Kinetics of the antioxidant response to salinity in the halophyte *Limonium bicolor*

Y. Li[1, 2]

[1]*Biology Department, Dezhou University, Dezhou, P.R. China*
[2]*Key Laboratory of Plant Stress Research, College of Life Science, Shandong Normal University, Jinan, P.R. China*

ABSTRACT

The fresh weight (fw) and dry weight (dw) of shoots and roots, the activity of anti-oxidant enzymes [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)], and parameters of oxidative stress of shoots – malondialdehyde (MDA) – were investigated in *Limonium bicolor*, a naturally salt-resistant halophyte. The seedlings of *L. bicolor* were treated with different (0, 100, 200, 400 mmol/l) NaCl concentrations. The results showed that NaCl played an important role in growth of *L. bicolor*. It made obviously promotion of a certain NaCl concentration to growth of *L. bicolor*, the seedlings of *L. bicolor* grew best under 100 mmol/l salt concentration, fresh weight and dry weight reached the maximum. MDA concentration of shoots slightly decreased under 100 mmol/l salt stress, then increased with increased NaCl concentration. The activities of SOD, POD and CAT increased with the increase of the concentration of NaCl in shoots of *L. bicolor*. The salt tolerance of this halophyte under salt stress conditions is probably due to its ability to exhibit high SOD, POD and CAT enzyme activities and low levels of oxidative stress.

Keywords: antioxidative enzymes; salt stress; malondialdehyde; seedlings growth

Soil salinization is one of the major factors responsible for soil degradation. Salinity limits CO_2 assimilation and induces many metabolic changes (Hernández et al. 2000). It also induces oxidative stress, which contributes to its deleterious effects (Hasegawa et al. 2000). Oxidative stress is a central factor in abiotic and biotic stress phenomena that occurs when there is a serious imbalance in any compartment between the production of reactive oxygen species (ROS) and antioxidant defense, leading to dramatic physiological challenges (Foyer and Noctor 2003). Reactive oxygen species have been considered mainly as dangerous molecules, whose concentrations need to be maintained as low as possible, but this concept has changed because of the multiple functions of activated oxygen (Gratao et al. 2005). Many reports have indicated that the negative effect of environmental stresses may be partially due to the generation of ROS and/or inhibition of the system which defends against them. Moreover, lipid peroxidation induced by ROS is considered to be an important mechanism of membrane deterioration (Santos et al. 2001).

Plants protect themselves by scavenging and disposing of these reactive molecules by use of an enzymic and non-enzymic antioxidant system present in several subcellular compartments. When these defenses fail to halt the self-propagating autooxidation with ROS, cell death ultimately results. The primary scavenger is superoxide dismutase (SOD; EC 1.15.1.1), which converts $O^{\cdot}_2^{-}$ to H_2O_2; it is eliminated by peroxidase (POD; EC 1.11.1.11). Hydrogen peroxide is also scavenged by catalase (CAT; EC 1.11.1.6). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Shalata and Tal 1998, Bor et al. 2003). The correlation between antioxidant capacity and salt tolerance was demonstrated in a large number of plants, including salt-tolerant glycophytes and true halophytes, such as *Mesembryanthemum crystallinum* (Broetto et al. 2002), *Lycopersicon pennellii* (Mittova et al. 2000), *Setaria italica* (Sreenivasulu et al. 2000), *Beta maritime* (Bor et al. 2003), *Cassia angustifolia* (Agarwal and Pandey 2004), *Helianthus annuus* (Di Baccio et al. 2004), and *Crithmum maritimum* (Ben Amor et al. 2005).

MATERIAL AND METHODS

Plant materials

Seeds of *Limonium bicolor* were used in this study. Seeds were collected from natural grassland located in the east of Dongying of Shandong province of China. The trial was conducted at Biology Department, Dezhou University, Dezhou, Shandong, China, in 2007. Seeds were sown in plastic pots containing washed sand. Each pot contained 6 seedlings and seedlings were sufficiently watered with ½ Hoagland nutrient solution every day. All pots were placed in a greenhouse. Temperatures during the experiment were in the range of 28–30°C during the day and 19–21°C at night.

Stress treatments

NaCl solutions of 0, 100, 200, and 400 mmol/l were prepared for the salt stress treatment. The seedlings were subjected to stress treatment when they were 6 weeks old. Twelve pots of uniformly growing seedlings were randomly divided into 4 sets, three pots per set. Each pot was considered a single replicate. Each set contained three replicates. One set was used as an untreated control. The remaining 3 sets were treated with different stress treatments. Stress treatments were performed daily at around 5–6 p.m. by thoroughly watering treated plants with 500 ml of treatment solution per pot, in three portions. Control plants were maintained by watering with nutrient solution. On the first day, all pots were treated using the 50 mmol/l treatment solutions. Concentrations of treatment solutions were increased daily by 50 mmol/l increments, as appropriate for sets with higher designated concentrations. As each set reached the designated concentration, that concentration was maintained until the end of the experiment. After the concentrations of the set with the highest concentration, 400 mmol/l, were reached, treatment continued for another 3 weeks.

Physiological indices measurements

All plants were harvested the morning after the final treatment. The plants were first washed with tap water, and then with distilled water. Roots and shoots were separated and the fws were determined for each plant. Portion of the fresh samples were taken to measure the physiological indices. The remainders of the samples were oven-dried at 105°C for 10 min, then vacuum-dried at 80°C to constant weight and the dws were recorded. The extent of lipid peroxidation was estimated by determining MDA formation using the thiobarbituric acid method described by Zhao et al. (1994). Shoot tissues were homogenized under ice-cold conditions (liquid nitrogen) in 50 mmol/l phosphate buffer (pH 7.8), containing 0.1 mmol/l ethylenediaminetetraacetic acid (EDTA), 4% (w/v) polyvinylpolypyrrolidone (PVPP), and 0.3% (v/v) Triton X-100. The homogenate was centrifuged at 14 000 g for 20 min at 4°C. The supernatant was used for assays of enzyme activity.

Superoxide dismutase (EC 1.15.1.1) activity was estimated according to the method of Beauchamp and Fridovich as modified by Giannopolitis. Absorbance was recorded at 560 nm. One unit enzyme activity (U) was defined as the quantity of SOD required to produce a 50% inhibition of reduction of nitroblue tetrazolium (NBT) and the specific enzyme activity was expressed as nmol/mg protein. Activities of CAT (EC 1.11.1.6) were assayed spectrophotometrically according to Chance and Maehly with modifications. One unit of CAT activity is defined as 1 mol of H_2O_2 consumed at 240 nm/g fw/min. Activities of POD (EC 1.11.1.7) were determined spectrophotometrically by measuring the oxidation of guaiacol at 470 nm. One unit of POD activity is defined by the increase in absorbance at 470 nm for 1 min due to guaiacol oxidation as 1 mol/g fw/min. All the experiments were performed at least for three times with three replicates each time.

Statistical data analysis

Statistical analysis of the data, which involved data processing and variance analysis (ANOVA), was performed using the statistical program SPSS 14.0. All the acquired data were represented by an average of the three replicate measurements and standard errors (SE). Significance was tested at the 5% level. The results were equal to the molar concentration of each solute in the living plants.

RESULTS AND DISCUSSION

Effect of salt stress on seedlings growth

The seedlings of *Limonium bicolor* grew best under 100 mmol/l salt stress, fresh weight and dry

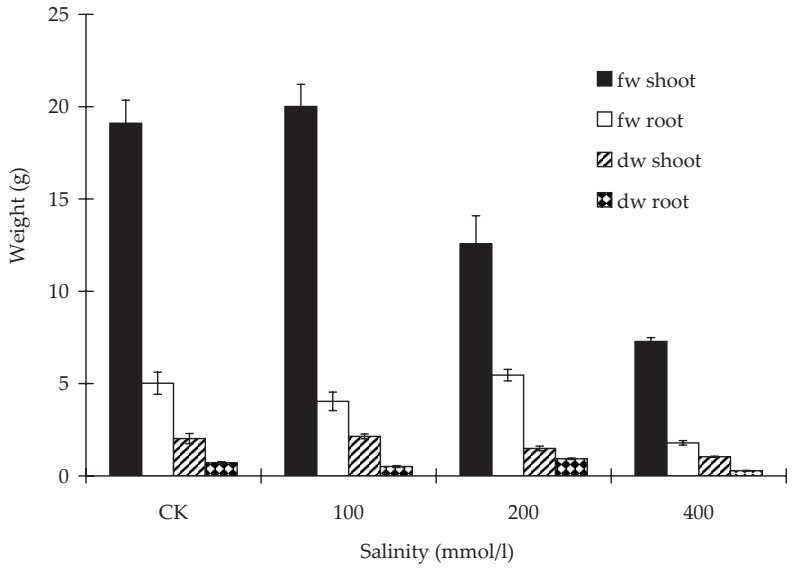

Figure 1. Effect of salt stress on seedlings growth of *Limonium bicolor*. The 6-week-old *L. bicolor* seedlings were treated with salt stress (NaCl solution of 0, 100, 200, and 400 mmol/l) for 21 days. The values are means (±SE) of triplicate samples

weight reached the maximum; they were 104.8% and 105.9% compared with control plants at the end of the experimental period. With increasing salt stress, fw and dw of shoots decreased respectively. Reductions under 200 mmol/l salt stress treatment were 65.8% and 73.8% in fw and dw compared with control plants. The growth of shoots was inhibited significantly under 400 mmol/l salt stress treatment ($P < 0.01$), fw and dw were 38.1% and 51.5% compared with control plants (Figure 1).

There were slight differences in change trend in roots than shoots; fresh weight and dry weight reached the maximum under 200 mmol/l salt stress, they were 108.8% and 131% compared with control plants at the end of the experimental period. The growth of roots was inhibited significantly under 400 mmol/l salt stress treatment ($P < 0.01$), fw and dw were 35.7% and 39.4% compared with control plants (Figure 1).

Effect of salt stress on malondialdehyde

The MDA concentration changed with increasing salt concentration in shoots of *Limonium bicolor*. It decreased slightly and reached 98.03%

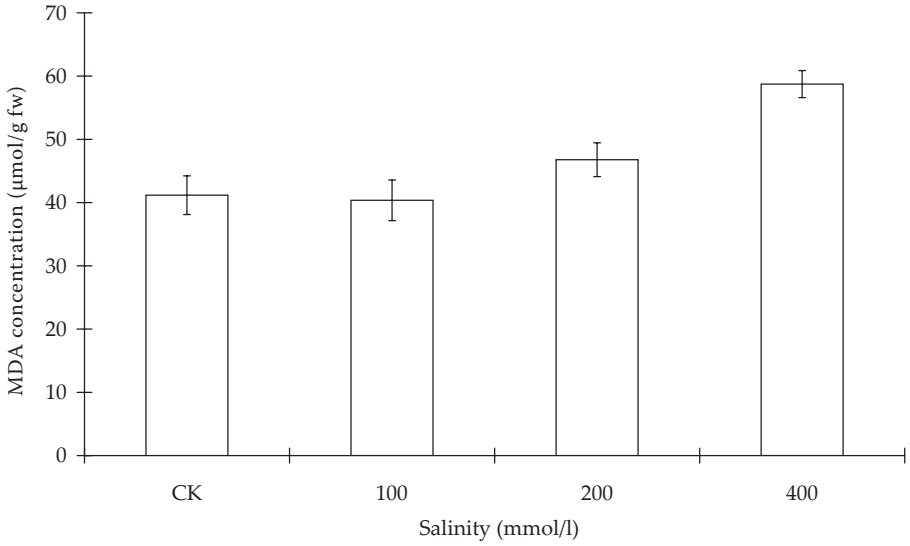

Figure 2. Effect of salt stress on malondialdehyde of *Limonium bicolor*. The 6-week-old *L. bicolor* seedlings were treated with salt stress (NaCl solution of 0, 100, 200, and 400 mmol/l) for 21 days. The values are means (±SE) of triplicate samples

compared with control plants under 100 mmol/l salt stress and it increased to 113.63% compared with control plants under 200 mmol/l salt stress. It significantly increased to 142.68% compared with control plants under 400 mmol/l salt stress ($P > 0.05$) (Figure 2).

Effect of salt stress on antioxidant activities

The activity of SOD increased with the increase of the concentration of NaCl in shoots of *Limonium bicolor*. Reductions under 100, 200, and 400 mmol/l salt stress treatment caused a 31.41% ($P < 0.01$), 43.94%, and 47.88% ($P > 0.05$) increase in SOD activity, respectively compared with control plants at the end of the experimental period (Figure 3). SOD activity in shoots is very important to protect *L. bicolor* from injuries.

The activity of POD increased with the increase of the concentration of NaCl in shoots of *L. bicolor*. Reductions under 100, 200, and 400 mmol/l salt stress treatment caused an 80.30%, 124.03%, and 170.32% increase in POD activity, respectively compared with control plants at the end of the experimental period ($P > 0.05$) (Figure 3). POD activity in shoots is also important to protect *L. bicolor* from injuries.

The activity of CAT increased with the increase of the concentration of NaCl in shoots of *L. bicolor*. It slightly increased and had a 4.62% increase compared with control plants under 100 mmol/l salt stress ($P < 0.001$). Reductions under 200 and 400 mmol/l salt stress treatment caused an 84.71% and 138.75% increase in CAT activity respectively, compared with control plants at the end of the experimental period ($P > 0.05$) (Figure 3).

It is believed that Na^+ is essential element in growth of most halophytes, they cannot grow normally without Na^+ (Zhao and Fan 2000). The results show that NaCl played an important role in growth of *Limonium bicolor*. It made obviously promotion of a certain NaCl concentration to growth of *L. bicolor*, the seedlings of *L. bicolor* grew best under 100 mmol/l salt stress, fresh weight and dry weight reached the maximum.

Lipid peroxidation is the symptom readily ascribed to oxidative damage and is often used as an indicator of oxidative stress (Hernandez et al. 2000). The present results showed it slightly decreased under 100 mmol/l salt stress, then increased in the MDA level of shoots with salt concentration increased. It suggests that *L. bicolor* shoots are better protected from oxidative damage under

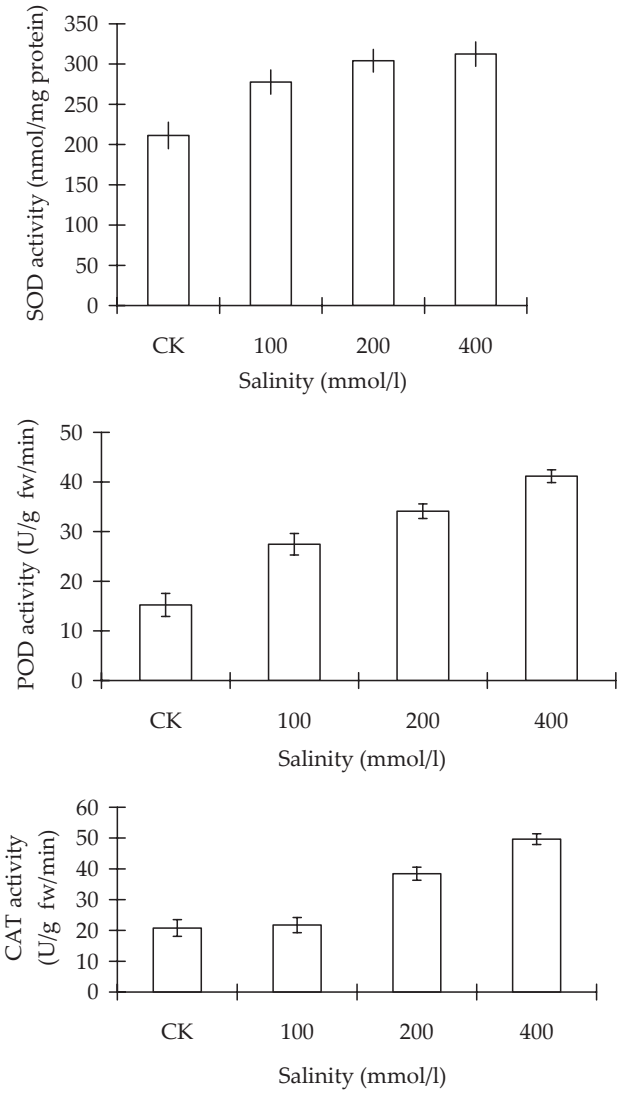

Figure 3. Effect of salt stress on anti-oxidant activities of *Limonum bicolor*. The 6-week-old *L. bicolor* seedlings were treated with salt stress (NaCl solution of 0, 100, 200, and 400 mmol/l) for 21 days. The values are means (±SE) of triplicate samples

salt stress, the concentration of 100 mmol/l NaCl proved to be the best for growth of *L. bicolor* seedling. It is in accordance with the results of SOD, POD and CAT activity in this study. The result is in good agreement with *Suaeda sals*a (Lu et al. 2003).

Salt tolerance is often correlated with a more efficient antioxidative system (Bor et al. 2003). The activities of SOD, POD and CAT increased with the increase of the concentration of NaCl in shoots of *L. bicolor*. The results of the present study also indicated that similar to SOD, POD and CAT activities coordinate with SOD activity to play a central protective role in $O_2^{\cdot -}$ and H_2O_2 scavenging processes at moderate salt concentrations. The activities of antioxidant enzyme

in shoots are important in protecting *L. bicolor* from injuries under salt stress. The specific and salt-dependent changes observed for different antioxidative enzymes and antioxidants showed that salt is abiotic elicitor of phytopathological and antioxidative defenses in *L. bicolor*. The salt tolerance of this halophyte under salt stress condition are probably due to its ability to exhibit high SOD, POD and CAT enzyme activities and low levels of oxidative stress. Little is known about the physiological mechanism of plants resisting to salt stress. *L. bicolor* grows perennially in highly salt-alkalinized habitats, forcing evolution to form special mechanisms for antioxidative enzyme activities under the salt stress.

Acknowledgements

The author sincerely thanks Mrs. Zhang Xia (Key Laboratory of Plant Stress Research, College of Life Science, Shandong Normal University, Jinan, Shandong province, P.R. China) for technical assistance.

REFERENCES

Agarwal S., Pandey V. (2004): Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plant., *48*: 555–560.

Ben Amor N., Ben Hamed K., Debez A., Grignon C., Abdelly C. (2005): Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. Plant Sci., *168*: 889–899.

Bor M.F., Özdemir F., Türkan I. (2003): The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Sci., *164*: 77–84.

Broetto F., Lüttge U., Ratajczak R. (2002): Influence of light intensity and salt-treatment on mode of photosynthesis and enzymes of the antioxidative response system of *Mesembryanthemum crystallinum*. Funct. Plant Biol., *29*: 13–23.

Di Baccio D., Navari-Izzo F., Izzo R. (2004): Seawater irrigation antioxidant defense responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. J. Plant Physiol., *161*: 1359–1366.

Foyer C.H., Noctor G. (2003): Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol. Plant., *119*: 355–364.

Gratao P.L., Polle A., Lea P.J., Azevedo R.A. (2005): Making the life of heavy metal-stressed plants a little easier. Funct. Plant Biol., *32*: 481–494.

Hasegawa P.M., Bressan R.A.P., Zhu J.K., Bohnert H.J. (2000): Plant cellular and molecular responses to high salinity. Plant Mol. Biol., *51*: 463–499.

Hernández J.A., Jiménez A., Mullineaux P., Sevilla F. (2000): Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell Environ., *23*: 853–862.

Lu C.M., Qiu N.W., Wang B.S., Zhang J.H. (2003): Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. J. Exp. Bot., *54*: 851–860.

Mittova V., Volokita M., Guy M., Tal M. (2000): Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii*. Physiol. Plant., *110*: 42–51.

Santos C.L.V., Campos A., Azevedo H., Caldeira G. (2001): *In situ* and *in vitro* senescence induced by KCl stress: Nutritional imbalance, lipid peroxidation and antioxidant metabolism. J. Exp. Bot., *52*: 351–360.

Shalata A., Tal M. (1998): The effect of salt stress on lipid peroxodation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennelli*. Physiol. Plant., *104*: 169–174.

Sreenivasulu N., Grimm B., Wobus U., Weschke W. (2000): Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiol. Plant., *109*: 435–441.

Zhao K.F., Fan H. (2000): Comparative study on osmotica and their contributions to osmotic adjustment in euhalophytes and recretohalophytes. Chin. J. Appl. Environ. Biol., *12*: 99–105.

Zhao S.J., Xu C.C., Zou Q. (1994): Modification of determination method of MDA in plant organization. Plant Physiol. Lett., *300*: 207–210.

Received on March 6, 2008

Corresponding author:

MSc. Yan Li, Dezhou University, Biology Department, 566 Daxue Lane, Dezhou 253023, Shandong Province, P.R. China
phone: + 865 348 982 127, fax: + 865 342 355 088, e-mail: lylxy0524@yahoo.com