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Antioxidant Responses of Two Barley Varieties to Saline Stress

F. Khosravinejad, R. Heydari and T. Farboodnia
Department of Biology, Faculty of Science, University of Urmia, Iran

Abstract: In this study, we investigated antioxidant responses of activities of Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX) and Guaiacol Peroxidase (GPX) to saline stress in two barley varieties named *Hordeum vulgare* L. var. Afzal and var. EMB82-12 treated with 50, 100, 200, 300 and 400 mM NaCl for 3 days. The MDA content of Afzal plants grown under different salt regimes remained nearly constant but it largely increased in EMB82-12 plants under the same conditions. There was a linear and significant correlation in CAT, APX, SOD, GPX activities in Afzal plants in response to increased salt concentration. The strong and positive correlation between antioxidant enzymes and salt concentrations, may account for the MDA level of Afzal plants remaining constant in response to different salt regimes. In general, the activities of antioxidant enzymes were increased in the root and shoot under saline stress. But the increase was more significant and consistent in the root. Among the antioxidant enzymes, CAT activity was increased the most drastically.

Key words: Antioxidant enzymes, salinity, barley, oxidative stress

INTRODUCTION

Increased use of fertile agricultural lands for human activities other than crop production pushes crop cultivation to less productive lands, including saline areas. Plants in saline areas are easily exposed to multiple abiotic stresses. Among these stresses, high salinity is the most severe factor limiting plant growth in the areas. Equipped with an array of enzymatic and non-enzymatic antioxidant molecules to alleviate cellular damage caused by Reactive Oxygen Species (ROS) (Foyer and Noctor, 2000; Apel and Hirt, 2004). Multiple antioxidant enzymes systems are involved in the enzymatic scavenging of ROS. Superoxide Dismutases (SOD) react with the superoxide radical to produce H_2O_2. Hydrogen peroxide is scavenged by Catalases (CAT). Among peroxidases, Ascorbate Peroxidases (APX) and Guaiacol Peroxidase (GPX) which use ascorbate and a guaiacol electron donors, respectively, are well known for their role in H_2O_2 detoxification in plants. A large body of evidence has shown that the antioxidant enzyme systems are altered under abiotic stresses, including salinity. The quantitative and qualitative aspects of changes are often related to the levels of resistance to salinity. In rice, the salt-tolerant varieties have higher SOD activity and lower lipid peroxidation than the salt-sensitive varieties (Dionisio-Sese and Tobita, 1998). In tomato and citrus, salt-tolerance is attributed to the increased activities of SOD, APX and CAT (Gueta-Dahan *et al*., 1997; Mittova *et al*., 2004). Further supporting evidence on the involvement of antioxidant enzymes in salt tolerance has been provided by transgenic plants with a reduced or an increased expression of antioxidant enzymes. The antisense plants with reduced CAT activity are hypersensitive to salt and other oxidative stresses (Willekens *et al*., 1997). Increased protection to salt stress has been demonstrated by the overexpression of cytosolic APX (Torsethaugen *et al*., 1997). Enhanced oxidative stress tolerance was also observed in the plants overexpressing Fe-SOD (Van-Camp *et al*., 1996). Barley is relatively tolerant to salt stress compared to other crop plants and considered to be used at the early stage of cultivation trial of newly acclimated tideland. Even though much supporting evidence on the role of antioxidant enzymes in salt tolerance is available, there is little information on barley antioxidant enzymes under salinity stress.

MATERIALS AND METHODS

This study was conducted at Biochemistry Laboratory, Department of Biology, Urmia University, Iran, during the winter of 2007.

Plant materials and growth conditions: Two genotypes of Barley (*Hordeum vulgare* L.) were used: var. Afzal and var. EMB82-12 which was obtained from the Agricultural Research Center of West Azerbaijan, Iran. Seeds were

Corresponding Author: Fariba Khosravinejad, Department of Biology, Faculty of Science, University of Urmia, Urmia, Iran

surface sterilized in 0.5% sodium hypochloride solution for 20 min and grown in pots containing Vermiculite. Plants were watered every second day using half strength of Hoagland nutrient solution in controlled growth room for 4 days (Hoagland and Arnon, 1938), then seedlings were subjected to treatment with 50, 100, 200, 300 and 400 mM NaCl for 3 days. Leaves and roots to be used for biochemical determinations were frozen and stored in liquid nitrogen immediately after harvest until enzyme extraction.

Measurement of MDA content: MDA content was measured using a 2-thiobarbituric acid reaction (Heath and Packer, 1968). Thus 1 g of fresh tissue was homogenized in 5 mL of 5% (w/v) trichloroacetic acid and the homogenate was centrifuged at 10000 g fr 15 min at room temperature. The supernatant was mixed with an equal volume of 2-thiobarbutic acid (0.5% in 20% (w/v) trichloroacetic acid) and the mixture was boiled for 25 min at 95°C, followed by centrifugation for 5 min at 7500 g to clarify the solution. Absorbance of the supernatant was measured at 532 nm.

Determination of enzyme activity: The activities of APX, CAT, GPX and SOD were determined spectrophotometrically. APX activity was determined following the oxidation of ascorbate to dehydroascorbate, as described by Asada and Chen (1989). CAT activity was assayed by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules (Beers and Sizer, 1952). GPX activity was determined following the oxidation of guaiacol, as described by Upadhaya *et al.* (1985). SOD activity was assayed by determining the inhibition rate of nitroblue tetrazolium reduction with xanthine oxidase as a hydrogen peroxide generating agent (Obeley and Spitz, 1984). Assays were conducted for the four-replicated treatments and the enzyme activity and MDA content data were analyzed using the SPSS program.

Statistical analyses: Mean values were calculated from measurements of four replicates and the SE of the means were determined. One-way ANOVA and Tukey HSDs multiple range test ($p<0.05$) was applied to determine the significance of the result between different treatments. All statistical analyses were done using the statistical Package for Social Sciences (SPSS) for Windows (version 13.0.0).

RESULTS

Effect of salt stress on lipid peroxidation (MDA content): MDA is one of the end products which is produced as a result of lipid peroxidation damage by free radicals. In two varieties, MDA content significantly increased about 4 times in response to 400 mM NaCl compared to control (Fig. 1). MDA content of Afzal plants increased more than it of EMB82-12 plants grown at same NaCl levels. In two varieties, MDA content was in roots more than shoot.

Effect of salt stress on enzymes activities: Under saline stress, protective enzymes (APX, CAT, GPX and SOD) in barely roots and shoots increased in both varieties. APX activity increased in response to increasing NaCl concentration of soil in two varieties and its activity was 4-folds higher in presence of 400 mM NaCl compared to control (Fig. 2). APX activity in Afzal plants increased

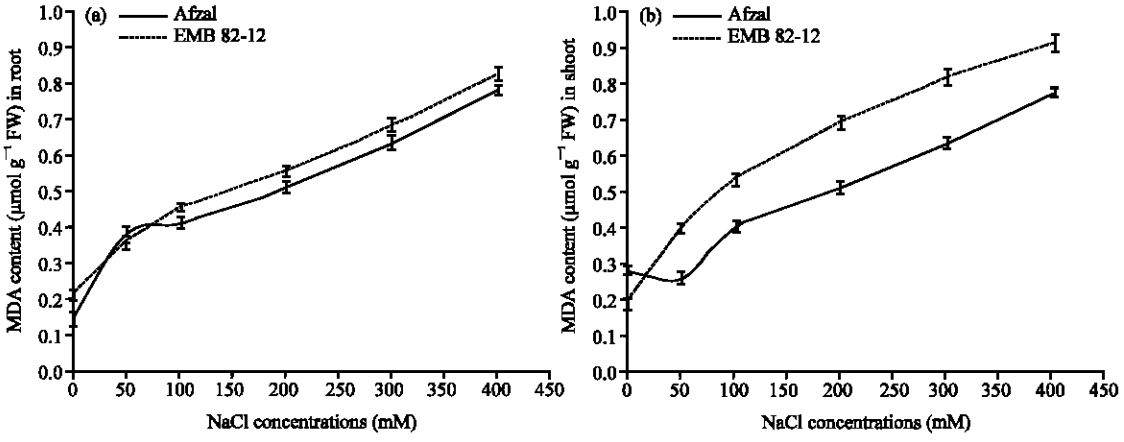

Fig. 1: Effect of different NaCl concentrations on lipid peroxidation (MDA content) in roots (a) and shoots (b) of two barely cultivars. Results are shown as mean±standard error ($p<0.05$), obtained from four replicates

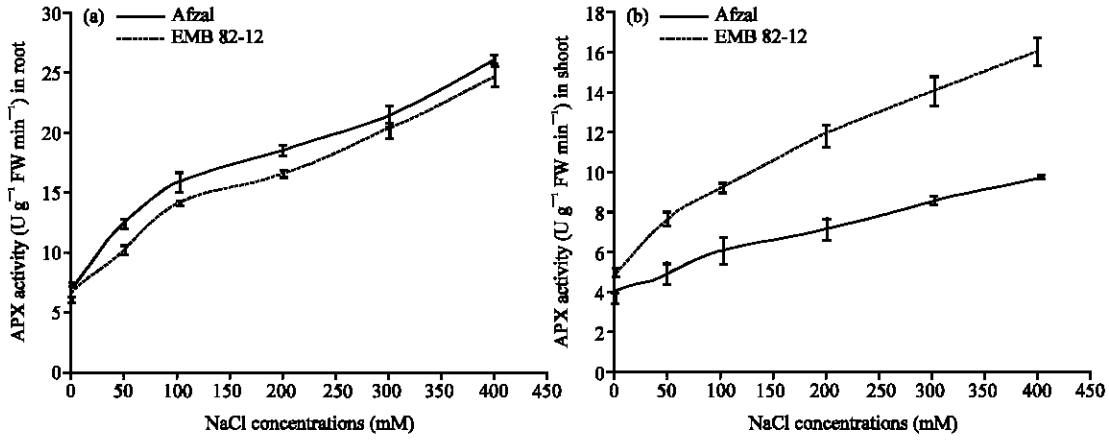

Fig. 2: Effect of different NaCl concentrations on APX activity in roots (a) and shoots (b) of two barely cultivars. Results are shown as mean±standard error ($p<0.05$), obtained from four replicates

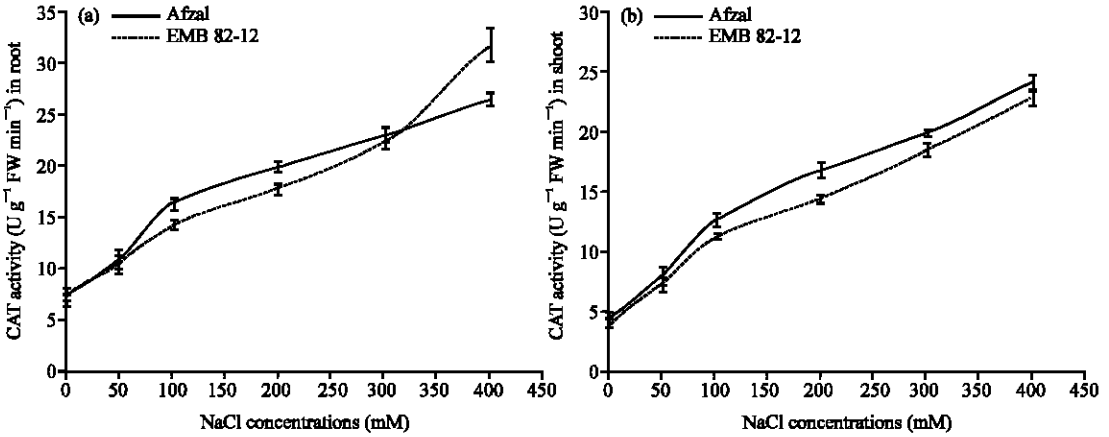

Fig. 3: Effect of different NaCl concentrations on CAT activity in roots (a) and shoots (b) of two barely cultivars. Results are shown as mean±standard error ($p<0.05$), obtained from four replicates

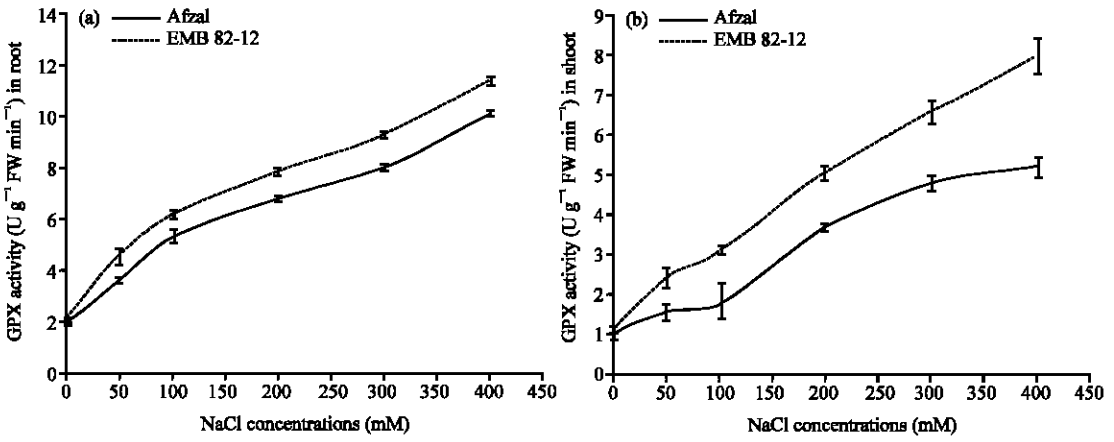

Fig. 4: Effect of different NaCl concentrations on GPX activity in roots (a) and shoots (b) of two barely cultivars. Results are shown as mean±standard error ($p<0.05$), obtained from four replicates

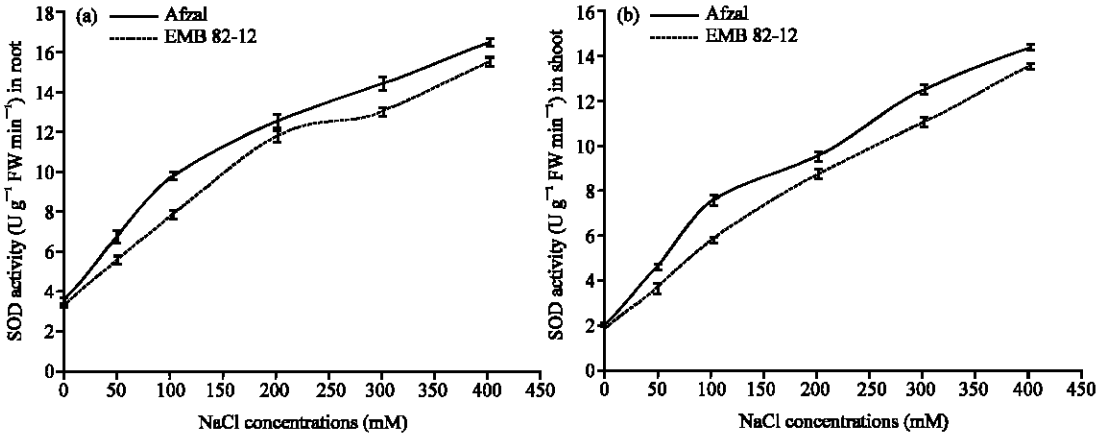

Fig. 5: Effect of different NaCl concentrations on SOD activity in roots (a) and shoots (b) of two barely cultivars. Results are shown as mean±standard error ($p<0.05$), obtained from four replicates

more than that in EMB82-12 plants in the various NaCl concentrations. The activity in the root was more than that in the shoot.

CAT activity in Afzal plants significantly increased as increased in NaCl concentration and plants grown at 400 mM NaCl showed greater CAT activity (more than 5-times increased compared to control) and the increase was same in shoot and root (Fig. 3). In EMB82-12 plants CAT activity increased following various NaCl treatment. CAT activity was increased in the root and shoot by the NaCl treatment, but the activity in the root was about 2.5-times higher than that in the shoot.

In two varieties, GPX activity increased in response to increasing NaCl concentration, but the increase was greater in Afzal plants. GPX activity was increased significantly in the root and shoot by NaCl treatment (Fig. 4).

SOD activity increased significantly in both varieties in response to 400 mM NaCl and the increase was greater in Afzal (less than 7-times in Afzal and more than 6-times in EMB82-12). SOD activity was increased in the root and shoot under salt stress, but its response patterns were variable in the tissues. The activity was in roots more than shoot (Fig. 5).

DISCUSSION

The activities of barley antioxidant enzymes in both varieties were increased in the root and shoot under the NaCl stress. But the increase was more significant and consistent in the root (Sang Yong *et al*., 2005). The activities of SOD, CAT, APX and GPX were increased significantly in the root in response to increasing NaCl stress, indicating rapid responses of antioxidant enzymes to salt stress in barley roots. In glycophytes, the root is the primary site of salt stress and the ability to maintain ion homeostasis and redox potential is critical for the normal root growth and function under saline stress and often related to salinity resistance (Greenway and Munns, 1980; Hasegawa *et al*., 2000). The observations also strongly imply a possibility that antioxidant enzyme systems are also utilized in barley to alleviate oxidative stress caused by salinity, thus protecting the cells from oxidative damage. Detoxification of excess ROS produced during stress is important to reduce ROS-induced membrane lipid peroxidation, enzyme inhibition and nucleic acid damage (Mittler, 2002). Enzymatic scavenging of ROS could be efficiently achieved through the complex, but elaborate coordination among the enzymes involved (Foyer and Noctor, 2000; Apel and Hirt, 2004). The increased activities of the antioxidant enzymes upon salt stress are often related to the enhanced tolerance to salt stress (Gueta-Dahan *et al*., 1997; Mittova *et al*., 2004). Furthermore, each enzyme showed specific quantitative and qualitative responses under salt stress. The major ROS scavenging mechanisms of plants include SOD, APX and CAT (Mittler, 2002). Increase of APX activity was relatively low compared with that of CAT in the NaCl-treated barley root (Fig. 4, 5). A rapid and continued increase in CAT activity might indicate that CAT is a major enzyme detoxifying hydrogen peroxide in barley under salt stress. Since ROS are produced through the multiple pathways including the SOD reaction under salt stress, over 2-fold higher increase in CAT activity than in SOD could better contribute in maintaining steady-state levels of cellular hydrogen peroxide. Also, direct evidence from CAT-deficient mutant barley demonstrates the essential role of CAT in stress resistance of barley. The CAT-deficient barley plants develop severe necrotic lesions and barely survive field stress conditions (Acevedo *et al*., 2001).

Based on the presents results, it appears that plants of the Afzal variety have a better tolerance to salinity as compared to EMB82-12 variety.

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