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Effects of NaCl Stress on Antioxidative Enzymes of Glycine *Soja sieb*

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Abstract: The activity of anti-oxidant enzymes (Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) and parameters of oxidative stress malondialdehyde (MDA) of shoots were investigated in *S. sieb* naturally salt-resistant halophyte. The seedlings of *S. sieb* were treated with varying (0, 80, 160 and 240 mM) NaCl stress. The results showed that NaCl played an important role in growth of *S. sieb*. It made obviously promotion of certain NaCl concentration to growth of *S. sieb*, the seedlings of *S. sieb* grew best under 80 mM salt stress. MDA concentration of *S. sieb* obviously decreased under 80 mM salt stress then increased with salt concentration increased. The activities of SOD, POD and CAT increased with the increase of the concentration of NaCl in *S. sieb*. 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 Soluble Sugar (SS) concentration.

Key words: Anti-oxidant activities, salt stress, malondialdehyde, soluble sugar

INTRODUCTION

Soil salinization is one of the major factors responsible for soil degradation. Salinity, which is a major constraint to agriculture throughout the world, reduces the ability of plants to absorb water, causing rapid reductions in growth rate, ion imbalance and toxicity. Salinity limits CO₂ assimilation and induces many metabolic changes (Hernandez *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. 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 (Gratão *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. During the reduction of O₂ to H₂O, a transfer of one, two or three electrons to O₂ can occur to form superoxide (O₂^{•-}), hydroxyl radicals (•OH), hydrogen peroxide (H₂O₂) and singlet oxygen (¹O₂). The superoxide radical is produced at the membrane level in most plant cell organelles and hydrogen peroxide is the product of superoxide dismutase and of several oxidases of the peroxisomes. These reactive molecules, especially

•OH, are highly destructive to lipids, nucleic acids and proteins. Nevertheless, ROS such as O₂^{•-} and H₂O₂ are required for lignification and function as signals in the defense response to pathogen infection (Gratão *et al.*, 2005).

ROS are toxic and result in a variety of injuries to plant metabolism. Reactive oxygen species damage photosynthetic components, inactivate proteins and enzymes and permeabilize membranes by causing lipid peroxidation (Meloni *et al.*, 2003). Two facts are known about lipid peroxidation: first, it occurs on polyunsaturated fatty acids located in cell membranes and intracellular organelles that are highly susceptible to reaction with free radicals; second, it proceeds as a radical chain reaction (Djordjevic, 2004). 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 O₂^{•-} to H₂O₂ which 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 anti-oxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage. The correlation between anti-oxidant capacity and salt tolerance has been demonstrated in a large

number of plants, including salt-tolerant glycophytes and true halophytes, such as *Cassia angustifolia* (Agarwal and Pandey, 2004), *Helianthus annuus* L. (Di Baccio *et al.*, 2004) and *Crithmum maritimum* (Ben Amor *et al.*, 2005).

MATERIALS AND METHODS

Plant materials: Seeds of *S. sieb* were used in this study. Seeds were collected from a 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 2008. Seeds were cut and sown in plastic pots containing washed sand. Each pot contained 6 seedlings and seedlings were sufficiently watered with 1/2 Hoagland nutrient solution every day. All pots were placed in 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 solution of 0, 80, 160 and 240 mM 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 the various stress treatments. Stress treatments were performed daily at around 5-6 pm 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 80 mM treatment solutions. Concentrations of treatment solutions were increased daily at 80 mM 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 sets with the highest concentration, 240 mM, 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, then distilled water. A portion of the fresh samples were taken to measure the physiological indices. The contents of total SS were measured using ninhydrin and anthrone. The extent of lipid peroxidation was estimated by determining MDA formation using the thiobarbituric acid method. shoot tissues were homogenized under ice-cold conditions (liquid nitrogen) in 50 mM phosphate buffer (pH 7.8),

containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 4% (w/v) polyvinylpyrrolidone (PVPP) and 0.3% (v/v) Triton X-100. The homogenate was centrifuged at 14000 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 (1971). 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 NBT and the specific enzyme activity was expressed as nmol mg⁻¹ protein. Activities of CAT (EC 1.11.1.6) were assayed spectrophotometrically according to Chance and Maehly (1955) with modifications. One unit of CAT activity is defined as 1 mol of H₂O₂ consumed at 240 nm g⁻¹ FW min⁻¹. Activities of POD (EC 1.11.1.7) was determined spectrophotometrically by measuring the oxidation of guaiacol at 470 nm (Chance and Maehly, 1955). 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⁻¹ FW min⁻¹. All the experiments were performed at least for three times with three replicates each time.

Statistical 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 SE. Significance was tested at the 5% level. The results were equal to the molar concentration of each solute in the living plants.

RESULTS

Effect of salt stress on soluble sugar content: With increasing stress intensity, the SS content increase then decrease, but the SS content is higher at NaCl stress than control. It reached maximum at 80 mmol L⁻¹ NaCl stress (increased 127%, respectively compared with control plants at the end of the experimental period). It increased 35 and 2.5% at 160 mmol L⁻¹ and 240 mM salt stress compared with control plants at the end of the experimental period (Fig. 1). The above results indicated that the SS concentration is important to growth of *S. sieb* under salt stress.

Effect of salt stress on malondialdehyde: The MDA concentration changed with increasing of salt concentration in *S. sieb*, it decreased then increased. It decreased significantly and reached 66% compared with control plants under 80 mM salt stress (p<0.05). It

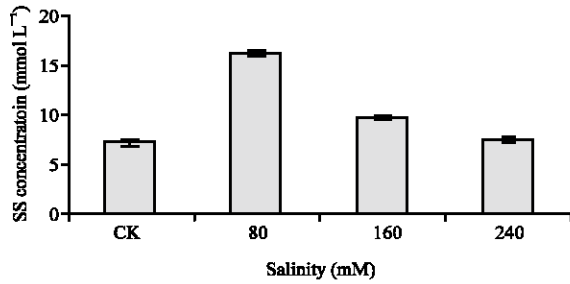


Fig. 1: Effect of salt stress on soluble sugar of *S. sieb*

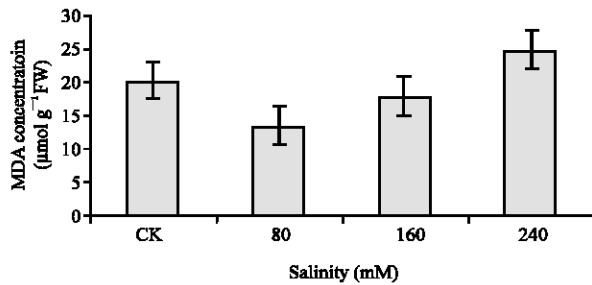


Fig. 2: Effect of salt stress on malondialdehyde of *S. sieb*

decreased to 88% compared with control plants under 160 mM salt stress. It lightly increased to 123% compared with control plants compared with control plants under 240 mM salt stress ($p>0.05$) (Fig. 2).

Effect of salt stress on anti-oxidant activities: The activity of SOD increased then decreased with the increase of the concentration of NaCl in *S. sieb*. Reductions under 80 and 160 mM salt stress treatment caused a 36.41% ($p<0.01$) and 15.68% ($p<0.05$) increase in SOD activity, respectively compared with control plants at the end of the experimental period. It decreased to 82.69% compared with control plants compared with control plants under 240 mM salt stress ($p>0.05$). It is very important of the SOD activity in shoots to protect *S. sieb* from injuries (Fig. 3a).

The activity of POD increased then decreased with the increase of the concentration of NaCl in *S. sieb*, but the activity of POD is higher at NaCl stress than control. Reductions under 80, 160 and 240 mM salt stress treatment caused a 80.30% ($p<0.05$), 45.24% ($p<0.05$) and 12.94% ($p>0.05$) increase in POD activity, respectively compared with control plants at the end of the experimental period. It is very important of the POD activity in shoots to protect *L. bicolor* from injuries (Fig. 3b).

The activity of CAT lightly increased then decreased with the increase of the concentration of NaCl in *S. sieb*.

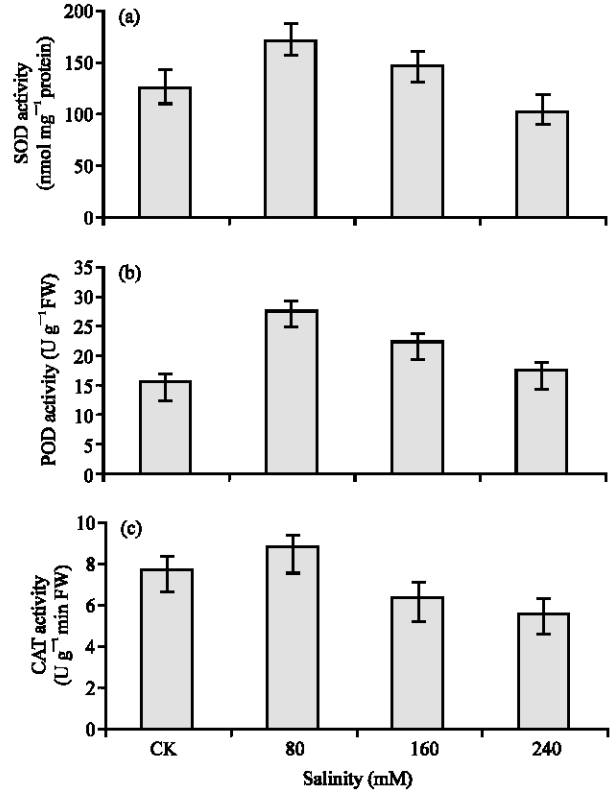


Fig. 3: Effect of salt stress on anti-oxidant activities of *S. sieb*. (a) SOD activity, (b) POD activity and (c) CAT activity

It had a 12.05% increased compared with control plants under 80 mM salt stress ($p>0.05$). Reductions under 160 and 240 mM salt stress treatment caused a 17.70% ($p>0.05$) and 27.44% ($p<0.05$) decrease in CAT activity, respectively compared with control plants at the end of the experimental period (Fig. 3c).

DISCUSSION

The deleterious effects of salt stress result primarily from osmotic stress and ion toxicities (De-Lacerda *et al.*, 2003). People believe that Na⁺ is essential element in growth of most halophyte (Brownell and Crossland, 1972), they can't normal growth without Na⁺ (Zhao and Fan, 2000). The results show that NaCl played an important role in growth of *S. sieb*. It made obviously promotion of certain NaCl concentration to growth of *S. sieb*, the seedlings of *S. sieb* grew best under 80 mM salt stress.

It has been demonstrated that salt treatment increases lipid peroxidation or induces oxidative stress in plants tissues (Hernandez *et al.*, 1994). 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 significantly decreased under 80 mM salt stress then increased in the MDA level with salt concentration increased. It suggest that *S. sieb* are better protected from oxidative damage under salt stress, it was the best of 80 mM NaCl to growth of *S. sieb* seedling. It is agree with the results of SOD, POD and CAT activity in this study. The result is in good agreement with *Suaeda salsa* (Lu *et al.*, 2003).

Salt tolerance is often correlated with a more efficient antioxidative system. The level of responses depends on the species, the development and the metabolic state of the plant, as well as the duration and intensity of the stress. This seems to occur also in *S. sieb*, although the specific regulation of enzyme activities in response to salinity and the interrelationships between them is a complex problem that requires further investigation before it can be elucidated.

The activities of SOD, POD and CAT increased with the increase of the concentration of NaCl in *S. sieb*. 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 $\cdot\text{O}_2^-$ and H_2O_2 scavenging processes at moderate concentrations of salt. It is very important of the activities of antioxidant enzyme in plants to protect *S. sieb* from injuries under salt stress. The specific and salt-dependent changes observed for the different anti-oxidative enzymes and anti-oxidants showed that salt abiotic elicitor of phytopathological and anti-oxidative defenses in *S. sieb*. 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 SS concentration. Little is known about the physiological mechanism of plants resisting salt stress. Understanding the mechanism of native halophytes resisting salt stress is important for the ecological recovery and exploitation of the saltalkalinized soil, discovering natural salt resistant genes and developing salt-resistance biotechnology. *S. sieb* grows perennially in highly saltalkalinized habitats, forcing evolution to form special mechanisms for anti-oxidative enzymes activity under the salt stress.

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