigating the irrigation requirements of quinoa in the San Luis Valley was conducted in 1987 and 1988 (Flynn, 1990). *In vitro* callus production in quinoa was conducted by Tamulonis (1989), with the goal of developing male sterile lines for hybrid production. Researcher Dr Sarah Ward investigated the use of cytoplasmic male sterility in quinoa with the aim of generating saponin-free hybrid quinoa with improved yields. Three sources of cytoplasmic male sterility were identified in varieties 'Amachuma', 'Apelawa' and 'PI 510536'. The usefulness of 'Amachuma' in hybrid production was limited by the lack of exerted stigmas (Ward, 1991). 'Apelawa' lacked readily available sources of fertility restoration genes, while accession 'PI 510536' was found to have exertable stigmas combined with readily available fertility restoration genes (Ward, 1994).

In 1997, there was controversy over a patent placed on a source cytoplasmic male sterility found in the cultivar 'Apelawa'. The patent covered hybrids derived using CMS from 'Apelawa' and any hybrids created derived from this source of CMS. The patent was formally protested by Rural Advancement Foundation International (RAFI) and *Asociación Nacional de Productores de Quinua* (ANAPQUI). The dispute came to a close when the patent lapsed in 1998.

Alternative methods for saponin removal were investigated (Sweet, 1994). In 2002, levels of heterosis between varieties were determined using male sterile lines (Watson, 2002). Research continued at Colorado State University until 2003. During the course of the programme, there was collaboration with programmes in Bolivia and Ecuador, and collaboration and germplasm exchange with European quinoa programmes. Seeds and guidance were provided to quinoa programmes in Nepal, Mongolia and China (S. Ward, personal communication, 31 May 2013).

Texas A&M University

Dr Hugh Wilson conducted extensive research on the relationship among quinoa ecotypes and between species in the genus *Chenopodium*. Wilson surveyed quinoa in Argentina and in central and southern Chile and collected important germplasm from these areas. Chilean lowland quinoa was determined to be conspecific with Andean types through isozyme analysis (Wilson, 1978). Later, through the use of controlled crosses, it was determined that quinoa and the Mexican chenopod domesticate (*Chenopodium berlandieri* subsp. *nuttalliae*) were separate species. Of particular note was quinoa's ability to form partially self-fertile F_1s with *C. berlandieri* var. *zschackei.* (Wilson and Heiser Jr, 1979). In a later experiment involving interspecific crosses within *Chenopodium*, quinoa formed hybrids with *C. berlandieri* var. *nuttalliae*, *C. berlandieri* var. *berlandieri* and *C. bushianum* (Wilson, 1980). Later work focused on analysing the diversity within the South American tetraploid *Chenopodium* and quinoa through the use of isozymes and morphological analysis. Weedy type quinoas were found to be conspecific with cultivated quinoa (Wilson, 1988a). Among cultivated populations, quinoa from the southern Altiplano contained the greatest level of diversity. Andean and Coastal Chilean populations of quinoa were found to be distinctive groups (Wilson, 1988b; Wilson, 1988c).

Brigham Young University

Extensive research on quinoa has been undertaken at BYU, primarily in the area of quinoa genetics. Researchers Dr Jeff Maughan and Dr Eric Jellen are currently leading work in this area. A recent publication synthesized much of their work on the genus *Chenopodium*, its taxonomy, and the potential genetic resources available in the genus for improving quinoa (Jellen *et al*., 2011). An overview of the available biotechnology tools (many of which developed at BYU) and their potential contribution for quinoa improvement is provided by Jellen *et al*. (2013).

The earliest published research focused on mineral and protein characterization of 162 quinoa accessions (Burgener, 1992) and quinoa's suitability as a broiler feed (Improta, 1993). The potential of interspecific and intergeneric crosses with *Chenopodium berlandieri*, *C. berlandieri* subsp. *nuttalliae* and *Atriplex* spp. was investigated by Alejandro Bonifacio. In one of the earliest deployments of molecular markers in quinoa, RAPD markers were developed and used to screen for successful hybrids (Bonifacio, 1995; Bonifacio, 2004).

A large number of molecular markers have been developed by BYU researchers. Maughan *et al*. (2004) developed AFLP markers, and this was followed by the development of microsatellite markers (Mason *et al*., 2005; Jarvis *et al*., 2008). Both AFLP markers and microsatellite markers were deployed in characterizing the genetic diversity of quinoa accessions in the USDA germplasm database and in the CIP-FAO international nursery (Pratt, 2003; Christensen *et al*., 2007). SNP markers were developed from an EST library developed by Coles *et al*. (2005). More recently, KASPar genotyping technology was used by Maughan *et al*. (2012) to generate 14 178 putative SNPs and 511 SNP assays.

Several genetic libraries have been created. An EST library using immature seed and flower tissue was generated by Coles *et al*. (2005). Later, Reynolds (2009) constructed an EST library from seed tissue of a bitter saponin-producing quinoa variety. A custom microarray was developed and used to measure transcriptional differences between sweet and bitter quinoa varieties. Candidate genes potentially responsible for saponin biosynthesis in quinoa were also identified. A BAC library was constructed by Stevens *et al*. (2006) using *Eco*RI and *Bam*HI restriction enzymes and was probed for seed protein storage genes. Maughan *et al*. (2009) used this library to extensively characterize *SOS1* homologs in quinoa.

Quantitative expression of particular genes of interest has been investigated. The gene coding for 11S globulin seed storage protein, thought to contribute to the amino acid balance of quinoa protein, was investigated by Balzotti *et al*. (2008) using RT-PCR. Accumulation of the 11S globulin seed protein was measured using SDS-PAGE. Morales (2011) quantified expression of *SOS1*, *NHX1* and *TIP2* genes thought to potentially mediate salt tolerance, using RT-PCR on root and leaf tissue of different quinoa ecotypes. Ricks (2005) attempted to find a marker linked to the bitter saponin locus and uncovered a marker 9.4 cM distant from it. However, no completely linked markers were identified (Reynolds, 2009).

Research has also focused on the evolutionary origins of quinoa and its relatedness to other species within *Chenopodium*. Maughan *et al*. (2006) determined sequence variation of intergenic spacers in the nucleolus region and of 5s rRNA spacers. Fluorescence *in situ* hybridization was used to quantify the number of 45s and 5s rRNA loci in quinoa (*Chenopodium berlandieri* var. *zschackei* and *C. berlandieri* subsp. *nuttalliae*). The results indicate that quinoa and *C. berlandieri* share a common diploid ancestor. Sederburg (2008) used FISH to examine the 5s and 45s RNA gene loci in New World *Chenopodium* spp. No definitive ancestors to quinoa were located, but a few potential ancestral species were determined. Later, Kolano *et al*. (2011) used FISH for the DNA clone 18-24J on *C. quinoa* and a range of other *Chenopodium* spp. Results indicate that *C. quinoa*, *C. berlandieri* and hexaploid *C. album* share a common ancestor.

Downy mildew, the major pathogen of quinoa, has been studied by BYU researchers. Swenson (2006) examined the genetic diversity of Bolivian strains of downy mildew (*Peronospora variabilis*) collected across Bolivia in 2005 and 2006. Using AFLP markers, a high level of genetic diversity was found within the species. Quinoa resistance to the pathogen was found to be generally dominantly inherited. Kitz (2008) developed an inoculation method for downy mildew and characterized its infection pattern using scanning electron microscopy.

Washington State University

Work on quinoa at Washington State University began in 2010 with observational trials across Washington State. A total of 44 quinoa accessions from the National Plant Germplasm System were tested at three locations representing climatic conditions ranging from maritime to semi-arid. Varieties of Chilean lowland origin were among the best performing varieties. At the Evergreen State College Organic Farm in Olympia, Washington, a location characterized by an oceanic climate with high rainfall, pre-harvesting sprouting due to early rains was problematic. Lygus bugs and aphids were identified as common pests (unpublished data).

The author conducted a greenhouse experiment examining salinity tolerance among four quinoa cultivars of Chilean origin. Quinoa's unparalleled salinity tolerance is of great interest for areas facing soil salinization problems. Knowledge about variability in salinity tolerance and the mechanisms behind it have been extensively explored in recent years, and this subject is analysed in detail in chapter 2.3. As many regions in the United States of America with soil salinity problems experience high summer temperatures, investigation into the salinity tolerance of the more heat-tolerant Chilean varieties is important. Salinity tolerance significantly differed between varieties originating from different latitudes within Chile, reflecting existing research indicating a latitude gradient in salinity tolerance (Ruiz-Carrasco *et al*., 2011). Additionally, salinity tolerance differed when sodium chloride and sodium sulphate were applied, with sodium sulphate having a less detrimental effect on varieties (Peterson, 2012). As both chloride and sulphate salts are problematic salts over large areas in North America, any difference in tolerance between the two salts is of great importance.

In 2011, the author conducted a field experiment investigating the yield response of 16 quinoa varieties to nitrogen fertilization. High summer temperatures acted as a confounding factor and no meaningful data on nitrogen response were obtained. However, important information on varietal heat tolerance was attained.

Collaboration began with BYU, and the following year, approximately 700 lines developed as mapping populations were grown out at Washington State University for extensive phenotyping. Traits examined include successful seed set, height, date of flowering, plant colour, and downy mildew resistance (Walters, unpublished).

Additional Research

In addition to the work by CSU, BYU and WSU, quinoa has been tested at other locations in North America. The earliest of these attempts was in Alaska over a century ago (Caldwell, 2013). In 1985 and 1986, quinoa varieties from Colorado were grown in trials at the University of Idaho. However, the crop was a failure due to the late maturity of the varieties grown (Kephart *et al*., 1990). Quinoa trials in Minnesota in the late 1980s and early 1990s also resulted in crop failure, blamed on high temperatures (Robinson, 1986; Oelke *et al*., 1992). Poor results were also obtained in trials in North Dakota, and insects were cited as the major concern (Berti and Schneiter, 1993).

Mixed results were observed in Virginia. British quinoa varieties successfully set seed in 1992, but failed to do so the following year due to higher temperatures (Bhardwaj *et al*., 1996).

More recently, quinoa was tested in northeast America. In contrast to failures in the Midwest and the South, quinoa performed relatively well under challenging conditions in western Maine in 2002. Eight quinoa varieties from Chile, Bolivia and Colorado were grown (Conant, 2002). In contrast, a trial in 2012 in western New York on four lowland quinoa varieties exhibited low seed set under abnormally high temperatures (Dyck, 2012).

A range of non-agronomic studies on quinoa have been conducted at various institutions, including research into quinoa pathogens at Pennsylvania State University (Testen *et al*., 2012, 2013a and b). Archaeological work has investigated the domestication of quinoa: *Chenopodium* seeds excavated from Chiripa, Bolivia, revealed seeds of both quinoa and its weedy form, suggesting both were harvested together; however, by 800 B.C., almost all *Chenopodium* seeds were of quinoa, suggesting selection against the weedy form (Bruno and Whitehead, 2003). More recently, a novel morphotype of domesticated *Chenopodium* was discovered during excavations in the Bolivian Altiplano (Langlie *et al*., 2011).

Researchers at Pennsylvania State University analysed chloroplast DNA from Mexican *Chenopodium* domesticates (*C. berlandieri* subsp. *nuttalliae*), wild North American tetraploid *Chenopodium* species, and the extinct eastern North American domesticate *C. berlandieri* subsp. *jonesianum*. Their results suggest independent domestication of Mexican and eastern North American *Chenopodium* (Kistler and Shapiro, 2011).

Work is currently underway at UW Madison on *Chenopodium* phylogenetics and on a comparison of sequences of *C. berlandieri* subsp. *jonesianum* and *C. berlandieri* subsp. *nuttalliae* (Walsh, pers. comm., 25 Oct).

Quinoa Breeding

Quinoa breeding work in North America has been limited. Colorado State University performed a selection on a Chilean landrace and released the cultivar 'Colorado 407' in 1987 (Johnson, 1990). Work continued on breeding male sterile lines at Colorado State University, examining the inheritance of saponins and male sterility (Ward, 1991 and 1994). However, since the release of 'Colorado 407', no additional varieties have been released by a public university. Members of Sierra Blanca Associates collected the original germplasm used in breeding efforts in Colorado. Much of this germplasm continues to be grown and selected at White Mountain Farm under the care of Paul New and early quinoa pioneer John McCamant (J. McCamant, personal communication, 19 May 2013).

A range of quinoa varieties have been maintained and bred by private seed companies. Most significantly, several varieties were bred by Frank Morton of Wild Garden Seed, selected for the growing conditions of the Willamette Valley of Oregon. The main breeding objectives are improved heat tolerance and *Lygus* sp. resistance. The original germplasm in this programme traces its origins to material from the Colorado breeding programme (F. Morton, personal communication, 28 May 2013).

Early quinoa pioneer and farmer John McCamant has continued on-farm variety selection and development with heat tolerance a major breeding goal. Spontaneous hybrids between quinoa and wild *Chenopodium* spp. were developed into a black-seeded quinoa cultivar (J. McCamant, personal communication, 19 May 2013).

The Northern Quinoa Corporation in Canada has conducted variety trials and breeding, developing varieties better adapted to Canadian conditions. The variety 'Norquin 94-815' is currently undergoing the process of registration (M. Dutcheshen, personal communication, 28 May 2013).

Work is ongoing at Washington State University to develop varieties with critically important traits such as improved heat tolerance, lack of saponins and pre-harvest sprouting tolerance. The aim of this programme is to develop better adapted cultivars for the Pacific and Mountain West, while tackling common challenges to quinoa cultivation found throughout temperate North America. Experimental crosses are also being conducted between quinoa and related *Chenopodium* spp.

Germplasm

A wide range of quinoa germplasm has been tested and grown for research and production purposes in North America. Given the recent history of quinoa in North America, much of this germplasm can be traced back to the place of collection and the collector.

Currently, the North Central Regional Plant Introduction Center at Ames, Iowa, part of the National Plant Germplasm System, holds the largest quinoa collection in the United States of America with 164 publicly available quinoa accessions and a wide range of *Chenopodium* spp. accessions. These accessions represent a wide range of quinoa germplasm of diverse geographic origin. David Brenner, curator of the *Chenopodium* collection, has played a crucial role in supplying much of the quinoa germplasm used in programmes in the United States of America and internationally.

Several large subcollections exist within the quinoa collection. The first of these to be donated was the subcollection donated by Dr Hugh Wilson. These accessions were collected at various locations in Chile and Argentina (Wilson, 1978), and include accessions with important traits such as heat tolerance and pre-harvest sprouting resistance. The second and largest subcollection was donated by the early quinoa researcher Emigdio Ballón of the Talavaya Center in northern New Mexico. This subcollection represents approximately a quarter of the GRIN quinoa collection and was developed following heat tolerance screening. High temperatures sterilized pollen from Andean types and resulted in their subsequent pollination from Chilean types (Bertero, 2013, personal communication, 16 Oct.). This is confirmed by Christensen *et al*. (2007), who either grouped Ballón accessions with the Chilean lowland accessions or found that they were genetically intermediate between lowland and highland accessions.

Most recently, Dr Sarah Ward contributed a subcollection of Bolivian accessions in 1992. Marisol Berti Díaz from the University of Concepcion donated a smaller subcollection of lowland Chilean varieties in 1994.

Several varieties originally collected from South America continue to be grown commercially by both farmers and private seed companies. In addition to these commercially available sources of germplasm, other instances of quinoa variety collection have been reported. John Marcille, a farmer who grew quinoa commercially in the northern part of Washington State for several years, grew seed collected independently by an associate in South America (J. Marcille, personal communication, 19 May 2013). The earliest reported instance of a quinoa variety successfully setting seed in North America is by Gabriel Howearth, who successfully grew quinoa in Southern Oregon in 1978 (Wood, 1989). The contribution of these introductions to the current pool of North American quinoa germplasm is unknown.

Current Range of Cultivation

At present, quinoa cultivation in North America is limited to two areas (Figure 1). The first area is the San Luis Valley, a high altitude valley in southern Colorado where quinoa was first introduced on a commercial scale. Here, quinoa cultivation is run by White Mountain Farm, where it is grown on 20–40 ha. In recent years, the cultivated area has expanded (J. McCamant, personal communication, 19 May 2013).

The largest-scale quinoa cultivation in North America covers 650 ha in the Canadian Prairies, and has been managed by the Northern Quinoa Corporation since 2005 (Alberta Agriculture, 2005). The crop is grown approximately 100 km north of the American border in Alberta, Saskatchewan, and Manitoba (M. Dutcheshen, personal communication, 28 May 2013).

Possible Areas of Expansion

Although it has been tested in many regions in North America, quinoa has had limited success outside of high altitude locations in the American Rockies and the Canadian Prairies. Given the limited area of current quinoa cultivation, there is still scope for expansion within these two regions.

Several other regions show promise for quinoa cultivation, in particular, high altitude locations outside the Rocky Mountains, and quinoa was successfully grown in the Okanogan Highlands of northern Washington State for several years (J. Marcille, personal communication, 19 May 2013). High altitude areas in the foothills of the Sierra Nevada in California and the Cascades in the Pacific Northwest may also provide suitable microclimates. Winter planting in the Central Valley and other mild locations in California could be a way of avoiding high summer temperatures.

The next major areas identified for expansion are the coastal lowlands of the Pacific Northwest. However, pre-harvest sprouting is a challenge due to the relatively late maturity of quinoa. Additionally, for some parts of the Willamette Valley of Oregon and southwest Washington, high summer temperatures may be a problem.

Quinoa could expand into areas of the northern Great Plains and the Midwest if issues of heat tolerance, insect pressure and downy mildew are addressed. One of the few regions outside the western United States reported to successfully grow quinoa is western Maine (Conant, 2002). The northeast, Atlantic Canada, southeast Ontario and southern Quebec may be promising regions for further development.

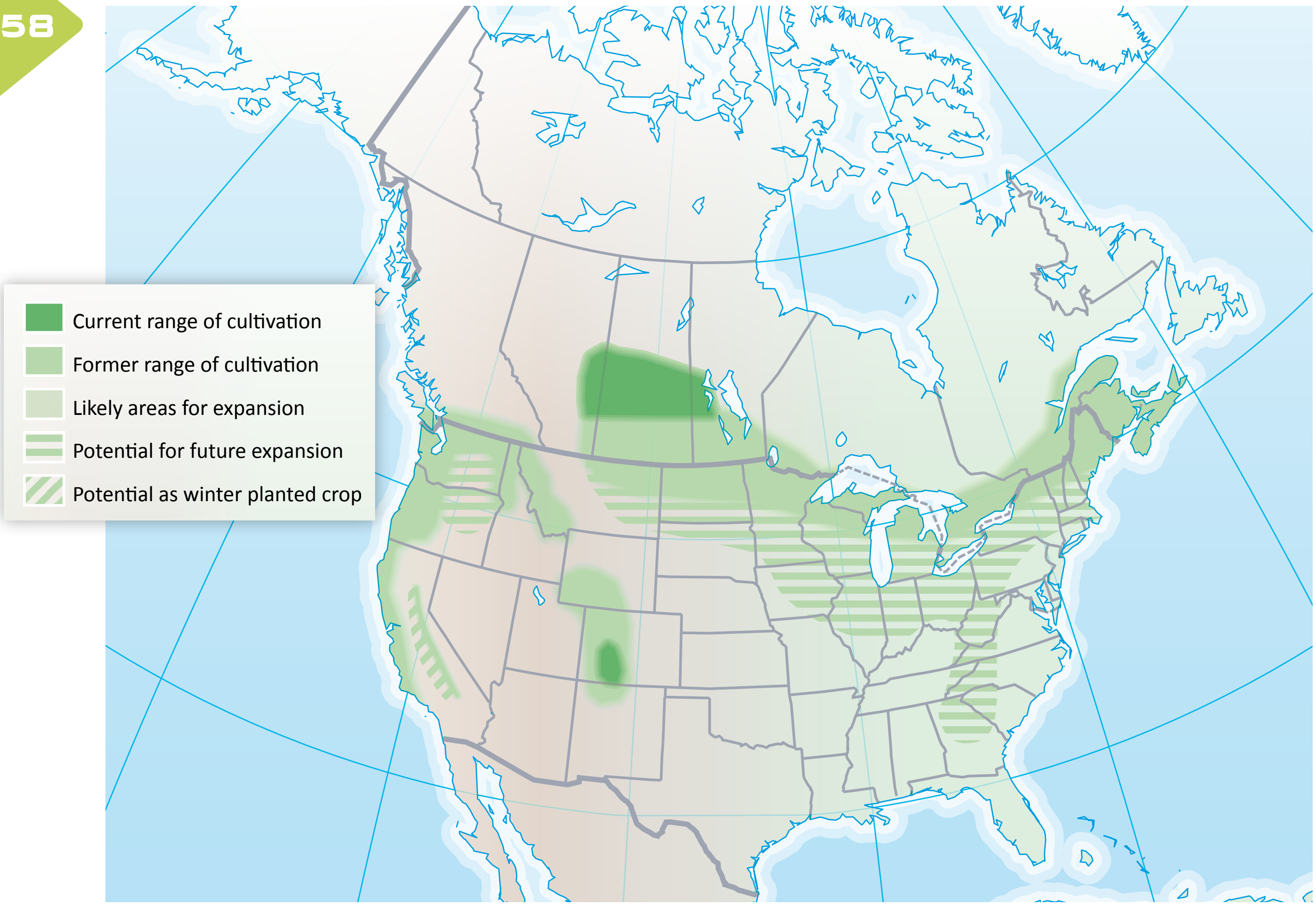

Figure 1: Areas of quinoa cultivation in the United States of America

Economics and Current Market

There have been few detailed studies of the North American quinoa market as a whole. However, a study of quinoa market dynamics is currently underway at WSU. The predominant market dynamic relies on major imports from South America, with a small contribution from domestic production. In 2005, estimated quinoa consumption in the United States of America and Canada was 3 000 tonnes (Alberta Agriculture, 2005).

In recent years, there has been a rising demand for more locally grown foods, including quinoa. There has also been increased concern for the socio-economic consequences of rising quinoa demand (Romero and Shahriari, 2011). Locally produced quinoa remains a large unfilled niche in the North American market, and both farmers and distributors have expressed great interest in meeting this unmet demand.

In both the San Luis Valley and the Canadian Prairies, quinoa has been reported to give greater profitability in recent years (J. McCamant, personal communication, 19 May 2013; J. Dutcheson, personal communication, 17 May 2013).

There are limited data available on the relative costs and returns for Colorado quinoa farmers beyond the initial years of cultivation. However, there is indication that production costs decreased as infrastructure improved and agronomic practices were refined (Johnson, 1990).

The most extensive economic analysis was conducted by Alberta Agriculture and Rural Development, which focused on current production of Canadian quinoa and considered the crop's economic viability. Quinoa was found to be more cost-effective than wheat, broad bean and canola. Quinoa production was more cost-effective in Canada than in the United States of America, but not as cost-effective as in South America. At the time of the study, quinoa imports were cheaper than domestic products, but domestic production was nevertheless identified as profitable. The major constraints were high risk and variable yields, and improved agronomy was identified as necessary (Alberta Agriculture, 2005).

Conclusion

Despite the initial challenges faced in establishing quinoa production in North America, quinoa continues to be grown over a significant area three decades after its introduction as a commercial crop. Although obstacles exist in terms of production and environmental challenges, there are identifiable routes to overcome these problems. Increased investigation into quinoa agronomy can boost production in growing environments where quinoa is currently most adapted. Additionally, breeding to develop new varieties can result in increased yields, and the introduction and strengthening of valuable traits, such as heat tolerance, can facilitate quinoa's expansion to new areas.

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Chapter: 6.4.2

Advances and Challenges for Quinoa Production and Utilization in Brazil

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Abstract

Brazil has become a major grain producer, favoured by the climatic conditions and by technologies for acid soil amendment and crop improvement in the savannah. The first attempts to introduce quinoa as a second crop began in the 1990s, when breeding lines from hybrids among day-length responsive, Andean Altiplano cultivars were evaluated. These progenies yielded more than in their centre of origin, and had small seeds and high saponin content. In view of the potential of quinoa, the germplasm collection was enlarged by introducing a wide range of variability from the Andean countries and the United States of America, including saponin-free, large-seed valley accessions. A high cross-pollination rate in the savannahs allowed hybrid recovery from which single plant progenies were derived, tested in uniform trials and evaluated for nutritional quality. These efforts resulted in the cultivars, 'BRS Piabiru' and 'BRS Syetetuba', in 2000 and triggered the interest in quinoa in Brazil. Outstanding genotypes have been hybridized to study the genetic inheritance of plant type, seed size and yield, and organic and mineral components to improve selection efficiency. Quinoa cropping has been limited by its intrinsic seed quality, i.e. becoming unviable in the short term and rapidly deteriorating in the field under high moisture and temperature. Enriched food has now been developed following pioneer experimentation, although most of the grain and by-products are imported, due to limited local supply. Unlike major crops, quinoa has received little support for research and development, making it difficult to make advances. However, the achievements reported herein may represent an opportunity for public institutions and universities to collaborate and perfect the relative technology. Producers potentially involved in quinoa commercial cropping are large to medium-sized mechanized and family farmers; they adopt two different approaches, and family farmers are more geared towards organic production. With increasing world demand, Brazil's contribution is expected to increase in the next 20 years, reducing the pressure on Bolivia and Peru, where quinoa has become the sole grain crop export, depriving the local populations of this valuable ancestral food.

1. The role of quinoa in the Brazilian savannah agriculture

The natural environment of the Brazilian savannah, although restricted by soil and climate conditions, shows no incidence of pests and epidemics. The savannah is inhabited by numerous indigenous living species, which occupy small niches in great numbers – typical of the tropics. This results in discontinuity and increases the distance between plants and animals of the same species, ensuring a good population balance among organisms (Spehar, 2007).

The soils are acidic, devoid of nutrients and in need of amendments for use in agriculture, while the climate is rainy in spring/summer and dry in autumn/winter. There is surplus water in the rainy season, and a deficit during the 6-month dry season (Spehar and Rocha, 2011).

The main chemical soil problems have been solved by experimentation and research. Liming and fertilization with P, K and micronutrients have turned the savannah into a major agricultural land, where the soil is dependent on chemical and physical improvement and soybean is adapted to tropical low latitudes (Spehar *et al*., 2011).

However, since the occupation of the savannah, plant sanitary and soil management problems have increased. In general, the land tenure is shaped by large farming areas, covered with a small number of highly specialized crops, with soybean and hybrid maize predominating. Given the narrow genetic background of these crops, repeated monocropping has become vulnerable to serious pests and diseases (Czepak *et al*., 2013). Continuous monoculture causes phytosanitary problems and a major negative environmental impact, compromising the future of agriculture and cancelling the technological gains barely made in the last 40 years (Spehar and Trecenti, 2011).

In order to develop and reach stability, production systems in such environments need to imitate, as closely as possible, the diversity found in nature. The lack of temperature limitation for plant growth means that quinoa can be developed for use in rotation, succession and association with other crops (Spehar *et al*., 2011). A combination of species with varied botanical constitution could mitigate the negative biological impacts of year-round plant growth. However, diversification is still only practised on a small scale, and is not sufficient for sustainable exploitation.

Introduction of zero tillage leads to more efficient sowing and better utilization of moisture. The planting schedule is maintained and crop yield potential accomplished, leaving for a second crop that both protects the soil and increases income prospects (Spehar *et al*., 1997; Spehar and Trecenti, 2011); a third crop may even be grown in the irrigated dry season. This represents an opportunity for quinoa to innovate cropping systems.

Interest in quinoa's potential to improve production systems and increase food quality has grown in Brazil, following pioneer studies in the savannah environment, and its cultivation has been extended to other regions of the country – the direct result of research and development projects aimed at crop plant adaptation for grain production, soil protection and diversification of the agricultural system (Spehar, 2007).

Quinoa was first introduced as a follow-up crop in the savannah, adopting late summer sowing and using residual moisture from the rainy season (Spehar and Souza, 1993). In addition to its high nutritional value, quinoa also has mechanisms for drought tolerance, useful in a cropping sequence (Jacobsen *et al*., 1998). After 20 years of continuous efforts in genetics, breeding, crop husbandry and soil management, results are promising. Interaction between agronomic and food research has led to the identification of new and strategic uses for quinoa, and the relevant results are reported here.

The crop has a short but interesting history in Brazil, and the production chain is being developed as demand increases. To stimulate farmers and other participants in the chain, emphasis has been placed on the utilization and not only the agronomic synergetic function of quinoa. Using quinoa in diversification helps create opportunities for farming and food quality improvement. Diversified production systems need to be biologically and economically sustainable.

2. Historical perspective of research and development and disciplinary interactions to promote quinoa in Brazil

Until the first half of the last century, quinoa cultivation was limited to the Andean mountains of South America, where it was carried out as subsistence farming in its centre of origin and domestication. There were no reports of its growth or use in Brazil at that time. The reduced importance of quinoa for food outside its region of origin was put down to the rural exodus and urban dwellers' disdain for indigenous food crops (Risi Carbone, 1986). However, in the mid-1970s, there was the opportunity to realize the value of quinoa as a potential crop in Brazil, based on findings about the nutritional composition of its grain protein relative to cereals (Spehar, 1976).

The last 20–30 years have seen a rising interest in quinoa for high nutritional value: high protein content, better balance of essential amino acids compared with cereals, and its high content of minerals, vitamins and other organic compounds (Borges *et al*., 2010; Ascheri *et al*., 2002). These properties have made the grain and its by-products popular among people seeking alternative, low-cholesterol inducing food (Spehar and Santos, 2002). Worldwide demand has created a prosperous market, stimulating the cultivation of quinoa for export in the Andean countries, in particular in Bolivia and Peru (Bonifacio, 1999).

Quinoa was first introduced in Brazil in the early 1990s with the objective of selecting new options for savannah agricultural systems. Efforts were directed to local progeny selection of individual plants in segregating populations from hybrids generated in the University of Cambridge, United Kingdom (Spehar and Souza, 1993). These progenies were preliminarily evaluated in the savannah highlands and grouped according to their maturity – early, mid-cycle or late (Spehar and Santos, 2005). By testing across locations for yield and agronomic stability, it was possible to select high-yielding lines, classified by maturity, seed size and saponin content.

In 1996, the best-performing lines were included in demonstration plots with the support of the Savannah No-till Association (APDC), Goiânia, GO, Brazil. It was the first contact between the novel grain crop and the public (Spehar *et al*., 1997). Food preparation using quinoa grains during promotional events brought the crop to the attention of people related to agriculture and food. Similar media events in subsequent years then brought quinoa to be known all over the country. The demand for quinoa grew as its value in terms of both agricultural diversification and food quality was demonstrated, and there was increasing innovation on both fronts.

Complementary research on breeding, plant husbandry, mineral nutrition and sowing dates contributed to develop the agronomical aspects of quinoa and promote its adoption by producers (Spehar, 2007). Grain composition was studied and food products developed. In general, these actions were jointly conducted, involving the Brazilian Organization for Agricultural Research and Development (Embrapa), University of Brasilia (UnB), and other research and extension partners. Joint efforts involved guidance to university graduate and postgraduate students, resulting in cultivar acquisition (Spehar and Santos, 2002; Spehar and Rocha, 2009).

Quinoa was introduced as a second crop (after soybean or maize) in no-till cultivation aimed at soil protection. The grain produced was used in the food and ration industry (Ascheri *et al*., 2002; Spehar, 2002), and the whole plant could be employed as silage for livestock (Spehar and Santos, 2002). The search for uses and applications of quinoa has contributed to increased interest in research and development investment and quinoa is ready to be inserted into Brazilian agriculture.

Considerable biomass is produced by the quinoa plant, creating the opportunity for inclusion in no-till systems to protect the soil (Spehar, 2009; Spehar and Lara Cabezas, 2000). Moreover, it has been shown that selected genotypes are adaptable to fully mechanized cropping, and the estimated production costs and operational income ensure a competitive profit margin (Spehar, 2007).

All the research has resulted in a pool of technologies that have been promoted to initiate commercial production. Embrapa organized a seed distribution scheme which led to the first experience of quinoa farming in Brazil. Farmers' interest has increased, despite the fact that production has not yet been accomplished at commercial level for reasons presented below.

3. Genetic resources and advances in selection

Quinoa's selection and release for cultivation is a recent event in Brazil (Spehar and Souza, 1993; Spehar, 2007; Spehar and Rocha, 2010). In cultivar acquisition, plant introduction continues, followed by selection in segregating populations. In the early 1990s, new accessions carrying large grains were introduced from the United States of America germplasm collection in Iowa. These were previously screened in a controlled environment and grown in the field in Planaltina, DF, located in the savannah highlands. Most accessions segregated and were subject to individual plant selection, originating new progenies and enlarging the existing collection.

Selected progenies originating from valley types are more likely to yield promising commercial cultivars in the savannahs (Spehar and Rocha, 2010). Evaluation of Altiplano, valley and locally selected types cultivated in the savannah highlands showed that, at the same latitude (15–18°S), Altiplano are early (80–100 days), valley are mid-cycle (120 days), and the local types take 150 days from emergence to maturity (Santos, 1996; Spehar and Rocha, 2010; Spehar *et al*., 2011). In contrast, the savannah early-maturing types, when grown in the high altitude Andean plains, increase considerably the number of days to maturity and this is directly related to low mean temperature (Mujica-Sanchez *et al*., 2001).

Quinoa has been defined as short-day plant, based on its response to photoperiod (Risi Carbone, 1986). Accessions from valleys, however, differ in response compared with high altitude accessions (Bertero, 2001). In tropical regions, where there is no frost, quinoa can be cultivated all year, depending on water availability for plant growth and reproduction. In the savannahs, quinoa grows from rainfed late summer to irrigated autumn/winter (Rocha, 2007).

The savannah lands are located at 800–1 200 m asl, where quinoa has been studied as a second crop in late summer, autumn and winter (Spehar, 2007; Spehar and Rocha, 2010). Plant growth and architecture have been used in selection for mechanized cropping and high-yielding performance, irrespective of the sowing season. Crop feasibility was demonstrated by early research on agronomic performance, and selection has profited from the high variability within genotypes for number of days to maturity (Spehar, 2007) originating from natural hybridization (Spehar, 2001; Spehar *et al*., 2011).

The genetics of maturity must be understood in order to help selection and characterize germplasm (Spehar, 2007). Breeding strategies must aim at high-yielding genotypes of different maturity groups, producing relatively large, saponin-free grains, with compact inflorescence, absence of lodging and dehiscence (Rocha, 2011).

With regards to these characters, great advances have been made in the last 20 years (Spehar and Rocha, 2009; Spehar and Santos, 2002). Late-maturing, saponin-free and small grain genotypes and cultivars contrast with the mid-cycle, larger grains acquired during selection. However, there are limitations on desirable plant characteristics, justifying continued investment in selection. The objective is to obtain genotypes with 100–120 days from emergence to maturity, long reproductive phase and high-yielding grains possessing the quality and size demanded by the market. These types suit no-till, double-cropping in many regions of the country, and reach good market value (Spehar, 2009).

Research on genetic differences for maturity, grain size and yield has been conducted, providing information to guide quinoa breeding (Rocha, 2011). The first recommended cultivar, 'BRS Piabiru', takes 145–150 days from emergence to physiological maturity (Spehar and Santos, 2002), whereas 'BRS Syetetuba', selected from the same genetic background, matures within 120 days. In the savannahs, the two cultivars present similar performance irrespective of sowing date, whether in summer or autumn/winter. Although, in higher latitude, cool winter areas, the number of days to maturity increased at low temperatures (Spehar *et al*., 2011; Vasconcelos *et al*., 2012). Knowledge about inheritance for days to maturity is required to understand its direct relation to maturity and grain yield (Santos, 1996). Other selection characters include extended reproductive phase and seed quality. The former could be a limiting factor for quinoa production in the tropics (Souza, 2013).

Observation in progeny outcome, occurring from natural crosses, has led to hypothesize that lateness is a recessive trait. It has been observed that early-maturing plants, when self-pollinated from segregating populations, show late types in their offspring (Spehar, 2001). It could be argued that low fitness mutants occurring in the environment of quinoa origin may have survived thanks to partial allogamy, and they express themselves in the warm temperature Andean valleys.

Varying rates of cross-pollination have been reported in different environments and related to floral biology and pollinators (Lescano, 1980; Rea, 1969; Spehar, 2001). In Brazil, the most frequent pollinating agent is *Apis mellifera* bee, although other indigenous bees have been associated with quinoa, visiting the flowers in high numbers at anthesis (Rocha, 2011). Data on cross-pollination in the savannahs indicate an average of 15% hybrid seeds between varieties grown adjacently and simultaneously, in terms of saponin, stem colour, inflorescence type and maturity (Spehar, 2001). In commercial cropping areas, however, at the pollen release stage, bee populations were scarce in contrast to the density of 10–20/m^2/hour in insecticide-free areas (Rocha, 2011).

As part of the effort to define the genetic factors conditioning grain yield related traits (e.g. days from first flower to maturity), experiments were conducted in the savannahs (Spehar and Santos, 2005). The aim was to evaluate the genetic inheritance for number of days to maturity. It was calculated on the basis of the frequency of early and late types within F_2 hybrids, obtained by cross-pollination between early 'BRS Syetetuba' and late 'BRS Piabiru' quinoa genotypes. The data indicated the presence of two dominant alleles for earliness in this hybrid.

Selection of progeny per panicle and within progenies, generating families, was the first step in the improvement of quinoa. The procedure was utilized for acquiring pioneer varieties for low-altitude acidic-soil environments, a turning point in the agricultural diversification of the savannahs (Spehar and Souza, 1993; Spehar and Santos, 2005).

Segregating populations from hybrids among wide range quinoa varieties revealed considerable genetic differences in terms of: plant cycle (number of days between emergence and maturity); architecture (length, size, shape and colour of panicle); and grain size, colour and saponin content (Santos, 1996). Following successive progeny selection cycles, genotypes with phenotypic uniformity and adaptable to savannah improved soils have been obtained (Spehar and Santos, 2005).

Mass selection is one of the most widely used methods in handling recombinants from existing varieties and in standardization for desirable phenotypes (Mujica-Sanchez *et al*., 2001). The method *per se* has restrictions, one of which is reduced variability as a result of the endogenous process of fixing desirable characters. Progeny tests have shown that genetic gain is maximized when characters derive from crosses of local outstanding genotypes (Spehar, 2001; Spehar and Santos, 2002; Spehar and Rocha, 2011).

In temperate climates, the phenotype is a result of day-length and temperature conjugated effects, which determine flowering onset and maturation in quinoa (Bertero, 2001). Winter experiments in the savannah highlands gave genotypic records for maximum grain and biomass yields as 4.2 and 12.3 tonnes/ha, respectively, confirming the lack of response to day length for quinoa grown in mild climates (i.e. similar to the Andean valley varieties) (Bertero, 2001). This growth pattern is found in savannah-selected genotypes at low latitudes, irrespective of sowing date (Spehar and Santos, 2005).

The present collection maintained by Embrapa comprises original introductions and the recombinants selected from cross-pollination. Considerable variability in saponin content, seed colour and plant size, type and colour is found in genotypes, and a sample is presented (Figure 1). The characters are retrieved from selected individuals generating progenies, within which families are formed. To fix genetic purity, self-pollination of desirable individuals is practised. In total, over 1 000 different accessions were obtained, by combining desirable adaptability characters to the savannahs (Spehar, 2011). Further research is required to understand whether these genotypes represent the species diversity found in the centre of origin. However, these new genetic combinations may be useful in quinoa breeding programmes conducted in similar environments.

4. The Value of International Collaboration on Quinoa Research in Brazil

The first experiments with quinoa in Brazil were made possible thanks to the support of the University of Cambridge, United Kingdom, in 1988. A research project aimed at quinoa adaptation to the United Kingdom comprised hybridizations among cultivars that originated from a wide range of segregating populations (Risi Carbone and Galwey,

Figure 1. Sample of Brazilian quinoa genotype collection: plant and inflorescence types, with respective seed colour and size.

1984). Individual plant selections were made in the field, based on the assumption that extremely late maturity types at high latitude (Cambridge, 52°12'N) are adaptable to the tropics (Planaltina, DF, 15°36'S). Therefore, field-selected individuals originated progenies introduced and tested in the Brazilian savannah highlands. Following experimental evaluation in 1989, these progenies generated the first information on quinoa performance in the Brazilian savannahs (Santos, 1996; Spehar and Souza, 1993), thanks to the joint efforts of Embrapa and the University of Brasília. Little was known about quinoa and its value, making it difficult to prioritize the allocation of resources; early work on quinoa was conducted with great effort in the face of all sorts of difficulties.

The results of studies on quinoa in Brazil were presented during the IX *Congreso Internacional de Cultivos Andinos*, in Cuzco, Peru (UNSAAC, 1997), supported by the Food and Agriculture Organization of the United Nations (FAO). Contacts were made with researchers from Peru and Bolivia, some accessions were exchanged, and opportunities arose for collaboration between Embrapa, University of Brasília and universities and institutes in Peru and Bolivia to share information and accessions.

Subsequently, contacts were made with Latinreco, the research and development institute of Nestlé, Quito, Ecuador. Latinreco created opportunities for cooperative work, on the basis of the promising performance of quinoa in mechanized commercial production (Wahli, 1990). Accessions from these countries and institutions were introduced in Brazil to enrich the germplasm, and were revealed to be segregating population when evaluated in the field.

Under the auspices of FAO, during the Technical Meeting and Workshop to Formulate a Regional Project on Production and Human Nutrition based on Andean Crops, agreements were reached on the promotion of quinoa (Spehar, 1998). Relationships among institutions were formalized, creating opportunities for quinoa production to supply the growing demand.

Additionally, through a project supported by the International Potato Center (CIP) and the Danish International Development Agency (DANIDA), with the inclusion of a Brazilian partnership, it was agreed to improve and utilize quinoa production in the Andes and outside the region of origin, including in the Brazilian savannah (Jacobsen *et al*., 1998).

The collection was substantially increased with the support of the Germplasm Resources Information Network (GRIN), Ames, Iowa. Accessions possessing variable agronomic characteristics (e.g. large grains and absence of saponin) were acquired with considerable help from David Brenner (Spehar, 2007). GRIN has engaged in extensive exchange of information for the benefit of all in agriculture and food: an example of free exchange.

In addition, internships from Peruvian, French and German universities at graduate and post-graduate levels created new opportunities. Genotype comparative evaluations were performed, helping to select, generate information and add variability to existing germplasm. A wide range of genotypes were tested for their biological potential and limitations, indicating the lines of research for quinoa improvement in the savannahs.

5. Experimentation with Quinoa in Tropical Environment Under zero tillage

5.1 Initial experiments

5.1.1 Acquisition of adapted genotypes

Quinoa is responsive to day-length and temperature variations when grown in the temperate zone and these factors are genetically controlled (Risi Carbone, 1986). On the assumption that cultivation in the tropics would be based on selection for lateness, a similar approach to soybean was used (Spehar *et al*., 2012). Progenies from individual, late-maturing plants, selected in Cambridge, United Kingdom, were grown in field experiments at the Brazilian Savannah Research Centre, Embrapa Cerrados. Selections within progenies of originating families, additionally selected for phenotypic uniformity, underwent preliminary evaluation and were classified in maturity groups in 1990. A total of 64 progenies were tested in a partly balanced lattice design, with two repetitions. Each experimental plot was made of five rows equally spaced (0.20 m) with a population density of 500 10^3 plants/ha. The harvest comprised the three central rows, discarding 0.25 m at each end. The experiment was repeated for three sowing dates: spring/summer, summer/autumn and autumn/winter, on savannah fertilized soil, according to recommended technology (Santos *et al*., 2008).

The lines differed in number of days to maturity, plant height and yield, and the average yield was 2.4 tonnes/ha. Despite the small grains and the saponin content, the results were sufficiently promising to continue research on quinoa for ample adaptation in the savannahs (Spehar and Souza, 1993).

5.1.2 Agronomic evaluation

A total of 26 breeding lines, selected from individual plant progenies of hybrids among the cultivars 'Amarilla de Marangani', 'Blanca de Junín', 'Chewecca', 'Faro 4', 'Improved Baer', 'Kancolla', 'Real' and 'Salares-Roja', had their agronomic characters evaluated in Planaltina, DF, Brazil (15°36'S, 47°12'W, 1 005 m asl), in randomized complete blocks, on a Latosol (Ferralsol, according to FAO's classification) previously limed and fertilized.

Grain yield was positively associated with plant height, inflorescence length and diameter, and plant cycle. Genetic gain can be attained by selection based on these characters for commercial production of quinoa in tropical regions (Spehar and Santos, 2005).

The practice of zero tillage, which depends on soil cover, includes a small number of species from just two botanical families, i.e. maize, millet and sorghum (Poaceae), and soybean and common bean (Fabaceae). Quinoa was introduced in the savannah to utilize residual moisture in double-cropping, for mulch and grain production. Selected genotypes were grown in autumn, following the soybean harvest, in Planaltina, DF, Brazil (15°36'S, 47°12'W, 1 005 m asl). Experiments were conducted on randomized complete blocks and three replications under residual moisture and supplemental irrigation regimes. Grain yield for 'Q18' and 'Q24' was 2.2 tonnes/ha under irrigated conditions and 1.153 tonnes/ha under stress (Spehar and Santos, 2006).

Grain yield was positively associated with plant height and plant cycle. Early maturity genotypes had higher yields than late maturity under stress, with the exception of 'Q24', which yielded 56% of its respective performance with sufficient water supply. Selection for drought tolerance and vigorous growth, combined with early maturity in the main crop to be able to anticipate sowing, should have a positive impact on commercial cultivation of quinoa in the tropics (Spehar and Santos, 2006).

For agricultural systems based on soybean or maize grown in summer, weed management might be a problem for successive crops. The effect of herbicide residue on quinoa was studied in relation to herbicides recommended for major savannah crops. Treatments of trifluralin, pendimethalin, clomazone, imazaquin, trifluralin + imazaquin and control were applied to soybean, cv. 'BR 9 Savana' in summer cultivation. Soil samples were collected at 15, 38, 100, 145 and 206 days after herbicide application, and stored in the freezer at -5°C. Bioassays were conducted, sowing 'Q18' genotype in the greenhouse. Imazaquin proved the most damaging to quinoa seedlings, with residue still active 206 days after application, while with Clomazone the residual effect lasted 30 days. These results indicate the need for herbicide screening to select for effectiveness of weed control in production systems including quinoa (Santos *et al*., 2003).

The results reported confirm quinoa's adaptability to grain production in the Brazilian savannah. Its fruits (achene type) are cylindrical, flat and germinate quickly in the presence of moisture, once physiological maturity is reached. At its early stage of development, quinoa can be confused with the weed *Chenopodium album*, which becomes a problem in winter cultivation. The morphological differences become more visible after flowering: profuse branching, with the axillary and terminal racemes in *C. album* different to those in *C. quinoa*. In the latter, the terminal panicles are similar to sorghum, and the pericarp has a light colour, in contrast to the black in *C. album*.

The results showed morphological differences between 'BRS Piabiru', the first quinoa cultivar in Brazil and *C. album*. 'BRS Piabiru' had height of 190 cm, physiological maturity 145 days from emergence, resistance to lodging and average grain weight of 2.42 g/1 000. In *C. album*, plants were smaller, branched with open panicles and the seeds were very small (0.52 g/1 000). They have dormancy with gradual germination, and can remain in the soil, infesting crops for many years. Differences in number of chromosomes are a natural barrier against crosses between species.

Morphological differences detected during experiments demonstrated that the two species are distinguishable and that quinoa displays adaptability characteristics for commercial cultivation, contrasting with *C. album*, which presented typical weed behaviour (Spehar *et al*., 2003).

5.2 Genotype selection and agronomic performance

On the available accessions, screening was carried out for large grains and absence of saponins. A total of 17 saponin-free quinoa genotypes, selected for agronomic characters and yield, were evaluated in summer and winter sowings, for phenotypic stability. They were obtained through single plant-progeny selection from hybrids, as part of the breeding efforts to adapt the crop to Brazilian savannah no-till cropping. The soils – dark red Latosol and red yellow Latosol (both classified as Ferralsols) – were limed and fertilized prior to cultivation. Experiments were sown on two dates: 20 December 2006 (summer) and 30 April 2007 (autumn/winter), at 15°39' and 16°14'S, 47°27' and 47°44'W, 976 and 1 110 m asl.

Mean summer temperature was 23.0°C and total rainfall 1 435 mm. In winter, under controlled irrigation, the mean temperature was 2.9°C lower than in summer, when plants were exposed to spells of waterlogging. Early-maturing 'Kancolla' from the Peruvian Altiplano, and the savannah-selected 'BRS Piabiru' and 'BRS Syetetuba' cultivars (intermediate and late maturity, respectively) were used as controls. Comparisons were based on plant height, maturity, biomass, grain yield, harvest index (HI) and 1 000-seed weight.

Experiments were conducted on complete randomized block design, with three replications. Analysis of variance was performed for each experiment and jointly. In winter, most selected genotypes exhibited yield, grain size and HI higher than the control cultivars, maturing in 120 days from emergence. Plant height was suited to combine harvest, although in the summer genotypes had higher biomass production and smaller seeds compared with winter sowing. Yield-stable progenies across the two environments came from plant selections in populations of 'Q79', 'Q80' and 'Q82'.

Genotype selection in the Brazilian savannah has been effective, with higher HI than in late 'BRS Piabiru' and early maturity 'Kancolla' cultivars. Genotypic differences are reflected in plant height, grain and biomass production. Populations from hybrids between yield-stable genotypes could originate superior recombinants, eligible for use in breeding programmes (Spehar and Rocha, 2010).

Commercial cultivation of quinoa in the Brazilian savannah depends on crop adaptation and plant husbandry. Selected genotypes should be managed on suitable population density for maximal grain yield. The experiment was conducted to determine the population density that results in best use of water, light and nutrients, with ground cover during the biological cycle (Rocha, 2009).

Quinoa, in its region of origin, is grown under cold nights and low moisture availability, spreading out to the Andean valleys. It has reached high temperature tropics, where crop husbandry is not known. The experiment with 'BRS Syetetuba' – 120 days of biological cycle in a savannah farm – aimed at understanding the effect of population densities on agronomic characteristics and yield. Densities varied between 100 10^3 and 600 10^3 plants/ha.

There was a negative impact on plant height, which is negatively associated with density increase, but grain and biomass yield, harvest index and 1 000-grain weight were not affected. Stand uniformity was achieved with 30 010^3 plants/ha. These results are explained by the extraordinary capacity of quinoa to compensate for missing plants. At low density, plant branching and vigour increased, which was reflected in the higher number of days to maturity (Spehar and Rocha, 2009).

5.3 Morphology of progenies selected from 'BRS Piabirù

Double-cropping in the savannahs has been improved to exploit yield potential and maximize farmers' income, and represents an opportunity for quinoa. Early-maturing individual plants from segregating 'BRS Piabiru' were selected in Embrapa Savannah Research Centre, for cultivation in February–May (second crop) and May–September (winter, irrigated crop). The growing period of 'BRS Piabiru' is 145 days from emergence to maturity, while selected genotypes mature in 90–100 days.

Morphology was evaluated in April, 70 days after emergence, on 968 'BRS Piabiru'-derived progenies, with 10 repetitions for each of the following characteristics (descriptors): stem stripes and colour, with respective intensities (Figure 1); branch number and position in the plant; form of basal leaf (Figure 2); plant height and lodging. Plants were grouped into short (1.10–1.44 m), medium (1.45–1.64 m) and high (1.65–2.50 m). Leaf spots were recorded.

Differences in progenies (Table 1) confirmed the high cross-pollination reported in an earlier study (Spehar, 2001). New recombinants were selected and their description is useful in agronomic performance evaluation. The same procedure could be used with 'BRS Syetetuba', containing high morphological variations for market-desirable traits, prior to progeny selection and acquisition of high yield, early-maturing cultivars.

Table 1. Frequency (%) of morphological descriptors in 968 genotypes originated from 'BRS Piabiru'. Embrapa Cerrados, Planaltina, DF, 2012

Descriptor		Frequency (%)
Stem Stripe Colour	Present	99.5
	Absent	0.5
	Green	94.9
	Red	1.4
	Green + Red	3.6
Stem Colour Intensity	Light	39.3
	Medium	44.7
	Dark	16
Stem Branching	Absent	84.8
	Present	15.2
Primary Branching	Number/Plant	3
Position of Primary Branch	Slant from Stem	73.3
	Strait from the Stem Base	26.7
Leaf Border	Smooth	21.1
	Peaked	78.9
Peak/Leaf	< 3	76.7
	3 A 12	23.3
Leaf Type Variation	Yes	25.2
	No	74.8
Lodging	Yes	8
	No	92
Plant Height	Short (1.10–144 m)	21.7
	Medium (1.45–164 m)	67.9
	High (1.65–2.50 m)	10.3
Disease Leaf Spot	< 5%	69.4
	5–10%	23.9
	10–20%	4.1
	20–50%	2.2
	> 50%	0.4

Figure 2. Stem colour in the stem base found in selected progenies from 'BRS Piabiru'.

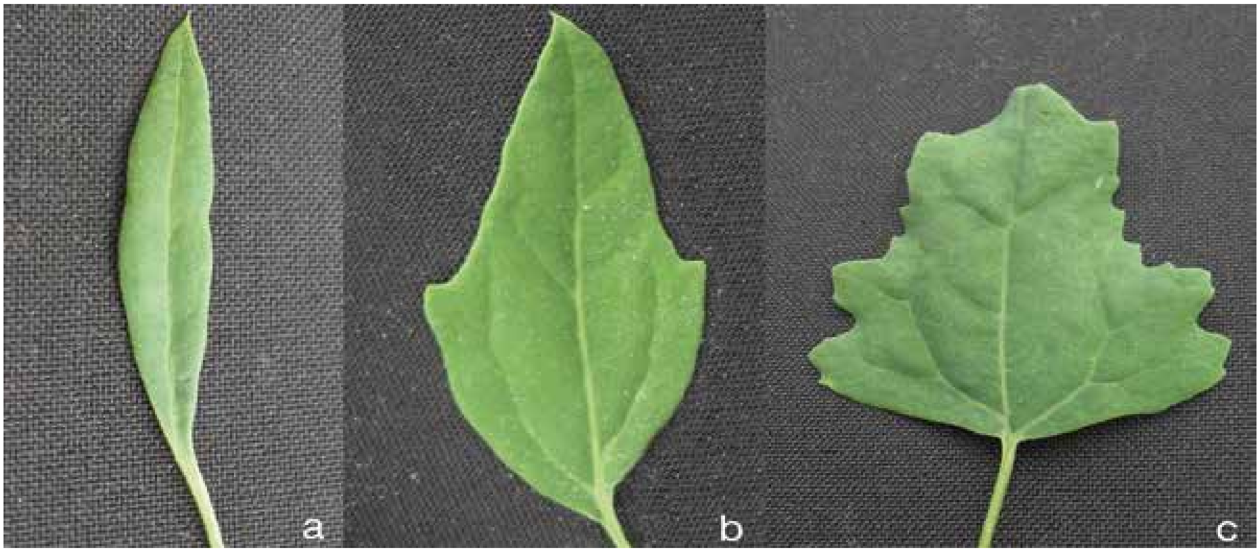

Figure 3. Predominant basal leaf types in selected progenies from 'BRS Piabiru'.

5.4 Cultivar Release

5.4.1 'BRS Piabiru' – Agronomic performance and characteristics

'BRS Piabiru' was a pioneer cultivar released to diversify production systems in the savannahs and to improve farmers' income. Additionally, when sown as a second crop, it could synergize the management of pests and diseases in the main summer crops, reducing production costs. Its stubble, remaining on the soil after harvest, could improve weed management in a no-till system (Spehar and Trecenti, 2011).

Moreover, a diversified system with quinoa as an alternative crop could improve mineral nutrient cycling and soil protection, based on the various requirements (Spehar, 2007). A source of raw material for products already in demand in Brazil, quinoa could contribute to developing the food and cosmetics industry. Quinoa has benn able to be inserted in Brazilian agriculture thanks to its drought tolerance, high protein quality, high content of compounds conditioning low cholesterol and absence of gluten, as well as the range of possible uses. Maturity in the species varies between 80 ('Kancolla') and 145 days ('BRS Piabiru') under savannah conditions.

'BRS Piabiru' was a breeding line selected from progenies of EC 3, originating from a plant population of Quito, Ecuador. After being tested for 2 years in central Brazil, it was standardized in terms of its agronomic characteristics. Results obtained when cultivated after soybean using residual moisture, and in the dry season under irrigation, revealed an average grain yield of 2.517 tonnes/ha (Table 2). Performance and the range of possible uses, as well as market demand, all stimulated interest in quinoa.

Characteristics

'BRS Piabiru' has hypocotyl with a colour varying between green and pink. Its leaves show polymorphism, with tips numbering > 12. Granules of calcium oxalate are abundantly present in leaves. The stem is erect, plain green or striped. Panicle is separated from stem and terminal, amaranth type and lax, turning yellow at physiological maturity. The grains, aquene fruit type, have a flat cylinder white pericarp, devoid of saponin. The perigon, the structure involving the fruit, is green and becomes yellow at maturity.

Average plant height in experiments was 1.90 m, of which the panicle accounted for 25%. Flower differentiation occurred 30 days after emergence, with anthesis initiating at 45 days. Plants showed lodging resistance and grains contained 130 g/kg protein. It was the first option available to farmers, released in 2002 with seed samples distributed to farmers together with cultivation guidelines.

Table 2. Grain yield (kg/ha) of cultivar 'BRS Piabiru', compared with 'Q15' and 'Q2', in two locations, 1998-1999(1). Source: Spehar *et al*., 2002.

Year	Location	Genotype		
		BRS Piabiru	Q15	Q2
1998	Planaltina, DF1	2832	2735	1920
	Rio Verde, GO2	3472	3247	2362
Mean		3152	2991	2141
1999	Planaltina, DF1	2665	2331	1983
	Cristalina, GO1	2370	2430	1832
Mean		2517	2380	1907

Second crop, with residual rainfall: 1250–350 mm; 2300–450 mm

Table 3. Grain yield (kg/ha) of cultivar 'BRS Syetetuba', compared with 'BRS Piabiru' and 'Kancolla', in two locations, 2006–07. Adapted from: Spehar and Rocha, 2011.

Year	Location	Genotype		
		BRS Syetetuba	BRS Piabiru	Kancolla
2006	Planaltina, DF1	2011	1221	1402
	Planaltina, DF1	2605	2425	921
2006-2007	Planaltina, DF2	2431	1812	1613
	Cristalina, GO3	2341	1823	2102
Mean		2347	1820	1509

1Summer/autumn, 250–300 mm rainfall; 2summer, 700 mm rainfall; 3winter irrigated, 450 mm

Thousands of seed samples of 'BRS Piabiru' were distributed free of charge and, in many parts of the country, small plots were grown. Feedback was provided by farmers, guiding the continuation of research resulting in new genotypes. The experience with the first quinoa cultivar was rewarding, increasing research and development in many parts of Brazil.

5.4.2 'BRS Syetetuba' - Agronomic performance and characteristics

Originating from the 'Q4' population in the Ecuadorian valleys, the line 4.5 gave a better performance than late maturity 'BRS Piabiru' and early 'Kancolla' (controls). During the trials, it was standardized in terms of agronomic characters and named 'BRS Syetetuba', meaning "large and abundant grains" in the indigenous language. In summer/autumn (rainfed) and winter (under irrigation), it reached 2.347 tonnes/ha grain yield in 120 days between emergence and maturity (Table 3). The harvest index (0.31) was higher than in the controls, explaining its superiority to late maturity 'BRS Piabiru'. 'BRS Syetetuba' has the desirable characteristics for commercial production in the savannahs, although it is necessary to continue selection for larger grains, using genetic variations in the cultivar, to suit the market and to standardize for phenotypic uniformity. Released seeds of 'BRS Syetetuba' are expected to attract the interest of farmers and consumers.

Characteristics

'BRS Syetetuba' has light coloured hypocotyl and its leaves are polymorphic, containing calcium oxalate. The upright stem is green with stripes, although plants with a purple stem sometimes occur in small numbers. Panicles are terminal, amaranth type and lax-branched, becoming yellow at maturity. Perigon is green, becoming yellow and opening at maturity, exposing the fruits. Phenotypic variations are associated with natural crosses that may have occurred during the evaluations.

The plants, resistant to lodging, are 1.80 m tall, and the panicle is 0.60–0.70 m. The period between emergence and maturity is 120 days. The saponin-free grains weigh 2.5 and 3.3 g/1 000, in summer and winter, respectively, containing 18 g/kg protein.

In the field, insects associated with soybean (e.g. *Nezara viridis*, *Pyezodorus guildinii* and *Euschistos heros*) have been found on quinoa plants, although no damage has been reported to date. When sown in no-till areas, the soil should be covered by stubble or after pasture desiccation to avoid weed infestation. Additionally, alachlor, setoxydin and metamitrona herbicides could be used in weed control when narrow leaf plants predominate.

Maintenance fertilization is based on plant composition and expected yields. Assuming the soil was previously limed and fertilized, phosphorus and potassium are recommended at rates of 80 and 100 kg/ha of P_2O_5 and K_2O, respectively, for yields of over 2.0 tonnes/ha. Nitrogen should be split into 20–30 kg/ha at sowing and 40–50 kg/ha 30–50 days after emergence (Spehar and Rocha, 2010).

5.5 Genetic evaluation and selection for agronomic traits and grain composition

Quinoa crop improvement for commercial production relies on selection efficiency for key agronomic characters. Moreover, the physical and chemical composition can also be modified by exploiting genetic variability. Some traits of interest in selection fit into single gene models, such as the presence of saponins in grains (Rivero, 1994). However, some characters are of complex inheritance and could be influenced by the environment. Grain size, plant type and number of days to maturity are examples requiring comprehensive study.

Understanding the genetics for qualitative characters of phenotypic expression is essential in quinoa improvement (Jacobsen *et al*., 1998). Likewise, Mendelian, morphological gene markers are useful in hybrid plant identification and progeny selection. However their number is limited and colour in various plant parts is frequently used.

The mode of inheritance for pigment in the quinoa plant was determined on the basis of hybrids proportion in F2. Crosses and respective genotypes were: ‘BRS Syetetuba’ × ‘34ZL’, ‘BRS Syetetuba’ × ‘37ZL’, ‘BRS Syetetuba’ × ‘40ZL’, ‘BRS Syetetuba’ × ‘44ZL’ and ‘BRS Syetetuba’ × ‘9542L’. Cultivar ‘BRS Syetetuba’, without pigment, was used as female, whereas the male genitors had pigment in calcium oxalate, leaf axil, stem stripes and inflorescence.

Genotypic frequency in F_1 showed 100% plants with red pigment, with intra-allelic interaction of full dominance type, confirmed in F_2 generation with a ratio of 3:1 red to green. The ratio was highly significant in the chi-square test, fitting into the Mendelian expected proportion. The pigmentation is found in the same proportion in all plant parts, defined as the pleiotropic effect. Hybrid plants could be identified in an early growth stage and used as a tool in selection (Rocha, 2011).

Genetic parameters and phenotypic and genotypic correlations were determined in populations of the same hybrids for plant height, yield and 1 000-seed weight, in order to develop cultivars for the growing conditions of the Brazilian savannah. F_2 hybrids were evaluated in randomized blocks with three replications, from the crosses ‘BRS Syetetuba’ × ‘34ZL’, ‘37ZL’ × ‘BRS Syetetuba’, ‘BRS Syetetuba’ × ‘40ZL’, ‘BRS Syetetuba’ × ‘44ZL’ and ‘9542L’ × ‘BRS Syetetuba’. The high heritability and genetic coefficient of variation for plant height in all crosses suggested the use of this parameter for efficient selection. The ‘BRS Syetetuba × 9542L’ hybrid showed best performance for all characters, favouring the inclusion of its progenies in subsequent generations.

Based on the intrinsic value of quinoa grains and the demand expressed by both consumers and industry, it is necessary to investigate further the genetics of quality and aim to improve each of its various compounds. A study to identify genetic variability in quinoa hybrids for physical-chemical composition was conducted. Genitors and their respective F_2 hybrids were analysed for lipids, proteins, crude fibre, carbohydrate, moisture, dry matter and ashes in order to carry out agronomic selection and improve quality.

Protein varies considerably between the F_2 progenies, and ‘BRS Syetetuba’ has the highest protein level among the genitors. Crosses for grain quality improvement can be made to achieve genetic improvements, using hybrids of high-protein selected genotypes (Rocha, 2011).

5.6 Tools in selection

5.6.1 Association of grain colour and saponin

The study involved a sample from the breeder variety collection at Embrapa Savannah Research Centre, Planaltina, DF, Brazil. A group of washed seeds and 35 genotypes of quinoa were screened using the soap column method and were classified according to the RGB Colour Model (R, red; G, green; B, blue) with the objective of determining the influence of the saponin content in the grain colour. Yellow seeds presented high levels of saponin. There was negative correlation ($p > 0.05$) among the soap column method and bands R ($r = -0.751$), G ($r = -0.660$) and B ($r = -0.594$). Four groups were identified. Tests confirmed group 4 as bitter (yellow seeds) and group 1 as sweet (white seeds). The range of standards represents probable differences in gene frequency, reflected by the colour and rate of saponin (Souza *et al*., 2004).

5.6.2 Cloning quinoa hybrid plants

Acquisition of hybrid seeds could be a limiting factor in quinoa breeding. A major setback in variance component analysis is the amount of F_2 seeds needed. Often the F_1 hybrid may be excluded from evaluations, because of the small number of individuals. Although the quinoa plant produces high numbers of seeds for field experiments, inclusion of F_1 allows generation mean evaluations.

Cloning quinoa plants could help solve the problem. Cuttings of five hybrids treated with three indolbutiric acid doses were grown on plant growth substrate, using a complete randomized experiment design. The quinoa cuttings rooted, irrespective of hormone treatment, as their survival was more dependent on substrate and high relative moisture. Differences in plant growth were probably more related to the age of the cutting. Mother plants at an advanced stage in the reproduction phase had reduced growth and yield. The cloning of quinoa plants is a potential tool to support breeders in increasing stocks of hybrid seeds (Rocha, 2011).

6. Current State and Perspectives of Quinoa Dissemination in Brazil

Pioneer research and development actions over the last 20 years have become a reference for quinoa cropping in Brazil. As a result, the public has already incorporated the use of its grains and derived products, although most of the supply comes from the Bolivian and Peruvian Andes, in the various forms demanded by consumers.

This situation contrasts with the existing technology for production in the savannahs. The technical information currently available is sufficient for the agricultural sector, although there are still limitations, for example, the high rates of cross-pollination in the Brazilian environment, causing a direct negative impact on crop uniformity of available cultivars (Spehar, 2001). It is necessary to invest in genetic and foundation seed production. However, due to the limited extent of cultivation, it is not attractive to the private sector, and public institutions need to play a major role, forming partnerships for seed production.

Another restricting factor is the intrinsic low seed quality found in quinoa. Even when seeds are produced according to recommended technologies (Spehar, 2007), there are problems related to conservation of germination and vigour. Quinoa seed loses germination rapidly in the savannahs when kept at prevailing room temperatures. Sensitivity to high temperatures has been demonstrated in storage condition studies (Souza, 2013). Irrespective of moisture levels, seed germination is maintained at 4.4°C and ceases to be viable at 25°C. This could represent a potential setback for small-scale and smallholder family farmers, who would need to invest in seed production and storage.

On the basis of market value, the high prices of quinoa and its by-products have favoured its insertion in Brazilian agriculture. This, however, depends on trading opportunities. Quinoa is in low demand compared with other grains, and the market is limited by the small volume. Therefore, it is easier for farmers to move towards other grain crops, when prices are attractive, rather than invest in a lesser-known crop with a limited and, therefore, risky market.

Moreover, the introduction of quinoa into production systems forces farmers to master a specific technology, which may still need to be perfected and popularized (Spehar, 2007). The technology for major grain crops is disseminated by input traders who are focused on production scale, while for minor crops like quinoa there are different considerations.

Research and development must continue to fill the existing gaps, prioritizing genetics and breeding, husbandry improvement, soil fertility and plant nutrition management, technology validation and market prospects. Comprehensive solutions are required for the development of quinoa in Brazil.

For concrete accomplishments in quinoa production, integrated teams involving universities and institutions must work together to maximize use of limited resources. The experience by the University of Brasilia and Embrapa, in association with producers, contributed information and technology, leading to the launch of production in Brazil (Spehar, 2006). Most of what is reported herein originated from partnerships that require further strengthening and additional participation.

Teams of experts should be formed to carry out the task of turning quinoa into a commercial crop in Brazil. Available information must be enriched and improved, so that it can be used in farming, the grain retail market and the transformation industry.

Once solutions to the existing problems are found, production will gradually increase in the next 5 years and the national product will have to compete with the imported one. The challenge will be to produce locally certified, organic quinoa, bearing similar quality standards to the imports to suit internal sophisticated demand. If proper government incentives are provided, this will represent an opportunity for family farmers, rather than large-scale farms.

In large farms, where chemical fertilizer and pesticide usage is high, there is the potential to rapidly achieve a large volume of produce. This represents an opportunity to bring the quinoa market to the less demanding, lower income public and popularize its use in Brazilian cooking. Moreover, it is an opportunity to alleviate the monoculture system, which is already causing disruption in cropping systems and leading to food supply and environmental problems in the Andean producing countries (Echalar and Torrico, 2009; Jacobsen, 2011).

On the basis of earlier projections for the savannahs, Brazil can play a major role in supplying quinoa internally and to the world in the next 20 years. There will be two markets: one to suit mass demand, using quinoa from large-scale farming; another to suit demand for organic, certified grains and by-products, coming from family farming. Quality versus price will define the trading relations, keeping niches of market to maintain a good balance and create opportunities.

As quinoa's role in the improvement of cropping systems is demonstrated, – costs reduced, income increased and food for consumers improved, – production will increase. High demand and opportunities for profit will attract private sector interest, leading to development of trade and industry. Better quality food will lead to better physical health among the population. Thus, the availability of quinoa and other valuable and less exploited grain products in Brazilian agriculture will contribute to improving food security at local and global level.

7. Uses and markets for quinoa in Brazil

The various uses of quinoa in Brazil derive from the organic and mineral composition of its grains, in addition to its functional properties. As with soybean and maize (major agricultural grains in world agriculture), finding new uses for quinoa has contributed to increasing demand, creating an opportunity for its insertion in the Brazilian market. In the 1990s, quinoa was first introduced as an alternative health food, stimulating the interest of producers and consumers (Spehar, 2007).

In the Andes quinoa has been used for thousands of years as a valuable food to enrich the daily diet. During its long history, domestication took place leading to multiple associated uses, some of which remain unknown in other parts of the world. The composition of essential amino acids is close to that in milk casein and it has been used to feed infants during and after weaning in rural areas (a common tradition among local people) (Ascheri *et al.*, 2002). Considering the similarities in infant feeding in Brazil, quinoa products could suit demand and add nutritional value.

Quinoa grains and its products are used by adults in Brazil in existing dishes to increase quality and enhance flavour. The exquisite taste of quinoa was first experienced by the public in the form of simple preparations as part of the effort to introduce the grain and popularize its uses (Spehar, 2007). In animal feeding, the grain and the plant are both used instead of similar agricultural products. Special diets combining quinoa and other food sources have been developed for elderly people and patients recovering from illness. In addition, it is used to mitigate disorders caused by coeliac disease (allergy to gluten). These various uses have contributed to the increasing interest in quinoa, consolidating demand.

Other organic compounds, such as unique lipids and starch, in addition to B and E vitamins and minerals, have created new opportunities for quinoa-derived and processed products attractive to the public and industry (Nascimento *et al*., 2003; Ascheri *et al*., 2002). It is expected that once a critical level of demand is reached, local agriculture will begin to include quinoa as an alternative crop in the production chain.

7.1 Properties and uses of plant and grain

The quinoa plant, in all growth stages, can be consumed by humans and livestock and many new opportunities have been identified in Brazil. At early growth, sprouts can be harvested and used like spinach, while at flower differentiation, the buds are used like broccoli (Spehar, 2007). These plant parts, however, contain a high level of calcium oxalate that is reduced by boiling. Genetic variations for calcium oxalate in germplasm could be used in selection for low content and direct use as vegetables. During the reproductive phase, the whole plant can be used as silage for livestock. In late maturity genotypes, plants can be chopped before flowering and allowed to resume growth and reproduction – double-purpose cropping (Tavárez *et al*., 1995).

Saponins are considered undesirable in quinoa grains. Although they are present in some genotypes, cultivars in Brazil are free of these glycosides and can be used directly. Harvested grains, maintained under the same conditions as other crops, can be used in various forms: i) boiled in water and seasoned as salad; ii) fried and boiled with spices (as is done with rice); iii) added to soups and sauces. These preparations are easily performed in family farms and by urban consumers. Moreover, quinoa flour can be used in infants' porridge and in desserts, enriched bread, pancakes, biscuits and beverages.

Cooking preparations were developed early on as part of the effort to introduce quinoa in Brazil (Spehar, 2007). They are based on local foods and are quite different from those in the Andes. Quinoa has thus been made more popular, thanks to its quality and exquisite flavour (Table 4).

Simple forms of preparation, associated with public interest in health conditioning food, form the basis of quinoa consumption in Brazil (Spehar, 2007). Salad has been modified by restaurants and food suppliers into more sophisticated recipes. Original enriched bred has been made in different creative forms by individuals, restaurants and the food industry. Similarly, biscuits have been improved using the recipe presented in Table 4. Other recipes have been adopted by people and restaurants to prepare home-made nutritious pancakes and crepes.

When these home-made food recipes were developed, there were no uses for quinoa in Brazil. The variety of derived food coming into market in the last 10 years surpassed all expectations. The enriched bred is currently available in many forms in the Brazilian market and contains quinoa and several other grains. The nutritional value of quinoa-enriched bred has been demonstrated (Stikic *et al*., 2012).

Research and development, as well as promotion, popularized quinoa with a positive impact on food security. The search for new forms (e.g. noodles) continues, seeking new uses and derived products (Caperuto *et al*., 2001). The crop's nutritional properties should encourage consumers and industries to continue food innovation with quinoa. It is expected that, as the grain is produced in large quantities, prices will diminish, increasing access by large numbers of people.

7.2 Quinoa processing and value-added products

Quinoa processing can be done at smallholder level, on family farms, by combining with the other grains and products available in the property. This could lead to an enriched diet for rural populations, and the excess can be destined for market. Moreover, quinoa trading is expected to increase the income of small farmers. Communitarian industries developed by farmers' associations could improve processing on a small, artisan scale. On the other hand, big industries would absorb the bulk from large-scale commercial farming.

Table 4. Basic recipes using quinoa grains to suit Brazilian food habits.

INGREDIENT	PREPARATION
ENRICHED BREAD[1]	
Quinoa and wheat flour (1:3), water, sugar, salt, leavening, vegetable oil	Heat water, add sugar, salt (to taste), leavening; mix with flours and oil, wait until doubles volume; smash, prepare loaves, wait; bake at 180°C for 15 min.
SALAD[1]	
Quinoa grains (2 cups), water (2 cups), garlic cloves, half onion, chives, chopped tomatoes and cucumber, lemon juice, olive oil	Wash grains, add water bring to boil for 5–8 min; wait until cool; add other ingredients; lemon juice, olive oil and salt to taste
BISCUIT[1]	
Quinoa flour, grains, corn starch (1:1:2); water or milk, eggs, butter, sugar and salt (to taste), baking powder	Soak grains overnight; mix with water, eggs, salt and sugar in blender; place in bowl, add starch, flour and baking powder; pour into greased trays and bake at 180°C for 20–30 min.
CREPE/PANCAKE[1]	
Quinoa grains, wheat flour or corn starch (2:1), butter, eggs, water or milk, salt. For pancake, add baking powder	Soak grains overnight; grind in blender until liquid; mix with other ingredients, salt to taste, blend; heat pan and add little oil; pour to cover, wait 1 min., turn. Serve with syrup, jam or honey; alternatively use with salty toping

[1]Developed by E.C. Spehar

Source: Spehar *et al*., 2007.

On family farms, enriched food can be prepared using simple, basic recipes, adjusting the peculiar taste to the existing food sources. Examples of recipes adapted to Brazilian cuisine are presented in Table 4. These recipes promote the use of quinoa in the country and provide new ideas for different forms of food processing.

Another possibility on family farms is use of market-rejected low-standard quinoa grains, originating from harvest cleaning products and used in feed for livestock (Cardozo and Bateman, 1961; Jacobsen *et al*., 1996). Animal products, originated from quinoa feeding, can be traded advantageously over those coming from animals fed on artificially balanced rations.

At communitarian level, other products can be obtained in the local processing industry. One simple preparation is popped quinoa grains, made using an extrusion cannon. These expanded grains could become a basic ingredient in special foods. Popped grains can be used to make instant flour, flakes, chips and other elaborate products for human nutrition. Table 5 shows some preparations which suit Brazilian tastes, and all of them are good for the health.

7.3 Opportunity for new products

The use of quinoa in the food industry has increased and has a direct impact on public health. In terms of agronomic traits, the composition of quinoa grains produced in Brazil and those produced in the Andes has been compared (Rocha *et al*., 2010a, b). The desirable qualities of quinoa are not lost when the crop is produced in the Brazilian savannah. Given the high quality of the grains and the range of potential uses, support should be given to public policies to introduce quinoa into school meals to improve pupils' nutrition and to develop a taste for new food early in life.

Table 5. Quinoa extrusion and uses in food preparations

INGREDIENT	PREPARATION
POPPED QUINOA	
Quinoa grains	Place grains in extrusion cannon, calibrate adjusting temperature and pressure for popping
HEALTH BAR[1]	
Popped quinoa (15 cups); honey or molasses (1 cup); raisins (1 cup); corn flakes (2 cups)	Heat honey or molasses in pan; add popped grains, raisins and flakes; place in tray, press firmly, wait until consistent, cut into bars
GRANOLA[1]	
Popped quinoa, corn or oat flakes (5:2), molasses or honey, gritted coconut, raisins	Place honey or molasses in pan, moisten with water and heat; add flakes, coconut grits; place in oven at 120°C for 15 min.; add raisins and mix
CREPE/PANCAKE[1]	
Quinoa grains, wheat flour or corn starch (2:1), butter, eggs, water or milk, salt. For pancake, add baking powder	Soak grains overnight; grind in blender until liquid; mix with other ingredients, salt to taste, blend; heat pan and add little oil; pour to cover, wait 1 min., turn. Serve with syrup, jam or honey; alternatively use with salty toping

[1]Adapted from B. Pelizzaro and H. Pelizzaro, Celeiro Alimentos, Brasília, DF, Brazil

Source: Spehar *et al*., 2007.

The current trend among urban dwellers to demand healthy and functional food in Brazil began with quinoa. Both consumers and industry are attracted by its properties, and it occupies an outstanding position among innovative foods. A wide range of food has become available containing quinoa, following the research and development efforts reported. Given the high biological value of quinoa protein and its content of starch resisting freezing temperatures (making quinoa good for use as thickening in food), new applications continue to increase the possibilities for quinoa (Ascheri *et al*., 2002; Wahli, 1990).

Comparison of processed food by extrusion of grains produced in Brazil has revealed quinoa's superiority over rice and maize in trems of lipids, protein and fibre (Ascheri *et al*., 2002). Quinoa can be used in industry to enrich foods and to produce instant flour of better value than that made from cereals (Tables 6 and 7). Its properties of stability and biological value make it suited to numerous applications (Spehar, 2002).

Promoting quinoa as an alternative for health and food security in Brazil has produced formidable results. It is important to also include other innovative, less exploited grain crops, in terms of agronomy and food.

The peculiar food properties of quinoa, an outstanding plant and grain, have been confirmed in adapted cultivars grown in the Brazilian savannah (Spehar, 1976). It is hoped that its gradual incorporation into new food will help increase demand and, consequently, lead to production and market growth. The trends for new rations conditioning animal health will increase and bring additional opportunities for quinoa. High sanitary performance has been observed in livestock fed with rations containing controlled quantities of saponins (Cheeke, 2001). The high methionine content of quinoa flour (Table 7) means that use of and demand for quinoa in the intake of milking cows may rise.

Table 6. Instant flour centesimal composition obtained from extruded grains of quinoa, maize and polished rice, with respective caloric value (kcal/100 g).

Flour	Moisture	Protein	Lipids	Fibre	Carbohydrates	Ash	Caloric Value
Quinoa	4.8	12.2	5.6	4.4	70.5	2.3	396.0
Maize	12.1	7.6	1.2	0.5	78.1	0.5	355.6
Rice	11.1	7.5	0.3	2.1	78.9	2.1	349.3

Source: Ascheri *et al*., 2002

Table 7. Amino acid composition of instant flour obtained by extruded quinoa, maize and polished rice grains.

Flour	Amino-acid (mg/100 g)								
	ASP	GLU	SER	HIS	GLY	THR	ALA	ARG	TYR
Quinoa	1.160	1.962	578	387	681	452	562	1.133	359
Maize	400	899	209	152	167	149	322	251	152
Rice	758	1.253	301	166	230	184	324	501	2.231
	CYS	VAL	MET	TRP	PHE	ILE	LEU	LYS	PRO
Quinoa	422	560	210	N.D.[1]	505	458	623	710	480
Maize	151	242	74	N.D.	234	219	610	141	754
Rice	359	339	119	N.D.	318	260	502	194	331

[1]N.D. = non determined

Source: Ascheri *et al*., 2002

8. Questions and Problems for Dissemination of Quinoa in Brazil

Undoubtedly, Brazil will increase its participation in the world's agriculture as a major grain supplier. The potential to produce grains will expand to include crops which are unique sources of nutrients, vitamins and minerals, such as quinoa. Other grains demanded in some countries or regions of the world, such as grain amaranth, buckwheat, tef, chickpea and sesame, could fit into windows of the savannah cropping systems, rotating with quinoa (Spehar, 2009).

There is no simple solution to the increasing threat of pests and diseases associated with monoculture in the tropics. Even though the scale and pattern of agriculture in the savannahs are similar to that of the temperate zones of the world, there are great differences in the climate (Spehar, 2009). Continuous plant growth multiplies pathogens and damaging insects, allowing new virulent strains and varieties to appear. There are examples of striking pests of recent introductions in Brazil, such as *Helicoverpa armigera* (Czepak *et al*., 2013). Like many other insects, it is polyphagous, attacking various crops, and it is difficult to control.

Several diseases have adapted their biology to crop and non-crop species, attacking and damaging plants, compromising yield and quality of the final products. This is the case with rice blast, which causes damage to most cereals and indigenous host plants associated with variability of pathogen (Choi *et al*., 2013). Diseases typical of quinoa have not yet been found in the Brazilian environment, perhaps because the climate differs greatly from that in the Andes. Diseases are, nevertheless, a concern, especially quinoa downy mildew, *Peronospora farinosa* (Danielsen *et al*., 2003).

Temporary solutions to the dilemma of a warm climate – e.g. sanitary void, eliminating spontaneous crop plants originated from seed loss during harvest – will become ineffective. The inclusion of genetically modified soybean, maize and cotton in production systems is effective for weed and pest control only when there is crop rotation. Repeated monocropping will destroy these expensive technologies because weeds and pests will, inevitably, develop resistance. Thus, diversification with crops of distinct botanical families is needed and should be high priority in research and development. The initiative must come from public research institutions, influencing government actions.

In this scenario, quinoa is a potentially effective rotation crop. However, farmers' decisions are highly influenced by market forces where big traders play a major role. On top of sanitary problems, homogenous crops with little rotation have been exposed to two major constraints: climate and prices. The less diverse a production is, the more vulnerable it is, regardless of the technology available for predominant soybean and maize crops.

Public policies must be directed to crop diversification, focusing on the future, observing and learning from the dynamics of life. It is worth emphasizing the balance in diversity, exhibited by the exuberant vegetation in the tropics. This should guide planning and coordinated actions for sustainable agriculture. Alternatives should become available and production technology needs to be implemented.

Increasing the number of crops optimizes production factors, reduces risks and costs and maximizes revenues. This will not happen on its own: as reported herein for quinoa, it is important to demonstrate the facts and make them known to the public through effective communication channels. Research and development and the promotion of innovative plant species must receive the support of federal and local governments in the form of incentives to agricultural and food diversification.

New products should be promoted and made available to improve diet and food security (Spehar, 2009). Diversified systems require the support of public policies in order to manage capacities and provide technical training for extension agents and famers. Joint actions are required to consolidate quinoa and other novel crops in Brazil.

The Brazilian market for quinoa is expected to increase given its appeal in terms of nutrition and health. Several products have been developed innovating consumers' taste, following the crop improvement initiative. Market products include yogurt, flours, flakes, health bars and noodles, and they serve to stimulate consumers and industry to invest in a range of combinations, using quinoa as a food innovator. This initiative should be applied also to other less exploited crops.

There are windows in production systems and quinoa represents a promising option in zero tillage. A survey in the savannahs in southwestern Goiás State revealed that 100% of grain producers base their cropping on soybean (Levin and Fox, 2004; Jayme-Oliveira, 2013). With prolonged rainfall in that region, 80% of them grow maize as a second crop from February to June, using residual moisture. However, pests and diseases have increased in the soybean–maize system (Toledo-Souza *et al*., 2008). Both the farmer economy and the environment are threatened by increased production costs and yield reduction.

The survey concluded that farmers would be ready to introduce quinoa into cropping systems on the basis of expected income and relative drought tolerance. They were not aware of the benefits to the system and were surprised by the plant growth and other agronomic characters, useful in the crop–livestock integrated system. The many possible uses for grazing, silage or post-harvest residue stimulated farmers to introduce quinoa in their production schemes (Spehar, 2006).

Additionally, saponins present in quinoa have shown effectivenes in reducing nematodes and white mould (*Sclerotinea sclerotiorum*) infestations in plants (Ferraz and Freitas, 2013). Quinoa could also be used – in addition to grain and biomass production – to protect the soil, minimizing exposure to solar radiation. Organic matter loss in bare soil can impact negatively on the soil's physical, chemical and biological characteristics (Spehar and Trecenti, 2011). It is the essential basis of no-till development.

Given the scenario and opportunities for innovation, there is scope for FAO to play a major role, coordinating, promoting and supporting actions for diversification of agriculture and food. Governments, universities and institutions are key stakeholders, directing multidisciplinary team projects aimed at the introduction of new crops in modern tropical agriculture, taking the Brazilian experience as reference.

Once a virtually unknown crop outside its centre of origin, quinoa is on the verge of becoming a valuable member of the world's agriculture. It is an example that will awake opportunities for diversification on a sustainable basis. The authors hope the experience with quinoa in Brazil will help support development projects for similar environments worldwide.

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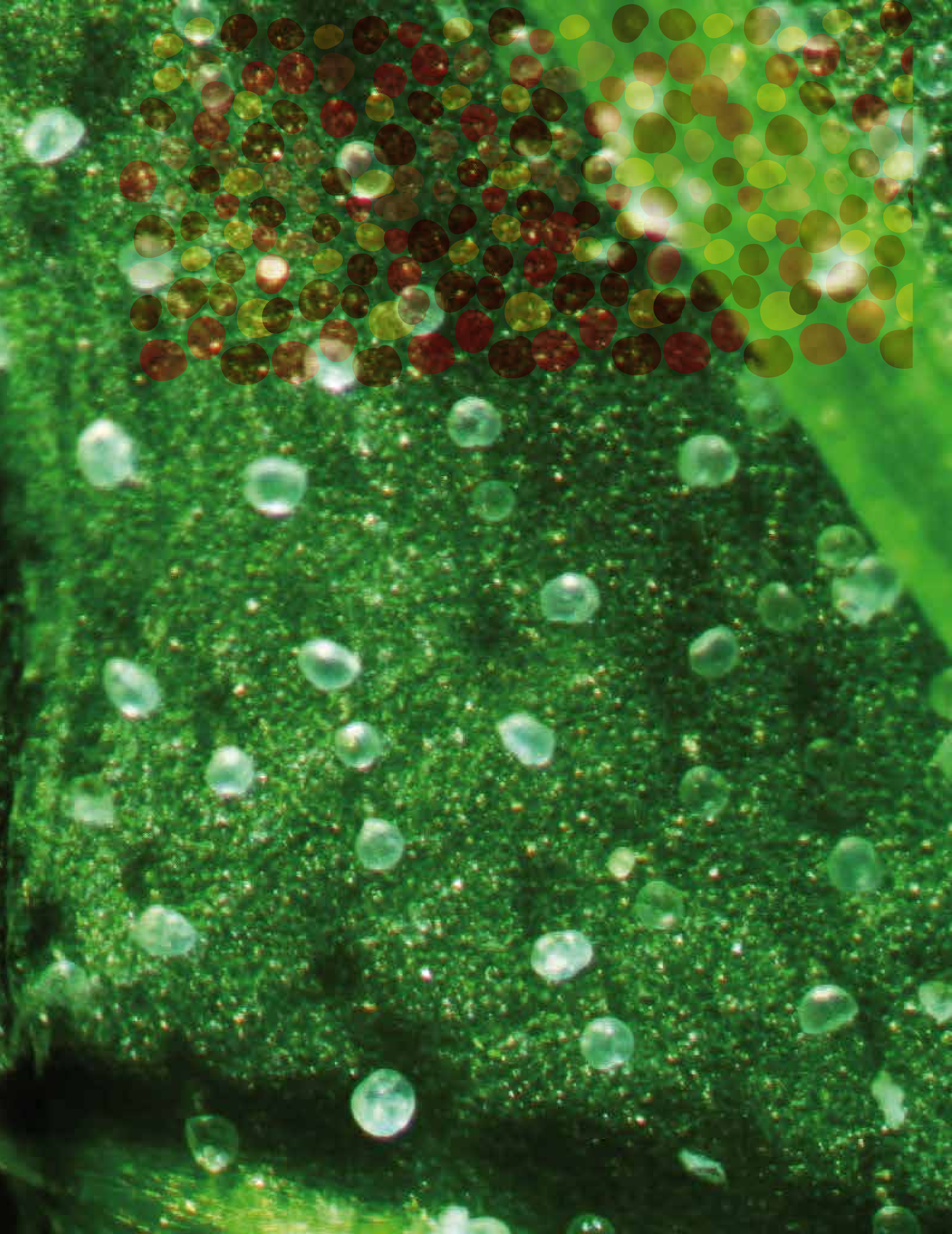

Conclusions

CHAPTER: 7.1

CONCLUSIONS: CHALLENGES, OPPORTUNITIES AND THREATS TO QUINOA IN THE FACE OF GLOBAL CHANGE

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The book entitled *State of the Art of Quinoa in the World 2013* is a joint publication by CIRAD and FAO. It compiles all relevant information on quinoa, generated by the world's foremost researchers, producer organizations, decision-makers and other actors involved with quinoa.

Quinoa has been grown in the Andes for over 5 000 years. During the Spanish Conquest, however, the crop was strongly discouraged, due to its important role in the indigenous culture. Fortunately, in the 1980s, quinoa's potential as a major crop was rediscovered, and there has been a surge in the number of countries growing or experimenting with quinoa. Between the 1980s and the 2000s, the number of quinoa-growing countries increased from just six in the Andean zone to 50 countries around the world with a variety of ecological contexts and climactic conditions. Quinoa's upward momentum is not expected to change – this year, at least another 20 countries have indicated an interest in quinoa cultivation once they have access to phytogenetic resources or improved seeds.

It is no coincidence that quinoa is experiencing this boom. Although many grains and legumes have high protein content, it is today recognized that quinoa has the ideal balance of amino acids essential to humans. What is more, quinoa's high levels of linoleic acid (omega-3) and the fact that most varieties are gluten-free make it an exceptional food. Quinoa's nutritional features mean it has enormous potential in the fight for worldwide food and nutritional security.

Nevertheless, the current heightened interest in quinoa's global expansion is in fact largely due to its resistance to numerous abiotic stresses, particularly drought and salinity. A large proportion of agricultural production around the world depends on the availability of water and irrigation. Intensive use of limited water resources has led to the excessive pumping of groundwater, resulting in saltwater intrusion in coastal areas and soil salinization. In the face of worsening climate conditions due to climate change, a huge area of our planet is threatened by water shortages and soil salinization. These phenomena have intensified in many agricultural zones, particularly in semi-arid regions around the world. Quinoa's high genetic diversity offers a way to address the situation, by adapting to different ecological environments where these limiting factors are present. When evaluating quinoa's adaptation capacity, it is important to bear in mind that quinoa's main production zone worldwide is the southern Altiplano in Bolivia, where average annual rainfall is < 150 mm and frost occurs on > 200 days per year. The soil is saline, as the region borders on the Uyuni salt flats, and the altitude is about 4 000 m asl. In such extreme conditions, Andean peasants selected quinoa's phytogenetic resources for generations, resulting in a high level of genetic diversity in quinoa landraces.

In order to better appreciate quinoa's global potential, this book presents a series of scientific papers on the state of the art of quinoa in the world. The book is primarily aimed at scientists, students and decision-makers, for whom the information may be relevant and necessary in the implementation of large-scale projects in the fight against hunger. The book is also intended for quinoa producer organizations, which may also benefit from the wide range of material gathered here. The objective of this project is not to provide a comprehensive history of quinoa, but to disseminate the latest information available on this "golden grain" of the Andes. To this end, 22 countries in North and South America, Europe, Africa and Asia contributed to the book. A total of 165 co-authors were convened to work directly on the writing process, and half of them are from the five Andean countries: Argentina, Bolivia, Chile, Ecuador and Peru. The book includes 43 chapters, each of which is dedicated to a specific topic. It is divided into six thematic parts, as outlined below.

The first part is dedicated to the botany and phylogeny of quinoa, and sets out to understand the relative dynamics of its domestication and dissemination leading up to its current area of distribution. The authors provide detailed information on how indigenous communities have preserved quinoa's high levels of biodiversity for centuries. These chapters also present an international perspective on the current risks related to seed flow regulation at various levels. The movement of seeds between human groups contributes to the dynamic evolution of a species by maintaining its capacity to adapt in the face of global changes. In the seven chapters of this first part of the book, the authors share new ideas about quinoa's high levels of diversity, and the innovative genome tools available to characterize the crop's phytogenetic resources.

Rick Jellen and Jeff Maughan, both researchers at Brigham Young University in the United States of America, describe the most recent molecular markers. The range of genetic marker tools developed may be freely accessed by anyone who needs to conduct research on quinoa. A complete review of the state of the conservation of quinoa's genetic resources, coordinated by Wilfredo Rojas of Bolivia, underscores the importance of both *ex situ* quinoa collections found in seed banks in the Andean countries, and the 25 seed banks outside quinoa's zone of origin. This phenomenon is explained by quinoa's current global expansion, with the latest varieties bred for temperate climates developed mainly in Europe and the United States of America. Insofar as the origin of phytogenetic resources and their use to generate innovation, Marco Chevarria-Lazo, a lawyer from Peru, opens a stimulating debate on North-South relations in today's context. He compares the case of quinoa with the expansion of the potato 200 years ago, when there were no national or international seed standards to protect the rights of farmers in the indigenous communities of the Andes. Contributing to this debate, Unai Pascual from the United Kingdom presents the experiment carried out in Peru and Bolivia, on incentive payments (or subsidies) for the *in situ* conservation of quinoa's diversity, exploring the concept of ecosystem services applied to genetic resources in agriculture. The first part of this book also provides a summary of the information available to explain the evolutionary dynamics of the *Chenopodium quinoa* Willd. species, from its centre of origin in relation to its wild relatives. In order to optimize conservation, it is vital that existing phytogenetic resources, both *in situ* and those in gene banks (or *ex situ*), complement each other. There is also an examination of the limitations of regulatory instruments to conserve and protect without impeding innovation.

The second part of this book is an in-depth study of quinoa's biology. Temperature, day length, water availability and sunlight are key factors in quinoa's development. Argentine quinoa specialist, Daniel Bertero, describes the close relationship between quinoa's development and the four environmental components controlling plant growth.

Quinoa's agricultural potential for dissemination and expansion to other regions of the world is linked to its great capacity to adapt in the face of climate change and its effects. Stefania Biondi, a biologist at the *Università di Bologna* (University of Bologna) in Italy, describes quinoa's tolerance and adaptation to saline conditions. Andres Zurita-Silva, of the Chilean *Instituto Nacional Agropecuario* (National Agricultural Institute) addresses in detail quinoa's response and adaptation to drought. Alejandro Bonifacio from Bolivia and Luz Gómez from Peru provide a historical review of quinoa breeding programmes, aimed at maintaining drought and salinity tolerance, while also increasing seed yield, improving disease and pest resistance and maintaining kernel quality. This second part also presents a comprehensive view of agronomic and ecological issues, and each chapter includes a review of scientific literature on a specific biotic or abiotic factor, with the goal of better understanding how quinoa's adaptation to a broad range of ecological contexts in the Andes can be extrapolated to establish the crop in other regions around the world.

The third part of this book begins by describing various processes carried out to eliminate saponins from quinoa seeds to make them fit for human consumption. Jacopo Troisi, an Italian chemist, explains that the saponins present in quinoa may have value as by-products for medicinal or cosmetic purposes, or as natural cleaners. In consideration of the high nutritional content of quinoa seeds currently used for human consumption, Antonio Blanco, an agronomist from Bolivia who works at the *Universidad Católica del Maule* (Catholic University of Maule) in Chile, demonstrates quinoa's value as animal feed. He includes a description of the different parts of the plant that can be used for various kinds of animals in marginal livestock-producing zones. Francisco Fuentes, a Chilean geneticist working at the University of New Jersey in the United States of America, reviews the latest research on quinoa's biological properties as an anti-oxidant, anti-inflammatory or anticarcinogen. Victor Zevallos, a gastro-enterologist at King's College in London, describes quinoa's potential role in a gluten-free diet for patients suffering from coeliac disease. Chapters in the third part of this book unveil quinoa's wide range of potential uses, among which human consumption is simply the most visible.

Given the diversification of and potential for quinoa-based products, it is necessary to examine how legal instruments on food and agriculture are adapted to consider quinoa crops and quinoa-based products.

The fourth part of this book reviews domestic and international quinoa markets. The current Chilean Minister of Agriculture, Carlos Furche, Mexican agricultural economist at FAO, Salomón Salcedo, and others analyse past production and current international quinoa demand. They include the implications of recent price fluctuations on quinoa's international expansion. Peru and Bolivia, still the world's two largest quinoa producers, are developing new links with importers, but new producer countries are also emerging, including the United States of America, Canada, France, China and Morocco. These countries will compete with traditional exporter countries and the small- and medium-scale producers who live there. There is a clear risk in the international quinoa markets that new producer countries will corner the niche markets currently dominated by large Andean producers, especially in Bolivia and Peru, and also take over markets where Ecuador, Chile and Argentina are looking for a foothold. The chapter coordinated by Aurélie Carimentrand, economist at the *Université de Bordeaux* (University of Bordeaux) in France, analyses how the different quinoa certifications (organic, fair trade etc.) in the supply chain may add value to the product, increasing farmers' incomes and promoting local development.

The fifth part of this book contains chapters focused on the Andean countries where quinoa is grown. For each country, major quinoa specialists from the past 50 years review quinoa production system features and dynamics. These chapters are not thematic, but they provide a holistic vision of quinoa in each country, at various levels, taking into account the wide range of stakeholders involved in research, production, sale, and conservation. Quinoa's outlook in each of the five Andean countries is explored in the light of new stakeholders and new public policies aimed at developing quinoa crops. These chapters describe the success of quinoa production in fragile systems, and indicate the conditions necessary to maintain the sustainability of these agro-ecosystems.

The sixth and final part of this book comprises 11 chapters presenting examples of new countries or regions where quinoa is being produced – in Europe, Asia, Africa and North America. For example, Sven Jacobsen of the University of Copenhagen in Denmark describes Europe's initial forays into quinoa production and the crop's subsequent importance in the United Kingdom, the Netherlands and Denmark, within the framework of crop breeding programmes. Atul Barghava describes the agricultural potential of quinoa's biodiversity in the face of agricultural land salinization in India and Pakistan. Ouafae Benhabid of Morocco reviews the last 10 years of experimentation with quinoa in the marginal areas of the Atlas Mountains, and considers how quinoa might benefit small farmers. Larger-scale quinoa production programmes are also being developed with farmers who own large areas of land in the plains of Marrakech. The immense range of research projects being implemented in a variety of contexts is a reflection of the numerous objectives set by programmes and projects: drought resistance, tolerance to salinity, food security, the fight against poverty, export markets, family farming diversification, breeding varieties etc. While each situation presented relates to a specific development issue at various levels, the vital role of research networks in facilitating quinoa's sustainable global expansion remains a crosscutting theme. This means that research and the new knowledge it produces must be disseminated, or at least made accessible, to all. Regulations on phytogenetic resources have the power to promote or halt quinoa's current expansion, either building inclusivity or excluding certain stakeholders.

State of the Art of Quinoa in the World 2013 presents a snapshot of the current available knowledge, to enable us to reflect on potential short- and long-term scenarios, within which quinoa will continue to expand based on limited access to genetic resources in Andean countries, and restricted property rights to modern varieties developed in the North.

In the short term, the dominant trend of agricultural intensification focusing on genome advancements follows the industrial agricultural model that is driving us ever further away from sustainable food production and access to healthy foods. The *State of the Art of Quinoa in the World 2013* contributes to changing this scenario by forcing us to engage in dialogue with stakeholders and prevent quinoa's potential being lost in the face of conflicts over access to genetic resources and seeds. Rather, this book acts as a tool to develop joint innovations and share conservation costs. The full implications of quinoa's global expansion must be considered. Otherwise, both quinoa's biodiversity and the future of the Andean communities that depend on quinoa crops for local development in their regions will be threatened.

The international challenge of building an equitable long-term solution is tied to the geopolitics of quinoa. Today, new experimentation centres are opening in countries that previously did not even import quinoa. This leads to new competition on the global market, where small Andean producers will find that organic or fair trade certification is not enough in the face of competition from large-scale producers who, unlike them, have access to the financial capital necessary to invest in new forms of intensification. Against this backdrop, quinoa must be promoted not only as a crop, but also as an efficient and inclusive food and agricultural system to develop the most vulnerable sectors where South-South Cooperation has to play a central role.

This implies that the various stakeholders involved in quinoa's food and agricultural system must promote:

a) Monitoring of market behaviour, particularly the international markets, so as to predict supply and demand imbalances with a negative impact on prices;

b) Public policies that build the conditions necessary for fair trade and equitable distribution, to benefit farmers and local organizations;

c) Public policies that promote sustainable quinoa production, and agro-ecosystems where it is produced, while strengthening the food system;

d) Social inclusion policies that ensure quinoa contributes to territorial development and promotes the recognition of other Andean grains;

e) Monitoring of the expansion of growing areas at international level, and its impact on biodiversity depending on the agricultural models chosen;

f) Creation and implementation of international and national instruments for the protection, sustainable use and exchange of quinoa germplasm and seeds;

g) Strengthening of research networks to continue generating and sharing information on quinoa, making research available in the local languages of the quinoa-growing areas.

It is hoped that this book serves as a tool to foster the development of inclusive, respectful, responsible and ethical quinoa programmes and projects in the world, making a real difference in the fight against hunger and poverty, while recognizing and valuing the traditional knowledge and practices of indigenous peoples in the Andean region, who have maintained and preserved quinoa's biodiversity for generations.

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