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EFFCTS OF SALT STRESS ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF POMEGRANATE (*PUNICA GRANATUM* L.) CUTTINGS

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Abstract

With 2-years-old pomegranate (*Punica granatum* L.) cv. 'Tunisia' cuttings as materials, the growth properties, salt injury indexes, leaf membrane permeability, Chlorophyll (Chl) content, malondialdehyde (MDA) content, proline and soluble protein content, and activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were investigated under different NaCl concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6%, w/w) treatments after 7, 21 and 35 days. The results showed that: the height and ground diameter of pomegranate were increased at low salinity (0.1%), and they were significantly inhibited at high salinity (≥ 0.5%). With increased NaCl concentrations, Chl a, Chl b, and Chl (a + b) contents were decreased and chlorophyll a/b were increased. Leaf membrane permeability was seriously enhanced and the amount of MDA was markedly increased at high salinity (≥ 0.5%). The proline and soluble protein were significantly accumulated and quickly responded to NaCl stress. The activities of SOD, CAT, and POD showed a trend of first rising and then decreasing, with the maximum appearing at 0.4% salinity. In addition, the adverse effects on these physiological indexes aggravated gradually over time. Our study suggested that pomegranate 'Tunisi' was a moderately salt tolerant cultivar (0.4% NaCl) with a promoting effect on the growth below 0.1% salinity. This cultivar presents a mechanism of alleviating the detrimental effects of salt stress through improving the proline content, soluble protein content and the activities of antioxidant enzymes. But the protections of antioxidant enzymes are in a limited range of salinity.

Key words: Pomegranate; Salt stress; Growth; Physiological characteristics

Introduction

In recent years, nearly 7% of the global cultivated lands are affected by salinity, and soil salinization aggravating has been a greater threat to healthy and sustainable development of agriculture all over the world (Li *et al.*, 2012). In China, the saline soils distributed mainly in the north and coastal areas, are about 3.6×10^7 hectares, accounting for 4.88% of the available land areas (Yang, 2008). Plants exposed to saline environment suffer from osmotic stress, ion toxicity, nutrient deficiencies, physiological and biochemical alterations (Jampeetong & Brix, 2009; Gomes *et al.*, 2011). Many studies showed that salt stress interfered the growth and development of plants, all the major processes such as photosynthesis, protein synthesis, ion balance, substance and energy metabolisms were affected (Munns & Tester, 2008; Zhang *et al.*, 2013). Salinity tolerance is a complex feature and depends on both genetical and physiological properties. At present, more and more researchers pay close attention to further study the mechanisms of salt resistance and how to improve plant salt tolerance.

Pomegranate (*Punica granatum* L.) is an important commercial fruit tree belonging to the family *Punica ceae* (Aseri *et al.*, 2008), which is referred to as a 'super fruit' (Silva *et al.*, 2013) and widely grown in arid and semi-arid soils (Holl *et al.*, 2009; Cao & Hou, 2013). It is worthy to note that pomegranate has a set of economic, nutrition, medicinal, ornamental and ecological values (Yuan, 2016), and is considered to be moderately tolerant to salinity (Allen *et al.*, 1999; Tavousi *et al.*, 2014; 2015). Researches reported that the pomegranate survival percentage, plantlet height, node number, stem diameter, fresh and dry weight are decreased with increasing levels of salinity (Khoshgoftarmanesh, 2006; Elagamy *et al.*, 2010; Khayyat*et al.*, 2014). At present, the effects of salt stress on pomegranate focused mainly on the growth and ions balance (Akça & Samsunlu, 2012; Karimi & Hasanpour, 2014; Ibrahim, 2016), but those on the physiological and biochemical responses of pomegranate to salt stress were still insufficient. Therefore, the study aimed to determine the effects of salt stress on physiological and biochemical characteristics in pomegranate cuttings, in order to better understand the salt resistance of pomegranate and offer a reference for pomegranate cultivation on saline-alkali lands.

Materials and methods

Plant materials and stress treatments: Pomegranate 'Tunisia' (2-years-old cutting plants, collected from Pomegranate Repository of Nanjing Forestry University. China) were transplanted into plastic pots (32 cm height × 25cm diameter) containing 2.5 ± 0.01 kg mixed perlite and turfy soil (1:1) on March 1, 2016. The plants were grown in a greenhouse of Nanjing Forestry University (119°12′ E, 31°36′ N) with temperature at 28/24 ± 3°C day/night in natural light. Before starting salinity treatments, the plants were irrigated with Hoagland nutrient solution (Feng *et al.*, 2014) once a week and water twice a week.

The experimental treatments were arranged in a complete randomized blocks design. The plants of total 105 pots were selected and randomly divided into 7 blocks, with 5 pots per block and each set of three biological replications on April 1, 2016. The plants were treated with NaCl at 0(Control), 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%, respectively. All the treatments were watered Hoagland nutrient solution once a week. Each treatment was carried out once after watering corresponding to NaCl content thoroughly. Leaking was

not allowed. The control group was thoroughly irrigated with fresh water. Every three days, at 10:00 a.m. all cuttings were poured with 1 L fresh water, and the leached solution in trays were collected and put back into pots. At the end of experiment (35 days), plants growth situation was observed. After salinity treatment 7, 21 and 35 days, the middle-upper part functional leaves of each treatment plants were collected and frozen in liquid nitrogen and stored at 80℃ refrigerator for subsequent use.

Plant growth parameters: At the beginning and the end of the experimental period, the following determinations were studied: the length of the main stem of each plant (from the soil surface to the tip of the main stem), the size of the ground diameter (10 cm off the ground), the salt injury rate (P) and salt injury classification (D). The leaves of plants in salt stress which became perished and shriveled were considered injured, and the salt injury rate (P) of each treatment was recorded and the values were expressed in percentages as following formula:

$$P = \frac{\text{Number of salt injured plants}}{\text{Total number of plants each treatment}} \times 100\% \quad (1)$$

The salt injury classification (D) of each treatment were also recorded. Salt injury classification (D) was divided into 5 levels based on the degrees of salt injury symptoms (Du *et al.*, 2002): Level 0 (No salt injury symptoms), Level 1(Mild salt injury, a little part of leaves margin and tip turned yellow), Level 2 (Moderate salt injury, about 1/2 of leaves margin and tip turned yellow and witling), Level 3 (Severe salt injury, most of leaves became withered or fallen), Level 4 (Extremely severe salt injury, branches became shriveled, with leaves fell and then finally died).

Chlorophyll content: Fresh leaves (0.2 g) were cut into small pieces and then added in 10 mL 80% acetone solution for 24 hours at room temperature in darkness. The optical density values (OD value) of extract (3ml) were determined at 663nm and 645 nm. The concentrations of chlorophyll *a* (*Ca*), chlorophyll *b* (*Cb*), and total chlorophyll (*Ct*) were calculated with following formulas (2), (3), (4) respectively.

$$Ca = 12.7OD_{663} - 2.69OD_{645} \quad (2)$$

$$Cb = 22.9OD_{645} - 4.68OD_{663} \quad (3)$$

$$C_t = C_a + C_b \quad (4)$$

Cell membrane permeability: The relative electrical conductivity is an important indicator for the membrane permeability evaluation (Mccann & Solomon, 2000; Qi *et al.*, 2014). In this study, the capacity of membrane permeability of samples was expressed as the values of relative electrical conductivity. Firstly, leaves were washed clearly with double-distilled water and cut into 0.5 mm strips, and then they were soaked with double-distilled water (10 mL) for 3 hours at room temperature with R1 determined. The samples were sealed with parafilms with heating to boiling for 30 min with R2 determined. The relative conductivity was calculated as follows:

$$\text{Relative conductivity} = (R1/R2) \times 100\% \quad (5)$$

The R1 is the conductivity measured before heating and R2 is the conductivity measured after boiling.

Malondialdehyde (MDA) content: MDA contents were determined by the method of Heath & Packer (1968) with little modifications (Guo *et al.*, 2015) using a spectrophotometer (UVmini-1240 ultraviolet spectrophotometer, Shimadzu Corporation, Suzhou, China). Fresh leaves (0.5 g) were homogenized in 5 mL 10% trichloroacetic acid (TCA) (w/v) then centrifuged at 4000 rpm for 20 min. A quantity of supernatant (1 mL) was added to 3 mL 6% thiobarbituric acid (TBA) (w/v). The mixture was boiled at 100°C for 15 min and then cooled rapidly in ice. The OD values at 450nm, 532nm and 600 nm were measured, respectively. MDA contents were calculated using the following formula:

$$C\ (\mu mol/L) = 6.45(OD_{532} - OD_{600}) - 0.56OD_{450} \quad (6)$$

$$\text{MDA content}\ (\mu mol/g) = (C \times V_t)/(W \times V_i) \quad (7)$$

The V_t is the total volume of supernatant, the Vi is volume of supernatant used for analysis.

Proline content: Free proline was measured according to the method of Bates *et al*., (1973) and Esan & Olaiya (2016). The fresh leaves (0.2 g) were homogenized with 5 mL 3% aqueous sulphosalicylic acid. The mixture was centrifuged at 4000 rpm for 15 min. The supernatant (2 mL) was added to 4mL 2.5% acid ninhydrin and 2 mL glacial acetic acid and then the mixture was heated at 100℃ for 1 h. The mixture was extracted with 5mL toluene and the absorbance of fraction with toluene from liquid phase was read at 520 nm. Proline concentration was calculated using t calibration curve (0-50 μg/mL) and expressed as $\mu g \cdot g^{-1}$ FW.

Protein content: The soluble protein content was determined by the method of Bradford (1976) with bovine serum albumin (BSA) as the standard. Samples (0.5 g) were homogenized with 10 mL 50 mM phosphate buffer (pH 7.0) and then centrifuged at 4000 rpm for 20 min. After adding 2 mL Coomassie Brilliant Blue G-250 to 2 mL supernatant for 3 minutes, the OD values of the mixture were measured at 595nm.

SOD, POD, CAT activities: The extraction of antioxidant enzymes were assayed by the method of Xu (2011) with some modifications. Frozen leaf samples (0.5 g) were extracted with 10 mL ice-cold 50 mM phosphate buffer (pH 7.8) and then the extracts were centrifuged at 4°C for 20 min at 4000 rpm. The supernatant referred to as enzyme crude extract was collected and used for assay.

SOD activity was determined by monitoring the decrease of photochemical reduction nitrobluetetrazolium (NBT) by the enzyme at 560 nm (Winterbourn *et al.*, 1975). Enzyme activity was calculated as $U \cdot g^{-1}$ FW (unit of enzyme activity per gram of fresh weight). The reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM l-methionine, 75 μM NBT, 10 μM ethylene diaminetetraacetic acid (EDTA) -Na_2, 2 μM riboflavin, and distilled water (15: 3: 3: 3: 3: 2.5 v/v). About 3 mL reaction solution was taken in test tubes in duplicate, with one tube added by 0.01 mL enzyme extract and another tube without enzyme extract being taken as the control group. The tubes were placed in an illumination incubator at 4000 Lux light for 30 min. At the end of reaction the light was turned off and the tubes were putted in the dark for determination.

POD activity was determined by measuring the guaiacol oxidation at 470 nm (Iori *et al.*, 1995). The reaction started with 20 μL enzyme extraction added to mixture solution containing 50 mL of 0.1M phosphate buffer (pH 6.0), 28 μLguaiacol and 19 μL 30% H_2O_2. After 3min, the mixture of absorbance were measured. One unit of POD activity was expressed as the change of absorbance per min and was calculated as $U \cdot g^{-1} \cdot min^{-1}$ FW.

CAT activity was determined by monitoring decrease in the absorbance due to decomposition of H_2O_2 at 240 nm (Greenwald, 1987). The 3.1 mL reaction mixture contained 2.4 mL of 0.1M phosphate buffer (pH 7.0), 0.6 mL of 0.1M H_2O_2, and 0.1 mL enzyme extract. One unit of CAT activity was expressed as the change of absorbance per min and was calculated as $U \cdot g^{-1} \cdot min^{-1}$ FW.

Statistical analysis: The results were obtained from three independent repeated experiments and were presented as a mean average. ANOVA (Analysis of Variance) analysis ($p<0.05$) and Duncan multiple comparison ($p<0.05$) were determined using the SPSS program (Version 19.0. Chicago, IL, USA). The results were graphed using Excel 2010.

Results and Discussion

Pomegranate growth under salt stress: Fig. 1a showed the growth curves of pomegranate at different salinity. Compared with the control group, the inhibitory effects on growth of net stem height and ground diameter were non-significant when NaCl concentration was less than 0.4%, and the low salinity (0.1%) acted as a facilitator to the growth of pomegranate. At high salinity (≥0.5%), significant inhibitions were found in growth of net stem height and ground diameter compared with the control. The salt injury classification (D) and salt injury rate (P) both increased with increasing NaCl concentration. Under high salt stress (≥0.5%), D was up to level 1, mild salt injury, with a few leaves becoming yellowed and the margins curled or withered, and P was more than 65% of pomegranate cuttings (Fig. 1b).

The net growth and salt injury index are the major indications of plant morphological changes under salt stress, and are also the obvious characteristics to determine salt resistance of plant. The previous studies suggested that the growth of some plants exposed to hypersaline conditions were inhibited (Siddqi, 2007), while that of others were promoted in hyposaline conditions (Woolley, 1957; Lu *et al.*, 2014), which was possibly due to that the sodium is a 'functional nutrient' in some plants and could be helpful to growth (Brownell, 1979; Subbarao *et al.*, 2003). There was reports that some pomegranate cultivars presented higher tolerance to salinity than others (Okhovatianardakani *et al.*, 2010). The length of the main stem and the number and length of internodes of pomegranate cv. 'MalasShirin' increased at low salinity levels (Karimi & Hasanpour, 2014). Our findings were consistent with these results. There was no obvious symptoms of salt injury in leaves in moderate salinity (0.4%) indicating that pomegranate 'Tunisi' has an ability to adapt saline environment.

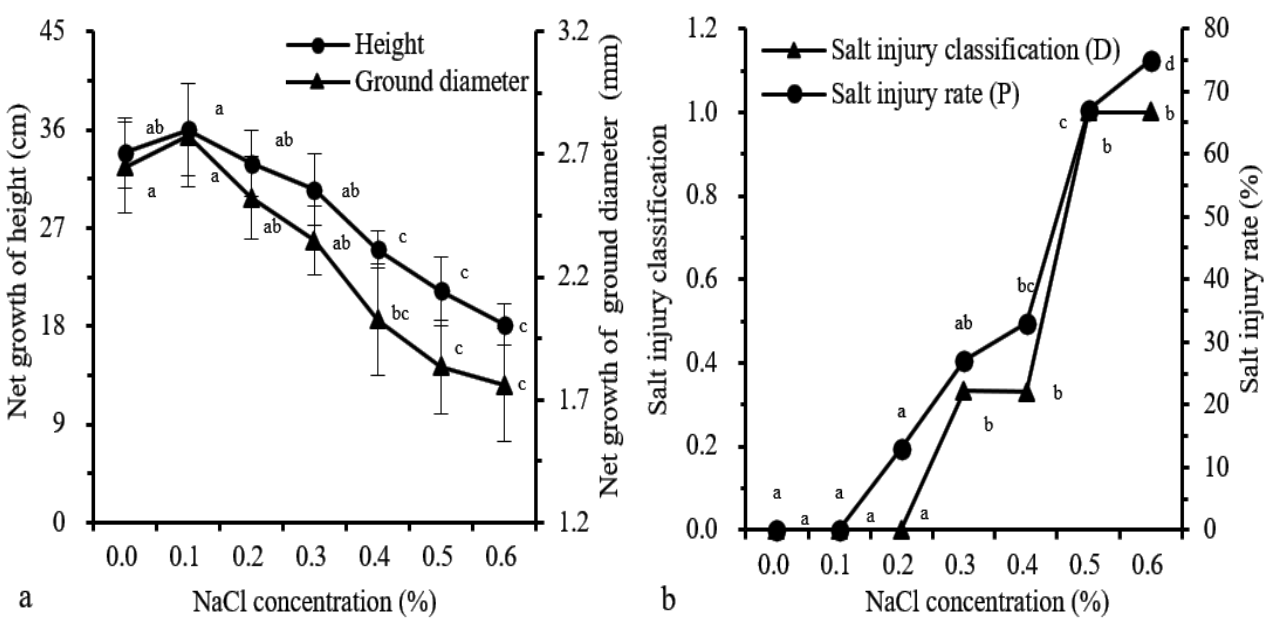

Fig. 1. Effects of NaCl stress on the net growth of height, ground diameter of pomegranate (a), and salt injury indexes (b). The values are the means of three replicates. Means followed by different letters in the same line are significantly different at $p<0.05$ among NaCl treatments.

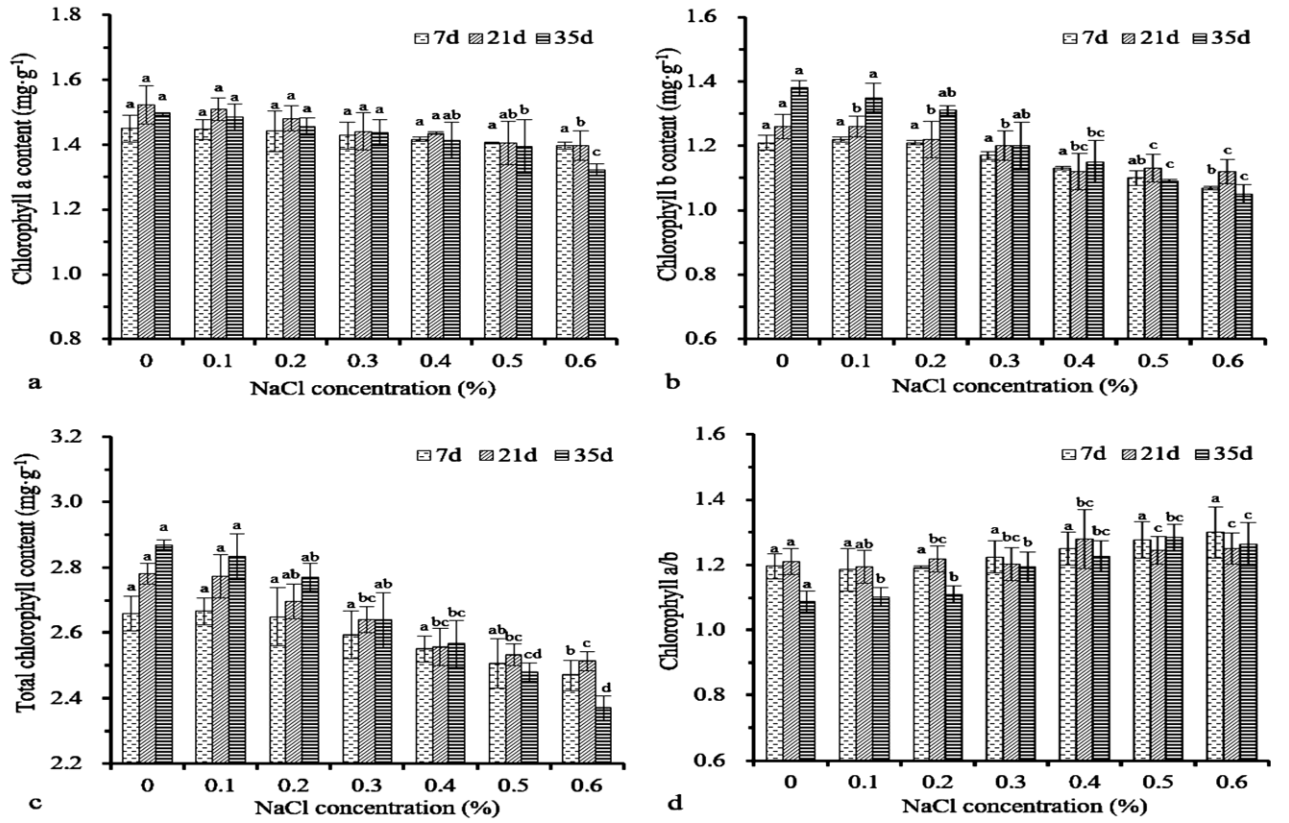

Fig. 2. Effects of NaCl stress on leaf Chl a (a), Chl b (b), Chl (a+b) (c) contents and Chl a/b (d) of pomegranate after 7, 21, 35 days of treatment. Values are expressed as the mean ± SE (n = 3). The vertical error bars with different letters are significantly different at $p<0.05$ among NaCl treatments.

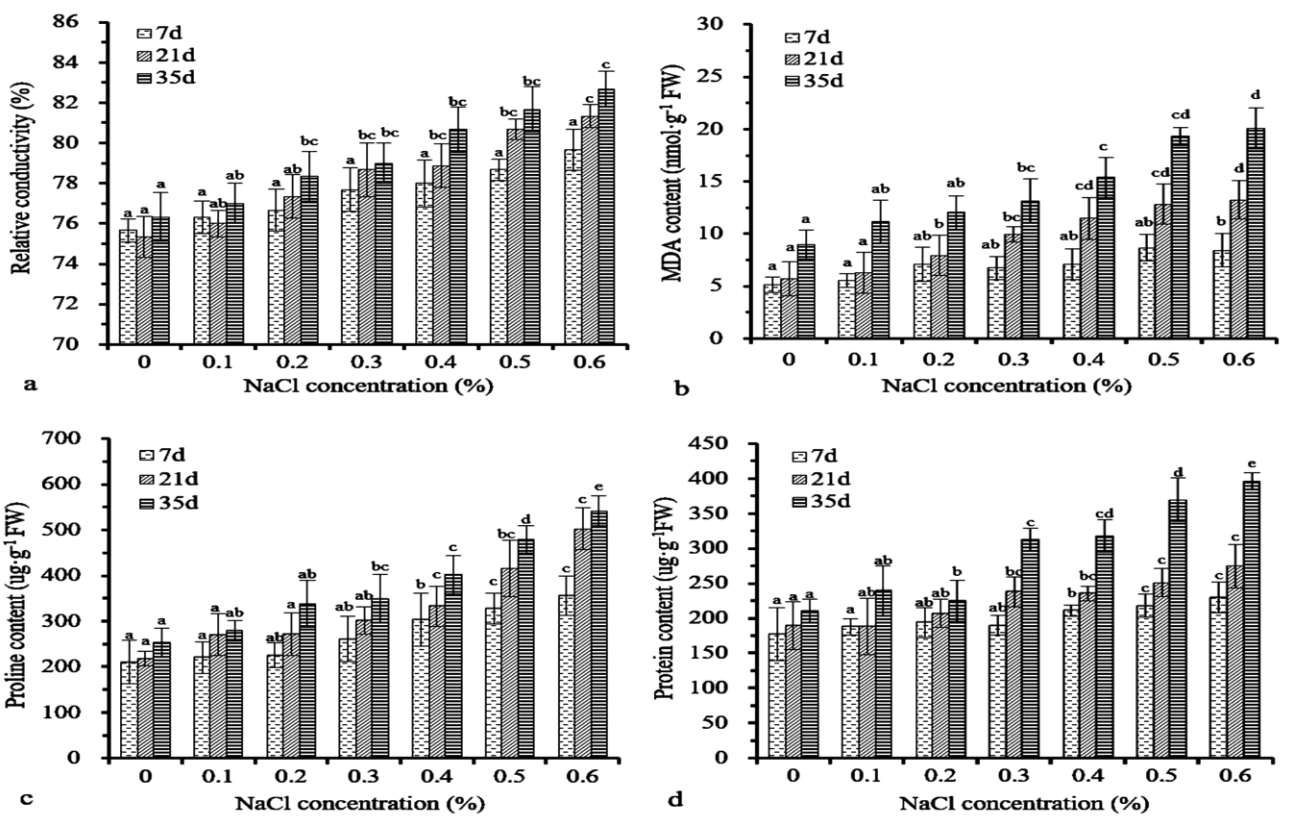

Fig. 3. Effects of NaCl stress on relative conductivity (a), MDA content (b), soluble proline content (c) and protein content (d) of pomegranate after 7, 21, and 35 days of treatment. Values are expressed as the mean ± SE (n = 3). The vertical error bars with different letters are significantly different at $p<0.05$ among NaCl treatments.

Table 1. Effects of NaCl stress on activities of SOD, CAT, and POD in pomegranate leaves after 7, 21 and 35 days

Antioxidant enzyme activity ($U \cdot g^{-1} \cdot min^{-1}$ FW)		NaCl concentration (w/w)						
		CK	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%
SOD	7d	757.5 ± 89.4c	798.0 ± 66.8bc	888.4 ±46.8ab	975.7±121.5b	1034.7±66.6ab	1198.1 ±97.2a	1084.6 ±48.6ab
	21d	783.8 ± 74.9c	886.4 ± 61.6bc	977.1 ±106.5ab	1094.0 ±46.0ab	1183.9 ±92.1a	998.9 ±94.0ab	883.7 ±37.8ab
	35d	845.9±101.8c	981.6 ± 47.1bc	1084.1±123.4b	1297.9 ±72.9ab	1387.1 ±96.4a	1181.6 ±63.3ab	1064.3 ±77.5bc
CAT	7d	928.0±398.5b	1169.5 ±453.1b	1862.5±198.4ab	2072.5±368.1ab	2172.1 ±264.7a	1996.5±481.6ab	1881.0±132.1ab
	21d	1038.5±260.9c	1669.0±357.1bc	2197.7±282.0ab	2361.0±191.5ab	2931.2 ±328.9a	2339.2±220.2ab	1904.0±319.7bc
	35d	1465.0±258.2d	1806.7±195.9cd	2323.2±370.8bc	2599.6±255.6ab	2967.7 ±375.9a	2507.1±379.7ab	2242.3±95.9bc
POD	7d	1026.7±10.0c	1135.6 ±9.6c	1557.8±16.8c	1855.6 ±13.7b	2207.8 ±3.9a	2195.6 ±12.6ab	2197.8 ±20.1b
	21d	1361.1±21.4d	1630.0 ±13.3cd	1981.1±10.1bc	2333.3 ±23.3ab	2665.6 ±12.6a	2221.1 ±20.1b	2192.2 ±25.5bc
	35d	1920.6±230.1d	2240.4 ±75.3cd	2635.0±378.0b	3303.2±395.7ab	3948.4 ±303.6a	3570.4±255.1ab	3212.3±225.3bc

Note: Values are expressed as the mean ± SE (n = 3). The different letters following error value are significant different at $p < 0.05$ among NaCl treatments.

Effects on chlorophyll content of pomegranate under salt stress: The contents of Chl a, Chl b, and Chl (a + b) in leaves of pomegranate cuttings were decreased with increasing salinity. The effects on these three indexes were all non-significant after 7 days under NaCl stress. After 35 days, the contents of Chl a, Chl b, and Chl (a + b) reduced significantly at ≥ 0.4% NaCl (Fig. 2). At 0.4% salinity, they decreased by 15, 24, and 18% compared with the control group, respectively. The lowest contents of Chl a, Chl b, and Chl (a + b) were all observed in 0.6% NaCl concentration (Fig. 2a, b, c) and the values were 69, 59, and 67% of controls, respectively. Consequently, Chl a/b showed a rising trend with increasing content of salinity, and had a significant raise under 0.4% NaCl (Fig. 2d). The contents of Chl a, Chl b, and Chl (a + b) were increased with the growth of pomegranate, however, the contents declined as stress time went on at more than 0.4% levels of salinity.

The effects of salinity appear to be dependent on the species and cultivars and on the stage of the plant development. Many pomegranate species showed a decline of chlorophyll content in response to an increasing salt stress (Melgar *et al.*, 2008; Mastrogiannidou *et al.*, 2016). Exposure of higher than 0.4% NaCl resulted in a significant decline of Chl a, Chl b, and Chl (a + b) contents in pomegranate leaves. This was probably due to the increasing activity of the chlorophylase (chlorophyll degrading enzyme) (Noreen & Ashraf, 2009), or the protein complexity structure of Chloroplast changes in cells (Bejaoui *et al.*, 2016). Appropriate Chl a/b inhibited the production of salt-induced O^{2-} in leaf, which was an important index of plant salt resistance. The increasing ratio ofChla/b with strengthening salt stress was possibly because the degradation of Chl b was more effective than that of Chl a (Zhao, 1993). However, other studies showed that the reduction of Chl a was greater than that of Chl b (Zhang, 2012) under salt stress. There was a controversial conclusion about the correlation between chlorophyll a/b value and salinity levels, and therefore it was worthy of further study.

Membrane permeability and malondialdehyde (MDA) content under salt stress: The membrane permeability of pomegranate was enhanced with increase of NaCl concentrations and stress times (Fig. 3a). When compared with the control group, the relative conductivity had no significant change in the leaves of pomegranate under moderate salinity, but obviously was enhanced by 6.9% and 8.3% under 0.5% and 0.6% NaCl treatments after 35 days (Fig. 3a). The membrane permeability of *Abelmoschus esculentus* (Dkhil & Denden, 2012), *Medicago sativa* (Wang *et al.*, 2009), and *Ziziphus jujube* cv. Jinsixiaozao (Xu *et al.*, 2011) were also enhanced with elevated salinity. The cell membrane is the first barrier for ions to penetrate into the cell, playing an important role in salt resistance of plants. The results suggest that the stability of cell membrane may be destroyed in some extent and the electrolyte solution could exude from cells in pomegranate leavesunder salt stress.

With the increases of salinity and salt stress time, an increase of MDA content was found in pomegranate leaves. After 35 days, the contents of MDA at 0.1%, 0.2% and 0.3% salinity levels had no significant differences compared with the controls (Fig. 3b). While the MDA massively accumulated in leaves under high NaCl stress (≥0.4%). The amount of MDA enlarged by 1.1 and 1.3 times at 0.5% and 0.6% NaCl levels compared with the control group (Fig. 3b), which suggested that high salt stress aggravated lipid peroxidation reaction in pomegranate leaves. MDA is an important product of lipid peroxidation in plants exposed to stress conditions (Jia *et al.*, 2012). In many studies the content of MDA increased with salinity (Gunes *et al.*, 2007; Stavridou *et al.*, 2017), which was consistent with our conclusion. The significant increase of MDA content at high salinity (0.5% to 0.6%) indicated that cells substances in the pomegranate was out-of-balance with the serious membrane peroxidation under high salt stress.

Effects of salt stress on proline and soluble protein accumulation: After 35 days the soluble proline accumulation in the leaves of pomegranate was observed for all treatments. Compared with the control group, at 0.1% and 0.2% salt treatments, proline amount had been less affected by NaCl stress, and when NaCl concentrations were higher than 0.3%, a significant increase of proline content was found in leaves (Fig. 3c). At the highest salinity (0.6% NaCl), the maximum content of proline in pomegranate was observed and 2.1 times higher than that of the control group. The protein accumulation in the leaves of pomegranate treated with NaCl solution. Compared with the control group, the mild concentration of NaCl (less than 0.2%) had little effect on soluble protein content, whereas significant changes of soluble protein accumulation were observed at moderate and high levels of salinity (Fig. 3d). Generally, the accumulation of soluble protein presented a rising trend with increases of NaCl contents and NaCl stress times.

When plants exposed to saline environment the non-toxic soluble substances were activated to play a role in regulating and protecting plants. Many studies showed that the contents of proline and soluble protein in plants raised with increasing salt concentration (Akça & Samsunlu, 2012; Rahdari *et al.*, 2012). In this study a rising trend was also observed with a significant increase at more than 0.3% NaCl concentrations. As the results of *Miscanthus* × *giganteus* strongly inducted to the osmoprotectant (Stavridou *et al.*, 2017), proline and soluble protein in pomegranate leaves were also sensitive and responsive quickly to salt stress and exerted relaxation effect. Plants exposed to saline environment regulate and alleviate osmotic stress by osmoprotectants, such as soluble proteins, proline, sugars, etc. Some specific ions, such as Na^+, K^+, Ca^{2+}, Cl^-, SO_4^{2-}, and NO_3^-, were also involved in osmotic stress regulation and alleviation (Yang *et al.*, 2009). For pomegranate, how the ions content change and whether these ions act as osmotic regulators involved in response to salt stress need a further study.

Effects of salt stress on activities of antioxidant enzymes SOD, CAT and POD: At same levels of salinity, the activities of SOD, CAT, and POD in leaves of pomegranate cuttings were significantly increased as stress time went on. Meanwhile, the activities of SOD, CAT, and POD showed a trend of first increasing and then decreasing, with the peak at 0.4% NaCl (Table 1). After NaCl treatments 35 days, a significant difference of the activities of SOD, CAT, and POD were observed at 0.2% NaCl compared with the control group. Besides, the maximum activities of SOD, CAT, and POD were 1.6, 2.0, and 2.1 times greater than than of control group respectively. But the values of antioxidant enzymes activities declined from 0.5% to 0.6% NaCl concentrations, suggesting that the antioxidative enzyme system may be damaged at high salinity levels.

Plants subjected to salt stress experience oxidative damages via reactive oxygen species (ROS) in cells, such as $^1O_2$, H_2O_2, O_2^- and $HO^{\cdot}$. ROS disrupted the normal metabolism of plants by oxidative damage to proteins, DNAs and lipids (Apel & Hirt, 2004; Miller *et al.*, 2010; Corpas *et al.*, 2015). In order to scavenge the ROS, plants possess protective enzymes like SOD, CAT, and POD, and they are the most important elements of the antioxidant defense system to remove ROS from injury (Afzal *et al.*, 2006; Dai *et al.*, 2009). The trends of antioxidant enzymes activities with the increased salinity levels in pomegranate were similar to the results of other studies (Li, 2009; Hui *et al.*, 2012). There are other antioxidants like GSH (glutathione), AsA (Ascorbic acid) and polyphenol also having potentials to scavenge various oxygen species, which need to be studied further. Previous researches indicate that pomegranate is considered to be moderately tolerant to salinity, 'Malas Shirin' pomegranate was tolerant up to 40mM of NaCl (Naeini *et al.*, 2006); Wonderful cultivar was found to be tolerant to high salinity than Manfalouty (Ibrahim, 2016). The activities of CAT, POD, and SOD declined at high salinity (0.5%-0.6%), indicated that they played a role in defensing salt stress, but the protection effect was limited. Our study demonstrated that pomegranate 'Tunisi' growth was not greatly affected under less than 0.4% salinity levels.

Conclusions

The growth of pomegranate cv. 'Tunisi' was increased under low salinity (0.1%), and the threshold value of salinity was 0.4% level of NaCl concentration. Our study showed that the adverse effects on these physiological indexes aggravated over stress time. We inferred that it was one of mechanisms for pomegranate alleviating the detrimental effects of salt stress through increasing contents of proline and soluble protein and activities of antioxidant enzymes. This study would provide a theoretical basis for the cultivation and utilization of pomegranate plants on saline soil.

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