Original article

Effects of NaCl stress on growth and ion homeostasis in pomegranate tissues

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Summary

Pomegranate is a 'super fruit' that is always adaptable to a saline environment. To elucidate the mechanism underlying the response to salinity, we investigated the effects of NaCl stress on growth and ion balance in six-month-old self-rooted pomegranate cuttings. The plants were fertigated with half-strength Hoagland's solution containing 0 (control), 100, 200, or 300 mM NaCl every six days. After 18 days, the growth parameter was determined, and dry samples of root, stem and leaf were analyzed for ion content. We found that the growth of pomegranate was not inhibited under 100 mM NaCl stress. With the increased salinity level, excessive accumulations of Na⁺ and Cl⁻ occurred in the pomegranate tissues and the net accumulations were ranked as leaf > root > stem. The uptake of K⁺, Ca²⁺ and Mg²⁺ was enhanced at low salinity (100 mM) and inhibited at high salinity (300 mM). With increasing salinity, the K⁺ contents in the roots and leaves initially increased and then decreased, with peaks at 200 mM NaCl. The Ca²⁺ and Mg²⁺ contents increased in the leaves and decreased in the roots. The absorptions of NO₃⁻ and SO₄²⁻ were inhibited by salt stress, but the H₂PO₄⁻ uptake had no obvious change. The ion ratio and transport coefficient of pomegranate tissues were significantly lower under stress than in controls, but the K⁺/Na⁺ and Ca²⁺/Na⁺ ratios of the roots and stems were higher than 1 at 100 mM. This study revealed that salinity affected the ion balance in pomegranate tissues through impeding the ion uptake, accumulation and transport. Furthermore, this pomegranate cultivar 'Taishanhong' can adapt to 100 mM NaCl stress by maintaining a stable or higher uptake of K⁺, Ca²⁺ and Mg²⁺ to mitigate the detrimental effects of Na⁺ and Cl⁻ in the cells.

Keywords

ion accumulation, *Punica granatum*, salt stress, uptake and transport

Significance of this study

What is already known on this subject?

- Many studies have found that some inorganic ions in plants play an important role in resisting salt stress. Very poor data are available in the literature on the uptake, transport and distribution of K⁺, Ca²⁺, Na⁺, Mg²⁺, NO₃⁻, and SO₄²⁻ in pomegranate tissues.

What are the new findings?

- Under 100 mM NaCl stress, the pomegranate growth was enhanced, and the uptake of K⁺, Ca²⁺ and Mg²⁺ was also enhanced to mitigate the detrimental effects of Na⁺ and Cl⁻ in the cells.

What is the expected impact on horticulture?

- High salinity affected the ion balance in pomegranate and 'Taishanhong' cultivar adapted to low saline lands.

4.88% of the available land area (Yang, 2008). Plants exposed to saline conditions suffer from osmotic stress, ion toxicity, nutrient deficiency, and the combined effects of these factors (Munns and Tester, 2008; Pandey et al., 2015). In terms of all major metabolism processes involved in plant growth and development, such as photosynthesis, protein synthesis, substance and energy metabolism and ion balance, are affected under salt stress (Munns and Tester, 2008; Zhang et al., 2013). An ion imbalance may result from salinity, which was due to the ion availability, selective uptake, and transport or partitioning within a plant. Currently, an increasing number of researchers are studying the mechanisms of ion balance in plants in saline environments.

Pomegranate (*Punica granatum* L.) is an important commercial fruit tree that belongs to the Lythraceae family (Yuan et al., 2018). Pomegranate has high nutritional, economic and ecological values, and is referred to as a 'super fruit' (Silva et al., 2013). Pomegranate is widely grown in arid and semi-arid soils (Bhantana and Lazarovitch, 2010). In China, cultivated land planted with pomegranate is threatened by salinization. Our studies have found that pomegranate can alleviate the detrimental effects of salt stress by increasing osmoprotectant contents and the activities of antioxidant enzymes (Liu et al., 2018). In addition, some inorganic ions in plants play an important role in resisting salt stress, such as K⁺, Ca²⁺, Na⁺, Mg²⁺, Cl⁻, SO₄²⁻, and NO₃⁻ (Silva et al., 2015). Plant salt tolerance was associated with the ability to extrude, transport and compartmentalize toxic ions (Apse and Blumwald, 2007; Munns et al., 2016). Researchers have reported

Introduction

Almost 20% of the global cultivated land and half of the irrigated areas are affected by salinity (Zhu, 2001). The aggravation of soil salinization has become a considerable threat to the healthy and sustainable development of agriculture worldwide (Li et al., 2012). In China, saline soils, which are distributed mainly in the north and coastal areas, cover an area of approximately 3.6×10^7 hectares and account for

that some pomegranate cultivars exhibit higher tolerance to salinity than others, and this tolerance was closely related to the selective uptake of ions and the ability to maintain vegetative growth (Karimi and Hasanpour, 2014; Okhovatianardakani et al., 2010). Under salt stress, the amount of Na⁺ and Cl⁻ in pomegranate tissues increased, but the amount of Ca²⁺, Mg²⁺ and K⁺ varied among different cultivars (Hasanpour et al., 2015; Mastrogiannidou et al., 2016; Naeini et al., 2005). The K⁺/Na⁺, Ca²⁺/Na⁺, and NO₃⁻/Cl⁻ ratios are crucial for tolerance or adaptation to salt stress (Apse and Blumwald, 2007; Khayyat et al., 2014). Changes in these ratios may provide evidence of disruption to the ionic equilibrium induced by salinity (Apse and Blumwald, 2007). We wondered what was the underlying relationship between the saline environment and ion balance in pomegranate tissues, therefore, we chose an excellent Chinese cultivar, 'Taishanhong', to study the effects of salt stress on the absorption, transport and distribution of ions. The study aimed to provide a reference for salt-tolerance studies and cultivation of pomegranate.

Materials and methods

Plant materials and stress treatments

Pomegranate ('Taishanhong') plants (one-year-old branches were collected from the Pomegranate Repository of Nanjing Forestry University, China) were rooted and transplanted on October 10, 2016, into plastic pots (32 cm high × 20 cm diameter) containing a medium comprising 1:1 perlite-turfy soil. The plants grown in a phytotron at Nanjing Forestry University (119°12'E, 31°36'N) with a 28/22°C day/night temperature, 70% humidity and 14-h light/10-h dark photoperiod. Hoagland's nutrient solution (Feng et al., 2014) was supplied (one liter per pot on a weekly basis), and water was supplied every three days.

The experimental treatments were arranged in a completely randomized block design. On April 1, 2017, six-month-old cuttings in vegetative growth from a total of 60 pots (one plant per plot) were selected and randomly divided into 5 blocks (12 pots per block), and each 4 pots was designed as a biological replicate (total 3 replicates), since the sample of one plant was not enough for analysis. At the beginning of the treatment, one block was harvested for determining the dry weight and ion content of the whole plant, defined as W1 and M1, respectively. Four blocks of plants were fertigated with half-strength Hoagland's solution containing 0 (control), 100, 200, or 300 mM NaCl every six days, respectively. Drainage was not allowed, and the substrate was washed every 6 days with plenty of distilled water before the treatments to avoid salinity accumulating. After 18 days of salt stress, we determined the growth parameters and then harvested all plants, which were thoroughly rinsed three times using distilled water. The plants were divided into roots, stems, and leaves to determine the growth and ion content (Table 1).

Dry weight and root-shoot ratio

The dry weight of the root, stem and leaf samples of each treatment was determined by drying in a drying and heating oven at 75°C for 48 h until a stable weight was reached. The root-shoot ratio was calculated as the dry weight of the root divided by the summed weights of the stems and leaves.

Ion contents

The samples were finely milled to a uniform size to pass through a 40-mesh sieve. Dry samples (0.4 g) were added to 10 mL HNO₃-HClO₄ (4:1) in a glass Kjeldahl flask and then placed in darkness for one night at 25°C. Using a microwave cooking apparatus (ETHOST-T640, Milestone, Italy), the samples were dissolved for 40 min, after which the acid was volatilized for approximately 1 h at 60°C, followed by dilution to 100 mL with deionized water (Jin et al., 2015). The K⁺, Na⁺, Ca²⁺ and Mg²⁺ contents were determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AES, Optima 7000, PE, Salem, MA, USA). Additional dry samples (0.4 g) were treated with 50 mL deionized water for 1 h in an ultrasonic extractor at room temperature, and the extracts were used to determine the contents of NO₃⁻, Cl⁻, H₂PO₄⁻, and SO₄²⁻ using ion chromatography (ICS900 ion chromatographic system; AS4A-SC ion-exchange column, CD M-II electrical conductivity detector, mobile phase: Na₂CO₃/NaHCO₃ = 1.7/1.8 mM; Dionex, Sunnyvale, USA) (Rui et al., 2010).

Ion uptake rate

To determine the ion uptake rate of K⁺, Ca²⁺, Mg²⁺, Na⁺, NO₃⁻, Cl⁻, H₂PO₄⁻, and SO₄²⁻ in pomegranate by the method of Silva (Silva et al., 2015), the absorption rate was calculated as follows:

$$J_s = \frac{(M2 - M1)}{(T2 - T1)} \times \frac{\ln(W2 - W1)}{(W2 - W1)}$$

J_s is defined as the rate of ion accumulation in the whole plant per unit of pomegranate root dry weight. M2-M1 are the ion accumulations in the whole plant from the beginning to the end of treatments; W2-W1 are the dry weight of roots from the beginning to the end of treatments, respectively. T2-T1 is the experimental period (18 days). The results are expressed as mg in the whole plant per g of root dry weight per day (mg g⁻¹ day⁻¹ DW).

The capacity of ion transport

The transportation coefficient (S_{X,Na} and S_{Y,Cl}) represented the capacity for transporting mineral ions from the roots to the leaves, which was compared with toxic Na⁺ and Cl⁻, respectively. The values were calculated with the following formulas:

$$S_{X,Na} = \frac{\text{Leaf } (X/Na)}{\text{Root } (X/Na)}; S_{Y,Cl} = \frac{\text{Leaf } (Y/Cl)}{\text{Root } (Y/Cl)}$$

TABLE 1. The dry weight and ion content of pomegranate tissues at the beginning of the experiment.

Weight (W1, g)	Cations	Content (M1, mg g ⁻¹)	Anions	Content (M1, mg g ⁻¹)
1.01 ± 0.09 (Root)	K ⁺	10.23 ± 0.76	Cl ⁻	9.31 ± 0.32
3.97 ± 0.12 (Stem)	Ca ²⁺	11.04 ± 0.47	NO ₃ ⁻	0.43 ± 0.01
3.56 ± 0.13 (Leaf)	Na ⁺	1.99 ± 0.08	H ₂ PO ₄ ⁻	1.02 ± 0.07
8.54 ± 0.29 (Total)	Mg ²⁺	2.32 ± 0.11	SO ₄ ²⁻	2.25 ± 0.25

Values are the means ± SE of three replicates (4 plants per replicate).

X represents K^+ , Ca^{2+} or Mg^{2+} , and Y represents NO_3^- , $H_2PO_4^-$ or SO_4^{2-} . X/Na and Y/Cl are the ratios of cations and anions in the roots and leaves.

Data analysis

The results were obtained from three independently repeated experiments (4 plants per replicate), and they were presented as the means of three repetitions for each treatment. All data were analyzed using one-way ANOVA (analysis of variance) at $P < 0.05$, and Duncan's multiple comparison ($P < 0.05$) test by the SPSS program (Version 19.0, Chicago, IL, USA).

Results

The growth of pomegranate cuttings

With increasing salinity, the dry weights of pomegranate roots and leaves showed a trend of an initial increase and

then decrease (Table 2). The dry weight was slightly higher at 100 mM than in controls, but there are no significant differences between each other ($P < 0.05$). At the 200 mM salinity level, the clear decrease in dry weight suggested that NaCl stress inhibited the growth of the pomegranate cuttings. The root cap ratio increased with elevated salinity (Table 2, $P < 0.05$), which was mainly due to the decrease in the dry weight of the aerial parts of the plants.

Cation accumulations in pomegranate tissues

Different accumulations of K^+ , Na^+ , Ca^{2+} and Mg^{2+} in the pomegranate roots, stems and leaves were observed (Figure 1). The Na^+ contents in the whole plant increased significantly with the elevated salinity ($P < 0.05$), and at 300 mM NaCl, the accumulations in the roots, stems and leaves were 13.3, 10.3 and 46.1 times higher than those in the control, respectively (Figure 1A). The Na^+ net accumulation (Na^+ content in treatments – Na^+ content in controls) in pomegranate tissues

TABLE 2. The dry weight and root cap ratio of pomegranate tissues under NaCl stress.

NaCl concentration (mM)	Root weight (DW/g)	Stem weight (DW/g)	Leaf weight (DW/g)	Total weight (DW/g)	Root shoot ratio (R/C)
0	2.30 ± 0.03 a	4.09 ± 0.02 a	5.09 ± 0.02 b	11.48 ± 0.26 a	0.25 ± 0.02 a
100	2.39 ± 0.02 a	4.09 ± 0.19 a	5.24 ± 0.12 a	11.72 ± 0.10 a	0.26 ± 0.01 a
200	2.20 ± 0.03 b	4.07 ± 0.08 a	3.66 ± 0.10 c	9.93 ± 0.36 b	0.28 ± 0.05 a
300	2.16 ± 0.09 b	4.08 ± 0.06 a	2.09 ± 0.03 d	8.33 ± 0.06 c	0.35 ± 0.05 b

Values are the means ± SE of three replicates (4 plants per replicate). Different letters indicate significant differences at $P < 0.05$.

FIGURE 1. The contents of Na^+ (A), K^+ (B), Ca^{2+} (C), and Mg^{2+} (D) in pomegranate tissues under NaCl stress. Values are the means ± SE of three replicates (4 plants per replicate). Bars with different letters within each panel are significantly different at $P < 0.05$.

FIGURE 2. The contents of Cl^- (A), H_2PO_4^- (B), NO_3^- (C), and SO_4^{2-} (D) in pomegranate organs under NaCl stress. Values are the means $\pm$ SE of three replicates (4 plants per replicate). Bars with different letters within each panel are significantly different at $P < 0.05$.

TABLE 3. Effects of different concentrations of NaCl on the ion ratio in pomegranate roots, stems, and leaves.

Tissues	NaCl concentration (mM)	Cation ratios			Anion ratios		
		K^+/Na^+	$\text{Ca}^{2+}/\text{Na}^+$	$\text{Mg}^{2+}/\text{Na}^+$	$\text{H}_2\text{PO}_4^-/\text{Cl}^-$	$\text{NO}_3^-/\text{Cl}^-$	$\text{SO}_4^{2-}/\text{Cl}^-$
Root	0	$10.72 \pm 0.29\text{a}$	$12.72 \pm 0.62\text{a}$	$4.72 \pm 0.11\text{a}$	$0.11 \pm 0.00\text{a}$	$0.39 \pm 0.01\text{a}$	$0.84 \pm 0.03\text{a}$
	100	$1.31 \pm 0.01\text{b}$	$1.02 \pm 0.03\text{b}$	$0.37 \pm 0.01\text{b}$	$0.06 \pm 0.00\text{c}$	$0.14 \pm 0.00\text{b}$	$0.26 \pm 0.03\text{b}$
	200	$0.99 \pm 0.01\text{c}$	$0.64 \pm 0.01\text{b}$	$0.21 \pm 0.01\text{c}$	$0.05 \pm 0.01\text{c}$	$0.08 \pm 0.00\text{c}$	$0.16 \pm 0.01\text{b}$
	300	$0.69 \pm 0.01\text{c}$	$0.52 \pm 0.01\text{b}$	$0.15 \pm 0.01\text{c}$	$0.07 \pm 0.01\text{b}$	$0.05 \pm 0.01\text{d}$	$0.15 \pm 0.00\text{c}$
Stem	0	$5.78 \pm 0.06\text{a}$	$6.42 \pm 0.04\text{a}$	$0.43 \pm 0.01\text{a}$	$0.53 \pm 0.05\text{a}$	$0.19 \pm 0.02\text{a}$	$0.63 \pm 0.06\text{a}$
	100	$1.35 \pm 0.01\text{b}$	$1.52 \pm 0.02\text{b}$	$0.14 \pm 0.01\text{b}$	$0.11 \pm 0.01\text{b}$	$0.03 \pm 0.00\text{b}$	$0.15 \pm 0.01\text{b}$
	200	$1.10 \pm 0.01\text{c}$	$1.40 \pm 0.01\text{c}$	$0.12 \pm 0.01\text{c}$	$0.15 \pm 0.01\text{b}$	$0.02 \pm 0.00\text{b}$	$0.10 \pm 0.01\text{bc}$
	300	$0.63 \pm 0.03\text{d}$	$0.70 \pm 0.02\text{d}$	$0.06 \pm 0.00\text{d}$	$0.08 \pm 0.00\text{c}$	$0.02 \pm 0.00\text{b}$	$0.05 \pm 0.01\text{c}$
Leaf	0	$25.74 \pm 1.14\text{a}$	$15.86 \pm 0.78\text{a}$	$3.11 \pm 0.19\text{a}$	$0.17 \pm 0.02\text{a}$	$0.04 \pm 0.00\text{a}$	$0.14 \pm 0.01\text{a}$
	100	$1.37 \pm 0.02\text{b}$	$0.80 \pm 0.01\text{b}$	$0.14 \pm 0.00\text{b}$	$0.03 \pm 0.00\text{b}$	$0.0019 \pm 0.00\text{b}$	$0.02 \pm 0.00\text{b}$
	200	$1.02 \pm 0.02\text{b}$	$0.51 \pm 0.01\text{b}$	$0.09 \pm 0.00\text{b}$	$0.03 \pm 0.00\text{b}$	$0.0022 \pm 0.00\text{b}$	$0.01 \pm 0.00\text{b}$
	300	$0.76 \pm 0.02\text{b}$	$0.47 \pm 0.01\text{b}$	$0.07 \pm 0.00\text{b}$	$0.02 \pm 0.00\text{b}$	$0.0020 \pm 0.00\text{b}$	$0.02 \pm 0.00\text{b}$

Values are the means $\pm$ SE of three replicates (4 plants per replicate). Different letters indicate significant differences at $P < 0.05$.

were ranked as leaf > root > stem. With increasing salinity, the K^+ contents in the roots and leaves increased initially and then decreased, with the peaks at 200 mM NaCl, but we observed no significant differences in the stems among treatments and controls ($P < 0.05$). The K^+ concentrations in the tissues were ranked as leaf > root > stem (Figure 1B). With increasing salinity, the Ca^{2+} and Mg^{2+} contents in the leaves increased initially and then decreased; the maximum values

were observed at 200 mM NaCl. Compared with the contents in the controls, the contents of Ca^{2+} and Mg^{2+} were slightly higher in the stems and lower in the roots ($P < 0.05$). Generally, Ca^{2+} has no difference among the tissues, but Mg^{2+} was mainly concentrated in the roots and leaves (Figure 1C, D). In conclusion, pomegranate maintained high or stable contents of K^+ , Ca^{2+} , and Mg^{2+} in the leaves and stems at a moderate salinity level (200 mM NaCl, $P < 0.05$).

Anion accumulations in pomegranate tissues

Under high NaCl stress (300 mM), the Cl^- concentrations in the pomegranate tissues increased considerably, reaching values were 2.8 times higher in the roots, 9.2 times higher in the stems, and 6.8 times higher in the leaves than that in the controls (Figure 2A, $P < 0.05$). The Cl^- contents were ranked as leaf > root > stem (except at 300 mM). The contents of H_2PO_4^- in the root and stem increased with increasing salinity, but no changes were observed in the leaf compared with the control ($P < 0.05$). Moreover, H_2PO_4^- was mainly accumulated in stems (Figure 2B). By contrast, the NO_3^- contents fell sharply in the pomegranate tissues under NaCl stress ($P < 0.05$) and they decreased by approximately 28% to 63% in roots when subjected to salinity (Figure 2C). In addition, the SO_4^{2-} concentrations in pomegranate also decreased sharply with increasing salinity (Figure 2D, $P < 0.05$). The NO_3^- and SO_4^{2-} contents were ranked as root > stem > leaf.

The change in the ion ratio in pomegranate tissues

The K^+/Na^+ , $\text{Ca}^{2+}/\text{Na}^+$, and $\text{Mg}^{2+}/\text{Na}^+$ ratios in pomegranate roots, stems and leaves significantly reduced under NaCl stress ($P < 0.05$). However, the K^+/Na^+ ratios were almost higher than 1 at the 200 mM salinity level. In addition, the $\text{Ca}^{2+}/\text{Na}^+$ ratios of the roots and stems were ≥ 1 at 100 mM NaCl (Table 3). The results indicated that the potential disturbance of ion balance was mitigated by the maintenance of the K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios at values > 1 in the pomegranate tissues at low salinity (100 mM). The $\text{H}_2\text{PO}_4^-/\text{Cl}^-$, $\text{NO}_3^-/\text{Cl}^-$, and $\text{SO}_4^{2-}/\text{Cl}^-$ ratios of the pomegranate tissues were clearly reduced in all NaCl treatments compared with those in the control (Table 3, $P < 0.05$). The K^+/Na^+ , $\text{Ca}^{2+}/\text{Na}^+$, $\text{Mg}^{2+}/\text{Na}^+$, $\text{H}_2\text{PO}_4^-/\text{Cl}^-$, $\text{NO}_3^-/\text{Cl}^-$, and $\text{SO}_4^{2-}/\text{Cl}^-$ ratios decreased sharply,

suggesting that the ionic equilibrium was disturbed by salt stress.

Ion uptake and transport in pomegranate tissues

Under salt stress, the Js of Na^+ rapidly increased, and the uptake of K^+ , Ca^{2+} and Mg^{2+} by the roots was enhanced at a low salinity level (100 mM) and inhibited at a high salinity level (300 mM). The capacity to transport K^+ , Ca^{2+} , and Mg^{2+} from the roots to the leaves ($S_{K,Na}$, $S_{Ca,Na}$, and $S_{Mg,Na}$) decreased under NaCl stress (Figure 3C). Notably, the $S_{K,Na}$, $S_{Ca,Na}$, and $S_{Mg,Na}$ increased slightly with the decrease in the Js of K^+ , Ca^{2+} and Mg^{2+} at the high salinity level when compared with those in the low salinity (100 mM NaCl). These findings suggested that pomegranate may maintain a stable or greater ability to transport K^+ , Ca^{2+} and Mg^{2+} into the leaves to alleviate the nutrient deficiency.

The uptake of Cl^- increased sharply; thus, Cl^- gradually accumulated in the leaves, leading to serious toxicity. The Js of NO_3^- and SO_4^{2-} decreased significantly compared with those in the controls (Figure 3B, $P < 0.05$). However, the Js of H_2PO_4^- did not show an obvious change under salt stress. The capacity of pomegranate to transport H_2PO_4^- , NO_3^- , and SO_4^{2-} from the roots to the leaves was significantly affected by NaCl stress ($P < 0.05$). The $S_{N,Cl}$ and $S_{S,Cl}$ values decreased initially and then increased with increasing salinity. In addition, $S_{P,Cl}$ decreased with increasing salinity (Figure 3D), which was mainly due to greater accumulation in the stems (Figure 2B). In summary, the decreased $S_{P,Cl}$, $S_{N,Cl}$, and $S_{S,Cl}$ values under NaCl stress indicated that the imbalance of nitrogen (N), phosphate (P) and sulfur (S) in the leaves was aggravated. Therefore, the normal metabolism in pomegranate leaves would be affected seriously by salinity.

FIGURE 3. The effects of NaCl stress on ion uptake rate and ion selective transport capacity in pomegranate. K, Ca, Na, Mg and N, P, S, Cl are the abbreviations of K^+ , Ca^{2+} , Na^+ , Mg^{2+} , and NO_3^- , H_2PO_4^- , SO_4^{2-} , Cl^- , respectively. Values are the means $\pm$ SE of three replicates (4 plants per replicate). Bars with different letters within each panel are significantly different at $P < 0.05$.

Discussion

Effects of NaCl stress on the growth of pomegranate cutting

The growth parameters are the major indicators to assess the plant's salt-resistance when plants are subjected to a saline environment. The effects of salinity appear to be dependent on the species and cultivars and on the stage of the plant development. Previous studies have suggested that many plants exposed to hypersaline conditions were inhibited to grow. Meanwhile, some plants were accelerated to grow under hyposaline conditions, which are most likely agreed that the sodium is a 'functional nutrient' in some plants and is beneficial for growing (Lu et al., 2014; Subbarao et al., 2003). We discovered that pomegranate grew normally under 100 mM NaCl stress (Table 1), which indicated that there was no significant inhibition at low salinity levels. Many studies have reported that pomegranate could be classified as a salinity tolerant plant (Tavousi et al., 2014, 2015). The plant's salt-tolerance were different among the cultivars (Sun et al., 2018), and some pomegranate cultivars could adapt to the low salinity stress (Karimi and Hasanpour, 2014; Okhovatianardakani et al., 2010), such as the 'Malas Shirin' cultivar (Naeini et al., 2006). Our findings are consistent with these conclusions. On the other hand, more comprehensive and further researches are needed to assess the pomegranate salt tolerance, since we only determined the dry weights of tissues.

Effects of NaCl stress on the ion balance of pomegranate tissues

Plants in a saline environment can regulate and alleviate osmotic stress through osmoprotectants, such as soluble protein, proline, sugar, and lysine. In addition, many inorganic ions are critical for plants to resist abiotic stress, such as K^+ , Ca^{2+} , Na^+ , Mg^{2+} , Cl^- , SO_4^{2-} , and NO_3^- (Silva et al., 2015). Salt stress caused by a high NaCl concentration mainly results in a perturbation of the Na^+ and Cl^- balance at both the cellular and whole plant level (Teakle and Tyerman, 2010). Moreover, salinity might affect the uptake and transport of other cations (such as K^+ , Ca^{2+} , and Mg^{2+}) and anions (such as $H_2PO_4^-$, NO_3^- , and SO_4^{2-}) (Ibrahim, 2016; Silva et al., 2015); hence, nutrient deficiency and growth inhibition have been observed in plants subjected to salt stress.

Excess Na^+ and Cl^- are the primary factors that cause adverse effects in plants under NaCl stress. Na^+ and Cl^- mainly affect important metabolic activities such as photosynthesis in the leaves and nutrient absorption in the roots (Teakle and Tyerman, 2010; White and Broadley, 2001). Plants respond to excess Na^+ and Cl^- with three strategies in the roots: (a) osmotic adjustment, (b) Na^+ and Cl^- exclusion from the stems and leaves, and (c) tissue tolerance to Na^+ and Cl^- accumulation (Apse and Blumwald, 2007; Chen and Polle, 2010; Munns and Tester, 2008). In halophytes, salt tolerance is mainly based on the inclusion rather than on the exclusion of salts. Thus, although some glycophytes such as *Glycine max* (Zhang et al., 2011), *Canarium album* (Chartzoulakis, 2005), *Citrus reticulata* (García-Sánchez et al., 2002) and *Solanum lycopersicum* (Estañ et al., 2005) limit ions to move into the shoots, halophytes readily transport and accumulate ions in the aerial parts (Flowers and Colmer, 2008; Orsini et al., 2011). Our study found that the contents of Na^+ and Cl^- in the pomegranate cv. 'Taishanhong' were ranked as leaf > root > stem (Figures 1B, 2A), which is probably due to the relatively strong ability to transport and accumulate toxic ions in the aerial parts (Munns, 2002). The results verify that 'Taishan-

hong' is a salt-tolerant cultivar. In addition, the Cl^- accumulation in the leaves was more than two-fold that of Na^+ (Figures 1B, 2A). Cl^- appeared to be more toxic than Na^+ for citrus (García-Sánchez et al., 2002; Moya et al., 2003), soybean (Luo et al., 2002; Zhang et al., 2011), and cashew (Silveira et al., 2012). However, whether pomegranate is more sensitive to Cl^- than Na^+ requires further study.

K^+ plays an important role in protein synthesis, photosynthesis, the maintenance of cellular osmotic potential, and the activation of enzymatic reactions (Wang et al., 2013). The ability of plants to maintain a high K^+/Na^+ ratio is confirmed as one of the key determinants of plant salt resistance (Apse and Blumwald, 2007; Munns and Tester, 2008). Reports show that the concentration of K^+ in plant cells required to maintain metabolic processes is approximately 100–150 mM, and when Na^+ concentration is higher than 100 mM, metabolic processes are restricted (Blumwald et al., 2000). Therefore, a K^+/Na^+ ratio of approximately 1 is the minimum value for normal metabolism. With the elevated salinity, the K^+ contents in the pomegranate tissues, especially the leaves, increased clearly (Figure 1A). On the other hand, although the K^+/Na^+ ratios in pomegranate decreased significantly, the values were higher than 1 at 100–200 mM NaCl (Table 2). These responses to salt stress are mainly related to the high ability of K^+ absorption to maintain a suitable K^+/Na^+ ratio in pomegranate tissues (Figure 3A, C) (Flowers et al., 2015; Hamdani et al., 2017; Silva et al., 2015). High salt stress (300 mM) restricted the uptake of K^+ and affected the balance of K^+ and Na^+ in the pomegranate tissues (Figure 1A). One reason is Na^+ competition at the transport sites for K^+ entry into cells (Apse and Blumwald, 2007). Another reason is a high Na^+ concentration in the cytoplasm results in competition at K^+ binding sites for metabolic processes that depend on K^+ (Munns and Tester, 2008). In a word, these results for pomegranate are contrary to the generalized expectation that salinity reduces K^+ in plants (Greenway and Munns, 1980), but are associated with some plants such as durum wheat (*Triticum turgidum*) (Dvořák et al., 1994) and chickpea (*Cicer arietinum*) (Kotula et al., 2015). These plants might also have enhanced K^+ concentrations in tissues because of the induction of a high affinity K^+ uptake at a high, but not immediately salinity, or due to the K^+ remobilization from senescent leaves and/or more content in unit biomass at some levels of salinity.

Ca^{2+} , one of the main components of the cell wall, participates in the protection of membrane integrity and the resistance of adverse effects of abiotic stress on the cell membrane (Sotiropoulos, 2007). A number of studies have attested that intracellular Ca^{2+} acts as a signal transduction messenger in plants and participates in transmitting the salt stress signal together with ABA (abscisic acid) and ROS (reactive oxygen species) (Martí and Webb, 2013; Xu et al., 2013). Mg^{2+} serves as component of chlorophyll, an activator of many enzymes involved in photosynthesis and respiration, and a catalyst of DNA and RNA polymerase (Sgrignani and Magistrato, 2012). Our study found that the concentrations of these elements increased in leaves and stems (Figure 1C, D), and the Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios decreased considerably under salt stress. These results indicated that NaCl stress upsets the balance between Na^+ , Ca^{2+} , and Mg^{2+} in the pomegranate tissues, and both Ca^{2+} and Mg^{2+} might be replaced by Na^+ in the roots and then transported to the stem and leaves during transpiration. These findings are similar to those observed for *Quercus virginiana* (Wang et al., 2010) and *Taxodium* cv. 'Zhongshanshan' (Yu et al., 2016). The uptake (Js) of Ca^{2+} and

Mg²⁺ by roots were enhanced at a low salinity (100 mM NaCl) and significantly inhibited at the high salinity (300 mM NaCl) (Figure 3A), indicating that pomegranate maintained a stable capacity to absorb Ca²⁺ and Mg²⁺ and transport them to the leaves to avoid severe ion disequilibrium caused by NaCl stress. On the other hand, Ca²⁺ and Mg²⁺ uptakes were decreased with increasing the K⁺ concentration in the tissues could be due to these ions are sufficiently similar to compete for absorption and transport sites on the plant root surfaces (Reis Jr. et al., 1999).

The macroelements N, P, and S are mainly absorbed by plants in the form of NO₃⁻, H₂PO₄⁻ and SO₄²⁻, respectively, in a saline environment (Guo et al., 2009). Plant roots take up Cl⁻, NO₃⁻ and SO₄²⁻ from the soil via specific anion channels under saline conditions (White and Broadley, 2001). The increases of the Cl⁻ content in pomegranate tissues were concomitant with decreases of the NO₃⁻ and SO₄²⁻ concentrations (Figure 3B, D), which could be explained by reductions in the uptake and transport of NO₃⁻ and SO₄²⁻ when high Cl⁻ concentration was applied to the root medium. An antagonism between Cl⁻ and NO₃⁻ has also been previously observed in other plants (Abdelgadir et al., 2005). The H₂PO₄⁻ contents increased in the pomegranate roots and stems (Figure 1D), suggesting that salt stress has no adverse effect on phosphate element. In plants, it has been observed that a low ratio of NO₃⁻/Cl⁻ may cause a reduction in growth (Grattan and Grieve, 1998). The NO₃⁻/Cl⁻, SO₄²⁻/Cl⁻ and H₂PO₄⁻/Cl⁻ ratios can also be used as indicators of plant salt resistance (Teakle and Tyerman, 2010). The decreases in these three ratios suggested that NaCl stress altered ion homeostasis, and under a saline condition pomegranate plants may suffer from nutrient deficiency and inhibited growth (Apse and Blumwald, 2007). Our study indicated that the imbalance of N, P, and S in the leaves was aggravated. Therefore, growth and normal metabolism in pomegranate leaves were seriously affected by salt stress.

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