Effects of different concentration of Ca $(NO_3)_2$ on quinoa-treated with salinity

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ABSTRACT

Salinity has some adverse effects on the morphology and physiology in many crops. To alleviate the damages of salinity, the applications of calcium nitrate on quinoa-treated NaCl (*Chenopodium quinoa* Willd.) were investigated under the supported-hydroponic environment. The plants were exposed to 200mM NaCl with 20mM and 150mM Ca $(NO_3)_2$ (EC 18.61~37.85 ds·m^{-1} and osmotic potential -0.89~-1.71MPa), and sampled for measurements of osmotic potential, stomatal characteristics, and root characteristics. The presence of 200 mM NaCl alone decreased the relative parameters in different degrees. In all treatments, the indexes on stomatal characteristic were decreased with increasing electrical conductivity (EC) levels except for stomatal density. Stomatal conductance decreased more markedly when osmotic potential reached -0.89Mpa. Increasing in stomatal density observed in higher $Ca(NO_3)_2$ level (150mM) might be caused by the inhibition of cell division in the epidermis, which was also due to reduction of osmotic potential of the solutions.A similar trend was observed for osmotic potentials in the same tissue, which were deceased with increasing EC of the solutions. Although no significant differences in the all treatments were observed for the average diameter of roots, the beneficial effect of $Ca(NO_3)_2$ application at the concentration of 20 mM was significant in projected area, surface area, and volume. The phenomenon showed that moderate reduction in osmotic potential was favorable to cell extension due to maintaining cell turgor pressure. Much lower osmotic potential possibly inhibited cell division of root apical meristem. From the above results, it might be concluded that the effects of $Ca(NO_3)_2$ applications depended on the concentration, while the significant differences between the stomata and root morphology represented the tissue-specific as well.

Subject Agricultural Science, Plant Science

Key words Quinoa, Salinity, Calcium nitrate, Stomatal characteristic, Osmotic potential, Root characteristic

INTRODUCTION

Salinity is one of the most vital environmental stresses affecting seed germination (Lovato, Filho & Martins, 1999), seedling growth, development, and crop productivity. More than 800 million ha of land is salt-affected, which is over 6% of the world's land area (Rengasamy, 2006). Approximately 20% of the world's arable land and 40% of the irrigated land are subjected to salinity erosion at different degrees (Sahi et al., 2006). It is estimated that 50% of all arable lands may become saline by the year 2050 (Seki et al., 2007). As a consequence, enhancing salinity tolerance by some means would be an important strategy to improve the crop productivity (Khan et al., 2010).

In general, the effects of salt on plants are attributed to the decrease in water uptake, and toxicity of specific ions (Alam, 1994), leading to membrane disorganization, increase in reactive

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oxygen species (ROS) levels and metabolic toxicity (Hasegawa et al., 2000). For many conditions, water status and ion effect are inextricably linked. The initial and primary effects of salt, especially at moderate concentrations, are due to osmotic stress (Munns & Termaat, 1986). Even in well-watered soils by decreasing the osmotic potential of soil solutes, it is difficult for roots to extract water from their surrounding media (Sankar et al., 2007). In such case, the plants should adapt to the environmental variation through the stomatal aperture for maintaining the water balance. Resistance for CO_2 diffusion and decreases in transpiration rates reduce carbon assimilation, which inhibits the photosynthesis and crop productivity. Consequently, the stomatal characteristics should be regarded as one of important physiological parameters. In addition, ion toxicity or nutrition imbalance is increased owing to passive absorption and accumulation of some specific ions. Sodium and chlorine are generally the dominant ions in saline environments (Tester & Davenport, 2003), and directly affect nutrient uptake, such as Na^+ reducing Ca^{2+} uptake or Cl^- reducing NO_3^- uptake (Grattan & Grieve, 1999). Most plants are very sensitive to Na^+ which can disturb intracellular ion homeostasis (Rengel, 1992), membrane dysfunction (Ghoulam, Foursy & Fares, 2002), and disorder of metabolic processes (Manaa et al., 2011). Accordingly, two cost-effective strategies of increasing crop yield are breeding tolerant genotypes, and application of chemical substances.

Quinoa (*Chenopodium quinoa* Willd.), an ancient crop of the Amaranthaceae family, has been cultivated in the Andean region for thousands of years (Jacobsen, Mujica & Jensen, 2003). It is a tetraploid species, a close relative of beets and amaranth (Maughan et al., 2006). As reported in previous literatures, quinoa is a highly nutritious seed crop which is rich in amino acid (lysine), unsaturated fatty acids (linolenic acid, linoleic acid), mineral composition as cofactors in antioxidant enzymes (calcium, magnesium, iron, copper, and zinc), tocopherols (vitamin E), saponins and phenolic compound with antioxidant power (Rengasamy, 2006; Vega-G´alvez et al., 2010). As a kind of grain crop, quinoa has an excellent stability under freezing and retrogradation due to carbohydrate accumulation. Another critical characteristic is that quinoa may give seed yield of 1.721 t ha^{-1} of remarkable quality in typical agro-climatic conditions of South Eastern Europe (Stikic et al., 2012). For these reasons, FAO has suggested it as one of the crops that should be used for food security in the next century (Izquierdo et al., 2003; FAO, 1998). Quinoa is also regarded as a crop with a high level of resistance to several of the predominant adverse factors, such as drought, salinity, frost, hail and poor soil fertility (Jacobsen, Mujica & Jensen, 2003). In the field, most of plants known as glycophytes are not capable of dealing with salt concentrations of EC > 4 $dS \cdot m^{-1}$. For example, rice will die during vegetable stage when the salinity rises to 10 $dS \cdot m^{-1}$ (Munns, James & Läuchli, 2006). As a moderately salt-tolerant crop, most of quinoa species may grow, develop and fruit under the mild saline conditions (10–20 $dS \cdot m^{-1}$). It is worthwhile to note that quinoa can also survive even at 400 mM NaCl (40 $dS \cdot m^{-1}$), which amounts to the seawater (Razzaghi et al., 2011). The special characteristics and higher nutrient values have received much attention from worldwide, and led to lots of research on the development of new food products in recent years.

Salt tolerance is concerned not only plant species but also the exogenous application of chemical substances (Jaleel et al., 2007). Exogenous application of nutrient elements is one of

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efficient strategies minimizing the effects of salinity on plant productivity, such as N (Wu et al., 2008), K (Chartzoulakis et al., 2006), Si (Liang, 1999), Ca and Mg (Asaeda et al., 2014). It is well known that calcium is a very important macroelement for plant metabolism, and the hypothesis of Ca^{2+} being a second messenger has been advanced for environmental stress. Several practices have showed that calcium is involved in many processes, including cell division and elongation (Kader & Lindberg, 2010), competition to Na^{+} uptake (Epstein, 1962), lipid peroxidation of cell membranes (Kaya et al., 2002; Marinos, 1962), antioxidant enzyme activities, and the plant hormone metabolism (Manaa et al., 2014). Under abiotic stresses, application of moderate amount of exogenous Ca^{2+} can increase stomatal conductance, improve plant photosynthesis through calmodulin (CaM) and Ca^{2+}-dependent protein kinases (Zhang et al., 2014). However, there are some disagreements about Ca^{2+} evaluation. Sohan, Jasoni & Zajicek (1999) indicated that calcium supplements of 10 mM were not able to ameliorate the adverse effects of NaCl on the plant-water relations of sunflower. Navarro, Martinez & Carvajal (2000) reported that the growth-reductions and physiological effects induced by Na^{+} (60 mM) were partially prevented by additional Ca^{2+} in the hydroponics solution. Note that these plant characteristics may not be affected until a critical threshold level of Ca^{2+} has been reached. It means that crop biomass may not decrease until a given threshold concentration of Ca^{2+} is reached, below which there is no significant influence in the total output. The beneficial effect of Ca^{2+} did not persist once Ca^{2+} supply exceeded the critical level because further Ca^{2+} supply increased soil salinity (Vaghela et al., 2010). Even as a kind of benefit macronutrient, higher concentration of Ca^{2+} lead to water stress and inhibition of enzyme activity. Reviewing the past literatures, most attention has focused on alleviating adverse effects of Ca^{2+} on salinity, less attention has been given to the critical toxic level of Ca^{2+}. The influence of Ca^{2+} is largely relevant to the relative concentration of Na^{+} to Ca^{2+} as well as the absolute concentration of calcium.

Transpiration, water uptake, and CO_2 entrance are partially controlled by the plant via its regulation of stomatal opening. So the effects of initial water deficit on photosynthesis may be observed directly by diffusion limitations through the stomata under salt stress condition. As for the root systems, it not only plays an important role in water and nutrient uptake, but also interacts directly with the biotic and abiotic components of the rhizosphere. Under stress condition, adjustment to the root systems might alleviate the effect of the stress on the intracellular environment. Thus, it is critical for the growth and survival of the plants to adjust morphology and physiology of stomata and root system under salt stress. The focus of this study was to provide additional information on the optimal concentration of calcium nitrate. The relative physiological indexes of quinoa were investigated in terms of osmotic potential of different parts, stomatal characteristic and root characteristic, and the possible role played by calcium nitrate in regulating salinity-induced variations in these parameters.

MATERIALS AND METHODS

Plant culture

The experiment was carried out from 30th May to 10th July 2013 in the greenhouse located in the Faculty of Science, University of Copenhagen. Plant material, a Danish bred cultivar (Titicaca)

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more adapted to Mediterranean condition, was provided by Prof. Jacobsen SE of the University of Copenhagen.

Seeds were sown in vermiculite-filled plastic trays in the greenhouse. The environmental conditions were as follows: average day/night temperatures of 22 ± 2 ℃ / 18 ± 2 ℃, 16 h light / 8h dark with a photosynthetic photon flux density (PPFD) of 600 $mol \cdot m^{-2}s^{-1}$, and a relative humidity of 60 ± 5%. Once seedlings emerged, they were watered with half-strength Hoagland's nutrient solution (pH 6.8) every two days. After 2 weeks of culture, healthy and uniform seedlings were transferred into 500 ml plastic pot (four seedlings per pot) containing full-strength Hoagland's nutrient solution continuously aerated.

Salt treatments

When the seedlings were at the sixth leaf stage, the different treatments were initiated. The different four treatments were applied as follow: (A) Hoagland's nutrient solution (control), without the addition of NaCl or Ca $(NO_3)_2$. (B) Hoagland's nutrient solution with 200mM NaCl; (C) Hoagland's nutrient solution with 200mM NaCl + 20mM Ca $(NO_3)_2$; (D) Hoagland's nutrient solution with 200mM NaCl + 150mM Ca $(NO_3)_2$. Each treatment included four replicates. NaCl concentration was gradually elevated by 50 mM daily in order to avoid salt shock. The concentration of Ca $(NO_3)_2$ were designed on the basis of previous published experiments (Vaghela et al., 2010). The solution was changed every other day until the end of the experiment, and the pH of the solution was adjusted to 6.5 by adding 0.1M KOH. The plants were watered twice every day in the early morning and the late afternoon according to the weight loss. At the same time, the electronic conductivity (EC) and the osmotic potential (Ψ_π) of the respective treatment solutions (Table 1) were determined by Conductivity Meter and Dew point microvoltmeter (HR–33T, Wescor Inc., Logan, UT, USA), respectively.

Table 1 The physical properties of the treatments.

The stomatal characteristics of quioa under the stress conditons

The stomatal conductance (g_s, $mmol \cdot m^{-2}s^{-1}$) was measured on the fourth fully expanded leaf between 10:00- 12:00 AM with a leaf porometer (Model SC-1, Decagon, Pullman, WA, USA). The result of the same leaf was repeated twice. The same leaf was used for the measurement of stomatal morphology. According to the method of nail polish impressions (Shabala & Volkenburgh, 2003), four leaves were taken from each of treatment. For each leaf, four microscopic observations were analyzed at 400 magnifications. Stomatal morphology was observed under a LEITZ DMRD microscope camera system (Leica Microscope and System GmbH, D 35530, Wetzlar, Germany) equipped with a digital camera, and the images were presented using image-editing software (Leica Microsystems, version 2.5.0, CMS GmbH (Switzerland) Limited) on a computer screen. Stomatal length and stomatal density were measured with the images using UTHSCSA ImageTool software (UTHSCSA ImageTool for Windows version 3.00).

The osmotic potential ($\Psi\pi$) of different tissues in quinoa under the stress conditons

The different parts of seedlings were frozen in liquid nitrogen for 20 min to disrupt cell membranes, and then transferred to a refrigerator of -80°C for later osmotic potential measurement. Before the osmotic potential was measured, the tissues should be equilibrated for

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at least 20 min at 25℃. The sample was ground by the tissue grinder, and the osmotic potential of the sap was measured with a Dewpoint Potential Microvoltmeter (HR– 33T, Wescor Inc., Logan, UT, USA). The value was calculated on the basis of the standard curve determined previously.

The root characteristics of quioa under the stress conditons

The root systems used for morphology measurements were spread out in a clear, water filled plate, and were scanned to high definition (600 dpi) using the WinRHIZO analysis system (WinRHIZO ver. 2004a, Regent Instruments Inc., Quebec, Canada). Total root length, projected area, surface area, average diameter, root volume, length per volume were automatically analyzed from the root images.

Statistical analysis

The experiment design was a randomized complete block with four replications. The data were analyzed with SPSS version 10 (SPSS, Chicago, IL, USA), using the one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD), p values ≤ 0.05 were regarded as significant.

RESULTS

The stomatal characteristics of quioa

Stomatal conductances, stomatal density, and length of stomatal pore were used to evaluate the effects of salinity and supplementary $Ca(NO_3)_2$ on the stomata characteristics. In most cases, the relevant parameters of abaxial surface were higher than those of adaxial surface in different degrees (Table 2). The presence of 200 mM NaCl alone decreased all the parameters on the stomatal characteristics. As compared to the control, stomatal conductance, stomatal density, and stomatal length of the adaxial surface decreased by 59.5%, 0.4% and 16.3 %, respectively. Moreover, the above parameters of abaxial surface decreased by 59.3%, 20.2% and 14.9%, respectively. In the identical NaCl level (200mM), the plants supplemented with different concentrations of $Ca(NO_3)_2$ exhibited reduction in stomatal conductance, and stomatal length of both sides ($p<0.05$) with increasing electrical conductivity (EC) levels except for stomatal density. Stomatal conductance decreased more markedly when osmotic potential reached -0.89MPa. The values of adaxial surface and abaxial surface in group C decreased by 81% and 87.3%, respectively. The same parameters in group D decreased by 86.5% and 87.9%, respectively. In the similar manner, stomatal length of adaxial surface and abaxial surface in group C decreased by 29.4% and 8.2%, respectively. The same parameters in group D decreased by 32.3% and 34.5 %, respectively. In contrast to the above results, the maximum values of stomatal density in both surfaces were recorded from group D. The related indexes in adaxial surface and abaxial surface increased by 70% and 0.4 %, respectively.

Table2 Effects of salt stress and calcium application on stomatal characteristics in quinoa grown under greenhouse conditions.

The osmotic potential ($\Psi\pi$) of different tissues in quinoa

The variations of osmotic potential in leaf, stem, and root of quinoa were shown in Table 3. The highest values of osmotic potential in all treatments were found in the roots of the seedlings, and the lowest values were found in the stems. The control treatment had less negative osmotic potentials compared to the other treatments. In most cases, the osmotic potential in leaf, stem and

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root of quinoa decreased gradually with decreasing osmotic potential of the different treatments. At the same parts of the seedlings, decrease in osmotic potential was less pronounced among the stress treatments (p>0.05). Compared with the control treatment, the osmotic potential of stress solutions were 4.1-fold (B), 4.5-fold (C), and 7.8-fold (D), respectively (Table 1). In the quinoa roots from different stress treatments, the osmotic potential was 7.6-fold (B), 8.1-fold (C), and 8.5-fold (D) higher than that of the control. In the quinoa stems of different stress treatments, the osmotic potential was 2.6-fold (B), 3.1-fold (C), and 3.7-fold (D) higher than that of the control. In the quinoa leaves of different stress treatments, the osmotic potential was 2.1-fold (B), 2.7-fold (C), and 2.4-fold (D) higher than that of the control.

Table 3 Effects of salt stress and calcium application on osmotic potential (Ψ_π) in quinoa grown under greenhouse conditions.

The root characteristics of quinoa

Root length, projected area, surface area, average diameter, and root volume were used to evaluate the effects of NaCl and $Ca(NO_3)_2$ on morphological variations of the root system (Table 4). The results showed that the presence of 200mM NaCl alone in growth medium decreased all these parameters of root characteristics (p>0.05). Although no significant difference was observed on the average diameter among all the treatments (p>0.05), the maximum values of the root parameters were observed in group C.

Table 4 Effects of salt stress and calcium application on root morphology of quinoa grown under greenhouse conditions.

DISCUSSION

As one of essential macronutrients, calcium makes the major contribution to signal transduction, structural integrity and permeability to cellular membranes (Mengel & Kirkby, 1987), slowing the degradation of cell wall (An et al., 2014), counteracting the harmful effects of Na^+(Lahaye & Epstein, 1971), availability and uptake of nutrients ((Pandolfi, Mancuso & Shabala, 2012), and so on. High levels of salinity might disturb absorb of nutrients, such as Ca^{2+} deficiency. Exogenous calcium should reduce the damage of Ca^{2+} deficiency. The conclusion has been confirmed by Agarwal et al. (2005) suggested that Ca^{2+} might be responsible for the activation of transcription factor associated with SOD, APOX and CAT under abiotic stress. Liu et al. (2014) also found that $CaCl_2$ application increased stomatal conductance, and adjusted chloroplast structure in LNT-stressed plants. Still other studies suggested that 3 or 10 mM Ca^{2+} supplements possibly damaged the metabolism of the blueberry because of higher Ca^{2+} concentrations (Wright, Patten & Drew, 1993). Plants differ in both the amounts of Ca^{2+} they require and their tolerance of Ca^{2+} in the rhizosphere (White & Broadley, 2003). Therefore, such improvement in calcium application was associated with the concentration of calcium.

Effects of salt treatments on the stomatal characteristics of quioa

Under the salt stress condition, decreases in water potential of the environment resulted in the limited uptake of water. Water deficit-induced stomatal closure has been regarded as the initial response. In the current experiment, stomatal density did not show significant differences except for the higher level treatment of $Ca(NO_3)_2$ (150mM). The finding agreed with the previous report (Jacobsen, Liu & Jensen, 2009) that leaf expansion is also sensitive to water deficit. The lower

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osmotic potential in the leave may be significant in inhibition of cell division. While the division of the epidermis was inhibited, the relative number of the stomata was increased at the same time. This is the reason for the result that the highest value of stomatal density was observed in the treatment of 150mM $Ca(NO_3)_2$ and 200mM NaCl.

Compared with the variations of stomatal density and length of stomatal pore, stomatal conductance was inhibited significant in previous conditions. Under low level of $Ca(NO_3)_2$ (20mM), it may be suggested that reduction in gs is favorable to better water retention (Pandolfi, Mancuso & Shabala, 2012). With increasing in the Ca^{2+} concentration, the effect on the stomata aperture became more and more seriously. The results were consistent with reports of Montesano and Van (2007), suggesting that reductions in stomatal indexes were probably attributed to osmotic effects rather than Na^+ specifically, especially in the identical NaCl condition. In short term, stomatal conductance may contribute to plant metabolism and growth. Thus, it is a more practical approach to maintain the higher stomatal conductance, improve salt tolerance through applying the optimal concentration of calcium.

Effects of salt treatments on the osmotic potential ($\Psi\pi$) of different tissues in quinoa

The presence of NaCl in the nutrient solution reduces the osmotic potential of the root environment, and inhibits water absorption. Results presented in this study indicated that the osmotic potential ($\Psi\pi$) of different tissues in quinoa was not influenced by the same way. There were no proportional variations in the osmotic potential between the solutions and the different tissues. This may be involved in employing internal exclusion (vacuolar sequestration) and external (sequestration in EBC) exclusion for the cytosolic Na^+ (Bonales-Alatorre et al., 2013), reducing the following accumulation in the cytoplasm of the shoot. And on the other hand, osmotic potential of the same tissues became more negative with decreasing the osmotic potential of all the treatments. Reduced values of osmotic potential may be due to externally supplied Ca^{2+} under the identical NaCl concentration. The low osmotic potential in tissues was favorable to maintain of the potential gradient for water uptake, delaying the physiological drought of the seedlings in salt-stressed plants. Once the osmotic potential belows a threshold level, physiological damages might occur. Therefore, it seemed that the beneficial effect of Ca^{2+} did not persist when Ca^{2+} supply exceeded the critical level (Vaghela et al., 2010).

Effects of salt treatments on the root characteristics of quinoa

Environmental conditions affected root development processes in different ways. Root architecture has been directly related to plant productivity (Lynch, 1995) under limiting edaphic conditions. As expected, better root morphology meant more salt-tolerant (Ashraf et al., 2005). In order to maintain the absorption function of root system, plant should adjust root morphology and physiology. In current work, the NaCl treatment alone reduced all the parameters of the root system (p>0.05) due to water-deficit and ion- excess effects. The results of $Ca(NO_3)_2$ application were consistent with the previous observation that maize - treated with low level of NaCl showed the reduction in root elongation, and partly restored by the addition of Ca^{2+} (Cramer, Epstein & Lauchli, 1988). Under the identical Na^+ concentration, low level of $Ca(NO_3)_2$ (20mM) maintained the optimal states of the root system. It indicated that the interaction between Na^+ and Ca^{2+} was significant for the lengths, surface areas, projected areas and volumes of the roots. At

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higher level (150 mM), serious effect of $Ca(NO_3)_2$ application on the root length was detected. The results also confirmed the hypothesis that the optimal concentration of calcium may be significant in determining the rate and the extent of Na^+ - related inhibition of cell elongation (Rengel, 1992).

CONCLUSION

In summary, it might be concluded that the synergic effects of NaCl and $Ca(NO_3)_2$ on quinoa seemed to depend on suitable calcium concentration level and the applied tissues. Even as a kind of beneficial element, the optimal concentration of calcium varied with the sensitivity of different tissues. The results of the present study may serve as a reference for future research on exogenous application of chemicals.

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423 **Table 1 The physical properties of the treatments.**

Treatment	Electrical conductivity ($ds \cdot m^{-1}$)	Osmotic potential (MPa)
A	1.87	-0.22
B	18.61	-0.89
C	22.65	-0.99
D	37.85	-1.71

424 Notes.

425 A, Hoagland's nutrient solution (control), without the addition of NaCl or Ca $(NO_3)_2$; B,
426 Hoagland's nutrient solution with 200mM NaCl; C, Hoagland's nutrient solution with 200mM
427 NaCl + 20mM Ca $(NO_3)_2$; D, Hoagland's nutrient solution with 200mM NaCl + 150mM Ca
428 $(NO_3)_2$.

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Table2 Effects of salt stress and calcium application on stomatal characteristics in quinoa grown under greenhouse conditions.

Treatment	Stomatal conductance (mmol·m^{-2} s^{-1})		Stomatal density (No·mm^{-2})		Length of stomatal pore (μ m)	
	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface	Adaxial surface	Abaxial surface
A	475.68±76.94 a	684.80±62.57 a	138.00±9.50 b	165.33±12.51 a	90.21±4.00 a	96.89±1.54 a
B	192.52±31.96 b	278.88±46.23 b	137.33±18.58 b	132.00±12.48 ab	75.50±7.22 ab	82.48±2.55 bc
C	90.38±12.67 bc	87.24±11.27 c	112.00±8.86 bc	138.67±13.80 ab	63.68±8.07 b	89.00±2.66 b
D	64.20±8.94 c	83.15±13.92 c	234.67±17.24 a	166.00±9.60 a	61.10±4.33 bc	63.50±2.04 d

Notes.

A, Hoagland's nutrient solution (control), without the addition of NaCl or Ca $(NO_3)_2$; B, Hoagland's nutrient solution with 200mM NaCl; C, Hoagland's nutrient solution with 200mM NaCl + 20mM Ca $(NO_3)_2$; D, Hoagland's nutrient solution with 200mM NaCl + 150mM Ca $(NO_3)_2$.

Different letters indicate significant differences according to LSD at $p<0.05$. Data were presented as the mean ± standard error (SE, n=4).

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Table 3 Effects of salt stress and calcium application on osmotic potential (Ψ_π) in quinoa grown under greenhouse conditions.

Treatment	Leaf (MPa)	Stem (MPa)	Root (MPa)
A	-0.79±0.11a	-0.71±0.05a	-0.18±0.05a
B	-1.63±0.19 b	-1.84 ±0.21 b	-1.37±0.32 b
C	-2.16±0.24 bc	-2.22±0.07 bc	-1.46±0.27 bc
D	-1.89±0.29 b	-2.62±0.10 c	-1.54±0.31 bc

Notes.

A, Hoagland's nutrient solution (control), without the addition of NaCl or Ca $(NO_3)_2$; B, Hoagland's nutrient solution with 200mM NaCl; C, Hoagland's nutrient solution with 200mM NaCl + 20mM Ca $(NO_3)_2$; D, Hoagland's nutrient solution with 200mM NaCl + 150mM Ca $(NO_3)_2$.

Different letters indicate significant differences according to LSD at $p<0.05$. Data were presented as the mean ± standard error (SE, n=4).

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447 **Table 4 Effects of salt stress and calcium application on root morphology of quinoa grown**
448 **under greenhouse conditions.**

Treatment	Root length (cm)	ProjectedArea (cm^2)	Surface Area (cm^2)	Average Diameter (mm)	Root Volume (cm^3)
A	4445.95 ab	112.75 b	354.21 b	0.68 a	2.27 b
B	3136.41 ab	90.77 bc	285.17 bc	0.56 a	2.19 bc
C	4648.39 a	161.80 a	508.33 a	0.70 a	4.44 a
D	2915.95 b	114.90 ab	360.98 ab	0.66 a	3.56 ab

449 Notes.

450 A, Hoagland's nutrient solution (control), without the addition of NaCl or Ca $(NO_3)_2$; B,
451 Hoagland's nutrient solution with 200mM NaCl; C, Hoagland's nutrient solution with 200mM
452 NaCl + 20mM Ca $(NO_3)_2$; D, Hoagland's nutrient solution with 200mM NaCl + 150mM Ca
453 $(NO_3)_2$.

454 Different letters indicate significant differences according to LSD at $p<0.05$. Data were
455 presented as the mean (SE, n=4).

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