Pak. J. Bot., 52(1): 1-7, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-1(8)

VARIATIONS IN WATER RELATIONS, STOMATAL CHARACTERISTICS, AND PLANT GROWTH BETWEEN QUINOA AND PEA UNDER SALT-STRESS CONDITIONS

HONG YAN[1,2], SYED SADAQAT SHAH[1,3], WEI ZHAO[4] AND FULAI LIU[2*]

[1]*School of life science, Northeast Normal University, Changchun 130024, China*
[2]*Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, HøjbakkegaardAllé 13, DK-2630 Taastrup, Denmark*
[3]*Department of Botany, Islamia College Peshawar, Peshawar, KPK, Pakista*
[4]*Department of Avigation Lifesaving, Avigation University of Air Force, Changchun 130022, China*
[*]*Corresponding author's email: fl@plen.ku.dk*

Abstract

Salinity is a primary restrictive factor for crop growth at both the cellular and whole plant levels. The effects of salinity on water relations, stomatal morphology and physiology, and seedling growth in quinoa and pea were investigated to compare the salt tolerance mechanisms of these two species. The seedlings of quinoa and pea were cultivated in Hoagland's solutions supplemented with different NaCl concentrations (0, 100 and 200 mM). For quinoa,the relative water content, transpiration ratio, osmotic potential, stomatal conductance, stomatal density, and stomatal length were all reduced significantly by salt stress. Interestingly, a greater stomatal conductance of the abaxial surface in quinoa was found during salt stress in comparison with the control. Similar trends (root > stem > leaf) were found for leaf water potential in quinoa and pea. For different organs, quinoa possessed greater leaf water and osmotic potentialsthan pea, indicating that quinoa might limit the translocation of inorganic ions to maintain the water balance. The turgor pressure in the two species increased significantly, which could play an important role in sustaining seedling growth. In conclusion, quinoa was less affected by salinity, which was verified by the different physiological responses of stomatal and plant water states.

Key words: Salt tolerant; Water potential; Osmotic potential; Turgor potential; Stomatal conductance; Stomatal density.

Introduction

Salinity is a critical factor affecting crop production and agricultural sustainability in dry regions (Paranychianakisa & Chartzoulakis, 2005; Essa, 2002). Soil salinity has affected more than 800 million hectares of global land (Rengasamy, 2006). With an increasing global population, the need for high quality crops is also increasing. Therefore, it is necessary to cultivate salt-tolerant crops using cost-effective strategies.

Quinoa (*Chenopodium quinoa* Willd.) has been bred in the Andes of South America for 7,000 years (Pearsall, 1992). It is a major grain crop of the family *Amaranthaceae*. Because of its various components, including vitamins and minerals, and good balance between protein and fat, quinoa is an excellent example of a 'functional food' (Vega-G´alvez *et al.*, 2010). Additionally, quinoa is tolerant to environmental stresses, such as drought, cold, and salinity (Jacobsen *et al.*, 2003). Several cultivars of quinoa (such as Titicaca, a Danish bred cultivar) can survive in 40 $ds \cdot m^{-1}$ of NaCl (Razzaghi *et al*., 2011).

Pea (*Pisum sativum* L.) is an annual herb in the family *Fabaceae*. As a plant species with protein-rich seeds, pea is considered as the fourth legume (Vidal-Valverde *et al*., 2003). The demand for plant protein sources has drawn attention to pea as an important economic crop. However, pea is relatively susceptible to extreme conditions, such as salinity. Because pea and quinoaare important in agriculturalproduction, they have recently attracted enormous attention worldwide. However, research has mainly focused on seedling productivity (Pulvento *et al*., 2012), nutrient contents (Stikic *et al.*, 2012), physiological parameters (Yooyongwecha *et al*., 2013), and the characterization of the *SOS1* (Salt Overly Sensitive 1) gene (Maughan *et al.*, 2009). Research on their salt tolerance levels, includingcomparativestudies of the two crops, should be undertaken.

Under salt-stress conditions, adverse factors limiting crop growth may result from the osmotic stress of water availability (dehydration) and the toxic effects of high concentrations of salt ions (Zhu, 2001; Misra & Dwivedi, 2004). The osmotic effect of salinity is the initial factor related to growth inhibition (Munns, 2005). It is important for plants to maintain a lower potential gradient for water uptake when the soil water potentialis reduced (Jensen *et al*., 2000). Consequently, seedling development in extreme environments is correlated with preventing water loss and maintaining a favorable water gradient (Gharbi *et al*., 2019). Photosynthesis, the only process for harvesting energy, is affected by salinity (Munns *et al*., 2006; Shi *et al*., 2015). To regulate water balance during salt stress, plants reduce evaporation by closing the leaf stoma. Thus, variations in stomatal morphology and physiology can be considered the first defensive reactions or acclimation mechanisms against salinity. Stomatal closure resulting from a water deficit can lead to a reduction in CO_2 acquisition and photosynthesis (Miyashita *et al*., 2005; Zouaoui *et al*., 2019), which directly influences plant growth. It is essential to understand stress injury, adaptation and acclimation mechanisms of plants for future agricultural development.

The objective of the current research was to compare the effects of NaCl on water relations, stomatal characteristics, and seedlings growth between quinoa and pea. The relevant indexes were measured as follow: (i) seedlings growth: including relative growth rate (RGR); (ii) stomatal characteristics: including stomatal length, stomatal density, andstomatalconductance (g_s); (iii) water relations, including leaf water potential (Ψ_w), osmotic potential (Ψ_m), turgor pressure (Ψ_p), relative water content (RWC), and transpiration ratio. The results may be used for analyzing the important mechanisms involved in water metabolism and plant growth, as well as for determining the physiological indexes that are useful in screening for salinity tolerant crops.

Materials and Methods

Plant materials and stress treatments: The experiment was conducted in a greenhouse at the Faculty of Science, University of Copenhagen on 1st March 2013. The seeds of two species (quinoa and pea) were potted into a vermiculite matrix and maintained at average 22/18°C day/night temperatures with 60 ± 5% relative humidity. At 14 d after germination, the seedlings were shifted into a hydroponics system, and cultivation continued in 1× Hoagland's solution under photoperiodic conditions (16-h day/8-h night). Once the plants were at the six true-leaf stage, salinity treatments (each having 8 seedlings) were initiated. The NaCl concentration was gradually increased in 50 mM increments per day. The final concentrations were 0, 100, and 200 mMNaCl, mixed in a 2× nutrient solution (pH 6.5). Each treatment was replicated four times. The plants were irrigated twice per day (early morning and late afternoon) to achieve full turbidity.

Meanwhile, the electrical conductivity and water potential of the solutions (Shown in Table 1) were measured usinga Conductivity Meter and Water Potential System (PSΨPRO^{TM}, Wescor Inc., Logan, UT, USA).

Plant water metabolism: The Ψ_w and Ψ_m values were measured using the same leaf. The former was measured directly using a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). After determination of Ψ_w the leaf was cut into two parts, one of which was frozen in liquid nitrogen for 20 min and then transferred to-80°C for later $\Psi_m$measurement. At that time, the frozen leaf was thawed for 15 min and pressed in a grinder. The sap of the sample was collected on a filter paper disc and incubated at room temperature for 5 min before $\Psi_m$was measured using the Water Potential System. The Ψ_p was calculated using the equation:

$$\Psi_p = \Psi_w - \Psi_m.$$

As described by Smart & Bingham (1974), the second half part of the leaf was immediately weighed to determine fresh weight (FW) and then placed into distilled water for 4 h at room temperature to reach full hydration. After blotting, turgid weight (TW) was immediately determined using an electronic scale. Dry weight (DW) was measured after samples were completely dried at 60°C in an oven for 48 h. The RWCs were calculated as follow:

$$RWC = (FW-DW)/ (TW-DW)$$

Daily transpiration was calculated as the difference in pot weight between a day and the previous day. Total biomass per plant was obtained at the end of the treatment. The dry matter of each seedling was weighed after being oven dried at 60°C. The transpiration ratio was calculated as daily transpiration divided by the total biomass (from planting to the end of the experiment).

Stomatal characteristics: The fourth fully expanded leaf starting from the apex of each plant was selected for measurement. The stomatal measurements were taken between 10:00 and 12:00 AM using a porometer (LI-COR Inc., Lincoln, NE, USA). The g_s of the same leaf were determined twice, and each treatment had four replicates.

For the stomatal density, the same fully expanded leaf was selected for measurement. A method previously described by Kardel *et al*., (2010) and Shabala *et al*., (2013) was used. Briefly, from each treatment, four leaves were taken and four replicates of each leaf were made for microscopic observations (400× magnification). Stomatal length and density were determined using nail polish impressions.

Relative growth rates: The method described by Kingsbury *et al*., (1984) for determining the RGR was followed. From each species, eight plants were initially harvested before treatment. The plant's FW (W_1) was immediately determined using an electronic scale. At the end of the experiment, the FWs (W_2) of the eight harvested plants were obtained. For the determination of RGR, the following formula was used:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1),$$

where$t_2 - t_1$ is the time interval in days between the harvest events.

Statistical analysis

A completely randomized block design was used in the experiment, consisting of four replicates of each treatment. The results were analyzed using SPSS version 18.0 (SPSS, Chicago, IL, USA). A two-way analysis was performed to examine cultivar, treatment, and interaction effects. A *p*-value less than 0.05 was considered statistically significant.

Table 1. Description of NaCl solution in the study.

Treatment NaCl (mmol·L^{-1})	Electrical conductivity(ds·m^{-1})	Osmotic potential (MPa)
0	1.8703a	−0.2155a
100	10.8433b	−0.5638b
200	18.6133c	−0.8919c

Table 2. Effects of salt stress on water potential (Ψ_w), osmotic potential (Ψ_m) and turgor potential (Ψ_p) in quinoa and pea grown under greenhouse conditions.

Genotypes Leaf	Treatment NaCl (mmol·L^{-1})	Ψ_w	Ψ_m	Ψ_p
Pea	0	−0.4950 ± 0.0759 a	−0.9081 ± 0.0408 a	0.4131 ± 0.0914b
	100	−0.7350 ± 0.0714 ab	−1.6041 ± 0.1108 ab	0.8353 ± 0.1890ab
	200	−0.7850 ± 0.0866 b	−2.4147 ± 0.4191 c	1.6297 ± 0.2567a
Quinoa	0	−0.0790 ± 0.0031 a	−0.8319 ± 0.1377 ab	0.7529 ± 0.1375ab
	100	−0.0600 ± 0.0130 a	−0.5956 ± 0.1077 a	0.5356 ± 0.1038b
	200	−0.0560 ± 0.0150 a	−1.1654 ± 0.1120 b	1.1094 ± 0.1020a
Stem				
Pea	0	−0.4850 ± 0.0991 a	−1.1024 ± 0.0586 a	0.6174 ± 0.1419bc
	100	−0.6950 ± 0.0359 a	−1.6680 ± 0.1226 ab	0.8859 ± 0.1468b
	200	−0.5550 ± 0.1005 a	−2.1467 ± 0.2720 c	1.5917 ± 0.2507a
Quinoa	0	−0.0325 ± 0.0097 a	−0.7687 ± 0.0822 a	0.7362 ± 0.0802bc
	100	−0.0265 ± 0.0046 a	−0.9606 ± 0.0340 b	0.9341 ± 0.0328b
	200	−0.0355 ± 0.0090 a	−1.3216 ± 0.1109 c	1.2861 ± 0.1125a
Root				
Pea	0	−0.2450 ± 0.0624 a	−0.5302 ± 0.0247 a	0.2852 ± 0.0487a
	100	−0.5450 ± 0.0236 bc	−0.6286 ± 0.0668ab	0.1336 ± 0.0538a
	200	−0.475 ± 0.0834 b	−0.7465 ± 0.0392b	0.2715 ± 0.0579a
Quinoa	0	−0.0265 ± 0.0057 a	−0.1818 ± 0.0724 a	0.1553 ± 0.0738b
	100	−0.021 ± 0.0031 a	−0.3628 ± 0.1064 ab	0.3418 ± 0.1046ab
	200	−0.0245 ± 0.0034 a	−0.6569 ± 0.1392 b	0.6324 ± 0.1380a

Different letters indicate significant differences according to LSD at $p<0.05$. Values are means ± SE (n = 4)

Results

Characteristics of the NaCl solution used in the study are shown in Table 1, including the electrical conductivity and the water potential, which increased or decreased proportionately with the sodium concentration. At less than 200 mMNaCl, the electrical conductivity reached 18.6 ds·m^{-1}. The water potential of the treatment solutions ranged from −0.22 to −0.89 MPa.

Effect of salt stress on plant water relations: For two species, the same Ψ_w trend occurred in the order: root > stem > leaf (Table 2). With the increasing salt concentrations, no significant Ψ_w-related variances were obtained for the different quinoaparts. However, the Ψ_w values of the different pea parts decreased remarkably. Moreover, the Ψ_w values of various quinoa parts were greater than those of pea. Under 200-mM NaCl conditions, the Ψ_w values of different parts (leaf, stem, and root) in quinoa dropped to −0.056, −0.0355, and −0.0245 MPa, respectively. Under the same conditions, the corresponding values in pea were −0.78, −0.555, and −0.475 MPa, respectively. The two-way analysis between treatment and species revealed that the difference was significant, especially for Ψ_w in the root ($p<0.01$).

For both species, significant reductions were observed in $\Psi_m$under salt-stress conditions (Table 2). The $\Psi_m$value decreased gradually owing to the effects of salinity. Like the Ψ_w of the treatment, the Ψ_m values of different quinoa parts were relatively greater, to different degrees, than those of pea. Under 200-mM NaCl, the lowest Ψ_m(−1.32MPa, decreased by 71.9%) was obtained in the stem of quinoa. Under the same conditions, compared with the former, the lowest Ψ_m (−2.41MPa, decreased by166%) was obtained in the leaf of pea. The interaction between cultivars and treatments was significant ($p<0.05$, Table 5).

In our study, Ψ_p was positive under salt-stress conditions (Table 2). In contrast to the Ψ_m level, Ψ_p increased gradually as the Ψ_w of salt solution (except for the stem of pea) decreased. Compared with the aerial parts of the two species, the Ψ_p values in the stems of quinoa were greater than those of leaves. However, the Ψ_p trend followed the order leaf > stem > root. After the 200-mM NaCl treatment, the greatest $\Psi_p$in the leaf of quinoa was 1.11 MPa, whereas it was 1.63 MPa in pea. The interaction between cultivar and treatment was significant ($p<0.05$, Table 5).

With increasing water potential, the RWC and Tr values in the two species also decreased to different degrees (Table 3). Under salt-stress conditions, the RWC of quinoa was slightly greater than that of pea. With 200 mM of NaCl, the RWC values in quinoa and pea decreased by 11% and 12%, respectively. With regard to Tr, the values in quinoa were much lower than those in pea. The Tr in pea ranged from 59 to 80 g water·g^{-1} DW, whereas it changed from 26 to 43 g water·g^{-1} DW in quinoa. The interaction between cultivar and treatment was significant ($p<0.05$, Table 5).

Table 3. Effects of salt stress on relative growth rate (RGR), relative water content (RWC), transpiration rate (Tr) in quinoa and pea grown under greenhouse conditions.

Genotypes	Treatment NaCl (mmol L^{-1})	Relative water content RWC (%)	Transpiration ratio Tr (g water·g^{-1} dry wt)	Relative growth rate RGR
Pea				
	0	87.10 ± 1.51a	79.99 ± 30.20ab	0.0976 ± 0.0074a
	100	80.22 ± 0.51b	86.54 ± 23.02a	0.0865 ± 0.0032ab
	200	76.68 ± 0.55c	59.19 ± 20.30b	0.0581 ± 0.0012b
Quinoa				
	0	87.12 ± 2.98a	43.19 ± 1.61a	0.2392 ± 0.0111a
	100	86.51 ± 2.48ab	38.31 ± 8.32ab	0.2319 ± 0.0120a
	200	77.51 ± 1.61c	26.14 ± 3.49b	0.2448 ± 0.0121a

Different letters indicate significant differences according to LSD at $p<0.05$. Values are means ± SE (n = 4)

Table 4. Effects of salt stress on stomatal density, stomatal length and g_s in quinoa and pea grown under greenhouse conditions.

Genotypes	Treatment NaCl (mmol·L^{-1})	Stomatal conductance (mmol·m^{-2} s^{-1}) Adaxial surface	Abaxial surface	Stomatal density (no·mm^{-2}) Adaxial surface	Abaxial surface	Stomatal length (μm) Adaxial surface	Abaxial surface
Pea							
	0	95.18 ± 7.56a	202.94 ± 30.34a	199.33 ± 21.13a	158.00 ± 17.20a	124.89 ± 8.54a	127.69 ± 8.10a
	100	76.13 ± 19.59ab	176.50 ± 17.00ab	156.00 ± 7.97b	103.33 ± 5.78b	111.43 ± 8.40ab	114.25 ± 5.11ab
	200	28.53 ± 6.81c	46.83 ± 25.74c	144.67 ± 7.97bc	100.00 ± 6.86bc	92.62 ± 2.41c	106.25 ± 5.15c
Quinoa							
	0	475.68 ± 76.94a	684.80 ± 62.57a	138.00 ± 9.50a	165.33 ± 12.51ab	90.21 ± 4.00a	96.89 ± 1.54a
	100	315.20 ± 55.82ab	351.30 ± 41.88b	154.67 ± 8.55a	178.00 ± 11.32a	80.60 ± 2.57a	79.83 ± 2.61bc
	200	192.52 ± 31.96b	278.88 ± 46.23bc	137.33 ± 18.58a	132.00 ± 12.48c	75.50 ± 7.22a	82.48 ± 2.55b

Different letters indicate significant differences according to LSD at $p<0.05$. Values are means ± SE (n = 4)

Effect of salt stress on stomatal characteristics

Stomatal conductance (g_s): With decreasing water potential, the g_s values decreased significantly in both varieties ($p<0.05$, Table 4). In both species, the $g_s$of the adaxial surface was lower than that of the abaxial surface. For quinoa seedlings, the highest salt treatment significantly reduced the g_s values of the abaxial and adaxial surfaces by 59.3% and 59.6%, respectively. The corresponding reductions in pea were 77.2% and 70.5%, respectively. Under experimental conditions, the g_s in pea was lower than that in quinoa (Table 4). The lowest g_s of pea was 28.53 mmol·m^{-2} s^{-1} in the adaxial surface, while the lowest value of quinoa was 192.52 mmol·m^{-2} s^{-1} in the adaxial surface. The interaction between cultivar and treatment was significant ($p<0.05$, Table 5).

Stomatal density and stomatal length: Saline treatments significantly reduced the stomatal density and the stomatal length in pea ($p<0.05$, Table 4). The stomatal density on the abaxial surface of the leaf was much lower than that of the abaxial surface. However, the highest salt treatment significantly reduced the stomatal density of the abaxial and adaxial surfaces by 36.7% and 27.4%, respectively. The lowest value of stomatal density (100 no·mm^{-2}) was observed in the 200-mM NaCl treatment. In addition, the opposite tendency was shown in the stomatal lengths of pea. The stomatal length of the abaxial surface was much greater than that of the adaxial surface. The highest salt treatment significantly reduced the stomatal lengths of the abaxial and adaxial surfaces by 16.7% and 25.8%, respectively. The shortest stomatal length (92.62 μm) was observed in the adaxial surface.

Although no effects of stress were observed on the stomatal density and stomatal length of the adaxial surface in quinoa, it significantly reduced these two parameters on the abaxial surface (Table 4). Interestingly, the stomatal density on the adaxial surface of quinoa increased slightly ($p>0.05$) with 100 mM of NaCl. The lowest value of stomatal density (132 no mm^{-2}), which decreased by 20%, was found on the abaxial surface of quinoa. The highest salt treatment reduced the stomatal lengths of the abaxial and adaxial surfaces by 14.9% and 16.3%, respectively. The shortest stomatal lengths on the abaxial and adaxial sides were 82.48 μm and 75.5 μm, respectively.

The differences in stomatal characteristics between the two species and treatments are shown in Table 5. The interaction between cultivar and treatment was significant ($p<0.05$) for stomatal density. However, for stomatal length, no significant correlation was detected between cultivar and treatment ($p>0.05$).

Table 5. F values and significance of two−way analysis of variance of physiological parameters.

	F−statistics		
Physiological parameter	**Cultivar (C)**	**Treatment (T)**	**C×T**
Leaf			
Ψ_w	3.26*	176.71***	4.48*
Ψ_m	11.82***	23.41***	4.95*
Ψ_p	6.86**	0.84*	1.95*
Stem			
Ψ_w	1.50*	125.73***	1.71*
Ψ_m	16.99***	30.91***	1.75*
Ψ_p	14.70***	0.42	1.13*
root			
Ψ_w	6.24**	124.17***	6.65**
Ψ_m	8.53**	11.64**	1.23*
Ψ_p	4.92*	5.23*	4.45*
Relative water content	15.03	2.50*	1.71*
Transpiration ratio	0.77*	7.19*	0.97*
Abaxial surface			
Stomatal conductance	7.21**	48.78***	3.11*
Stomatal density	3.00*	5.33*	4.84*
Stomatal length	7.38**	30.45***	1.14*
Abaxial surface			
Stomatal conductance	17.77***	57.55***	5.67*
Stomatal density	5.90**	0.60*	2.36*
Stomatal length	8.93**	59.15***	0.66
Relative growth rate	0.57	151.94***	1.25*

*Significant difference at $p<0.05$; **Significant difference at $p<0.01$; ***Significant difference at $p<0.001$

Effect of salt stress on RGR: To observe the growth potential of both species, RGR was determined under salt-stress conditions. As shown in Table 3, there was no significant effects on the RGR of the quinoa seedlings. However, the 100-mM and 200-mM salt treatments significantly reduced the RGR in pea by 11.4% and 40.5%, respectively. With 200 mM of NaCl, the RGR values in quinoa and pea were 0.2448 and 0.058, respectively. Thus, the decline in the RGR of pea was attributed to a decline in the water potential of the different NaCl solutions. The interaction between cultivar and treatment was significant ($p<0.05$, Table 5).

Discussion

Salinity is a problem in arid and semiarid areas worldwide. Plants growing under salt-stress conditions exhibit waterdeficiencies, photosynthetic declines and growth reductions when compared with growth under normal conditions. Here, two crops, pea and quinoa, were selected to compare physiological characteristics. Theresults could provide additional information on water metabolism, stomatal characteristics and plant growth between the salt-tolerant (quinoa) and the salt-sensitive (pea) species.

Water status: Under salt-stress conditions, aerial plant parts adjusted osmotically to accumulate organic and inorganic matter, maintaining a greater negative $\Psi_m$and $\Psi_w$than the surroundings. According to Yin *et al*., (2013), lower Ψ_m values indicate better osmotic adjustment capacities, and the tissues have a greater capacity to uptake and retain water. In agreement with the regularity of water absorption, the Ψ_w and Ψ_p of leaves and stems in the two species were lower than those of roots. As a halophyte, quinoa can maintain a critical level of inorganic ions to avoid the impact of salinity. In the current study, the Ψ_m in the stem of quinoa was lower than that in the leaf. Thus, quinoa had a better ability to decrease the inorganic ion contents in leaves. This observation is in general agreement with the conclusion of Eisa *et al*., (2012), confirming a greater Na^+ accumulation in the stems compared with in leaves.

The Ψ_p is a significant component of cell water potential. Cell expansion is a turgor-driven process. The most sensitive turgor-dependent activities under water deficits are root elongation and leaf expansion. A higher Ψ_p value drives cell wall expansion and also prevents the cell from contracting (Yin *et al*., 2013). The greater Ψ_p is important in minimizing the water loss by transpiration (Zarinkamar *et al*., 2013). Thus, the maintenance of Ψ_p and a lower Ψ_w contribute to the growth (Bassiri Rad & Coldwell, 1992; Jensen *et al*., 2000). Here, the variation in $\Psi_p$was consistent with Ψ_m. The accumulation of inorganic ions (calcium, potassium and sodium) in vacuoles of the leaves led to decreasing Ψ_m values, which may aid in turgor preservation at high sodium concentrations (Riccardi *et al*., 2014; Cocozza *et al*., 2013). It allows the metabolic processes to be conserved and enables the growth and durability of plants (McCree, 1986). Similar conclusions were proposed by Volkenburgh (1999) and Liu (2003).

Stomatal characteristics: Stomata are the entrances on leaves for CO_2 exchange and water evaporation. As described by Adolf *et al*., (2012), stomatal and non-stomatal limitations to photosynthesis are distinguished under salt-stress conditions. To regulate water balance, plant- under induced salt-stress conditions have to reduce overall transpiration and avoid excessive water loss through the stoma. Thus, the present research mainly focused on stomatal limitations by determining the stomatal density, stomatal length, and g_s of the abaxial and adaxial surfaces in the two species.

For the sensitive species (pea), salinity had significant effects on the stomatal characteristics. The tendency of stomatal length was consistent with that of g_s in pea. Both values were greater on the leaf abaxial surface than on the adaxial surface. The opposite trend was found for the stomatal density, with the adaxial surface having a greater value than the abaxial surface. Therefore, the influence of the stomatal density was low in the sensitive species (pea). When compared with quinoa, the sensitive cultivar (pea) had greater stomatal lengths on the abaxial surface. This agrees with Adolf *et al*., (2012), who suggested that the reduced stomatal diameter for the tolerant crop required less water to respond to the salt environment, indicating that this is an environment-related adaptive trait. Thus, the lengths of stomata could be a main anatomical characteristic for the determination and variability of g_s (Aasamaa *et al*., 2001).

However, there were no significant effects on the length and the density of stomata on the adaxial leaf surface of the tolerant species (quinoa) under salt stress. Irregular variations in the stomatal density of quinoa proved that the g_s of the abaxial surface was one of the key non-stomatal limitations to photosynthesis. This was consistent with the findings of Yooyongwech *et al*., (2013) in which g_s was reported as a key physiological index for drought tolerance in sweet potato. Significantly, Venora & Calcagno (1991) emphasized that the stomatal aperture size on the abaxial surface of wheat grown under water-stress conditions was a useful index for selecting tolerant genotypes. The opposite observations have been found in some halophytes. For example, *Kochiaprostrata* has a relatively high stomatal density under salt-stress conditions (Karimi *et al*., 2005). Stomatal responses vary depending on the plant species, the environmental sensitivity, culture conditions, stressintensity and developmental stage.

Growth indexes: The RGR is regarded as a daily average of the tissue present and reflects growth potential under the extreme conditions (Kingsbury *et al*., 1984). In the present study, quinoa growth was not affected significantly by salt stress owing to the moderate saline concentration. The increase in the salt concentration resulted in a RGR reduction in pea as a consequence of the reduced water absorption. A severe water deficit may result in decreased photosynthesis owing to morphological changes in stoma and variations in g_s. As salinity increases, the accumulation of dry matter decreased. Differences in RGRs between species and treatments were correlated with different adaptive mechanisms to extreme growth conditions.

The Tr is defined as the amount of water-evaporated per gram of dry matter. Quinoa had a relatively lower Tr than pea, indicating that quinoa should uptake less water while accumulating dry matter compared with pea. Consequently, quinoa has a better ability to increase the water-use efficiency.

Conclusions

In conclusion, quinoa showed significantly greater Ψ_w, Ψ_m and $g_s$values, and a lower Tr value, compared with pea under saline conditions. This suggested that quinoa could sustain water uptake, preventing ion transport and maintaining Ψ_p, compared with pea under salt-stress conditions. In addition, greater stomatal regulation and water-loss prevention in quinoa resulted in greater RGR and Tr values. Therefore, quinoa appears to be a more salt-tolerant crop compared with pea.

Acknowledgments

Grateful thanks to the Danmark-China Exchange Scholarship for financial support. Thanks to Faculty of Science, Department of Plant and Environmental Sciences, University of Copenhagen for technical assistance. We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

References

Aasamaa, K., A. Sõber and M. Rahi. 2001. Leaf anatomical characteristics associated with shoot hydraulic conductance, stomatal conductance and stomatal sensitivity to changes of leaf water status in temperate deciduous trees. *Aust. J. Plant Physiol.*, 28(8): 765-774.

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(1): 117-129.

BassiriRad, H. and M.M. Coldwell. 1992. Root growth, osmotic adjustment and NO_3^- uptake during and after a period of drought in *Artemesiatridentata*. *Aust. J. Plant Physiol.*, 19: 493-500.

Cocozza, C., C. Pulvento, A. Lavini, M. Riccardi, R. D'andria and R. Tognetti. 2013. Effects of Increasing Salinity Stress and Decreasing Water Availability on Ecophysiological Traits of Quinoa (*Chenopodium quinoa* Willd.) Grown in a Mediterranean- Type Agroecosystem. *J. Agron. & Crop Sci.*, 199: 229-240.

Eisa, S., S. Hussin, N. Geissler and H.W. Koyro. 2012. Effect of NaCl salinity on water relations, photosynthesis and chemical composition of quinoa (*Chenopodium quinoa* Willd.) as a potential cash crop halophyte. *Aust. J. Crop Sci.*, 6: 357-368.

Essa, T.A. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *J. Agron. & Crop Sci.*, 188: 86-93.

Gharbi, F., A. Guizani, L. Zribi, H.B. Ahmed and F. Mouillot. 2019. Differential response to water deficit stress and shade of two wheat (*Triticum durum* Desf.) cultivars: Growth, water relations, osmolyte accumulation and photosynthetic pigments. *Pak. J. Bot.,* 51: 1179-1184.

Jacobsen, S.E., A. Mujica and C.R. Jensen. 2003. The Resistance of Quinoa (*Chenopodium quinoa* Willd.) to Adverse Abiotic Factors. *Food Rev. Intern.*, 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. *Europ. J. Agron.*, 13: 11-25.

Kardel, F., K. Wuyts, M. Babanezhad, U.W.A. Vitharana, T. Wuytack, G. Potters and R. Samson. 2010. Assessing urban habitat quality based on specific leaf area and stomatal characteristics of *Plantago lanceolata* L. *Environ.Pollut.*, 158(3): 788-794.

Karimi, G., M. Ghorbanli, H. Heidari, R.A. KhavariNejad and M.H. Assareh. 2005. The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochiaprostrata*. *Biologia Plantarum*, 49(2): 301-304.

Kingsbury, R.W., E. Epstein and R.W. Pearcy. 1984. Physiological responses to salinity in selected lines of wheat. *Plant Physilology*, 74: 417-423.

Liu, F., C.R. Jensen and M.N. Andersen. 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. *Funct. Plant Biol.*, 30: 65-73.

Maughan, P.J., T.B. Turner and C.E. Coleman, D.B. Elzinga, E.N. Jellen, J.A. Morales, J.A. Udall, D.J. Fairbanks and A. Bonifacio. 2009. Characterization of Salt Overly Sensitive 1 (SOS1) gene homoeologs in quinoa (*Chenopodium quinoa* Willd.). *Genome*, 52(7): 647-657.

McCree, K.J. 1986. Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. *Aust. J. Plant Physiol.*, 13: 33-43.

Misra, N. and U.N. Dwivedi. 2004. Genotypic difference in salinity tolerance of green gram cultivars. *Plant Science*, 166: 1135-1142.

Miyashita, K., S. Tanakamaru, T. Maitani and K. Kimura. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environm. & Experim. Bot.*, 53: 205-214.

Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytol*., 167(3): 645-63.

Munns, R., R.A. James and A. Lauchli. 2006. Approaches to incerasing the salt tolerance of wheat and other cereals. *J. Experim. Bot.*, 57(5): 1025-1043.

Paranychianakisa, N.V. and K.S. Chartzoulakis. 2005. Irrigation of Mediterranean crops with saline water: from physiology to management practices. *Agricul. Ecosyst. & Environ.,* 106(2-3): 171-187.

Pearsall, D.M. 1992. The origins of plant cultivation in South America. In: *The Origins of Agriculture: An International Perspective*, (Ed.): Cowan, C.W. and P.J. Watson. Washington, London: Smithsonian Institution Press, pp. 173-205.

Pulvento, C., M. Riccardi, A. Lavini, G. Iafelice, E. Marconi and R. D'andria. 2012. Yield and quality characteristics of quinoa grown in open field under different saline and non-saline irrigation regimes. *J. Agron. & Crop Sci.*, 198(4): 254-263.

Razzaghi, F., S.H. Ahmadi, V.I. Adolf, C.R. Jensen, S.E. Jacobsen and M.N. Andersen. 2011. Water relations and transpiration of quinoa (*Chenopodium quinoa* Willd.) under salinity and soil drying. *J. Agron. & Crop Sci.,* 197(5): 348-360.

Rengasamy, P. 2006. World salinization with emphasis on Australia. *J. Experim. Bot.*, 57(5): 1017-23.

Riccardi, M., C. Pulvento, A. Lavini, R. D'andria and S.E. Jacobsen. 2014. Growth and ionic content of quinoa under saline irrigation. *J. Agron. & Crop Sci.*, 200(4): 246-260.

Shabala, S., Y. Hariadi and S.E. Jacobsen. 2013. Genotypic difference in salinity tolerance in quinoa is determined by differential control of xylem Na^+ loading and stomatal density. *J. Plant Physiol.*, 170(10): 906-914.

Shi, L.X., Z.L. Meng, S. Ma, Y.J. Wang, M. Xu and J.Y. Xu. 2015. Growth and photosynthetic characteristics of glycine gracilis seedlings under different types of saline stresses. *Pak. J. Bot.*, 47(3): 819-828.

Smart, R.E. and G.E. Bingham. 1974. Rapid estimates of relative water content. *Plant Physiology*, 53: 258-260.

Stikic, R., D. Glamoclija, M. Demin, B. Vucelic-Radovica, Z. Jovanovica, D. Milojkovic-Opsenicab, S.E. Jacobsenc and M. Milovanovica. 2012. Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. *J. Cereal Sci.*, 55(2): 132-138.

Vega-Gálvez, A., M. Miranda, J. Vergara, E. Uribe, L. Puente and E.A. Martínez. 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.), an ancient Andean grain: a review. *J. The Science of Food and Agricul*., 90(15): 2541-2547.

Venora, G. and F. Calcagno. 1991. Study of stomatal parameters for selection of drought resistant varieties in *Triticum durum* DESF. *Euphytica*, 57(3): 275-283.

Vidal-Valverde, C., J. Frias, A. Hernandez, P.J. Martín-Alvarez, I. Sierra, C. Rodríguez, I. Blazquez and G. Vicente. 2003. Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum* L.) seeds. *J. Sci. Food and Agricul.*, 83(4): 298-306.

Volkenburgh, E.V. 1999. Leaf expansion- an integrating plant behaviour. *Plant, Cell Environ.*, 22(12): 1463-1473.

Yin, L., W. Shiwen, L. Jianye, T. Kiyoshi and O. Mariko. 2013. Application of silicon improves salt tolerance through ameliorating osmotic and ionic stresses in the seedling of Sorghum bicolor. *Acta Physiol Plant*., 35(11): 3099-3107.

Yooyongwecha, S., C. Theerawitayab, T. Samphumphuangb and S. Cha-um. 2013. Water-deficit tolerant identification in sweet potato genotypes (*Ipomoea batatas* (L.) Lam.) in vegetative developmental stage using multivariate physiological indices. *Scientia Horticulturae*, 162: 242-251.

Zarinkamar, F., S. Ghelich and S. Soleimanpour. 2013. Toxic effects of Pb on anatomy and hypericin content in *Hypericum perforatum* L. *Bioremed. J.*, 17(1): 40-51.

Zhu, J.K. 2001. Plant salt tolerance. *Trends in Plant Science*, 6(2): 66-71.

Zouaoui, R., Y. Ammari, M. Abassi, H. E. L. A. Ben Ahmed, A. Smaoui and K. Hilali. 2019. Physiological and biochemical responses of rhus tripartita (Ucria) grande under water stress. *Pak. J. Bot.*, 51: 1215-1221.

(Received for publication 15 April 2018)