Bulgarian Journal of Agricultural Science, 20 (No 4) 2014, 883-887
Agricultural Academy

EFFECTS OF SALINITY ON ANTIOXIDANT ENZYMES AND PROLINE IN LEAVES OF BARLEY SEEDLINGS IN DIFFERENT GROWTH STAGES

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Abstract

TURKYILMAZ UNAL, B., L. Y. AKTAS and A. GUVEN, 2014. Effects of salinity on antioxidant enzymes and proline in leaves of barley seedlings in different growth stages. *Bulg. J. Agric. Sci.,* 20: 883-887

The participation of antioxidant defence under mild and severe salt stress conditions (120 and 240 mM) on barley (*Hordeum vulgare* L.) seedlings in different growth stages, antioxidant enzyme (superoxide dismutase, peroxidase and catalase) activities and proline content were determined. Plants grown in Hoagland solution served as control. Salinity induced proline accumulation in both 2- and 4-week-old-seedlings (up to 8.7-fold and 1.8 fold, respectively, as compared with control). The activities of antioxidant enzymes in leaves increased under NaCl stress, the seedlings in the early growing stage (2-week-old) being generally more responsive than 4-week-old ones. The highest peroxidase (POX) and superoxide dismutase (SOD) activities were 165 and 152 % of control, respectively. Catalase (CAT) activity reached about 7- fold increase in NaCl treatment of 2-week-old seedlings. This dramatic increase might indicate that CAT is a major enzyme among antioxidant enzymes examined in barley under salt stress. Thus, antioxidant defence system induced by salinity plays prominent role particularly in early growth periods and its efficiency decrease with age of the plants.

Key words: barley, catalase, *Hordeum vulgare*, NaCl, peroxidase, proline, superoxide dismutase

Introduction

About one-third of irrigated land is considered to be affected by salinity (Flowers and Yeo, 1997) and expanding salinization is posing a greater threat in the world (Xiaoli et al., 2009; Kausar et al., 2013). In Turkey, salinity and sodicity were detected on 1 518 722 ha of the land resource, indicating saline soils constitute a large part of the barren lands (74%) (Kendirli et al., 2005). Salinity represents one of the most important environmental stresses since it limits crop production.

Salinity can be alleviated through either soil reclamation or growing tolerant crops. In plant breeding studies, the use of some physiological and biochemical markers for improving the salt tolerance in plants is crucial. Antioxidant enzymes are related to the tolerance to various abiotic stresses including salinity. In barley, the salt tolerant varieties have higher antioxidant enzyme activities than the salt sensitive varieties (Xiaoli et al., 2009). Also, proline accumulation is one of the common characteristics in many monocotyledons under saline conditions (Tani and Sasakawa, 2006; Ashraf and Foolad, 2007).

Barley (*Hordeum vulgare* L.) is rated as salt tolerant among the crop plants; however, a great genetic variation exists for salt tolerance in its cultivars (Naseer, 2001; Madidi et al., 2004; Khosravinejad et al., 2008; Mahmood, 2011).

This study was designed to explore the participation of antioxidant defence under mild and severe salt stress conditions (120 and 240 mM) on barley (*Hordeum vulgare* L.) seedlings in different growth stages by measuring antioxidant enzyme (superoxide dismutase, peroxidase and catalase) activities and proline content.

Material and Methods

Salt tolerant cultivars (Anadolu 98, Efes 3, F1Gem, Suleyman Bey and Vamik Hoca) of barley (*Hordeum vulgare* L.) were chosen for this study based on data from previous work (Turkyilmaz et al., 2011). Seeds were sown in pots (20 x 30 cm^2, 50 seeds for each) filled with *perlite* and grown under controlled conditions (16-h photoperiod, irradiance at leaf level 350 $\mu mol\ m^{-2}\ s^{-1}$, temperature 19±1°C, relative humidity of 60-70%). After germination, seedlings were sup-

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plied every week with Hoagland nutrient solution added with 120 and 240 mM NaCl and irrigated with distilled water at two-day-intervals. Plants grown in Hoagland solution served as control. Seedlings were harvested reaching two- or four-week-old stage.

Proline content was determined according to the modified method of Bates et al. (1973). The concentration was calculated from a proline standard curve and expressed as µmol/g FW.

Harvested fresh leaf samples were frozen in liquid nitrogen. Leaves were homogenized in 0.05 M Na phosphate buffer pH 7.8 including 1 mM EDTA and 0.2 g Dowex 1 x 8 (200x 400 mesh). Homogenates were centrifuged and supernatants were used for enzyme activity and protein content assays. Total soluble protein content was determined according to Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase activity (SOD) assay was based on the method of Beauchamp and Fridovich (1971), which measures the inhibition in the photochemical reduction of nitroblue tetrazolium chloride (NBT) spectrophotometrically at 560 nm. Peroxidase activity (POX) was determined according to Herzog and Fahimi (1973) using diaminobenzidine tetrahydrochloride dihydrate (DAB) as a substrate. Catalase activity (CAT) was estimated according to the method of Bergmeyer (1970) by the determination of the destroying of H_2O_2, measuring the decrease of the absorbance at 240 nm.

The mean values were calculated from two independent experiments, each with four replicates. Experimental data were analyzed with Tukey test at $p<0.05$ level. Standard errors (±) are calculated.

Results and Discussion

Plant response to NaCl stress is a quite complex phenomenon comprising the changes from morphology to metabolism, depending on several factors such as intensity of the stress, developmental stage of plant and tolerance potential.

Salinity exposure was very effective in proline accumulation in leaves of many crop seedlings (Amirjani, 2010) including barley (Pirasteh-Anosheh et al., 2014). NaCl treatment caused a massive accumulation of proline in the leaves parallel to the rise of NaCl concentrations in 2-week-old- seedlings reaching up to an 8.7-fold increase in Efes 3 cultivar compared with control (Table 1), whereas in 4-week-old-seedlings NaCl caused a less pronounced proline accumulation in all cultivars. Shevyakova et al. (2009) suggest that NaCl- and paraquat-induced accumulation of proline had both osmoprotective and antioxidant functions. Proline ability to quench reactive oxygen species particularly OH^- has convincingly been demonstrated (Signorelli et al., 2013). Our data also showed that induced proline accumulation co-occurred with higher activities of antioxidant enzymes in younger seedlings proving the proline role in activation of antioxidant defence in plants (Rejeb et al., 2014). Proline implication in protection of protein integrity (Szabados and Savoure, 2009) may contribute to ability of seedlings to survive in early growth stage under high saline conditions.

The activities of antioxidant enzymes of the five barley cultivars were increased in leaves under NaCl stress (Figures 1, 2 and 3). The responses of SOD, POX and CAT were particularly significant in the leaves of two-week-old-seedlings, indicating a high defence capability of antioxidant enzymes to salt stress at the early growth stage of barley. The highest rate of SOD activity was measured in 240 mM NaCl treated 2-week-old Anadolu 98 (about 52% increases as compared with control). SOD activity was not significantly altered in 4-week-old-seedlings (Figure 1).

POX activity was almost the same in control groups of two- and four-week-old-seedlings of Anadolu 98, Efes 3, F1 Gem and Vamik Hoca cultivars and slightly different in

Table 1
Proline content (µmol g^{-1} fresh weight) of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl

Growth stage	NaCl, mM	Anadolu 98	Efes 3	F1 Gem	Suleyman Bey	Vamik Hoca
2-week-old	0	0.36±0.01^a	0.26±0.01^a	0.44±0.01^a	0.42±0.06^a	0.45±0.05^a
	120	1.47±0.21^b	1.62±0.19^b	1.42±0.16^b	1.73±0.09^b	1.22±0.05^b
	240	2.20±0.03^c	2.26±0.72^b	1.81±0.12^c	1.73±0.17^b	1.19±0.17^b
4-week-old	0	0.99±0.05^a	0.85±0.08^a	0.91±0.05^a	0.99±0.08^a	0.94±0.07^a
	120	0.94±0.11^a	1.34±0.74^a	1.26±0.03^{ab}	1.06±0.19^a	0.95±0.08^a
	240	1.78±0.25^b	1.40±0.33^a	1.61±0.37^b	1.47±0.55^a	0.99±0.04^a

The mean values were calculated from two independent experiments, each with four replicates. Standard errors (±) are indicated. Different subcript letters indicate significant differences ($p<0.05$)

Suleyman Bey (Figure 2). Two-week-old-barley seedlings showed significant increases in POX activity with 240 mM salinity treatment except Suleyman Bey. Although significant increase (65%) was measured in the enzyme activity of

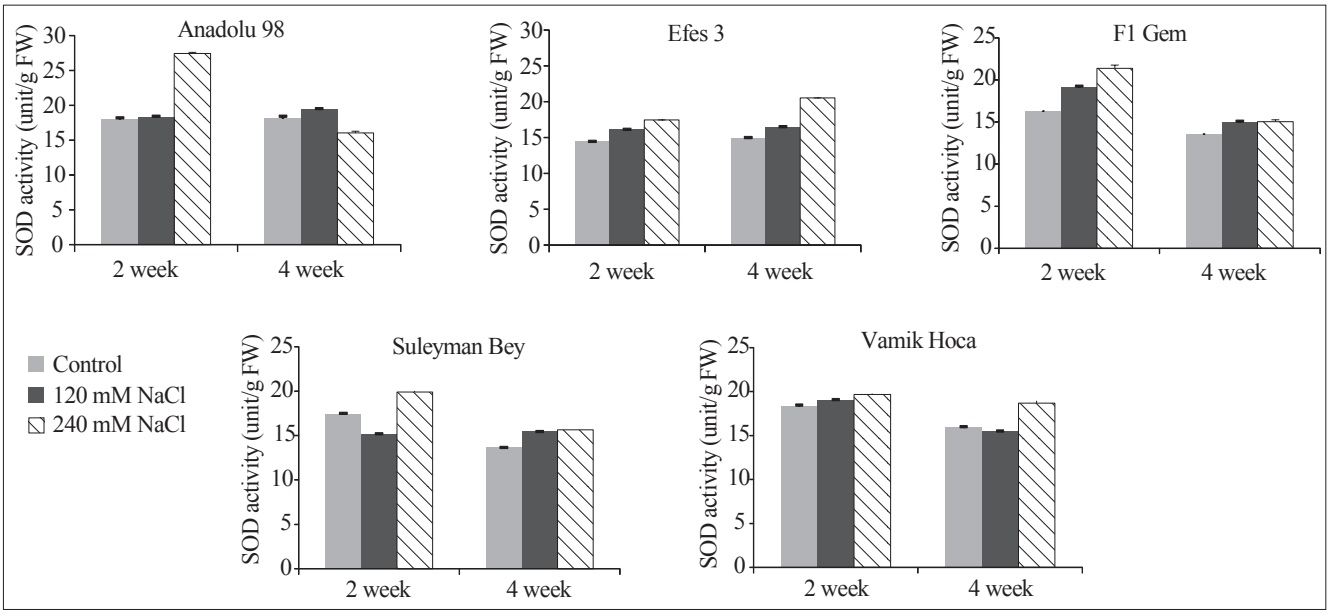

Fig. 1. Superoxide dismutase (SOD) activity of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl.

The mean values were calculated from two independent experiments, each with four replicates. Vertical bars indicate ± SE.

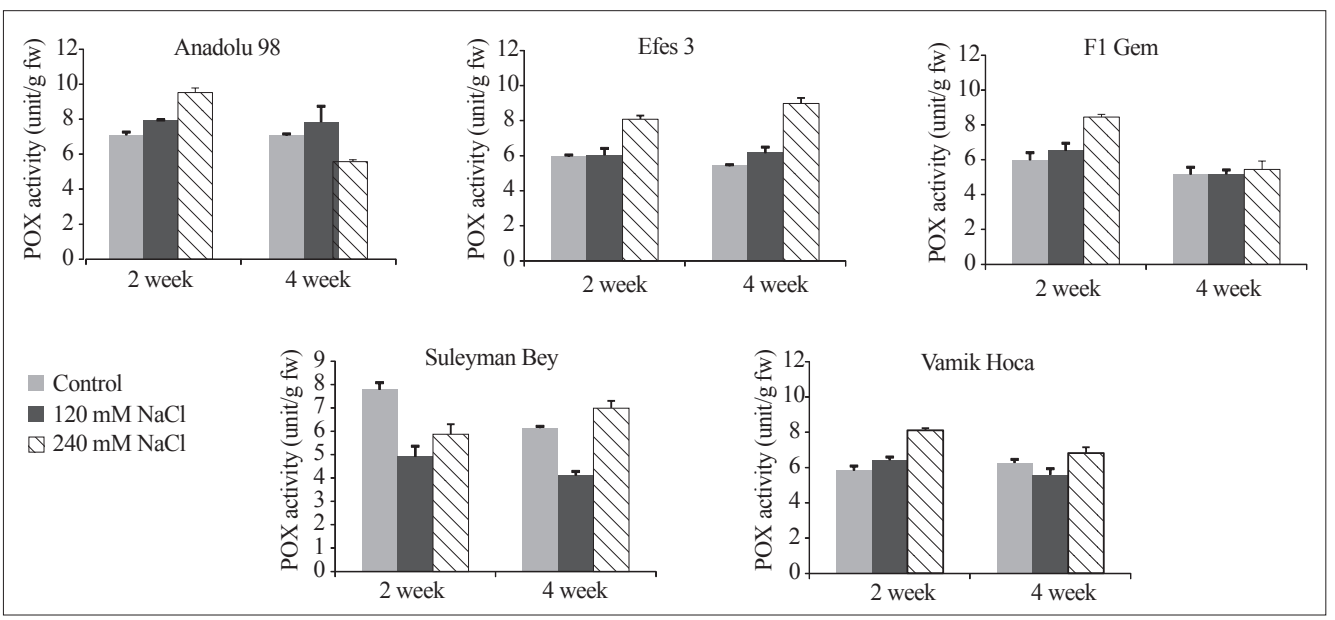

Fig. 2. Peroxidase (POX) activity of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl.

The mean values were calculated from two independent experiments, each with four replicates. Vertical bars indicate ± SE.

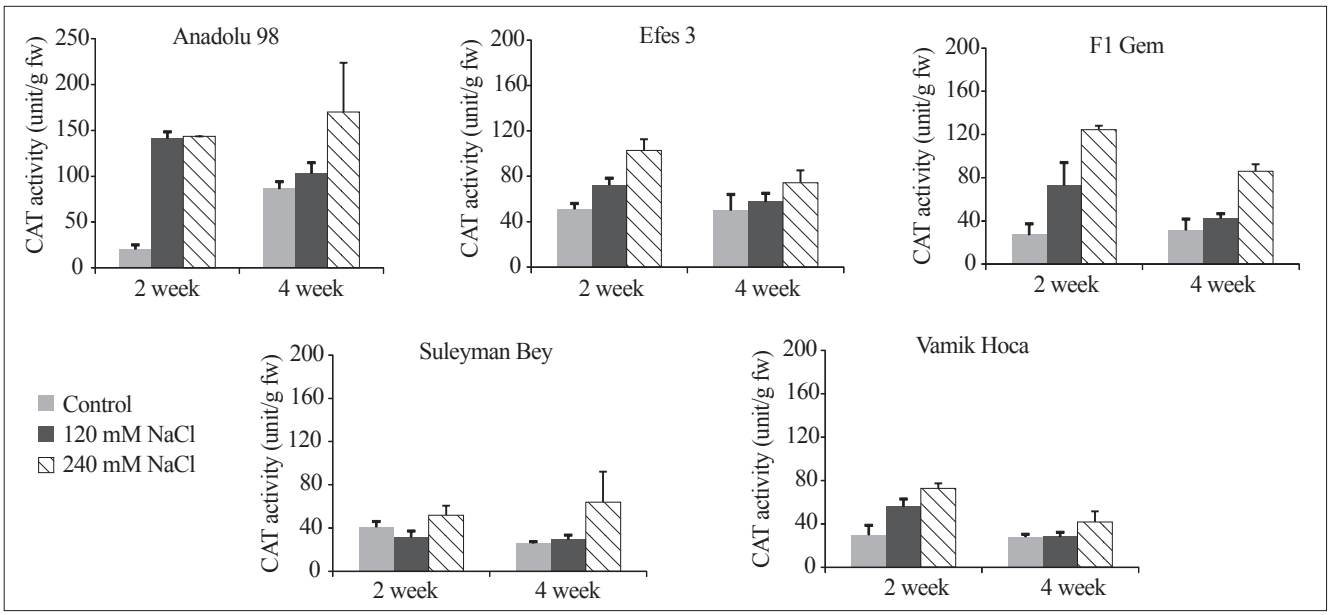

Fig. 3. Catalase (CAT) activity of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl.

The mean values were calculated from two independent experiments, each with four replicates. Vertical bars indicate ± SE.

the leaves of 240 mM NaCl treated four-week-old-seedlings of Efes 3, other cultivars exhibited slight and generally insignificant changes.

Another scavenger of H_2O_2, CAT activity, increased by increasing salt concentration. Especially in the early growth period changes were very dramatic reaching about 7-fold increase compared with control in Anadolu 98 cultivar in both NaCl concentrations (Figure 3). In four-week-old-barley seedlings, CAT activity was affected significantly only in 240 mM NaCl treatment. In this group, salinity caused significant changes in the activity of the cultivars Anadolu 98, Suleyman Bey and F1 Gem exposed to 240 mM NaCl concentration. The CAT activity of control groups was not changed during the growth period in Efes 3, F1 Gem and Vamik Hoca , in contrast with the big differences in Anadolu 98 in which CAT activity increased during the growth period almost 4-fold, whereas in Suleyman Bey a decrease of approximately 37% was observed.

Among the enzymes, CAT showed the highest rate of activity changes under salt stress in early growth period of barley seedlings in accordance with the experiments of Khosravinejad et al. (2008). Increases in SOD and POX activity were relatively low when compared with CAT activity in the leaves of NaCl treated barley cultivars, with this indicating the major role of CAT in the antioxidant defence of barley in salt stress conditions (Dai et al., 2009). While the highest POX activity rate measured was 165% and SOD activity was 152% of the controls, CAT activity increased about 7-fold, this pointing to the CAT contribution in maintaining steady-state levels of cellular hydrogen peroxide.

Conclusion

The results strongly imply a possibility that the antioxidant enzyme system is also utilized in barley to alleviate oxidative stress caused by salinity, thus protecting the cells from oxidative damage (Kim et al., 2005). 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).

Older seedlings of the same barley cultivars were affected more by ion toxicity than by oxidative stress (Turkyilmaz et al., 2011). Also, the idea that long term NaCl exposure causes Na^+ and Cl^- accumulation in older leaves and ion toxicity suggested by Munns (2002), Munns and Tester (2008) and Mane et al. (2011) supports our results. The antioxidant enzymes and proline accumulation undergo dramatic changes under NaCl stress in early growth period in comparison to four-week-old seedlings of five barley cultivars. This might suggest that oxidative stress caused by salinity is operative in early growth periods of barley plants.

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Received February, 26, 2013; accepted for printing December, 2, 2013.