

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 in-

vestigating 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’, ‘B078’). 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 (Delatorre-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% reduc-

tion 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 Marangani') 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

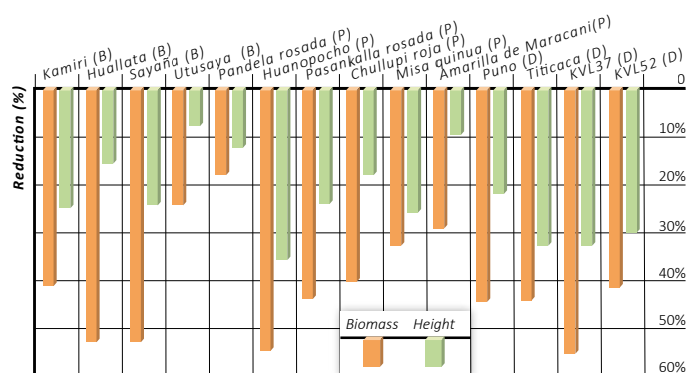


Figure 1: Name, origin (B = Bolivia, P = Peru, D = Denmark) and reduction in biomass and height under saline conditions relative to non-saline conditions of several quinoa genotypes. Adapted from Adolf *et al.* (2012).

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 distri-

bution 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-

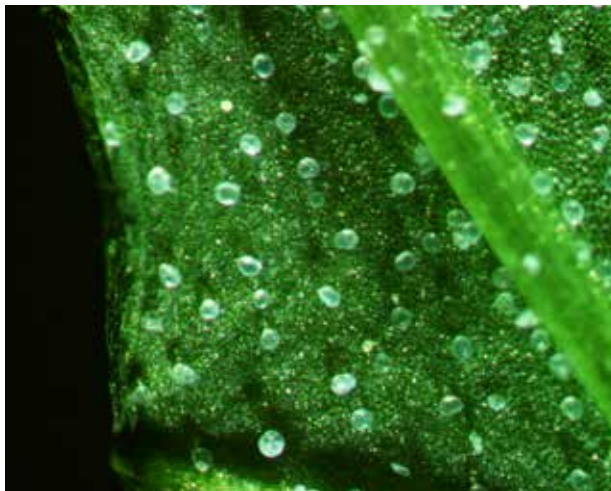


Figure 2: Salt bladders on the leaf surface of quinoa.

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 CO₂ 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 aper-

ture) 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 CO₂ entry. Stomatal conductance and

photosynthetic CO₂ 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 CO₂ 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 CO₂ 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

High 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 (Ca²⁺, Mg²⁺) 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 (MgSO₄, Na₂SO₄, NaCl and CaCl₂) 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 trig-

onelline 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 $\mu\text{mol/g DW}$, 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

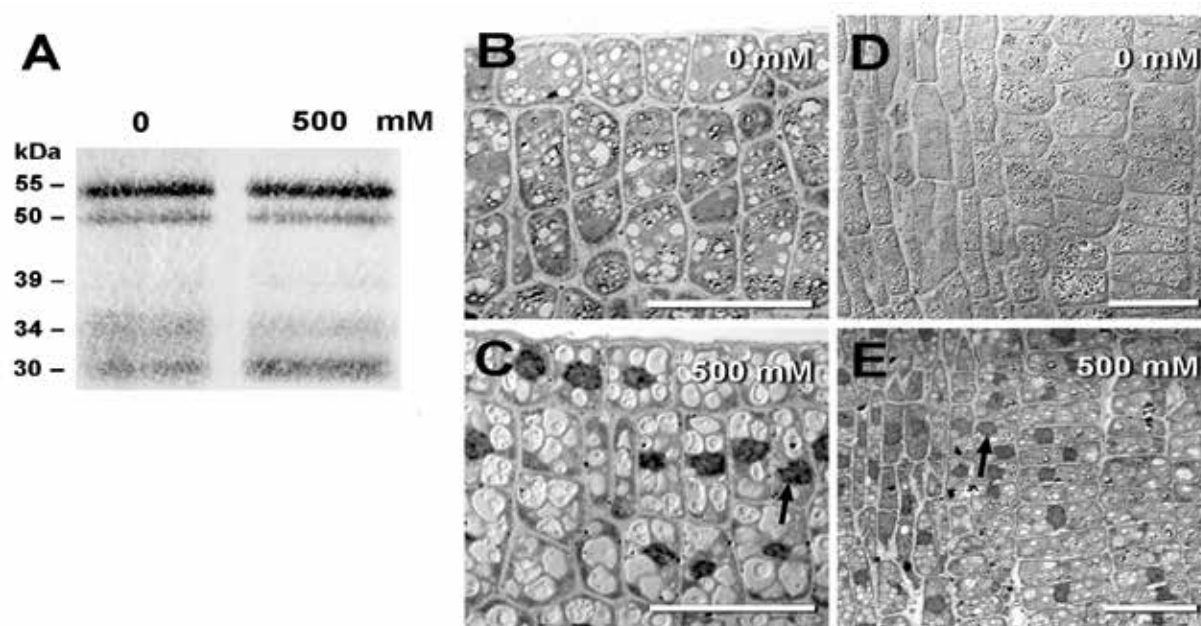


Figure 3: A. Western blot analysis of dehydrins in mature embryos of quinoa. Seeds were obtained from plants grown in 0 and 500 mM NaCl. B-E. Dehydrin in situ immunolocalization in the cotyledons (B, C) and embryo axis (D, E) observed under Differential Interference Contrast (DIC) microscopy. Labelling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

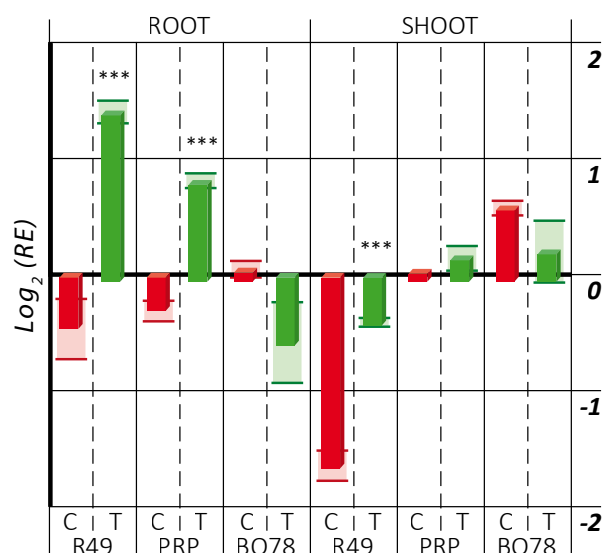


Figure 4. Expression of *CqNHX1* in roots (left) and shoots (right) of control (C) and 300 mM NaCl -treated (T) quinoa plants from northern (R49), central (PRP) and southern (BO78) Chile. The salt treatment was applied 60 days after germination in pots, and leaves were sampled 24 hours after the salt 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 (RUE_{seed}), straw ($\text{RUE}_{\text{straw}}$) and total dry matter (RUE_{DM}) as $\text{RUE} = \text{Yield}/\text{IPAR}$.

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 oth-

er 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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