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EFFECTS OF NACL ON PLANT GROWTH, ROOT ULTRASTRUCTURE, WATER CONTENT, AND ION ACCUMULATION IN A HALOPHYTIC SEASHORE BEACH PLUM (*PRUNUS MARITIMA*)

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Abstract

A pot culture experiment was conducted to evaluate the effect of NaCl on the growth, root ultrastructure, water content, and ion distribution in two - year - old beach plum (*Prunus maritima*) seedlings. The seedlings were subjected to different levels of NaCl addition (0, 50, and 150 mM) for 120 days under natural conditions. Under high salinity (150 mM NaCl), the ultrastructure of root cells in *P. maritima* showed the earliest sign of salt injury: including partial fuzzy nuclear membrane and plasma membrane invagination in the root cells of *P. maritima*. The root cells were unaffected by low salinity (50 mM NaCl). Their root cells exhibited complete karyotheca, obvious nucleolus, numerous organelles, and normal cell wall structure. However, *P. maritima* maintained higher water content in plant tissues under salinity conditions compared with that of the control. Meanwhile, the salt - treated seedlings also maintained high concentrations of Ca^{2+}, Mg^{2+}, and K^+ in leaf tissues. Furthermore, high salinity led to an increase in K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ selectivity ratios from stems to leaves in *P. maritima* seedlings. In addition, the growth rate, fresh and dry weights of *P. maritima* were significantly promoted by low salinity, whereas the growth parameters were unaffected or even decreased at high salinity compared to that of the control. The results indicated that low salinity had a positive effect on the growth of *P. maritima* by keeping the normal ultrastructure of root cells and increasing the uptake of Ca^{2+}, Mg^{2+}, and K^+ ions, especially in leaf tissues. The negative effect of high salinity on plant growth could be ameliorated to some extent by increasing water content in leaves and selective transport of K^+, Ca^{2+} and Mg^{2+} from stems to leaves in *P. maritima* seedlings.

Key words: Beach plum; Root cell ultrastructure; Growth rate; Ion transportation; NaCl.

Introduction

Soil salinity occurs mainly along worldwide coasts or in arid and semi-arid regions with 3.1% (397×10^6 hectares) of the total land area of the world (Setia *et al*., 2013). It is one of the major environmental stresses that adversely affects plant growth and biomass production due to the osmotic or ionic stresses, or a combination of both stresses induced by high NaCl - salinity (Hajiboland *et al*., 2014; Cunha *et al*., 2016; Yarsi *et al*., 2017). Meanwhile, the world population will expand to about eight billion people in 2030, with a concomitant increase in agricultural products. Providing for these needs will require its expansion into marginal unproductive salt-affected lands. The use of halophytes, especially woody plants, is the most cost-effective strategy for saline soil remediation.

Given that NaCl is the most widespread salt in nature, many halophytes have evolved various mechanisms including ion homeostasis and an increase in the intake of water for the adaptation of plants to high salinity (Maimaiti *et al*., 2014; Isla *et al*., 2014; Vijayan *et al*., 2003). Based on these mechanisms, halophytes can be categorized into three groups: **euhalophytes** - large amounts of Na^+ and Cl^- ions absorb and accumulate in leaf vacuoles, **excluders** – accumulate Na^+ and Cl^- ions in root tissues, and export these ions from the root cells back to the soil, and **conductors** - secrete the absorbed ions by glands or bladders (Jouyban, 2012). Numerous studies have shown that great differences in the salt response exist among the different species (Maimaiti *et al*., 2014; Boughalleb *et al*., 2009; Isla *et al*., 2014). Vijayan *et al*., (2003) suggested that plant growth of a moderately salt-tolerant mulberry was enhanced under low salinity but inhibited at high salinity. A different result was reported by Maimaiti *et al*., (2014), who indicated that plant growth of *Elaeagnus oxycarpa* was unaffected by low salinity and decreased with increasing salinity. Recently, although salt tolerance is investigated in many crops (Asma *et al*., 2015; Huyen *et al*., 2015), the cellular responses in the roots of woody plants to high salinity remain largely unknown.

P. maritima is a very salt-tolerant shrub of coastal plant communities across North America. Moreover, it becomes popular and widespread because of its prolific bloom, prized fruit, and the adaptation to saline environments. Currently, *P. maritima* has been developed as a new multipurpose crop for coastal beach in the world. Thus several attempts have been made to bring this wild fruit tree into cultivation and to make use of its resistance to salt stress for the stabilisation and rehabilitation of degraded saline land (Uva, 2004; Zai *et al*., 2012). Recent studies have focused on fruit production and the cause-effect relationship between the aerial parts and the salt tolerance mechanism in *P. maritima* (Uva & Whitlow, 2007; Zai *et al*., 2012). Nevertheless, little is known about how salinity affects its roots, which directly suffer from soil salinity. Thus, root traits, especially the root ultrastructure, should be more sensitive to salt stress. Furthermore, the ultrastructural changes in root cells of plants would also disrupt the uptake and transport of water and ion in

plants as a result of growth arrest and cell death under salinity conditions (Lambers & Veneklaas, 2006; Russell & Clarkson, 2016). Meanwhile, the seedling stage is usually the salt-sensitive hypertension phase in woody plants. For this reason, young seedlings represent a valuable material for the detection of the early response of plants to salt. Our main hypothesis was, therefore, salt stress would affect the ultrastructure in root cells of *P. maritima* seedlings, and thus resulting in altered ion selectivity or water content in plant organs, with consequent growth reduction in the seedlings. To test the hypothesis, this study aimed to determine the effects of low (50 mM) and high (150 mM) salt concentrations on root ultrastructure, ion distribution, water status, and plant growth at the seedling stage and to evaluate the relative significance of these traits imparting salt tolerance in *P. maritima*.

Materials and Methods

Plant materials and growth conditions: An experiment was conducted using a completely randomized design with 3 replicates per treatment in early April 2010 under natural conditions at the Agricultural Research Station of Nanjing Forestry University, Jiangsu, China (118°48' E, 32°04' N). This study area has a subtropical humid monsoon climate with the average annual precipitation of 1,274 mm, the mean annual air temperature of 16.2ºC, and the relative humidity of 76%. Two-year-old *P. maritima* seedlings were transplanted into plastic pots (23 cm in diameter and 28 cm in depth) containing 15 kg of a sandy loam soil (pH = 6.88), 10.61 g kg^{-1} of organic matter, 0.64 g kg^{-1} of total N, 146.05 mg kg^{-1} of available K and 4.10 mg kg^{-1} of available P. On the 15th day after potting, 1000 ml per pot of different NaCl solutions (0, 50, and 100 mM) was directly applied every other day. The seedlings were harvested after a 120-day treatment.

Growth parameters and water content: The plant height of three randomly selected seedlings under each treatment was measured with a steel tape at the beginning (Day 0, corresponding to 15th day after potting, t_1) and at the end of treatment (Day 120, corresponding to the 135th day after potting, t_2). Relative growth rates of height, then, were calculated by $(h_2 - h_1)/(t_2 - t_1)$, where h_1 and h_2 are the heights of the seedlings at times t_1 and t_2, respectively.

Control and salt - treated plants were collected and separated into leaves, stems and roots after 120 days of salt-treatment. The different plant parts were washed with the deionized water and dried with towels before the fresh weight (FW) was determined. Dry mass (DM) was measured after the plant parts were oven dried at 105ºC for 15 min and then at 70ºC till a constant weight. Water content (WC) of those tissues was calculated, using the formula described in Liu *et al.,* (2016):

$$WC = (FW - DM)/DM \times 100$$

Electron microscopy: Root samples of approximately 2 mm length were cut with a razor blade in the root tips (<2 cm), and fixed with 4% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.2) for 4 h. After being washed three times with the buffer, the root tip was postfixed for 2 h with $Os0_4$ (2%, pH 7), dehydrated in a graded acetone series (30%, 50%, 70%, 90%, 100% [v/v]; 10 min each step) and embedded in Spurr's resin (Spurr, 1969). The resin was polymerized at 35°C for 24 h, 45°C for 24 h, and 60°C for 12 h, respectively. The embedded blocks were sectioned using an LKB Ultrotome I. The ultrathin sections were picked up on copper grids and double-stained with uranyl acetate and lead citrate. The ultrastructure of the root cells of *P. maritima* was observed and photographed with a Hitachi H-600 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

Ion analyses: The oven-dried roots, stems and leaves were ground. The ground samples (1 g each) were digested in the mixture of HNO_3 and $HClO_4$ (3:1, v/v) for cation analyses. The Na^+, K^+, Ca^{2+} and Mg^{2+} ion contents in extracts from plant tissues were measured by using an atomic absorption spectrophometer (AAS) (Unicam Solaar32, USA). In addition, a 250-mg tissue sample was extracted in the mixture of 900 ml deionized water, 100 ml 10 % CH_3COOH, and 6.4 mL 0.1 *M* HNO_3. Cl^- content in the extract was determined by titration with $AgNO_3$ (TDMFC, 1991). These ion (Cl^-, Na^+, K^+, Ca^{2+}, and Mg^{2+}) contents were expressed as mg g^{-1} DM.

The selectivity of ion transport for K^+, Ca^{2+} and Mg^{2+} over Na^+ ($S_{K,Na}$, $S_{Ca,Na}$ and $S_{Mg,Na}$) was estimated using the following formula (Gorai *et al.*, 2010), where K, Ca, Mg and Na are the contents of these ions in different organs, respectively.

$$[K, Ca \text{ or } Mg/Na]_{shoot} / [K, Ca \text{ or } Mg/Na]_{root}$$

$$[K, Ca \text{ or } Mg/Na]_{leaf} / [K, Ca \text{ or } Mg/Na]_{shoot}$$

Statistical analyses: Values are shown as the means ± SD. One-way analysis of variance followed by Duncan's multiple range test was used to determine whether the differences between the fertilization types were significant. The significance threshold was at $p<0.05$. These analyses were conducted with SAS 8.02.

Results

Effect of NaCl on growth parameters in *P. maritima* seedlings

Root ultrastructure: A compact cell in the roots of control plants exhibited complete karyotheca, finely granular and evenly distributed chromatin, obvious nucleolus, numerous organelles, amylase - rich starches, and normal cell wall structure (Fig. 1A). The ultrastructure of the root cells in *P. maritima* was unaffected by low salinity (Fig. 1B, D). However, the ultrastructure of root cells of *P. maritima* subjected to high salinity showed a decrease in chromatin density and starch content compared with those of control plants (Fig. 1C, E). Moreover, organelle degradation was observed under high salinity conditions (Fig. 1C, E).

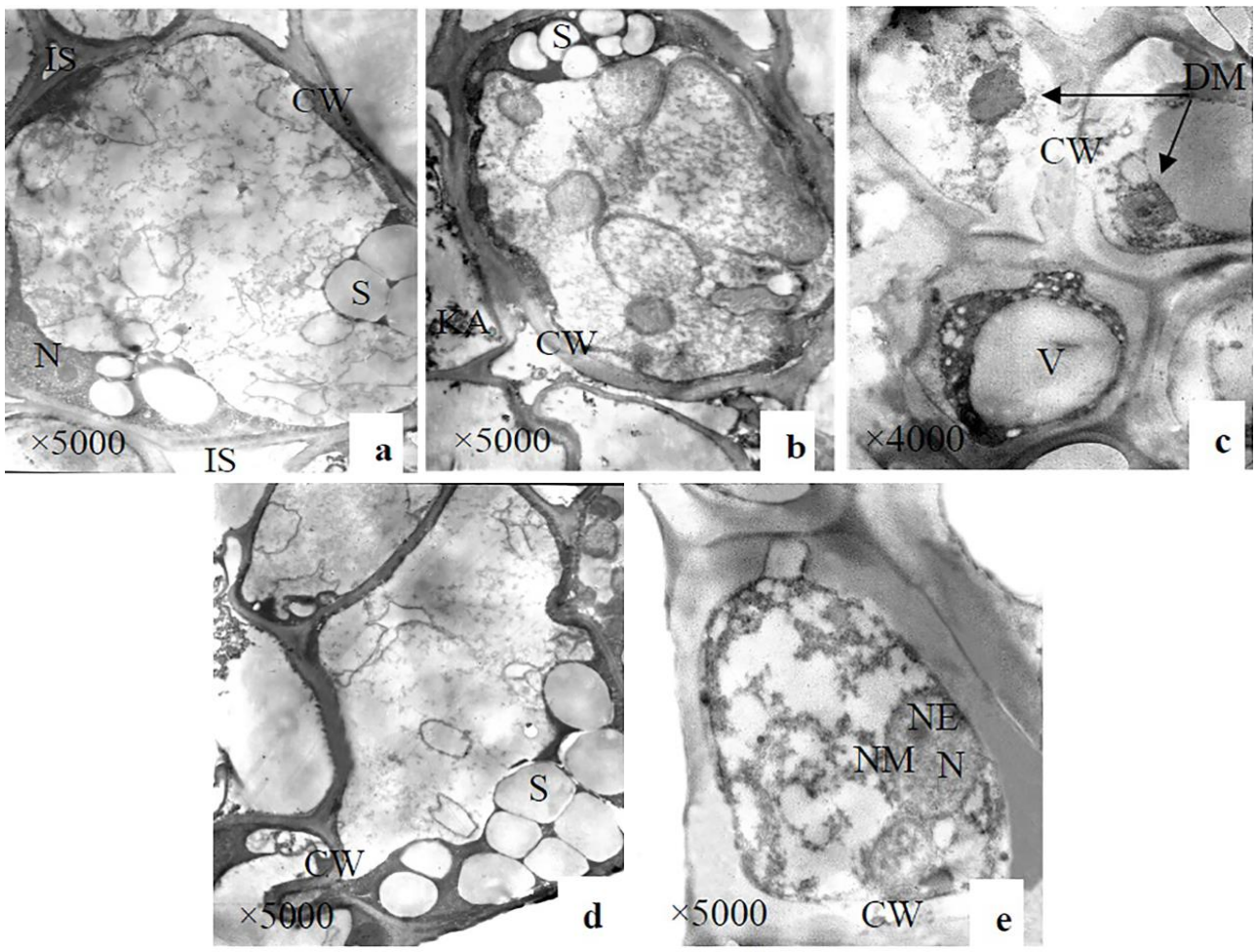

Fig. 1. The cell ultrastructure of *Prunus maritima* roots grown under different levels of NaCl in irrigation water of 0 (A), 50 (B, D), and 150 mM (C, E) for 120 days. CW - cell wall; IS - intercellular space; DM - degraded material; KA - karyotin; N - cell nucleus; NE - nucleolus; NM - nucleus membrane; S - starch; V - vacuole.

Table 1. Effect of NaCl on morphological characters of *P. maritima* seedlings after 120 days of treatment.

NaCl (mM)	Fresh weight /g plant^{-1}			Dry weight /g plant^{-1}			Growth rate /mm day^{-1}
	Root	Stem	Leaf	Root	Stem	Leaf	
0	20.06±1.42b(b)	25.26±1.69a(a)	5.46±0.27b(c)	14.62±1.16b(a)	15.81±0.42a(a)	2.33±0.13b(c)	0.38±0.05b
50	24.23±1.53a(a)	25.35±1.70a(a)	12.54±0.66a (b)	17.50±1.34a(a)	15.33±0.76a (b)	5.03±0.20a (c)	0.82±0.10a
150	14.12±1.12c(b)	20.42±1.49b(a)	5.98±0.33b (c)	9.89±0.23c (b)	11.70±0.54b (a)	2.44±0.15b (c)	0.37±0.07b

Values (means ± SD) with dissimilar lower-case letters inside and outside the parentheses are significantly different between tissues or salinities at $p<0.05$ by Duncan's multiple range test, respectively

Plant growth rate, fresh and dry matter, and water content: Plant growth was significantly promoted by low salinity, whereas it was unaffected by high salinity compared to that of the control (Table 1). Fresh or dry matter yields in roots, stems, and leaves of *P. maritima* were generally increased at low salinity, while the yields was unaffected or even decreased by high salinity compared with those in the control plants (Table 1). In addition, the fresh and dry weights in tissues were ranked as follows: stem ＞ root ＞ leaf in high salinity, and the dry weight at low salinity was in the order: root ＞ stem ＞ leaf (Table 1). Water content in leaves, stems and roots was generally increased with increasing salinity (Table 2). Besides, water content was significantly higher in leaves than that in shoots and roots of *P. maritima* (Table 2).

Effect of NaCl on ion distribution in individual tissues of *P. maritima* seedlings

Contents of Cl^-, Na^+, K^+, Ca^{2+}, and Mg^{2+}: The content of Cl^- and Na^+ was significantly ($p<0.05$) greater in NaCl-treated plants than that in the control ones (Table 3). Additionally, *P. maritima* seedlings generally accumulated more Cl^- and Na^+ in the leaves and roots than those in the stems (Table 3). Moreover, *P. maritima* would preferentially take up K^+, particularly Ca^{2+} over toxic ions such as Na^+ and Cl^- in leaves, stems and roots under saline conditions (Table 3).

Selective transport coefficients and ratios of K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+: Salinity caused marked reductions in K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios in

roots and stems, and Mg^{2+}/Na^+ ratios in leaves of *P. maritima* (Table 4). In addition, the Ca^{2+}/Na^+ ratio was higher than K^+/Na^+ and Mg^{2+}/Na^+ ratios in the tissues of *P. maritima* (Table 4). The selective transport coefficients of K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ from stems to leaves were generally increased with increasing salinity (Fig. 2D-E). There were also higher selective transport coefficients for K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ from roots to stems in *P. maritima* seedlings at low salinity while lower selectivity coefficients at high salinity than those of the control, respectively (Fig. 2A-C).

Table 2. Effect of NaCl on water content in roots, stems and leaves of *P. maritima* seedlings after 120 days of treatment.

NaCl (mM)	Water content/%		
	Root	Stem	Leaf
0	37.21±2.11 b(c)	59.77±3.04 c(b)	134.33±4.03 c(a)
50	38.46±2.50 b(c)	65.36±3.17 b(b)	149.30±4.12 a(a)
150	42.77±3.09 a(c)	74.53±3.08 a(b)	145.08±4.06 b(a)

Values (means ± SD) with dissimilar lower-case letters inside and outside the parentheses are significantly different between tissues or salinities at $p<0.05$ by Duncan's multiple range test, respectively

Table 3. Effect of NaCl on the contents of Cl^-, Na^+, K^+, Ca^{2+}, and Mg^{2+} (mg g^{-1} DW) in roots, stems and leaves of *P. maritima* seedlings after 120 days of treatment.

NaCl (mM)	Root	Stem	Leaf
		Cl^-	
0	0.64±0.02c(b)	0.18±0.01c(c)	1.43±0.02b(a)
50	1.34±0.03b(b)	1.20±0.02b(c)	1.72±0.03b(a)
150	3.46±0.07a(b)	1.97±0.03a(c)	5.97±0.06a(a)
		Na^+	
0	0.18±0.01c(b)	0.11±0.01c(c)	0.52±0.07b(a)
50	0.55±0.06b(a)	0.19±0.02b(b)	0.54±0.05b(a)
150	1.97±0.23a(b)	1.55±0.14a(c)	2.31±0.26a(a)
		K^+	
0	1.68±0.11a(b)	1.51±0.09a(c)	6.76±0.44a(a)
50	1.49±0.04b(b)	1.42±0.02b(b)	6.77±0.38a(a)
150	1.16±0.05c(b)	1.30±0.02c(b)	6.76±0.40a(a)
		Ca^{2+}	
0	6.12±0.39b(c)	6.75±0.41c(b)	11.70±0.54c(a)
50	6.03±0.36b(c)	9.70±0.51a(b)	12.07±0.48b(a)
150	6.88±0.35a(c)	8.45±0.46b(b)	12.31±0.44a(a)
		Mg^{2+}	
0	1.25±0.03a(b)	1.12±0.04a(c)	1.64±0.51a(a)
50	1.15±0.03b(b)	1.15±0.02a(b)	1.65±0.07a(a)
150	1.15±0.05b(b)	1.08±0.02b(b)	1.66±0.06a(a)

Values (means ± SD) with dissimilar lower-case letters inside and outside the parentheses are significantly different between tissues or salinities at $p<0.05$ by Duncan's multiple range test, respectively. Abbreviations: DW, dry weight

Discussion

We found that there were no significant changes in root cells of *P. maritima* at low salinity compared with those of the control (Fig. 1A, B, D). However, under high salinity, there were accumulated degradation products in root cells of *P. maritima*, in which organelle degradation was ongoing, and nuclear chromatin condensation was increased (Fig. 1C, E). In addition, high salinity caused more serious damage to root cells of *Fraxinus Americana*, such as cytoplasmic agglomerates, obvious plasmolysis, and organelle degradation (Yang *et al*., 2009) in comparison to those of *P. maritima* (Fig. 1C, E). These results suggested that *P. maritima* was more tolerant to salt stress than *F. Americana*. Meanwhile, this might be also accompanied by differences in ion distribution, water status, and biomass accumulation in the tissues of *P. maritima* (Tables 1, 2, 3).

An increase in the growth rate of *P. maritima* seedling responding to low salinity was observed (Table 1). This result is in agreement with that of Nguyen *et al.*, (2015) in the salt-tolerant mangrove, *Avicennia marina* under salinity conditions, indicating that low salinity had a stimulating effect on plant growth in halophytes. In contrast, growth reduction was found in non-tolerant pomegranate exposed to salinity (Khayyat *et al*., 2014). Those findings indicated that plants showed a diversity of growth responses to increasing salinity because of the discrepancies in salt tolerance of plant species, the salt level and exposure time. Similarly, leaf fresh and dry weight of *P. maritima* was stimulated by low salinity but unaffected by high salinity (Table 1). In addition, fresh and dry weight of roots increased at low salinity while decreased at high salinity compared with that of the control (Table 1). It is known that high NaCl - salinity may adversely affect photosynthesis, which is a prerequisite for biomass production (Abideen *et al*., 2014; Koyro *et al*., 2013), because Cl^- toxicity disrupts the normal electron flow to photosystem II, and thus causes the electron leakage, increases the generation of reactive oxygen species and induces oxidative stress (Fariduddin *et al*., 2013). Moreover, the biomass of stems was generally higher than that in roots or leaves (Table 1). The results are consistent with the findings from studies of *Pterocarpus officinalis* and *pistachio* (Dulormne *et al.*, 2010; Hajiboland *et al*., 2014), and *Elaeagnus oxycarpa* seedlings (Maimaiti *et al*., 2014). In general, the salt-tolerant species showed better growth performance at low salinity than that under high salinity.

Table 4. Effect of NaCl on the ratios of K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ in roots, stems and leaves of *P. maritima* seedlings after 120 days of treatment.

NaCl (mM)	Root	Stem	Leaf
		K^+/Na^+	
0	9.33±1.13a(c)	13.73±1.03a(a)	13.00±1.26a(b)
50	2.71±0.11b(c)	7.47±0.54b(b)	12.54±1.27a(a)
150	0.59±0.01c(c)	0.84±0.05c(b)	2.93±0.16b(a)
		Ca^{2+}/Na^+	
0	34.00±2.25a(b)	61.36±3.49a(a)	22.50±1.75a(c)
50	10.96±1.31b(c)	51.05±3.22b(a)	22.35±1.46a(b)
150	3.49±0.24c(c)	5.45±0.37c(a)	5.33±0.44b(b)
		Mg^{2+}/Na^+	
0	6.94±0.65a(b)	10.18±1.15a(a)	3.15±0.23a(c)
50	2.09±0.07b(c)	6.05±0.45b(a)	3.06±0.19b(b)
150	0.58±0.03c(b)	0.70±0.04c(a)	0.72±0.06c(a)

Values (means ± SD) with dissimilar lower-case letters inside and outside the parentheses are significantly different between tissues or salinities at $p<0.05$ by Duncan's multiple range test, respectively

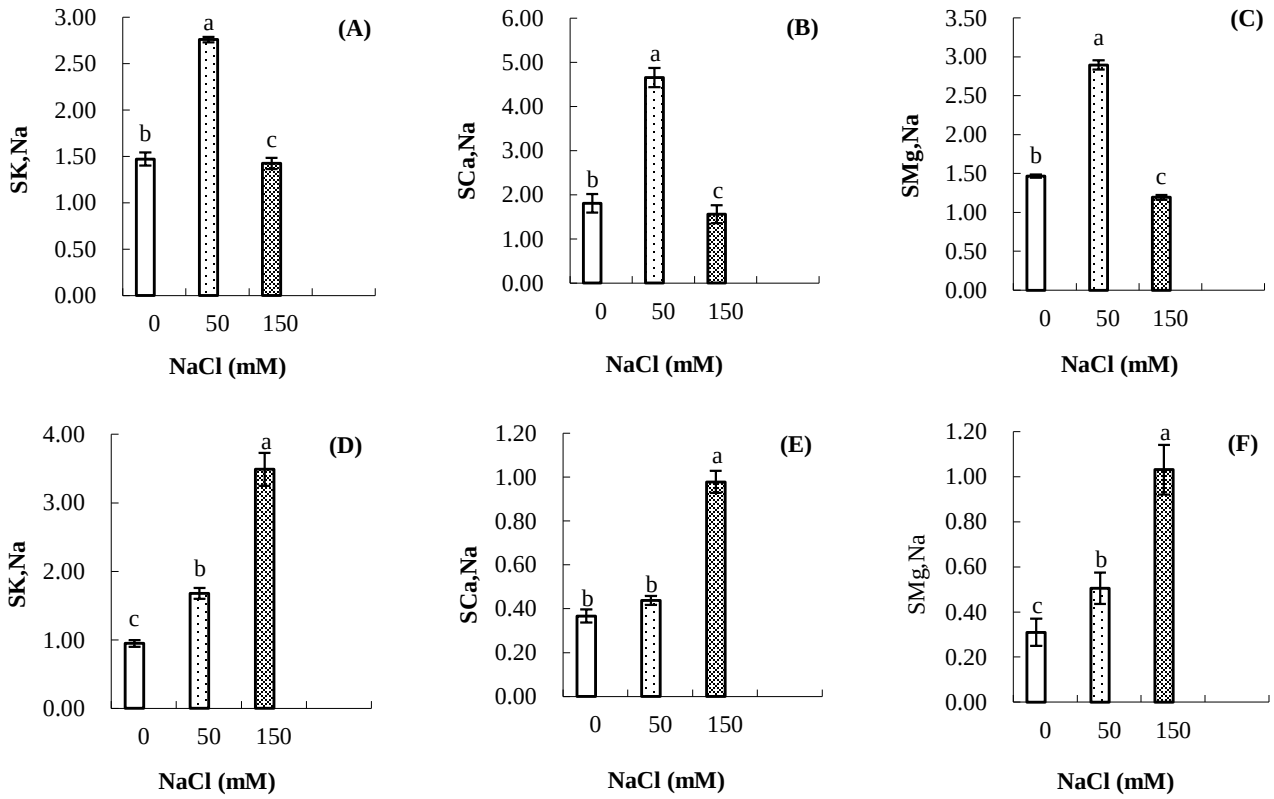

Fig. 2. Effect of NaCl on selective transport coefficients for (A) K^+/Na^+, (B) Ca^{2+}/Na^+, (C) Mg^{2+}/Na^+ from roots to stems, and (D) K^+/Na^+, (E) Ca^{2+}/Na^+, (F) Mg^{2+}/Na^+ from stems to leaves in *P. maritima* seedlings after 120 days of treatment. Values (means ± SD) with dissimilar lower-case letters are significantly different between salinities at $p<0.05$ by Duncan's multiple range test.

The water content in the root, shoot and leaf of *P. maritima* was generally increased with the increment of salinity (Table 2). Additionally, water content in leaves of *P. maritima* was higher than that in stems or roots (Table 2), suggesting that water transport from roots to leaves was not inhibited by salinity. Therefore, *P. maritima* tended to dilute an excess of Cl^- and Na^+ ions in their leaves and promote photosynthesis with the increase of water content, which might be an important trait that was required to overcome salinity - induced reduction in growth of *P. maritima* plants (Wise *et al.*, 2006). That contradicts the findings of Khayyat *et al.*, (2014), who reported a decrease in water content in leaves of 'Malas-e-Saveh' and 'Shishe-Kab' pomegranates under salt stress. These results also differ from those reported by Maimaiti *et al.*, (2014), in which water content in leaves under salinity remained unchanged in *Elaeagnus oxycarpa* seedlings. These differences in plant water status might be mainly related to the time of salt stress, the salt concentration, and plant salt tolerance.

The higher Cl^- and Na^+ contents in leaves of *P. maritima* were observed under salt treatment (Table 3). A similar conclusion was drawn by Storey *et al.*, (2003). This accumulation of Na^+ and Cl^- ions might be a double-edged sword. On the one hand, it could stimulate osmotic adjustment by lowering the osmotic potential at low salinity; on the other hand, the excessive accumulation of those toxic ions in leaf tissues has simultaneous detrimental effects on photosynthesis and plant growth despite osmotic adjustment in tissues at extreme salinity (Benzarti *et al.*, 2012). In particular, an increase in the accumulation of Na^+ can competitively prevent the uptake of the countercations (K^+, Ca^{2+}, and Mg^{2+}) through nonselective cation channels (Pottosin & Dobrovinskaya, 2014), thus resulting in an insufficient amount of these essential mineral ions necessary for plant growth and development (Desingh & Kanagaraj, 2007). However, our results showed that the increase in Na^+ content was not accompanied by a decrease in the contents of K^+, Ca^{2+} and Mg^{2+} in leaves of *P. maritima* (Table 3). Contrarily, leaf K^+, Ca^{2+} and Mg^{2+} contents were increased with increasing salinity (Table 3). The accumulation of these inorganic ions in plant tissues might be used as the osmotica to absorb water from saline soils by lowering their osmotic potential, which was a central feature of the salt tolerance of *P. maritima*. Interestingly, Kopittke (2012) reported that Ca^{2+} could not improve growth of salt-stressed plants in the absence of K^+, suggesting that Ca^{2+} did not directly reduce Na^+ toxicity, but rather enhanced the ability to retain K^+ in alleviating NaCl-induced salt stress on plant growth. Meanwhile, we found that *P. maritima* accumulated high amounts of Na^+ and Cl^- in their leaves, but both ions had no visible toxic effects on plant growth (Table 3). This could be possibly explained by the fact that Na^+ and Cl^- were compartmentalized in the vacuole of plant cells (Debez *et al.*, 2006). Conversely, two salt tolerant 'Malas-e-Saveh' and 'Shishe-Kab'pomegranates (Khayyat *et al*., 2014) as well as *Sumac* Species, *Rhus trilobata* (Liu *et al*., 2013), had relatively low leaf Na^+ and Cl^- contents due to a retention of both ions in shoots and roots of halophytes under salt stress, thereby preventing their translocation into the leaves to alleviate the salt - induced injury, which is typical of a halophytic species well adapted to salinity. Shabala & Munns

(2012) revealed that halophytes have developed different strategies to cope with salt stress, including Cl^- and Na^+ exclusion and/or the compartmentalization of both ions in leaf vacuoles. Overall, our findings suggested that the salt tolerance mechanism in *P. maritima* should belong to the latter case, but the preliminary conclusion was further verified at molecular levels.

The maintenance of high tissue K^+/Na^+, Ca^{2+}/Na^+, and Mg^{2+}/Na^+ ratios is also an important characteristic of salt-stress adaptation (Wei *et al*., 2003). However, these ratios in roots, shoots and leaves of *P. maritima* significantly ($p<0.05$) decreased with increasing salinity (Table 4)**.** Hence, the massive accumulation of Na^+ and Cl^- ions in plant tissues may cause ion imbalance. Additionally, the selective transport coefficients for K^+/Na^+, Ca^{2+}/Na^+, Mg^{2+}/Na^+ from roots to stems in *P. maritima* seedlings was increased with increasing salinity and then decreased at high salinity (up to 250 mM), whereas the selective transport coefficients for these ratios from stems to leaves was increased with increasing salinity (Fig. 2A-F). Thus high salinity led to the restriction on the selective transport of K^+, Ca^{2+} and Mg^{2+} from roots to stems but promotion on their selectivity from stems to leaves. As a consequence, the contents of K^+, Ca^{2+} and Mg^{2+} in stems would be reduced and cause growth inhibition of *P. maritima* with a much longer period of high salinity.

In conclusion, our results showed *P. maritima* could generally protect themselves from salt-induced osmotic and ionic stresses by maintaining higher selective transportation of K^+, Mg^{2+}, especially the enrichment of Ca^{2+} in leaves at the seedling stage as a distinctive trait of salt tolerance species. In addition, the growth responses of *P. maritima* to salinity were generally much later than changes in their root cell ultrastructure. This time lag may be an important factor in explaining the discrepancy between ultrastructural changes in root cells, ion distribution and grow rate in response to salinity. Meanwhile, the results supported the view that the root ultrastructure could be used as an early indication of salt injury to *P. maritima* seedlings.

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