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Full Length Article

Physiological and Growth Responses of Halophyte *Zoysia macrostachya* to Increasing Salinity

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Abstract

The current research aimed to evaluate the effects of various concentrations of NaCl (0.0, 1.0, 2.0 and 4.0%) on the physiological and growth behaviors of the halophyte *Zoysia macrostachya* collected from the coastal wetland in east China from 2017 to 2018. The relative water content, antioxidase activities and osmotic adjustment substances contents in leaf were determined on days 4, 8, 12, 16, 20 and 24 of the experiment. Besides, Na^+ and K^+ concentrations in the root, shoot and leaf, together with the biomass, were measured at the end of experiment. Our results showed that relative water content decreased, while the H_2O_2 content elevated; in addition, the activities of peroxidase, catalase and superoxide dismutase were higher under salinity stress. On the other hand, the contents of proline, soluble sugar and betaine were increased with the increase in the salinity concentrations. As the NaCl concentrations increased, the Na^+ concentrations in root, shoot and leaf increased significantly ($P \leq 0.05$), K^+ concentrations increased at first and then decreased, while K^+/Na^+ ratios were gradually decreased. Typically, the salinity levels of 1.0 and 2.0% improved root and aboveground growth, but that of 4.0% limited plant growth. These results indicated that the increase in antioxidase activities, the accumulation of osmotic adjustment substances, the absorption of a higher level of K^+, the maintenance of greater K^+ contents in leaf and shoot, and the lower Na^+ transfer from root to shoot and leaf, might account for the mechanisms of salinity tolerance in *Z. macrostachya*. © 2020 Friends Science Publishers

Keywords: *Zoysia macrostachya*; Salt stress; Relative water content; Antioxidase activities; Osmotic adjustment substances; Ion uptake; Growth traits

Introduction

Salinity, a leading environmental constraint, has affected the development and growth of plants worldwide (Parida and Das 2005; Gupta and Huang 2014). Salt stress can lead to metabolic and physiological disorders in plants through ion toxicity, osmotic stress, nutrient imbalance, and oxidative stress (OS), or the combinations of the above factors, which will result in damage to cells and reduce plant growth or even death (Shabala and Mackay 2011; Hamed *et al*. 2013; Flowers *et al*. 2015; Slama *et al*. 2015). During the evolution, plants developed numerous biochemical and physiological strategies to cope with stresses (Pastori and Foyer 2002), and the following strategies are included. (1) Accumulating the osmotic adjustment substances, such as the synthesis of soluble sugars, proline, betaine, glycine, together with other osmolytes for promoting the cellular osmotic balance in plants (Kishor *et al*. 1995; Garg *et al*. 2002; Taji *et al*. 2002). (2) Ion-selective absorption and compartmentalization; in plants, ion uptake, as well as Cl^- and Na^+ ions compartmentalization mainly takes place in vacuoles to adjust osmosis, and the compatible solutes are also generated for adjusting osmotic potential (Nanjo *et al*. 1999; Hong *et al*. 2000). (3) Scavenging of reactive oxygen species (ROS); typically, antioxidant enzymes, like catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), can scavenge the excessive ROS to protect the membrane integrity (Souid *et al*. 2016).

According to the F.A.O. (2007), about 20% of total agricultural lands (over 900 million hectares) have been under salt stress, which takes up over 6% total land area in the world. As in China, about 37 million hectares of lands are subjected to primary as well as secondary salinization stress (Zhang *et al*. 2007). Growing salinity-tolerant plants has been recognized as an effective way for exploiting the saline land resources. However, the salt tolerance of some crops is poor, and some researchers suggest that growing the salinity-tolerant grasses can be an alternative way in such regions (Roy and Chakraborty 2014). *Zoysia macrostachya*, one of the perennial turfgrasses growing in warm season, is originated from China, Japan as well as Korean Peninsula, and it mainly grows on the salt-affected soil along the sea-

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bank of Shandong, Jiangsu and Zhejiang provinces in China. *Z. macrostachya* can rapidly spread through the rhizomes and stolons, thus forming the dense turf with a deep root system. Therefore, it can be used as the soil–conserving, dike-protecting and sand-fixing turf. Importantly, *Z. macrostachya* is an euhalophyte, which has potent tolerance to salinity, and is promising to be used in landscaping of the saline-alkali lands. However, *Z. macrostachya* has low turfgrass quality, which is thereby rarely used as ornamental turf. Compared with other Zosia grasses, *Z. macrostachya* is relatively less investigated, to date, the activities of antioxidant enzymes, contents of osmotic adjustment substances, ion intake, and the growth traits of *Z. macrostachya* under salt stress remain unclear. Thus, this work aimed to examine the role of salinity in the physiology as well as growth of *Z. macrostachya*, so as to identify the traits related to higher salinity tolerance.

Materials and Methods

Collection of plants and growth conditions

Z. macrostachya was collected from the coastal wetland at about 45.0 Km east of Yancheng in Jiangsu province, was cut into fragments (5.0 cm in radius) and brought to our laboratory to grow in the plastic pots (20 cm × 30 cm) filled with washed sand. Each plant was watered at an interval of three days to reach the field capacity, and was irrigated weekly with 200 mL of the 1/2 Hoagland's nutrient solution before treatments. Each plant grew at the conditions of natural light, a photoperiod of 14/10 h, photosynthetic active radiation at 650–1500 $mmol \cdot m^{-2} \cdot s^{-1}$, daily minimum/ maximum air temperatures of 14°C/35°C, mean air temperature of about 26°C, and relative humidity of 80 ± 10% during the experiment. This research was carried out during the growing seasons in 2017 and repeated in 2018.

NaCl treatments

After 30 days of culture, plants were removed into the greenhouse to carry out four treatments, including (C) control (100% field capacity), (S1) 1.0% NaCl, (S2) 2.0% NaCl and (S3) 4.0% NaCl concentration treatment. Salt was incrementally increased by 0.5% every day to avoid the osmotic shock. On days 4, 8, 12, 16, 20 and 24 during the experiment, leaves were harvested to determine the relative water content (RWC), activities of antioxidant enzymes and contents of osmotic adjustment substances. On the completion of experiment, all plant samples were isolated as root, shoot and leaf to determine the ion content, root and aboveground dry biomass.

RWC

Ten leaves from plants of each pot were harvested to immediately determine their fresh weight (FW), followed by transfer into the deionized water in the Petri dishes for 4 h in dark to restore their turgidity. Later, the excess water was removed, turgid weight (TW) was recorded, samples were subjected to 30 min of drying at 105°C and 48 h at 80°C, respectively, so as to attain the dry weight (DW). Afterwards, RWC was computed according to the formula:

$$RWC\% = (FW-DW)/(TW-DW) \times 100\%$$

Enzyme extractions and assays

Enzymes were extracted at 4°C by the method proposed by Pereira *et al*. (2002). Briefly, 0.5 g fresh plant leaf samples were frozen using liquid nitrogen, followed by grinding using the ice-cold mortar and pestle, and extraction with the potassium phosphate buffer (100 m*M*, at pH=7.5) supplemented with 5% (w/v) insoluble polyvinylpyrrolidone (w/v, 1:3), 3 m*M* DL-Dithiothreitol, and 1 m*M* EDTA. Afterwards, the obtained homogenate was filtered using the four-layer cheesecloth, followed by 30 min of centrifugation at 14,000 × g to collect the supernatant, which was preserved as small aliquots under the temperature of − 80°C for POD, CAT, and SOD assays. Additionally, spectrophotometric analyses were performed using the spectrophotometer (Model 336001, Spectronic Instruments, U.S.A.).

SOD activity assay was carried out through inhibiting the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries 1977). An enzyme unit in SOD activity was deemed as the enzyme amount necessary for inducing 50% suppression of NBT reduction rate measured at the wavelength of 560 nm.

The activity of CAT was determined according to the method from Aebi (1984). In brief, 3 mL reaction solution was comprised of H_2O_2 (0.1 *M*), enzyme extract (20 μ*L*), and phosphate buffer (0.15 *M*, at pH=7.8). Specifically, the activity of CAT was detected after reducing H_2O_2 at the wavelength of 240 nm.

POD was measured using the substrate guaiacol (Kochba *et al*. 1977), and the elevation of absorbance was measured for every minute at the wavelength of 470 nm. One unit in POD activity was referred to as any alternation in the absorbance/min; meanwhile, the specific activity was regarded as the enzyme unit/mg soluble protein.

Determination of the proline, betaine and soluble sugar content (SSC) levels

0.2 g fresh leaf samples were collected and mixed with sulfosalicylic acid (10 mL, 3% w/v) to determine proline according to the 1.0 mL acid–ninhydrin method. Afterwards, the absorbance at 520 nm was measured by the spectrophotometer (Bates *et al*. 1973).

SSC was determined according to Buysse and Merckxs (1993) method. Briefly, dry leaf powders (20 mg) were extracted for 15 min for 4 times using 20 mL ethanol

(90% v/v), and centrifuged for 10 min at 3000 × g to collect the supernatant. Then, all supernatants were combined to result in a final volume of 40 mL. Afterwards, 2 mL supernatant was extracted, followed by transfer to the glass tube, and the addition of 5 mL concentrated sulfuric acid and 1 mL phenol solution (18%). Later, the resultant mixture was sufficiently shaken, and the absorption at 490 nm was read using the above-mentioned spectrophotometer.

Betaine was detected according to Grieve and Grattan (1983) method through predicting the betaine-periodite complex. In brief, 0.5 g dry ground leaves were subjected to 24 h of mechanical shaking at 25°C using 20 mL deionized water. Subsequently, the samples were filtered to collect the filtrates, which were then diluted with H_2SO_4 (1 mol·L^{-1}) at the ratio of 1:1. Later, 0.5 mL aliquots were determined within the centrifuge tubes, followed by 1 h of cooling within the icy water and addition of 0.2 mL cold KI-I_2 reagent under gentle stirring. Then, the tubes were preserved for 16 h under 4°C prior to 15 min of centrifugation at 10,000 × g under the temperature of 0°C. Afterwards, the resultant supernatant was collected with caution to measure the absorbance at 365 nm through the spectrophotometer at 2 h later. Notably, the betaine reference standards (50–200 μg·mL^{-1}) were prepared using H_2SO_4 (1 mol·L^{-1}).

Measurement of the hydrogen peroxide (H_2O_2) level

H_2O_2 level in leaf was measured based on the method proposed by Loreto and Velikova (2001). In brief, 0.5 g leaf sample was subjected to homogenization within the ice bath using 5 mL TCA (0.1% w/v), followed by 15 min of centrifugation of the obtained homogenate at 12,000 × g. Later, 0.5 mL supernatant was collected to mix with 1 mL potassium iodide (1 *M*, KI) and 0.5 mL potassium phosphate buffer (10 m*M*, at pH=7.0). Then, the absorbance of supernatant was determined at 390 nm, and H_2O_2 level was computed relative to the previously plotted standard calibration curve using H_2O_2 at various contents.

Measurement of Na^+ and K^+ concentrations

Root, shoot and leaf samples were ground to fine powders, followed by digestion using the HNO_3 solution (0.5%) to extract the ions. Subsequently, the contents of K^+ and Na^+ were measured using the flame photometer (Corning, London and UK).

Root and aboveground dry biomass

The residual root, shoot and leaf were dried for 30 min at 105°C, followed by 48 h of drying at 80°C to obtain their dry weights. The aboveground biomass represented the sum of dry weight of leaves and shoots.

Statistical methods

All experiments were performed under complete randomization condition, and were repeated for three times. Difference in data between 2017 and 2018 was not statistically significant, as a result, data collected at the sampling dates in these two years were combined, and the means were utilized in statistical analyses. The one-way ANOVA was used for data analysis by the S.A.S. software (S.A.S. Institute, Cary, NC, USA); in the meantime, the Duncan's multiple range test was performed to compare the averages, and the significance level was set as $P \leq 0.05$.

Results

RWC

Throughout the experiment, leaf RWC gradually deceased under the stress conditions (Fig. 1), and a higher salinity level resulted in a greater decrease in RWC. Under treatment at 1.0% salinity concentration, RWC was remarkably reduced by 8.98% on the 16th day compared with those in control plants; however, significant difference in RWC reduction appeared on the 8th day and the 4th day at 2.0 and 4.0% salinity concentrations ($P \leq 0.05$), respectively, which were reduced by 8.14 and 12.67%, respectively. At the end of experiment, the RWC under treatments at 1.0, 2.0 and 4.0% salinity concentrations were declined by 11.24, 31.59 and 63.18%, respectively.

Antioxidant enzyme activities

POD activity in the treated plants continued to increase under 1.0% salinity concentration, which was significantly increased (increased by 51.14%) compared with that in control plants from the 16th day. At the 2.0 and 4.0% salinity concentrations ($P \leq 0.05$), the POD activities were first increased and then decreased as the salinity stress prolonged, which peaked on the 16th day and were increased by 385.76 and 504.92% at two concentrations, respectively, compared with those in control plants. On the 24th day, POD activity remarkably increased relative to that in control plants (Fig. 2A).

The activity of SOD was elevated from day 0 to day 24 under three salinity concentrations. Under 1.0% salinity concentration, SOD activity was slightly increased, and difference in SOD activity was significant on day 24 between the treated and the control plants ($P \leq 0.05$). SOD activity was evidently elevated after 4 days under treatments at 2.0 and 4.0% salinity concentrations, and a higher NaCl concentration resulted in a higher SOD activity during the experiment (Fig. 2B).

Under 1.0% salinity concentration, CAT activity was increased slightly from day 0 to day 24, and significant difference in CAT activity between the treated and the control plants were found from the 12th day ($P \leq 0.05$). Specifically, the CAT activity of the treated plants was increased by 7.75% relative to that in control plants upon experiment completion. Under 2.0% salinity concentration,

plant CAT activity was increased, which was higher than that under 1.0% salinity stress. CAT activity was first increased and then decreased under 4.0% salinity concentration, which peaked on the 16th day and was increased by 75.06%. In addition, the CAT activity under 4.0% salinity concentration was higher than those under 2.0 and 1.0% concentrations during the experiment period (Fig. 2C).

Levels of osmotic adjustment substances

Throughout the experimental period, no significant difference was observed in the proline level of control plants, but proline content varied depending on the different salinity concentrations. Proline content displayed no obvious difference from day 0 to day 16, but it markedly increased after the 16th day under 1.0% salinity concentration compared with that in control plants. Specifically, the proline content increased by 44.00% on the 24th day. By contrast, the proline content was risen steadily under 2.0% salinity concentration during the experiment. On the 24th day, proline content was 18.35 $mg \cdot g^{-1}$, which was about 2-fold of that in control plants. For 4.0% salinity concentration, proline content was elevated from days 0–16, which was reduced after the 16th day. On the 24th day, the proline content at 4.0% salinity concentration was higher than those at 2.0 and 1.0% salinity concentrations (Fig. 3A).

In our experiment, the SSC level was always stable within control plants. Although the SSC content increased under 1.0% salinity concentration, no significant difference was observed compared with the control plants, except for that on the 16th day. In addition, the SSC contents kept increasing under 2.0 and 4.0% salinity concentrations as the salinity stress prolonged. On the 24th day, the SSC contents were increased by 172.86 and 215.04%, respectively. Moreover, the SSC content under 4.0% salinity concentration was always higher than those under 2.0 and 1.0% salinity concentrations (Fig. 3B).

The betaine contents dramatically decreased on the 8th and 12th days under 1.0% salinity concentration compared with those in control plants. Besides, betaine contents kept increasing under 2.0 and 4.0% salinity concentrations as the salinity stress prolonged, with the contents of 119.51 as well as 155.97 $mg \cdot g^{-1}$, respectively, which were increased by 132.91 and 203.98% on the 24th day, respectively. In addition, betaine content under 4.0% salinity concentration was always higher than those under the other two salinity concentrations (Fig. 3C).

H_2O_2 content

H_2O_2 contents in control plants and plants treated with 1.0% salinity concentration stress remained relatively constant during the experiment, and no significant difference was observed, except for that on the 24th day. Under 2.0 and 4.0% salinity concentrations, H_2O_2 contents were greatly

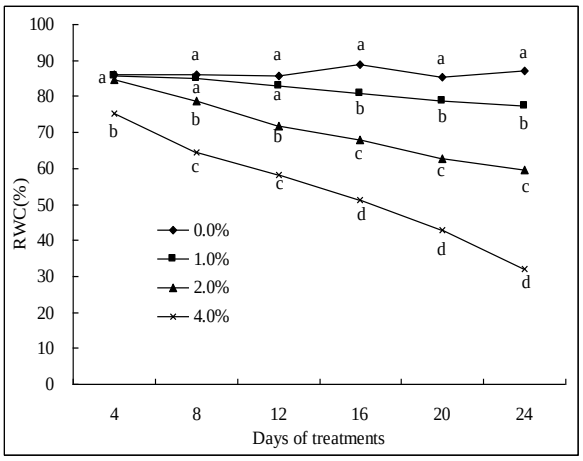

Fig. 1: Changes in RWC during salt stress
Note: The different letters in the same day suggest significant difference ($P \leq 0.05$), the same below

increased as the salinity stress prolonged, but the H_2O_2 content under 4.0% salinity concentration was greater than that under 2.0% salinity concentration (Fig. 3D).

Ion concentrations

Concentrations of K^+ and Na^+ in roots, shoots and leaves of *Z. macrostachya* are shown in Table 1. With the increase in NaCl concentration, Na^+ concentrations in root, shoot and leaf largely increased, under 4.0% salinity concentration, Na+ concentrations were 2.43, 2.98 and 3.36 times those of control plants, respectively. Besides, Na^+ concentrations in roots, shoots and leaves gradually decreased under the same salinity concentration. Changes in the K^+ concentrations in roots, shoots and leaves were different from those in Na^+ concentrations, which first increased and then declined as the salinity level increased. Typically, the K^+ concentrations in roots, shoots and leaves under 1.0% salinity concentration were increased by 17.59, 26.76 and 20.97%, respectively, compared with those in control plants; but the values were 24.45, 44.99 and 39.61 under 2.0% salinity concentration, as well as –1.5, 11.27 and –10.58% under 4.0% salinity concentration. K^+ concentrations in roots, shoots and leaves gradually increased under the same salinity level.

K^+/Na^+ ratios in roots, leaves and shoots upon experiment completion are shown in Table 2. The K^+/Na^+ ratios in control plants were 1.66, 2.87 and 4.40, respectively, while such values in treated plants decreased with the increase in salinity concentration. The K^+/Na^+ ratios in shoots, leaves and roots at 4% salinity concentration decreased by 59.63, 62.71 and 73.41% compared with those in control plants, respectively. At the same salinity concentration, the K^+/Na^+ ratios in roots, shoots and leaves increased gradually; typically, those at 1.0, 2.0 and 4.0% salinity concentrations in leaves were 1.55, 1.43 and 1.60 times of those in roots, and were 1.38, 1.43 and 1.09 times of those in shoots.

Table 1: Na^+ and K^+ concentrations in root, shoot and leaf of *Z. macrostachya*

Salinity concentration	Na^+ concentrations ($g \cdot kg^{-1}$)			K^+ concentrations ($g \cdot kg^{-1}$)		
	Root	Shoot	Leaf	Root	Shoot	Leaf
0.0%	6.40 ± 0.20	4.20 ± 0.10	3.50 ± 0.30	10.63 ± 0.15	12.07 ± 0.42	15.40 ± 1.11
1.0%	8.43 ± 0.81	6.67 ± 0.23	5.87 ± 0.21	12.50 ± 0.10	15.30 ± 0.62	18.63 ± 0.85
2.0%	10.98 ± 0.65	10.23 ± 0.25	8.77 ± 0.15	13.23 ± 0.21	17.50 ± 1.35	21.50 ± 0.36
4.0%	15.53 ± 0.15	12.53 ± 0.15	11.77 ± 0.15	10.47 ± 0.12	13.43 ± 0.42	13.77 ± 0.61

Table 2: K^+/Na^+ Ratios in root, shoot and leaf of *Z. macrostachya*

Salinity Concentration	Root K^+/Na^+ Ratio	Shoot K^+/Na^+ Ratio	Leaf K^+/Na^+ Ratio
0.0%	1.66	2.87	4.40
1.0%	1.48	2.29	3.17
2.0%	1.20	1.71	2.45
4.0%	0.67	1.07	1.17

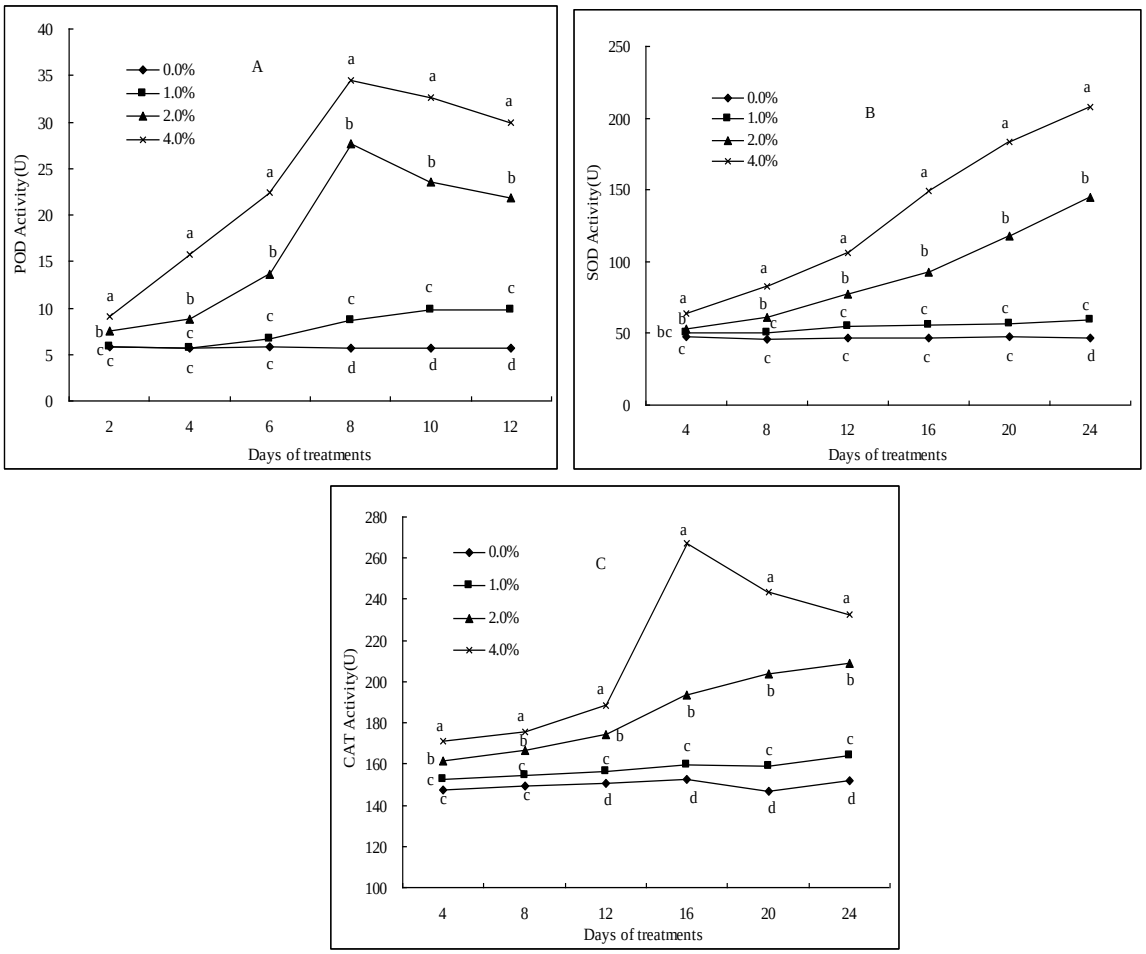

Fig. 2: Changes in antioxidant enzyme activities during salt stress

Root and aboveground dry biomass

Under 1.0 and 2.0% salinity concentrations, the root dry weights were evidently higher than that of control plants; however, that was markedly lower at 4.0% salinity concentration than that of control plants (Fig. 4A). Additionally, difference in the aboveground biomass between plants treated with 1.0% salinity concentration stress and control plants was not statistically significant, but the 2.0% salinity concentration apparently improved the growth of aboveground biomass, while the 4.0% salinity concentration limited shoot and leaf growth, and notably reduced the aboveground biomass (Fig. 4B).

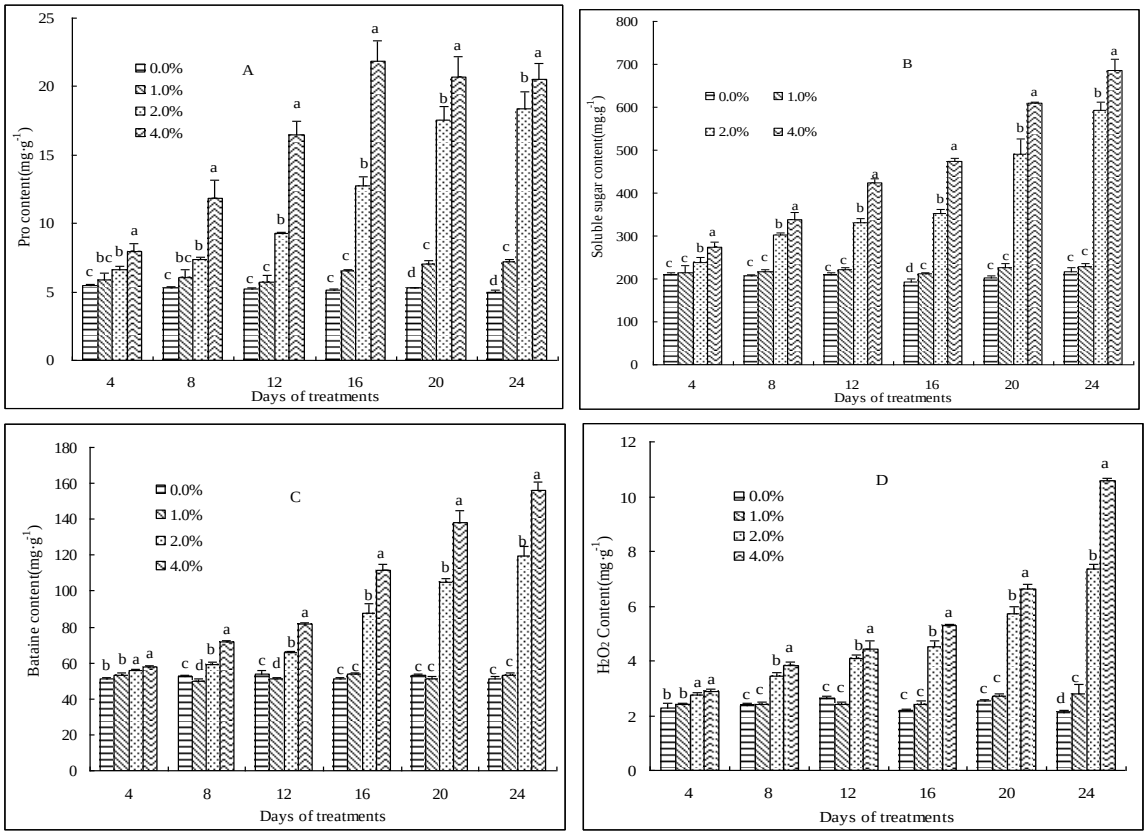

Fig. 3: Changes in osmotic adjustment substances and H_2O_2 content during salt stress

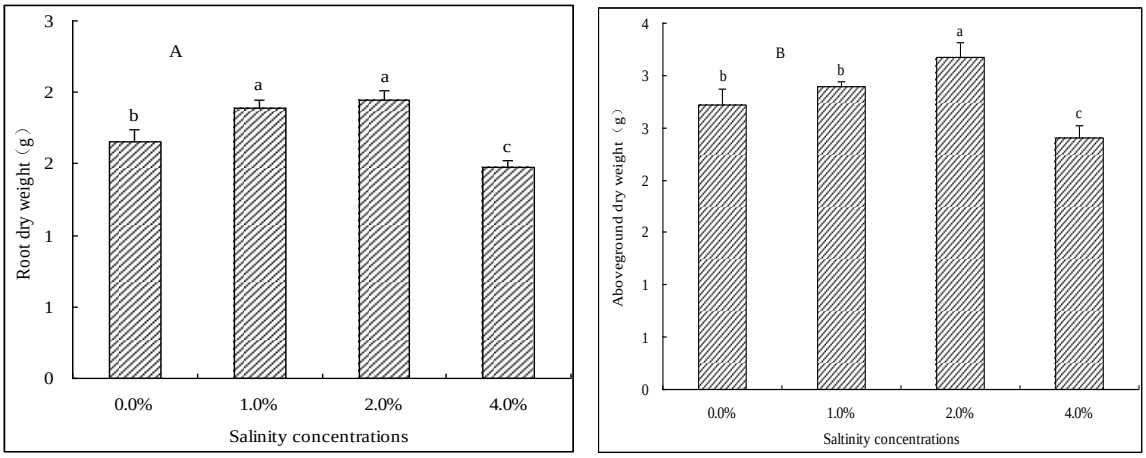

Fig. 4: Root and aboveground dry biomass of *Z. macrostachya* at the end of experiment

Discussion

The stress tolerating capacity in plant depends on several biochemical pathways, which can promote water acquisition and/or retention, remain cell membrane stability, and maintain ion homeostasis (Ashwani *et al*. 2016; Al-Maskri *et al.* 2010). RWC, a major creditable physiological index, can assess the salinity resistance for various genotypes (Hasheminasab *et al*. 2014). In our study, RWC decreased by salt stress throughout the experiment, but such effect was more pronounced in plants treated with 4.0% NaCl. Salinity is reported to decline the RWC for some turfgrass species, such as common bermudagrass (*Cynodon dactylon* [L.] Pers.) (Manuchehri and Salehi 2014), Iranian crested wheatgrass

(*Agropyron cristatum* L.) (Sheikh-Mohamadi *et al*. 2017) and tall fescue (*Festuca arundinacea* Schreb.) (Gao and Li 2012). Our finding was in line with these results.

Salinity stresses cause ROS production, while this subsequently promotes lipid peroxidation and increases the MDA content, which are the two indicators predicting oxidative damage in plants (Abbasi *et al*. 2007; Saruhan *et al*. 2012). H_2O_2 is one of the ROS, which is found to be increased within peroxisomes chloroplasts, as well as mitochondria responding to salt and drought stresses (Alscher *et al*. 2002; Pinheiro *et al*. 2004). According to our results, H_2O_2 content in the treated plants increased under different salinity concentrations, which indicated that the increased ROS resulted in severe injury to the crucial biomolecules, such as proteins, lipids, nucleic acids, and carbohydrates. To reduce the adverse effect of ROS, grasses will adapt to the antioxidant defence mechanisms, including the non-enzymatic and enzymatic mechanisms (Tahkokorpi *et al*. 2007). Specifically, the enzymatic antioxidant system is comprised of POD, CAT and SOD, which can remove H_2O_2 and superoxide in plants (Etemadi *et al*. 2015). Maintaining high activities of antioxidant enzymes increases the tolerance to salinity through enhancing the mechanisms to protect from OS (Jayakumar *et al*. 2008). Generally, salt tolerance has been identified to be associated with greater antioxidant enzyme activities of plants (Shalata *et al*. 2001). SOD has been identified to be the critical enzyme to keep the normal physiologic process and resist OS, which is achieved through the rapid conversion of O_2^- to O_2 as well as H_2O_2 (Quan *et al*. 2008). Our results suggested that, salt stress increased the activity of SOD in *Z. macrostachya*, indicating that ROS induced SOD activity in the case of salt stress, which thereby enhanced the ability of *Z. macrostachya* to scavenge O_2^-. Detoxification of H_2O_2 is mediated by CAT, a vital enzyme that can scavenge H_2O_2 through the direct decomposition of H_2O_2 into H_2O as well as O_2 in glyoxisomes and peroxisomes (Mittler 2006). On the other hand, POD is a leading enzyme to remove H_2O_2 from chloroplasts (Kyle *et al*. 1987), which can be activated under salt stress in plants to adapt to NaCl (Rahnama and Ebrahimzadeh 2005). In this study, salt stress resulted in higher activity of POD in *Z. macrostachya* confirmed the results of Hu *et al*. (2012). Therefore, increased POD, SOD and CAT activities might facilitate the possible antioxidant mechanisms in *Z. macrostachya* to resist salinity stress.

To mitigate the negative effect of stress on the plant osmotic equilibrium, some osmotic adjustment substances, such as proline, sugar and betaine, are synthesized in plants in response to stress (Ingram and Bartels 1996; Ashraf and Foolad 2007). These compatible solutes can finally restore the homeostasis and detoxification of cells, thereby rendering cell survival in the case of stress (Miller *et al*. 2010). Proline, one of the crucial osmolytes, can adjust osmosis in plants in the case of salinity-induced osmotic stresses, and free proline accumulation represents the general adaptation for mitigating abiotic stress severity within higher plants. At the same time, active proline accumulation has been recognized to be related to salinity tolerance among different types of plants (Mansour *et al*. 2005). In our experiment, we found that salinity induced the synthesis of free proline in *Z. macrostachya*, which conformed to the remarks by Akram *et al*. (2006) as well as Li *et al*. (2018). The SSC content in plants depends on the balance between carbohydrate consumption and production (Qian and Fu 2005). SSC accumulation in grass tissues can serve as an approach to detect the physiological state of turfgrass under stressful condition (Fu and Dernoeden 2008). According to prior research, the increased SSC level can improve the tolerance to drought and salinity stresses in plants through adjusting osmosis and maintaining turgor within the growing sites in roots and leaves (Streeter *et al*. 2001; Taji *et al*. 2002; Hameed and Ashraf 2008), which are consistent with our results. Betaine, a crucial determining factor of tolerance to salt stress (Zhang *et al*. 2009), participates in decreasing H_2O_2 content, boosting the antioxidant defense mechanism, and enhancing the tolerance to salt stress (Demiral and Turkan 2004; Banu *et al*. 2009). It has been suggested that betaine exerts a crucial role in the tolerance to salt stress (Grumet and Hanson 1986; Lutts and Bouharmont 1996; Liang *et al*. 2009). Our study showed that the betaine content in *Z. macrostachya* increased under various salinity concentrations. Therefore, proline, SSC and betaine accumulation within leaves might be related to salt tolerance in *Z. macrostachya.*

In a saline environment, restricting Na^+ from entering the roots and limiting its transport to the overground parts of plants (shoots and leaves) have been recognized as the crucial mechanism to increase the salinity tolerance in plants (Colmer *et al*. 2005). But this mechanism cannot prevent excessive Na^+ accumulation within roots, leaves and shoots in *Z. macrostachya*. Potassium nutrient, which represents a key factor in the development and growth of plants, has similar ionic radius to that of Na^+, thus there is competitive absorption between K^+ and Na^+ ions in abiotic stress conditions. Therefore, the K^+ absorption capacity and Na^+ transfer suppression (from roots to leaves) are of great importance to the salinity tolerance in plants (Guo *et al*. 2016). Our study showed that treatments under lower salt concentrations (1.0 and 2.0%) increased K^+ absorption and transportation, but treatment under higher salt concentration (4.0%) limited K^+ absorption, without affecting K^+ transportation from roots to shoots as well as leaves. Moreover, K^+/Na^+ ratios in roots, shoots and leaves also proved that salinity stress improved K^+ absorption and limited Na^+ transportation from roots to shoots and leaves. Thus, it was suggested in this study that, K^+ uptake and Na^+ transfer inhibition from roots to shoots and leaves were the crucial mechanisms in the salt tolerance in Z. *macrostachya*.

Salt stress severity would impact biometric response, affect plant growth, and markedly change the biomass (Pompeiano *et al*. 2016). Data from the present experiment showed that 1.0 and 2.0% salinity concentration treatments

improved the root and aboveground growth of Z. *macrostachya*, but the biomass of Z. *macrostachya* decreased under 4.0% salinity concentration. Such finding was ascribed to prior transient impact of osmotic stress following salinity tress. It has been reported that, intermediate salinity stress can boost root growth in bermudagrass, which can be a kind of adaptation to salt stress, finally leading to greater nutrient and water uptake levels (Marcum and Murdoch 1990).

Conclusion

According to our results, salt stress can reduce the RWC level, while increase the H_2O_2 content. In addition, the increased SOD, CAT and POD activities can improve the abilities of *Z. macrostachya* to scavenge ROS, accumulate proline, SSC and betaine, and alleviate the osmotic stress induced by salt stress. At the same time, *Z. macrostachya* can take up more K^+ and inhibit the transfer of Na^+, thus improving the salt tolerance of *Z. macrostachya*. Under the salinity concentrations of 1.0 and 2.0%, the root dry weight and aboveground biomass in *Z. macrostachya* increase, but those are reduced under treatment at 4.0% salinity concentration, indicating that *Z. macrostachya* can endure the 2.0% salinity concentration stress.

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