



Full Length Article

Exploring Potential of Well Adapted Quinoa Lines for Salt Tolerance

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Abstract

Losses of arable lands due to increasing soil salinization over the world are increasing and fresh water recourses are diminishing. Viable imperative option is to explore potential of halophytic plants like quinoa in semi-arid and arid agro ecologies where crop production is severely affected. Therefore, preliminary study was conducted using NaCl (0, 100, 200, 300 and 400 dS m⁻¹) amended soil in pots in wire house of Department of Crop Physiology, University of Agriculture Faisalabad during 2012–13 to examine the salt tolerance variations of four well adapted quinoa lines (Q1, Q2, Q7, Q9) with the purpose to identify line suitable for further cultivation on salt-affected soils. Data regarding different salt tolerance indices indicated that tolerance was linked to high leaf turgor potential, increased K⁺ over Na⁺ uptake and *de novo* accumulation of osmoprotectants (proline and total phenolics contents) in plant tissues. Lines Q7 and Q2 were more salt tolerant and Q7 gave maximum seed yield at 100 dS m⁻¹ by maintaining improved growth due to reasonable gas exchange relations under all salt regimes than other two. The large quinoa genetic variability in salinity tolerance opened new avenues to explore it further in different salt affected field conditions. © 2017 Friends Science Publishers

Keywords: Gas exchange; K⁺/Na⁺ ratio; Halophyte; Osmoprotectants; Quinoa; Salt tolerance

Introduction

Salinity is one of the major threat to crop-production world over, especially in semi-arid and arid areas (Schleiff, 2008). Salinity affects more in the areas of low rainfall, high evapotranspiration, elevated temperature, poor water and soil management practices (Azevedo *et al.*, 2006). Globally salinity affects more than 45 Mha of irrigated lands, 1.5 Mha are taken out of production as a result of high salinity each year (Munns and Tester, 2008). In cultivable land of Pakistan 6.8 Mha soils is salt affected and out of which 2.67 m ha are in Punjab province (Vashev *et al.*, 2010). The salt responsible for salinity includes sulfates, chlorides, carbonates and bicarbonates of sodium, potassium, magnesium and out of these sodium chloride (NaCl) is the main cause (Li *et al.*, 2006). Salt stress causes nutrient and ionic imbalance, ionic toxicity, production of reactive oxygen species and curtailed assimilation of ammonium and nitrate. Sodium replaces calcium bridging in membranes that hampers protein, nucleic acids and enzyme activities (Pattanagul and Maysaya, 2008). Similarly, soils having low fertility levels are responsible for yield reduction due to improper nutrients availability.

Generally, there are three approaches for increasing the production of crops from salt-affected soils: reclamation of salt-affected soils, use of salt tolerant germplasm and introduction of salt tolerant genotypes (Blumwald *et al.*, 2004; Yilmaz *et al.*, 2004) or alternate crops e.g.

acclimatization of salt tolerant plants (gaining popularity word wide) appropriate for highly saline conditions (Flowers, 2004). The first approach is not practicable due to wide salt-affected areas, insufficient availability of good quality water, soil permeability and high cost of amendments (Akhtar *et al.*, 2010). Limited salt tolerant germplasm is available due to several barriers during development i.e. variable environmental conditions, lack of information about genetic makeup of crops, their biochemical and physiological responses to salinity and complex polygenic salt tolerant traits. Salinity-tolerance will only be achieved when all key characters are collectively considered in a complementary way (Shahbaz and Ashraf, 2013). Therefore, we should be realized that there is no existence of such thing which we can considered as a “silver bullet” that can resolve problems of salinity; focusing only one character (gene) will not result any substantial improvements.

Halophytes can be raised in variety of salty environments, from coastal areas, mudflats and salt marches, to inland deserts steppes and salt flats. They occur in different plant families among them dominant is Chenopodiaceae (Flowers and Colmer, 2008). Halophyte can tolerate higher concentration of salts due to variety of adaptations. These include osmotic adjustment through ion compartmentalization in vacuoles, succulence, accumulation of organic compatible solutes, salt secreting bladders and glands (Shabala and Mackay, 2011).

Halophyte grows optimally even at high salt concentrations (100–200 mM NaCl for dicots and 50 mM for monocots) (Flowers and Colmer, 2008). For semiarid and arid agro ecologies, this suggests more wide irrigations with poor quality (saline) water. Yet again, halophyte species can be considered as “spotlight” being capable of not only surviving, but really gaining benefit from saline irrigation. Furthermore, there was not found significant yield reduction in some halophyte species even with sea water irrigations (e.g. *Chenopodium quinoa* Willd. Hariadi *et al.*, 2011). Any conventional known crop species is not capable to tolerate such extreme salt concentrations, considering halophyte ideal for saline agriculture.

Quinoa a grain crop belongs to Amaranthaceae family with superior nutritional profile, originated from Andean region of Latin America where its cultivation was found seven thousand years ago (Jacobsen *et al.*, 2003). Quinoa displays optimum growth in range of 100 to 200 mM NaCl, that why considered as *true halophyte*. Moreover, some genotypes can tolerate and grow with salt concentrations of seawater (40 dS m⁻¹) (Jacobsen *et al.*, 2003; Koyro and Eisa, 2008). The interest about this crop is increasing over the world, both due to abiotic stress tolerance and superior nutritional benefits (Stikic *et al.*, 2012).

Grain of quinoa is gluten-free, contain substantial amount of all essential-amino acid, vitamins, (A, B2 and E), minerals (Ca, K, Fe, Mn) and health supportive fatty-acids (omega 3) (Rep-Carrasco *et al.*, 2003). Recently in Pakistan quinoa has been successfully introduced, cultivated and basic production technology developed (Basra *et al.*, 2014). Since quinoa can be successfully grown on marginal soils (Jacobsen *et al.*, 2003) which was the main objective to introduce in Pakistan has yet to be tested under local salt affected conditions. Significant variations exist among quinoa accessions for physiological and agronomical variables when raised under different set of saline conditions (Adolf *et al.*, 2012).

Quinoa offers an alternate crop for the future due to its great properties as a food source. This study was therefore performed to examine the salt tolerance variations of four quinoa lines with the aim to identify germplasm suitable for cultivation on salt affected soils.

Materials and Methods

Experimental Details and Plant material

This pot study was conducted in wire-house (open natural environment), Department of Agronomy, University of Agriculture Faisalabad (UAF) during 25 November 2012 to 10 April, 2013 with following details. Four quinoa lines were collected from Department of Crop Physiology, (UAF). Details of lines are presented in Table 1. Lines are cited hereafter according to their respective local codes.

Imposition of Salinity Levels in Pots

The soil used in this study was analyzed according to standard procedures and its chemical and physical characteristics are presented in Table 2. Before filling soil, the pots were lined internally using polythene sheet and hole at bottom was also blocked with cork in order to stop leaching. Five salinity levels 1.51 (control; no salt added), 10, 20, 30 and 40 dS m⁻¹ were created by adding desired amount of NaCl salt in each pot and mixing was carried out with help of mechanical mixer one month prior to sowing according to protocol described in US Staff Handbook-60. Twenty-five seeds of each quinoa line were sown in every pot comprising 13 kg soil per pot. Every treatment was replicated four times. Five (1.51, 10, 20, 30 and 40 dS m⁻¹) reference pots were also maintained to notice the effect of irrigation water on developed salinity. ECe was recorded regularly in the reference pots and from pots containing plants at the end. No significant changes in levels of salinity were observed at end. Pots were placed in wire-house under natural light and temperature according to completely randomized design. Each pot was supplement with P and K @ 60 kg ha⁻¹ as basal dose using DAP and SOP fertilizers while N dose was applied @ 75 kg ha⁻¹ using urea half dose at sowing and half at flowering. Pots were provided with good quality water. After emergence, extra plants were uprooted to maintain equal plant population of five plants/pot. Data regarding Na⁺ and K⁺ concentrations in leaf and root, water relations, gas relations, leaf biochemical analysis and yield parameters were collected at different stages as mentioned below.

Determination of Na⁺ and K⁺ Concentrations in Leaves and Roots

Newly emerged fully expanded leaf; sixth leaf from the top (three leaves, from three plants and their respective roots) were collected from each pot and recorded fresh and oven dried weights. Dried leaf (0.1 g) and roots (0.1 g) were digested in 25 mL 1% HNO₃ solution on a hot plate at 85°C for 4 h. After digestion 1 mL of digested mixture was diluted to 20 mL volume to determine Na⁺ and K⁺ concentrations in leaf by flame photometer (Sherwood, UK, Model 360).

Determination of Water Relations

Early in the morning at 6:00 a.m after 45 DAS, leaf water potential (ψ_w) was measured using the youngest fully developed leaf from top from five plant/treatment with a Scholander type water potential apparatus (Arimad-2-Japan). The same leaf was kept in a freezer for one week at -21°C. the measurement of osmotic potential (ψ_s) after extracting the sap from the frozen leaf material, was determined with an osmometer (VAPRO, Model 5520, USA). Leaf turgor potential was calculated using the following equation:

$$\psi_p = \psi_w - \psi_s$$

Table 1: Details of quinoa lines

Code*	G. Line**	Origin
PI 596293	Q1	New Mexico, USA
Ames 13730	Q2	Colorado, USA
Ames 13737	Q7	New Mexico, USA
PI 634919	Q9	Chile

(*as per the germplasm database **coding of lines made for local identification)

Source: Main source was USDA since 2008, I collected from Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan

Table 2: Physical and chemical characteristic of soil used in pot study

Characteristics	Value
Sand (%)	52.1
Silt (%)	27.1
Clay (%)	19.2
Textural class	Sandy clay loam
Saturation percentage (%)	31
pHe	7.6
ECe (dS m ⁻¹)	1.41
Available phosphorous (Olson) (mg kg ⁻¹)	3.31
Extractable potassium (NH ₄ OAC) (mg kg ⁻¹)	82
Organic matter (%)	0.71
Total nitrogen (%)	0.06

Measurement of Gas Exchange Relations

Fifty days after sowing, net CO₂ assimilation rate (A), stomatal conductance (gs) and transpiration rate (E) were measured by putting leaf (young fully expanded, 3rd leaf from top) in leaf chamber of portable infrared gas analyzer (Analytical development company, Hoddeson, UK). Instrument was used between 10:00 am to 1:00 pm. Leaf chamber conditions were: temperature (25-28°C), ambient CO₂ (371 µmol/mol), leaf chamber molar gas flow rate (400 µmol/s) leaf chamber gas flow rate (296 ml/min), PAR at leaf surface up to 770 µmol/m²/s.

Determination of Antioxidants

Sixty days after sowing, fully expanded young leaves from five random plants, grown in each pot, were collected. Leaf samples were collected early in the morning before 6:00 am, packed in aluminum foil, put in thermo bottles containing liquid nitrogen, and transferred to plastic zipper bags, which were put in freezer (-80°C) for further analysis. Biochemical analysis was done within three days using spectrophotometer (UV 4000). Osmoprotectants, that is leaf total phenolic contents (TPH), were estimated according to protocol of Waterhouse (2001) at 765 nm wavelength using reference standards of gallic acid. The proline content in fresh leaf was determined by the method followed by Bates *et al.* (1973).

Growth and Yield Estimation

Plant height and main panicle lengths were measured at

maturity with the help of centimeter scale, while stem diameter was measured with the help of Vernier caliper at three points of stem i.e., upper middle and bottom than averages were calculated.

Harvesting was done from each pot on 10 April 2013 when plants were matured as according to suggestions cited in Jacobsen and Stølen (1993) article. Inflorescences and other plant parts were dried on filter paper at 25–30°C. After ten days' seeds were threshed manually. Remaining plant part was dried at 65°C for one week, and recorded the dry weight and added to seed weight to calculate total biomass. Later on, thousand seeds weights was also recorded using digital balance.

Statistical Analysis

Each treatment was replicated four times, and data were statistically analyzed by a two-way ANOVA analysis under a completely randomized design (CRD) comprising factorial arrangement using statistical software "Statistix" (ver. 8.1, Tallahassee, FL, USA). Salinity levels and quinoa lines were taken as factor.

Results

Plant Growth and Yield Responses

Data in Fig. 1(a, b) reveal that plant heights and main panicle lengths of all quinoa lines (Q1, Q2, Q7 and Q9) were significantly ($P \leq 0.001$) influenced by NaCl stress applied in pot soil. Salt stress of 10 dS m⁻¹ had a significant positive effect on increasing plant height and main panicle length of all lines, however further increase negative influence. Furthermore, Q2 and Q7 had larger main panicles and higher plant heights at maturity while growing at all salt regimes. Minimum values for these parameters were recorded for Q1 at 40 dS m⁻¹ salt stress while stem diameters were found statistically similar (Fig. 1c).

Data showed significant ($P \leq 0.001$) reduction in shoot biomass and seed yield of Q1 and Q2 at 30 dS m⁻¹, while these reductions started at 20 dS m⁻¹ for Q1 and Q9 (Fig. 3a, b). Furthermore, Q2 and Q7 produced more biomass and seed yield than Q1 and Q9 at normal and all salt regimes. Maximum shoot biomass and seed yield was produced by Q7 at 10 dS m⁻¹ NaCl level while minimum was found in Q1 at 40 dS m⁻¹ (Fig. 3a, b). 1000 seed weight of all lines reduced significantly at 40 dS m⁻¹ while lines Q2 and Q7 had more seed weights as compared to Q1 and Q9 at this extreme level of salt stress (Fig. 3c).

Leaf Gas Exchange

There was significant ($P \leq 0.001$) influence of varying levels of salt stress on leaf photosynthetic rate (An), stomatal conductance (gs) and transpiration rate (E).

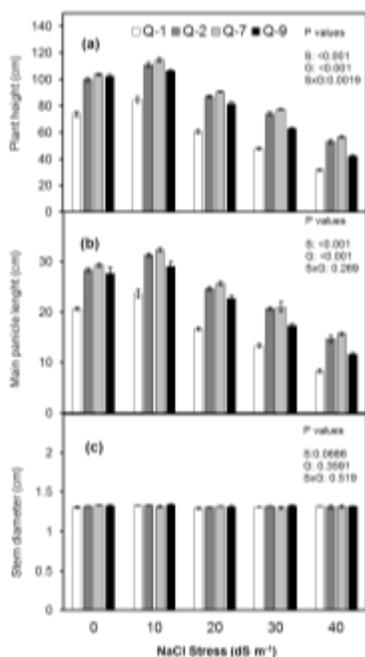


Fig. 1: Plant height (a), main panicle length (b) and stem diameter (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. ($n=4$)

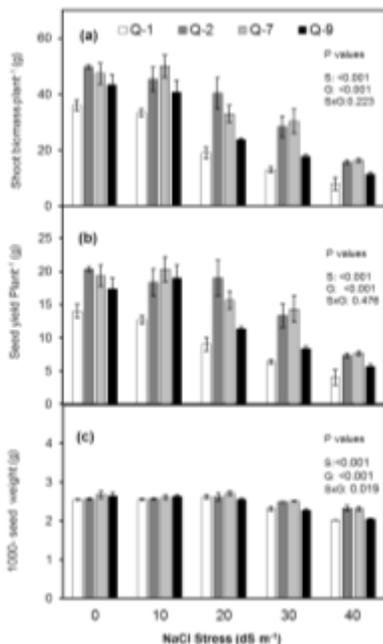


Fig. 2: Shoot biomass per plant (a), seed yield per plant (b) and thousand seed weight (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. ($n=4$)

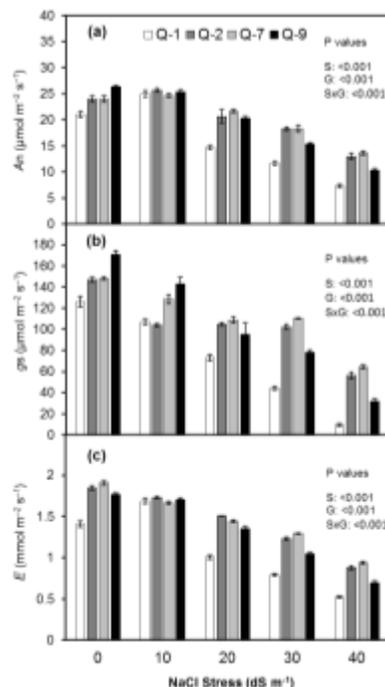


Fig 3: Leaf photosynthetic rate (An) (a), leaf stomatal conductance (gs) (b) and leaf transpiration rate (E) (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. ($n=4$)

NaCl stress in growth medium significantly decreased all these parameters (Fig. 3a-c). An was found significantly higher at 10 dS m⁻¹ in Q1, Q2 and Q7 than decreased with further increased salt stress. However, lines Q2 and Q7 had more An than An of Q1 and Q9 at every increased salt level (Fig. 4.2.3a). All lines showed decreasing trend for gs and E with increase in salinity levels except Q1 which had more E at 10 dS m⁻¹ while line Q2 and Q7 had more gs and E than other two lines (Q1 and Q9) at all salt regimes (Fig. 3b, c).

Leaf Water Relations

Fig. 4 depicts that all quinoa lines had significantly linear lower (more negative) leaf water potential (Ψ_l), leaf osmotic potential ($\Psi\pi$) and leaf turgor potential (Ψ_p) when grown at every incremented NaCl stress. However, lines Q2 and Q7 had significant better Ψ_l and $\Psi\pi$ compared with Q1 and Q9 at all salt regimes (Fig. 4 a, c).

Leaf Na⁺, K⁺ Contents and K⁺/Na⁺ Ratios

A significant linear increase was found for both leaf Na⁺ and K⁺ contents at every increased salt level in all lines (Fig. 5a, b). While significant less Na⁺ and higher K⁺ contents were found in leaves of Q2 and Q7 as compared to Q1 and Q9 at all salt regimes (Fig. 5a, b). Furthermore, leaf K⁺/Na⁺

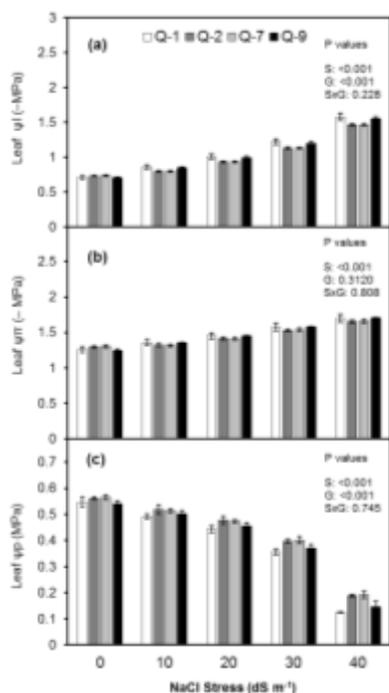


Fig 4: Leaf water potential (a), leaf osmotic potential (b) and leaf turgor potential (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. ($n=4$)

decreased significantly in all lines at every increased NaCl level. Q2 and Q7 had better leaf K^+/Na^+ ratios at all NaCl incremented growth regimes (Fig. 5c).

Root Na^+ , K^+ Contents and K^+/Na^+ Ratios

Higher root Na^+ contents were found in all lines at every increased salt level in pot soil (Fig. 6a). Furthermore, lines Q-1 and Q-9 had maximum Na^+ contents in roots at 400 mM NaCl salt stress. While root K^+ contents gradually decreased with increased in NaCl salt (Fig. 6b). Root K^+/Na^+ ratio also decreased drastically when plants were grown in all saline medium (Fig. 6c).

Leaf Free Proline and Phenolics

Total phenolic and free proline content in leaves were found significant higher at 10 dS m⁻¹ NaCl stress and onward stress in all quinoa lines with increasing trends compared to control. Furthermore, lines Q7 and Q2 showed maximum free proline content at 80% sea level salinity (Fig. 7a).

Discussion

Quinoa lines were evaluated in normal and saline soil in pots under natural wire house conditions of Faisalabad in

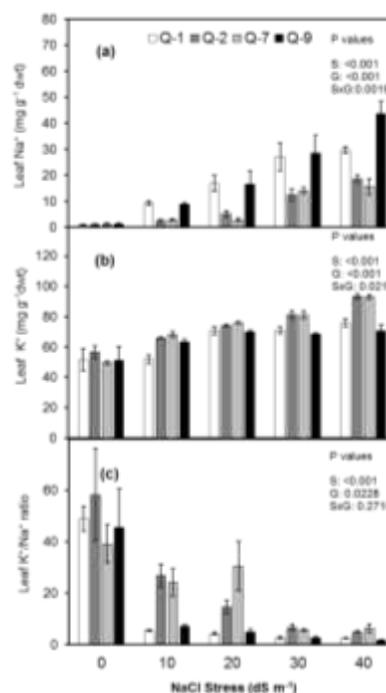


Fig 5: Leaf Na^+ concentration (a), leaf K^+ concentration (b) and leaf K^+/Na^+ ratio (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. ($n=4$)

order to explore physiological, growth and yield responses. Growing season was November 2012 to April 2013 same as growing season of quinoa in Pakistan (Basra *et al.*, 2014).

The growth and yield of (Fig. 1; Fig 2) of quinoa lines did not affected by 10 dS m⁻¹ NaCl salt stress but slightly increased. This level could be considered optimum therefore for growth in local conditions. This salt induced stimulation in growth response in quinoa and several other plant species has been found at moderate salinity levels (Hariadi *et al.*, 2011). This is might be due to increase in tissue water contents (Khan *et al.*, 2005). However, significant shoot biomass and seed yield reductions of Q1 and Q9 were found at 20 dS m⁻¹ while these reductions for Q2 and Q7 were recorded at 30 dS m⁻¹ (Fig. 3a, b). While drastic reductions were found at 80% sea level salinity in Q1 and Q9 lines. Thus, it shows wide variation among quinoa lines exists for salt tolerance when tested under new environmental conditions of Faisalabad, and confirms it as a facultative halophyte (Adolf *et al.*, 2012). Regardless of this variation, all lines completed life cycle and produced seeds even at 80% sea level salinity. Koyro and Eisa (2008) and Harriadi *et al.* (2011) also reported this type of trend in the past, it suggests that these lines could also be tested in other climatic conditions of Pakistan and other countries as well. Thousand grain weight

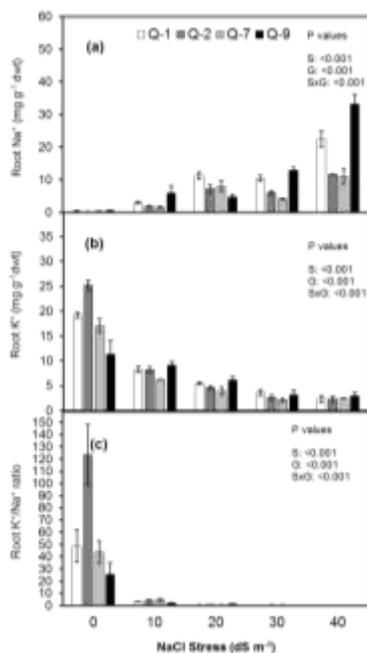


Fig. 6: Root Na⁺ concentration (a), root K⁺ concentration (b) and root K⁺/Na⁺ ratio (c) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. (*n*=4)

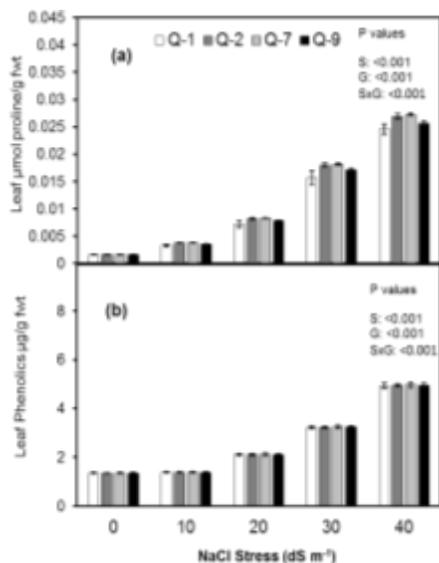


Fig 7: Leaf free proline concentration (a) and leaf soluble phenolic contents (b) of quinoa lines affected by NaCl salt stress. Q-1, Q-2, Q-7 and Q-9 indicate the quinoa lines. S and G indicate salinity treatments and lines respectively, and SxG indicates the interaction. Error bars indicate s.e. (*n*=4)

of all lines found less at higher salinity levels (30 and 40 dS

m⁻¹) might be due to more seed protein contents than carbohydrates (Koyro and Eisa, 2008).

The appropriate water relations for maintenance of turgor is very crucial for normal cell functioning and plant growth and has been recognized as a vital mechanism of salt tolerance in every plant (Jensen *et al.*, 2000; Bosque-Sanchez *et al.*, 2003). These attributes have been directly linked with plant gas/ionic relations and leaf morphological characteristics, as stomatal resistance is usually helpful to maintain plant water status (Adolf *et al.*, 2012).

In the current study, inverse relationships were found between stomatal conductance and leaf transpiration with NaCl concentration (Fig. 3b, c) which led to less growth and ultimately low yield (Fig. 1–2). Likely water relations were regulated in the tested lines through increased inorganic osmotic accumulation (K⁺ and Na⁺) in leaf, a hypertonic response (Fig. 5) in order to maintain internal osmolality of cell to extract water from high external osmolality of soil (Fig. 4). Furthermore, reduced transpiration as happened in all tested lines quinoa lines (Fig. 3c) at higher salinity levels, might be also helpful in regulating water relations, which could be possible due to quinoa morphological adaptations i.e. fewer and smaller stomata (Orsini *et al.*, 2011). These morphological adaptations have been reported as efficient strategy to cut down transpiration water loss but also responsible for reduction in overall stomatal conductance of leaf (Adolf *et al.*, 2012).

Salt stress of 20 dS m⁻¹ and onwards adversely affected net photosynthesis in quinoa lines especially in Q1 and Q9, which was linked to stomatal conductance (Fig. 3a, b). Although, stomatal closure under salt and drought stress is indicative of defense to prevent desiccation, but at the same hour, leads to a substantial reduction of CO₂ diffusion to the carboxylation sites (Ozgur *et al.*, 2013).

Other factors might be also responsible to non-stomatal reduction of photosynthesis; debatably most important of these is alteration in the leaf-K⁺/Na⁺ ratio. Potassium has been recognized for the activation of more than fifty enzymes (Shabala, 2003); including Rubisco and chlorophyll biosynthesis catalyzing enzymes. Similarities in physiochemical characteristics of K⁺ and Na⁺ (i.e., ionic radius and ion hydration energy), later contests with K⁺ for vital binding-sites of enzymes (Marschner, 1995). Therefore, a low leaf-K⁺/Na⁺ ratio in quinoa lines (Fig. 5c) especially Q1 and Q9, might be responsible to reduce dramatically plant's capacity of photosynthesis. Furthermore, like cellular-metabolic activities in animals (Hughes and Cidlowski, 1999), potassium depletions in leaf-mesophyll under saline-regimes (Shabala, 2000; Shabala *et al.*, 2005) may trigger programmed cell death by activating many caspase-like proteases (Shabala, 2009) and enhancing leaf drop.

In this scenario, plant's salt tolerance is highly linked

with prevention of shoot potassium decline and appropriate leaf-K⁺/Na⁺ ratio (Shabala and Cuin, 2008). In current study, lines Q2 and Q7 had better leaf-K⁺/Na⁺ ratios at all salt regimes (Fig. 5c), thus representing salt tolerance. This might involve an efficient Na⁺ dumping in leaf vacuole or Na⁺ translocation to older leaves. Na⁺ concentration was measured in young leaves in this study and usually young leaves had lower degree of accumulation (Adolf *et al.*, 2013). It seems that lines Q2 and Q7 responded at whole plant level by translocating Na⁺ in older leaves that leads to low Na⁺ loads in young leaves (Fig. 5a). It also seems to restrict Na⁺ at root parenchyma as root Na⁺ contents of Q2 and Q7 were higher while growing in saline solutions especially at 400 mM NaCl (Fig. 6a). This strategy of low accumulation of Na⁺ might be also linked with preferential K⁺ uptake at root parenchyma and translocation to leaf, as leaf K⁺ concentration was also found higher in Q7 and Q2 (Fig. 5b). Quinoa plants accumulate more K⁺ in leaves under salt stress (Adolf *et al.*, 2012), which was also confirmed in this study.

Further possible elucidation might be alteration in Na⁺ loading rate in xylem. It has been described that processes i.e. nutrient element uptake, their radial transport and movement to shoot are mostly uncoupled (Plett and Moller, 2010; Wegner *et al.*, 2011). It is recently argued that, thermodynamically Na⁺ loading in xylem is almost surely an active process, facilitated by SOS1-like Na⁺/H⁺ exchanger in halophytes (Shabala and Mackay, 2011). Meanwhile, xylem-K⁺ loading is a passive process happens through selective outward rectifying cation conductance (NORC) or K⁺-selective (SKOR) depolarization activated channels at xylem parenchyma (Shabala *et al.*, 2008). It seems that in quinoa xylem K⁺ and Na⁺ loadings are uncoupled and thus tolerant lines may exhibit better activities of Na⁺/H⁺ antiporters located at the xylem parenchyma border. Validation of this hypothesis may be included in future research programs.

Exposure to salt stress, proline concentration and leaf phenolic contents increased exponentially at every increased stress level in all quinoa lines (Fig. 7a–b). But, the levels of increase for both free proline and total phenoles were still not enough to contribute significantly to cell osmotic adjustment (Ruffino *et al.*, 2010). However, Cuin and Shabala (2007) proposed that these osmolyte may play indirect role in osmotic adjustment by regulation of K⁺ transport across the plasma membrane, thus preventing NaCl induced efflux of K⁺. In another study Ismail *et al.* (2016) reported that rutin; a phenol which accumulated approximately more than 27.5-times in young leaves of two quinoa cultivars under salt stress. Exogenous applications of rutin were also done by same group of scientists and found rutin can mitigate salt stress in quinoa plants. Furthermore, after detailed electrophysiological experiments after exogenous application of rutin in quinoa leaves, also revealed that these beneficial effects were attributed to improved potassium retention and increased rate of Na⁺

pumping from the cell.

The lack of correlation between rutin-induced changes in K⁺ and H⁺ fluxes suggest that rutin accumulation in the cytosol scavenges hydroxyl radical formed in response to salinity treatment thus preventing K⁺ leak via one of ROS-activated K⁺ efflux pathways, rather than controlling K⁺ flux via voltage-gated K⁺-permeable channels. So, the role of free proline and phenolics as osmoprotectant by mitigating Na⁺ toxicity warrants further detailed studies. Other important role of these compounds in quinoa, which has been widely accepted are very helpful to detoxify reactive oxygen species produced due to salt stress (Adolf *et al.*, 2012). Both these metabolites are indicators of salt toxicity, and their values were not increased significantly at 10 dS m⁻¹ in all quinoa lines (Fig. 7a–b), which confirms that 10 dS m⁻¹ level is not a big hazard for quinoa, as has been accepted for the halophytes (Shabala and Mackay, 2011).

Conclusion

All the quinoa lines not only survived but also produced seeds even at 40 dS m⁻¹ salt stress. Lines Q2 and Q7 performed better and produced 25–28% more seed yield as compared to Q1 and Q9 under control and saline conditions. With increase in salinity level osmoprotectants (free proline and phenolics) increased in all quinoa lines. Salt tolerance was linked with low Na⁺ and high K⁺ accumulation in leaves.

References

- Adolf, V.I., S. Shabala, M.N. Andersen, F. Razzaghi and S.E. Jacobsen, 2012. Varietal differences of quinoa's tolerance to saline conditions. *Plant Soil*, 357: 117–129
- Adolf, V.I., S.E. Jacobsen and S. Shabala, 2013. Salt tolerance mechanisms in quinoa (*Chenopodium Quinoa Willd.*). *Environ. Exp. Bot.*, 92: 43–54
- Akhtar, J., Z.A. Saqib, M. Sarfraz, I. Saleem and M.A. Haq, 2010. Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. *Pak. J. Bot.*, 42: 2857–2866
- Azevedo, N., J.T. Prisco, J. Eneas-Filho, C.E.B. Abreu and E.G. Filho, 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Env. Expt. Bot.*, 56: 87–94
- Basra, S.M.A., S. Iqbal and I. Afzal, 2014. Evaluating the response of nitrogen application on growth, development and yield of quinoa genotypes. *Int. J. Agric. Biol.*, 16: 886–892
- Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205–207
- Blumwald, E., A. Grover and G. Allen, 2004. Breeding for abiotic stress resistance: challenges and opportunities. In: *Proceedings of the 4th International Crop Science Congress*, 26 Sep–1 Oct 2004, Brisbane, Australia
- Cuin, T.A. and S. Shabala, 2007. Compatible solutes reduce ROS induced potassium efflux in Arabidopsis roots. *Plant Cell Environ.*, 30: 875–885
- Flowers, T.J., 2004. Improving crop salt tolerance. *J. Exp. Bot.*, 55: 307–319
- Flowers, T.J. and T.D. Colmer, 2008. Salinity tolerance in halophytes. *New Phytol.*, 179: 945–963
- Hariadi, Y., K. Marandon, Y. Tian, S.E. Jacobsen and S. Shabala, 2011. Ionic and osmotic relations in quinoa (*Chenopodium quinoa Willd.*)

- plant grown at various salinity levels. *J. Exp. Bot.*, 62: 185–193
- Hughes, F.M. and J.A. Cidłowski, 1999. Potassium is a critical regulator of apoptotic enzymes *in vitro* and *in vivo*. *Adv. Enzyme Regul.*, 39: 157–171
- Ismail, H., J.D. Maksimovic, V. Maksimovic, L. Shabala, B.D. Živanovic, Y. Tian, S.E. Jacobsen and S. Shabala, 2016. Rutin, a flavonoid with antioxidant activity, improves plant salinity tolerance by regulating K⁺ retention and Na⁺ exclusion from leaf mesophyll in quinoa and broad beans. *Funct. Plant Biol.*, 43: 75–86
- Jacobsen, S.-E. and O. Stølen, 1993. Quinoa—morphology and phenology and prospects for its production as a new crop in Europe. *Eur. J. Agron.*, 2: 19–29
- Jacobsen, S.E., A. Mujica and C.R. Jensen, 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. *Food Rev. Int.*, 19: 99–109
- Jensen, C.R., S.E. Jacobsen, M.N. Andersen, N. Núñez, S.D. Andersen, L. Rasmussen and V.O. Mogensen, 2000. Leaf gas exchange and water relation characteristics of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. *Eur. J. Agron.*, 13: 11–25
- Khan, M.A., I.A. Ungar and A.M. Showalter, 2005. Salt stimulation and tolerance in an intertidal stem-succulent halophyte. *J. Plant Nutr.*, 28: 1365–1374
- Koyro, H.W. and S.S. Eisa, 2008. Effect of salinity on composition, viability and germination of seeds of *Chenopodium quinoa*. *Plant Soil*, 302: 79–90
- Marschner, H., 1995. Saline soils. In: *Mineral Nutrition of Higher Plants*, pp: 657–680. Academic Press, New York
- Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651–681
- Orsini, F., M. Accorsi, G. Gianquinto, G. Dinelli, F. Antognoni, K.B.R. Carrasco, E.A. Martinez, M. Alnayef, I. Marotti and S. Bosi, 2011. Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: Functional elements of successful halophytism. *Funct. Plant Biol.*, 38: 818–831
- Pattanagul, W.T. and Maysaya, 2008. Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Ind. J. Exp. Biol.*, 46: 736–742
- Plett, D.C. and I.S. Møller, 2010. Na⁺ transport in glycophytic plants: What we know and would like to know. *Plant Cell Environ.*, 33: 612–626
- Ruffino, A.M.C., M. Rosa, M. Hilal, J.A. González and F.E. Prado, 2010. The role of cotyledon metabolism in the establishment of quinoa (*Chenopodium quinoa*) seedlings growing under salinity. *Plant Soil*, 326: 213–224
- Schleiff, U., 2008. Analysis of water supply of plants under saline soil conditions and conclusions for research on crop salt tolerance. *J. Agron. Crop Sci.*, 194: 1–8
- Shabala, S., 2000. Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant Cell Environ.*, 23: 825–837
- Shabala, S., 2003. Regulation of potassium transport in leaves: From molecular to tissue level. *Ann. Bot.*, 92: 627–634
- Shabala, S., 2009. Salinity and programmed cell death: Unravelling mechanisms for ion specific signalling. *J. Exp. Bot.*, 60: 709–712
- Shabala, S. and A. Mackay, 2011. Ion transport in halophytes. *Adv. Bot. Res.*, 57: 151–187
- Shabala, S. and T.A. Cuin, 2008. Potassium transport and plant salt tolerance. *Physiol. Plant*, 133: 651–669
- Shabala, S., L. Shabala, E. Van Volkenburgh and I. Newman, 2005. Effect of divalent cations on ion fluxes and leaf photochemistry in salinized barley leaves. *J. Exp. Bot.*, 56: 1369–1378
- Shahbaz, M. and M. Ashraf, 2013. Improving salinity tolerance in cereals. *Crit. Rev. Plant Sci.*, 32: 237–249
- Stikic, R., D. Glamoclija, M. Demin, B. Vucelic-Radovic, Z. Jovanovic, D. Milojkovic- Opsenica, S.E. Jacobsen and M. Milovanovic, 2012. Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. *J. Cereal Sci.*, 55: 132–138
- Waterhouse, A.L. 2002. Determination of total phenolics. *Curr. Protocols Food Analyt. Chem.*, : 11.1.1–11.1.8
- Wegner, L.H., G. Stefano, L. Shabala, M. Rossi, S. Mancuso and S. Shabala, 2011. Sequential depolarization of root cortical and stelar cells induced by an acute salt shock—implications for Na⁺ and K⁺ transport into xylem vessels. *Plant Cell Environ.*, 34: 859–869
- Yilmaz, K., I.E. Akinci and S. Akinci, 2004. Effect of salt stress on growth and Na, K contents of pepper (*Capsicum annuum* L.) in germination and seedling stages. *Pak. J. Biol. Sci.*, 7: 606–610

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