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

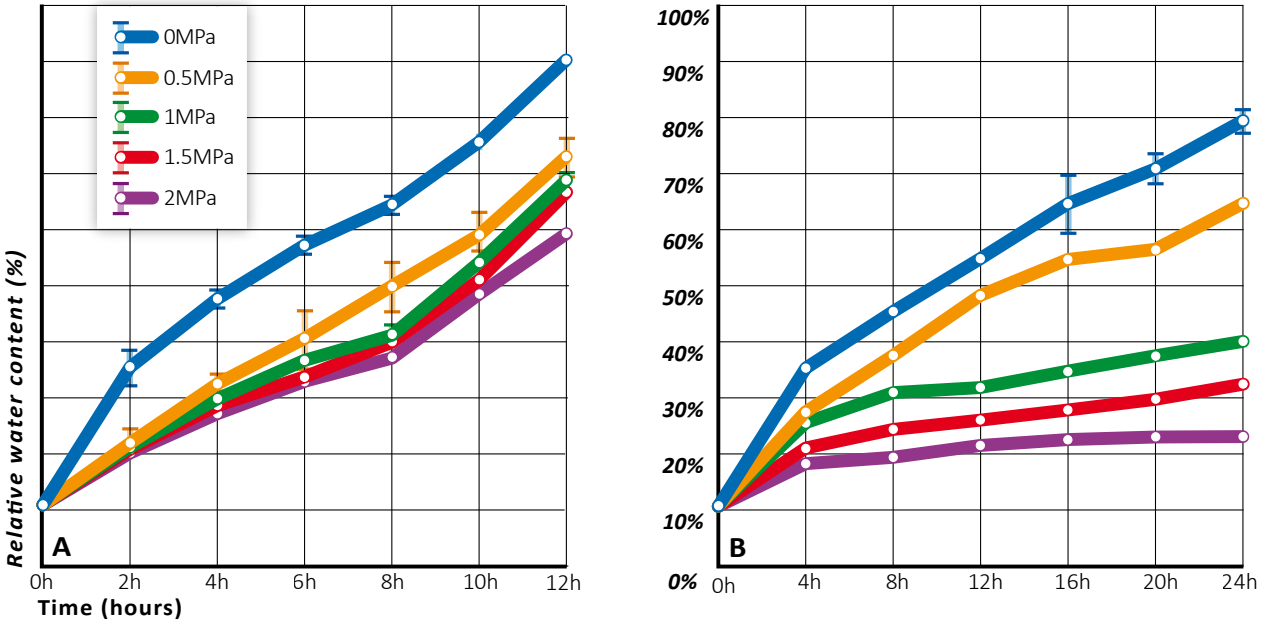

Figure 1: Kinetics of imbibition of quinoa seeds from Salare accessions: 'Amarilla' (A) and Coastal: 'Hueque' (B) treated with PEG 8000 at different osmotic potentials. Values correspond to the average of five repetitions. Vertical bars indicate the standard error (±). Different letters indicate significant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

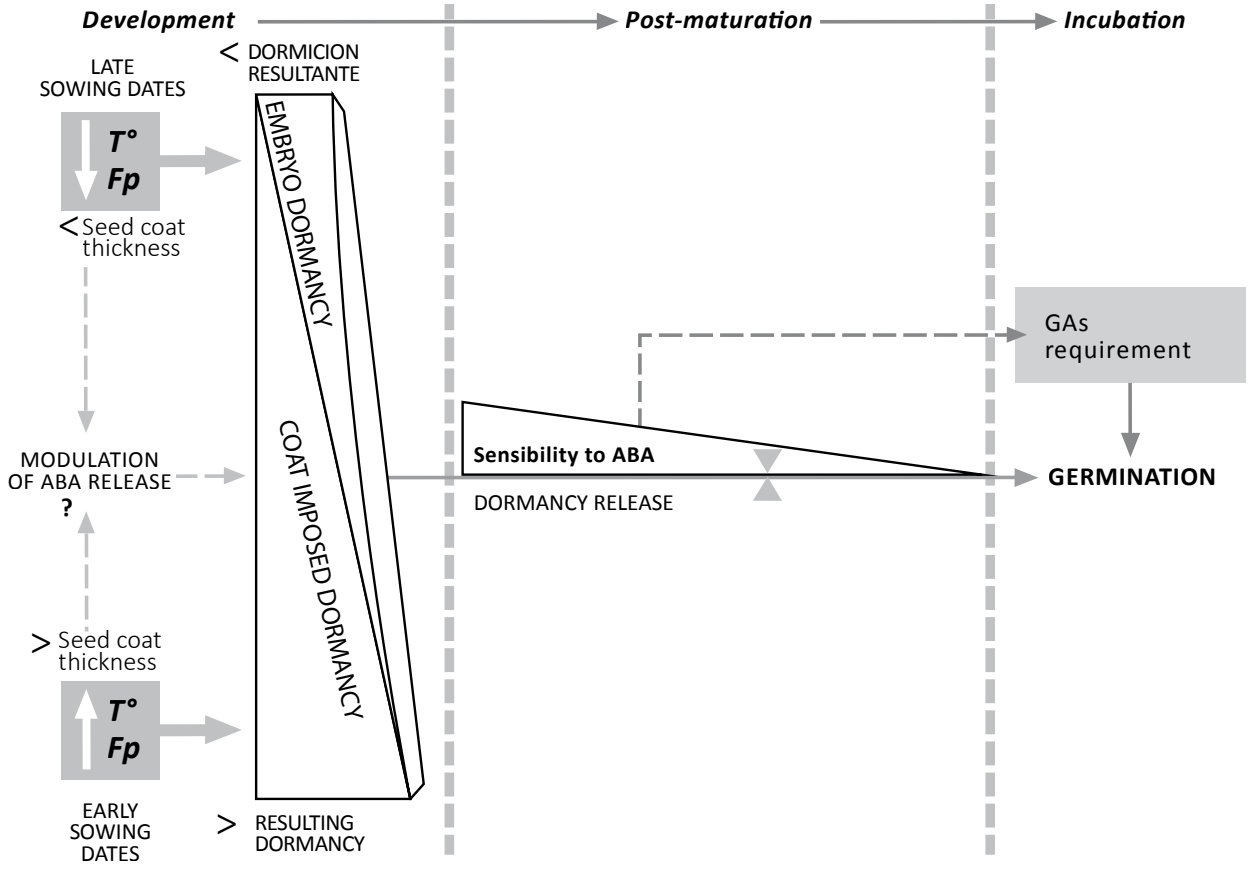

Figure 2. Conceptual schematic model of dormancy control in quinoa seeds, including the actual or hypothetical relationships between the different factors involved in its regulation. The dotted lines indicate regulation and the continuous lines indicate direct effects. During seed development, the environmental 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.

References

Allen, P., Benech-Arnold, R., Batlla, D. & Bradford, K. 2007. Modeling of seed dormancy. *In* K. Bradford & H. Nonogaki, eds. *Seed Development, Dormancy and Germination*, p. 72-112. Oxford, UK., Blackwell Publishing Ltd.

Batlla, D. & Benech-Arnold, R. 2007. Predicting changes in dormancy level in weed seed soil banks: Implications for weed management. *Crop Protection,* 26: 189-197.

Baker, E.H. & Bradford, K.J. 1994. The fluorescence assay for Maillard product accumulation does not correlate with seed viability. *Seed Science Research,* 4: 103-106.

Benech-Arnold, R. 2004. Inception, Maintenance and Termination of Dormancy in Grain Crops: Physiology, Genetics and Environmental Control. *In* R. Benech-Arnold & R. Sánchez, eds. *Handbook of Seed Physiology: Applications to Agriculture*, p. 169-198. Binghamton, NY, The Haworth Press.

Benech-Arnold, R., Sánchez, R., Forcella, F., Kruk, B. & Ghersa, C. 2000. Environmental control of dormancy in weed seed banks in soil. *Field Crops Research,* 67: 105-122.

Bennett, M.A. 2004. Seed and agronomic factors associated with germination under temperature and water stress. *In* R. Benech-Arnold & R. Sánchez, eds. *Handbook of Seed Physiology: Applications to Agriculture,* p. 97-123. Binghamton, NY, The Haworth Press.

Bertero, D., Aiscorbe, D., Piriz, S. & Benech-Arnold, R. 2001. *Susceptibilidad al brotado pre-cosecha en quinoa* (Chenopodium quinoa). Tenth International Congress of Andean Crops, Jujuy.

Bertero, D. & Benech-Arnold, R. 2000. Release from dormancy during seed development in quinoa: Variation associated to cultivar origin. Minnesota, USA, ASA (American Society of Agronomy), CSSA (Crop Science Society of America) & SSSA (Soil Science Society of America) Meeting.

Bewley, J. & Black, M. 1994. *Seeds: Physiology of Development and Germination*. New York, USA, Plenium-Press.

Boero, C., González, J. & Prado, F. 2000. Efecto de la temperatura sobre la germinación de diferentes variedades de quinoa (*Chenopodium quinoa* Willd.). *Lilloa,* 40: 103-108.

Bois, J.P., Winkel, T., Lhommee, J., Rafaillac, J.P. & Rocheteau, A. 2006. Response of some Andean cultivars of quinua (*Chenopodium quinua* Willd.) to temperature: Effects on germination, phenology, growth and freezing. *European Journal of Agronomy,* 25: 299-308.

Bove, J., Jullien, M. & Grappin, P. 2001. Functional genomics in the study of seed germination. *Genome Biology,* 3(1): reviews 1002.1-1002.5.

Bruno, M. 2005. ¿Domesticado o silvestre? Resultados de la investigación de semillas de *Chenopodium* en Chiripa, Bolivia (1500-100 A.C.). *Textos Antropológicos,* 15: 39-50.

Bruno, M. 2006. A morphological approach to documenting the domestication of *Chenopodium* in the Andes. *In* M. Zeder, D. Bradley, E. Emshwiller & B. Smith, eds. *Documenting Domestication: New genetic and archaeological paradigms,* p. 32-45. University of California Press.

Carter, L. & Chesson, J. 1996. Two USDA researchers develop a moisture-seeking attachment for crop seeders that is designed to help growers plant seed in soil sufficiently moist for germination. *Seed World,* 134: 14-15.

Castellión, M.L. 2008. Procesos de deterioro y mecanismos de protección y reparación involucrados en la pérdida diferencial de la viabilidad durante el almacenamiento en semillas de *Chenopodium quinoa* Willd. 116 p. University of Buenos Aires in the field of biological sciences. (thesis)

Castellión, M., Matiacevich, S., Buera, M.P. & Maldonado, S. 2010a. Protein deterioration and longevity of quinoa seeds during long-term storage. *Food Chemistry,* 121: 952-958.

Castellión, M., Maldonado, S. & Buera, P. 2010b. Molecular mobility and seed longevity in Chenopodium quinoa. *In: Water Properties in Food, Health, Pharmaceutical and Biological Systems*: ISOPOW 10.

Ceccato, D., Bertero, D. & Batlla, D. 2011. Environmental control of dormancy in quinoa (*Chenopodium quinoa*) seeds: two potential genetic resources for pre-harvest sprouting tolerance. *Seed Science Research,* 21: 133-141.

Ceccato, D. 2011. Efecto de las condiciones ambientales durante el desarrollo, maduración y almacenamiento sobre la dormición en semillas de quínoa (*Chenopodium quinoa* Willd.) con tolerancia potencial al brotado pre-cosecha. 203 p. Faculty of Agronomics, University of Buenos Aires. (PhD thesis)

Chilo, G., Vacca, M., Carabajal, R. & Ochoa, M. 2009. Efecto de la temperatura y salinidad sobre la germinación y crecimiento de plántulas de dos variedades de *Chenopodium quinoa. Agriscientia,* 26: 15-22.

Cortés-Bugueño, D. & Navarro-Honores, J. 2010. Evaluación de efectos simples y combinados de salinidad y nutrientes en la germinación y vigor de diez ecotipos de quinoa (*Chenopodium quinoa* Willd). Chile, University of La Serena. (thesis)

Delatorre, J. & Pinto, M. 2009. Importance of ionic and osmotic components of salt stress on the germination of four quinua (*Chenopodium quinoa* Willd.) selections. *Chilean Journal of Agricultural Research,* 69: 477-485.

Delatorre, J. 2008. Efecto de la salinidad sobre la germinación y la fotosíntesis durante los primeros estados de crecimiento y desarrollo de ecotipos nativos de quinua (*Chenopodium quinoa* Willd). University of Chile. 129 p. (thesis)

Delfino, I. 2008. Efecto de las bajas temperaturas sobre los estadios fenológicos en dos selecciones de quinua (*Chenopodium*

quinoa Willd.) del altiplano chileno. Iquique, Chile, University Arturo Prat. 120 p. (thesis)

Delouche, J. 2002. Germination and deterioration in seeds. *Seed News Review,* 6: 6.

Dorne, A. 1981. Variation in seed germination inhibition of *Chenopodium bonus-henricus* in relation to altitude of plant growth. *Canadian Journal of Botany,* 59: 1893-1901.

Eira, M.T.S., Walters, C., Caldas, L.S., Fazuoli, L.C., Sampaio, J.B. & Dias, M.C.L.L. 1999. Tolerance of Coffea spp. seeds to desiccation and low temperature. *Revista Brasileira de Fisiologia Vegetal,* 11: 97-105.

Ellis, R.H., Hong, T.D., Martin, M.C., Pérez-Garcia, F. & Goméz-Campo, C. 1993. The long-term storage of seeds of seventeen crucifers at very low moisture contents. *Plant Varieties and Seeds,* 6: 75-81.

Ellis, R.H., Hong, T.D., Roberts, E.H. & Tao, K.L. 1990. Low moisture content limits to relations between seed longevity and moisture. *Annals of Botany,* 65: 493-504.

Ellis, R. & Roberts, E. 1980. Improved equations for prediction of seed longevity. *Annals of Botany,* 45: 13-30.

Feurtado, A. & Kermode, A. 2007. A merging of paths: abscisic acid and hormonal cross-talk in the control of seed dormancy maintenance and alleviation. In Bradford K and H Nonogaki (eds). *Seed Development, Dormancy and Germination,* p. 176-223. Oxford, UK, Blackwell Publishing Ltd.

Giri, G.S. & Schillinger, W.F. 2003. Seed priming winter wheat for germination, emergence and yield. *Crop Science,* 43: 2135-2141.

González-Zertuche, L. & Orozco-Segovia, A. 1996. Métodos de análisis de datos en la germinación de semillas, un ejemplo: *Manfreda brachystachya*. *Boletín de la Sociedad Botánica de México,* 58: 15-30.

Gremillion, K. 1993a. Crop and weed in prehistoric eastern North America: The *Chenopodium*-example. *American Antiquity,* 58: 496-509.

Gremillion, K. 1993b. The evolution of seed morphology in domesticated *Chenopodium*: An archaeological case study. *Journal of Ethnobiology,* 13: 149-169.

Gubler, F., Millar, A.A. & Jacobsen, J.V. 2005. Dormancy release, ABA and pre-harvest sprouting. *Current Opinion in Plant Biology,* 8: 183-187.

Hilhorst, H. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research,* 5: 61-73.

Jacobsen, S.E. & Bach, A.B. 1998. The influence of temperature on seed germination rate in quinoa (*Chenopodium-quinua*-Willd). *Seed Science and Technology,* 26: 515-523.

Jacobsen, S.-E., Jørnsgård, B., Christiansen, J. & Stølen, O. 1999. Effect of harvest time, drying technique, temperature and light on the germination of quinoa (*Chenopodium quinoa*). *Seed Science and Technology,* 27: 937-944.

Jacobsen, S.E., Mujica, A. & Jensen, C.R. 2003. The resistance of quinoa (*Chenopodium quinoa* Willd) to adverse abiotic factors. *Food Reviews International,* 19(1-2): 99-109.

Jacques, R. 1968. Action de la lumière par l´intermédiaire du phytochromesur la germination, la croissance-et le développement de *Chenopodium polyspermum* L. *Physiologie Végétale,* 6: 137-164.

Jeller, H., Perez, S. & Raizer, J. 2003. Water uptake, priming, drying and storage effects in *Cassia excelsa* Schrad seeds. *Brazilian Journal of Biology,* 63(1): 61-68.

Johnston, M., Pinto, M., Fernández, G. & Riquelme, A. 1999. *Curso: Fisiología de la germinación de semillas*. 100 p. Department of Agricultural Production, Faculty of Agronomy, University of Chile.

Justice, O.L. & Bass, L.N. 1978. *Principles and practices of seed storage*. U.S. Government printing office, Washington, D.C.

Karssen, C. 1970. The light promoted germination of the seeds of *Chenopodium album* L. III. Effect of the photoperiod during growth and development of the plants on the dormancy of the produced seeds. *Acta Botanica Neerlandica,* 19: 81-94.

Karssen, C., Brinkhorst-van der Swan, D., Breekland, A. & Koornneef, M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta,* 157: 158-165.

Kermode, A. 2005. Role of abscisic acid in seed dormancy. *Journal of Plant Growth Regulation,* 24: 319-344.

Knowles, P.F. 1988. Recent advances in oil crop breading. *In* E.H. Applewhite, ed. *Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry*, p. 35-38. American Oil Chemists' Society.

Koornneef, M., Bentsink, L. & Hilhorst, H. 2002. Seed dormancy and germination. *Current Opinion in Plant Biology,* 5: 33-36.

Koyro, H.-W. & Eisa, S.S. 2008. Effect of salinity on composition, viability and germination of seeds of *Chenopodium quinoa* Willd. *Plant and Soil,* 302: 79-90.

Leopold, A.C. & Vertucci, C.W. 1989. Moisture as a regulator of physiological reaction in seeds. *In* P.C. Stanwood & M.B. McDonald, eds. *Seed Moisture*, p. 51-67. CSSA Special Publication Nº 14. Crop Science Society of America, Madison.

López Fernández, M.P. 2008. *Longevidad de las semillas de nueve cultivares de* Chenopodium quinoa *Willd., procedentes de regiones contrastantes: Ecuación de la viabilidad y rol de las cubiertas seminales*. 61 p. Faculty of Exact and Natural Sciences, University of Buenos Aires. (thesis)

Moncada, S. 2009. Hidro y osmoacondicionamiento de semillas de quinua y sus efectos sobre la germinación a temperatura subóptima. 110 p. University of Arturo Prat, Iquique, Chile. (thesis)

Mujica, A. 1988. Parámetros genéticos e índices de selección en quinua (*Chenopodium quinoa* Willd.). 158 p. Chapingo, Mexico, Colegio de Postgraduados. (thesis)

Mujica, A., Izquierdo, J. & Marathee, J.-P. 2000. Capítulo I. Origen y descripción de la quinua. (Available at: www.fao.org). Accessed on 15 October 2007.

Munns, R., Schachtman, D. & Condon, A. 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Australian Journal of Plant Physiology,* 22: 561-569.

Murthy, U.M.N., Liang, Y., Kumar, P.P. & Sun, W.Q. 2002. Non-enzymatic protein modification by the Maillard reaction reduces the activities of scavenging enzymes in *Vigna radiata*. *Physiologia Plantarum,* 115: 213-220.

Murthy, U.M.N. & Sun, W.Q. 2000. Protein modification by the Amadori and Maillard reactions during seed storage: roles of sugar hydrolysis and lipid peroxidation. *Journal of Experimental Botany,* 51: 1221-1228.

Ng, S.C., Anderson, A., Coker, J. & Ondrus, M. 2007. Characterization of lipid oxidation products in quinoa (*Chenopodium quinoa*). *Food Chemistry,* 101(1): 185-192.

Orsini, F., Accorsi, M., Gianquinto, G., Dinelli, G., Antognoni, F., Ruiz-Carrasco, K.B., Martínez, E.A., Alnayef, M., Marotti, I., Bosi, S. & Biondi, S. 2011. Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: functional elements of successful halophytism. *Functional Plant Biology,* 38: 818-831.

Paulsen, G.M. & Auld, A.S. 2004. Preharvest Sprouting of Cereals. *In* R. Benech-Arnold & R. Sánchez, eds. *Handbook of Seed Physiology: Applications to Agriculture,* p. 199-219. Binghamton, NY, The Haworth Press.

Pourrat, Y. & Jacques, R. 1975. The influence of photoperiodic conditions received by the mother plant on morphological and physiological characteristics of *Chenopodium polyspermum* L. seeds. *Plant Science Letters,* 4: 273-279.

Prego, I., Maldonado, S. & Otegui, M. 1998. Seed structure and localization of reserves in *Chenopodium quinoa* Willd. *Annals of Botany,* 82: 481-488.

Priestley, D.A. 1986. *Seed Aging: Implications for Seed Storage and Persistence in the Soil*. Comstock, Cornell, University Press.

Priestley, D.A. & Leopold, A.C. 1983. Lipid changes during natural ageing of soybean seeds. *Physiologia Plantarum,* 59: 467-470.

Roberts, E.H. & Ellis, R.H. 1989. Water and seed survival. *Annals of Botany,* 63: 39-52.

Rosa, M., Hilal, M., González, J.A. & Prado, F.E. 2004. Changes in soluble carbohydrates and related enzymes induced by low temperature during early developmental stages of quinua (*Chenopodium quinua*) seedlings. *Journal of Plant Physiology,* 161: 683-689.

Ruales, J. & Nair, B.M. 1992. Nutritional quality of the protein in quinoa (*Chenopodium quinoa* Willd.) seeds. *Plant Foods for Human Nutrition,* 42: 1-12.

Ruiz-Carrasco, K.B., Antognoni, F., Coulibaly, A.K., Lizardi, S., Covarrubias, A., Martínez, E.A., Molina- Montenegro, M.A., Biondi, S. & Zurita-Silva, A. 2011. Variation in salinity tolerance of four lowland genotypes of quinoa (*Chenopodium quinoa* Willd.) as assessed by growth, physiological traits, and sodium transporter gene expression. *Plant Physiology and Biochemistry,* 49: 1333-1341.

Soeda, Y., Konings, M.C., Vorst, O., Van Houwelingen, A., Stoopen, G., Maliepaard, C., Kodde, G., Bino, R., Groot, S. & Van Der Geest, A. 2005. Gene expression programs during *Brassica oleracea*-seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level. *Plant Physiology,* 137: 354-368.

Steinbach, H., Benech-Arnold, R. & Sánchez, R. 1997. Hormonal regulation of dormancy in developing sorghum seeds. *Plant Physiology,* 113: 149-154.

Sun, W.Q. & Leopold, A.C. 1995. The Maillard reaction and oxidative stress during ageing of soybean seed. *Physiologia Plantarum,* 94: 94-104.

Tarquis, A. & Bradford, K. 1992. Prehydration and priming treatments that advance germination also increase the rate of deterioration of lettuce seeds. *Journal of Experimental Botany,* 43(3): 307-317.

Tremolieres, A., Dubacq, J.P. & Drapier, D. 1982. Unsaturated fatty acids in maturing seeds of sunflower and rape: Regulation by temperature and light intensity. *Phytochemistry,* 21(1):41-45.

Vertucci, C.W. & Roos, E.E. 1993. Theoretical basis of protocols for seeds storage: II. The influence of temperature on optimal moisture levels. *Seed Science Research,* 3: 201-213.

Walters, C. 1998. Understanding the mechanisms and kinetics of seed ageing. *Seed Science Research,* 8: 223-244.

Wettlaufer, S.H. & Leopold, A.C. 1991. Relevance of Amadori and Maillard products to seed deterioration. *Plant Physiology,* 97: 165-169.

Zhu, J.K. 2003. Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology,* 6: 441-445.