Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 1, p. 350-357, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Changes in activity profile of superoxide dismutase in barley cultivars seedling under salt stress

Mohammad Behrouzi[1*], Mostafa Valizadeh[2], Mohammad Moghaddam Vahed[2]

[1]*Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tabriz University, Tabriz, Iran*

[2]*Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tabriz University, Tabriz, Iran*

Article published on July 26, 2015

Key words: ROS, SOD, Antioxidants, isozyme profile.

Abstract

Salt stress is one of the most important factors limiting barley cultivation in arid and semi arid regions. Meanwhile, salt induced production of reactive oxygen species (ROS) induces the activity of some antioxidant enzymes in order to protect plants against stress condition. This study was aimed to investigate the activity of superoxide dismutase (SOD) in ten Iranian indigenious barley cultivars in the seedling stage. A factorial experiment was conducted using three NaCl levels (0, 100 and 200 mM), in combination with three levels of proline (0, 5 and 10 mM) based on a completely randomized design with three replications. Seedlings shoots in each plot were mixed-harvested. Electrophoretic analyses were performed by using 8% slab polyacrylamide gels. Obtained results for three detected SOD isozymes activities revealed that the difference between barley cultivars were significant. Interaction of salinity × cultivar was also significant for SOD_2. In general, cultivar Torsh due to having more SOD activity could be introduced as the tolerant cultivar to salinity. Finally, it suggests an antioxidant analysis by gel electrophoresis as a useful tool for studying plant's tolerance to salt stress.

***Corresponding Author:** Mohammad Behrouzi ✉ behrouzi.m6880@gmail.com

Introduction

Salinity decreases growth and productivity of plants by reducing water uptake and causing nutrient disorders and ion toxicity. High salinity, mediated by NaCl, frequently constrains the production of most economically important crops in many arid and semi-arid regions in the world (Mourato *et al.*, 2012) and affects the plant growth and development through osmotic stress, specific ion (Na^+) toxicity as well as nutritional imbalance (Bartels and Sunkar, 2005), physiological and biochemical perturbations (Hasegawa *et al.*, 2000) and reduction of photosynthetic capacity (Zhao *et al*, 2007). Excessive presence of ions in the rhizosphere causes irreversible damage to plant roots, followed by their gradual accumulation in the aerial parts which typically culminates in severe interference with plant metabolism and diminished growth and production (Shannon, 1997). Increased production of reactive oxygen species (ROS), is another aspect of salt stress in plants (Panda and Khan, 2004). Based on biochemical and molecular studies, adverse conditions including salinity may lead to over-generation of both free radical including superoxide (O_2•-), hydroxyl (•OH) and molecular forms such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Scandalios, 2005; Gill and Tuteja, 2010). In plants, ROS are formed continuously as necessary accompaniments of various aerobic metabolic pathways in cellular compartments involving in photosynthesis, photorespiration and CO_2 assimilation (Rhoads *et al.*, 2006; Del Rio *et al.*, 2006). Chloroplasts in higher plants and algae, mitochondria and peroxisomes are considered the major sources of ROS (O_2•-, $^1O_2$ and H_2O_2). When applied exogenously, proline improved crop tolerance against salinity and drought by protecting crops against negative effects of ROS (Szabados and Savouré, 2009). When the plant is experiencing stress condition, the production of ROS may exceed the capacity of the plant's defense mechanisms, an imbalance in intracellular ROS content is established, which results in oxidative stress (Gill and Tuteja, 2010), and significant damage to cell structures through oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2002).

The plants' antioxidant defense system includes the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and few others (Apel and Hirt, 2004; Hossain *et al.*, 2011). Proline, an α-amino acid with an exceptional conformational rigidity, can be added to the group of non-enzymatic antioxidants that in different organisms counteract the inhibitory effects of ROS. It is now well established that Proline content increases dramatically following high salinity, drought and heavy metal stress (Yoshiba *et al.*, 1995; Choudhary *et al.*, 2005). In addition, proline proved an effective scavenger of OH and $^1O_2$ under stress conditions (Smirnoff and Cumbes, 1989).

Even though barley is considered a relatively salt-tolerant plant with so much variability among its cultivars, it undergoes a remarkable reduction (about 55%) in biomass when experiencing 150 mol/ m^3 NaCl (Garthwaite *et al.*, 2005). In Iran, located in a semi-arid zone with average annual precipitation less than 220 mm, barley cultivation plays an important role in agriculture as it is grown in many parts of the country for different purposes including livestock feed. So far, many studies have been done on the mechanism of tolerance to salt-induced oxidative stress, both in barley (Seckin *et al.*, 2010; Ahmed *et al.*, 2013) and other crops (Hossain and Fujita, 2010; Nounjana *et al.*, 2012), to identify and develop salt-tolerant, as well as high-yielded genotypes.

Salt stress induces gene expression alteration in a tissue time-related manner, therefore identifying tolerant plants as early as seedling stage of growth using biological and physiological approaches can shorten the selection process through identifying candidate enzymes, and genetic mechanisms involved. Horizontal electrophoresis can separate individual isozymes efficiently as opposed to spectrophotometry, which measures total enzyme activity.

In this article, it investigated the effects of different combination of salinity and proline treatments on SOD antioxidants profile in barley using horizontal electrophoresis as a means to investigate the correlation between salt tolerance and antioxidant enzyme activity.

Materials and methods

Plant growth and NaCl treatment

This experiment was conducted using 10 Iranian germplasm of barley cultivars (*Hordeum vulgare* L.) obtained from the Institute of Research center for Seed and Seedling, in Karag, Iran. These cultivars are as follows: Aras, Bahman, Yusof, Kavir, Sahra, Karoon, Makoii and Nosrat. The experiment was performed in a factorial experiment based on completely randomized design with three replicates. Uniform seeds of cultivars were surface-sterilized in 5% sodium hypochlorite and ethanol, rinsed with water and then planted in disposable plates in Lab condition. For the first 5 days, plants were irrigated with distilled water. After five days, plants were subjected to treatment combination of 0, 100 and 200 mM NaCl and exogenous application of 0, 5 and 10 mM proline, for another five days (Radyukina *et al*., 2008).

Enzyme Extraction and Electrophoresis

For enzyme extraction, the mixed leave samples were homogenized separately with mortar and pestle in a tris extraction buffer pH 7.5 (containing tris 50 mM, sucrose 5%, ascorbic acid 50 mM, sodium metabisulfite 20 mM, PEG 2% and 2ME 0.1% freshly before use (Valizadeh *et al*., 2011)with a ratio of 0.5 mg/µl (1W:2V), then centrifuged at 4°C and 10,000g for 10 minutes.

The supernatants were immediately absorbed onto 3×5 mm wicks cut from Whatman 3 MM filter paper and loaded onto 8% horizontal slab acrylamide gel (0.6×15×10 cm) according to poulik gel buffer (Poulik, 1957) using TBE (Tris-Borate-EDTA) electrode buffer (pH= 8.8).

Electrophoretic separation was performed at 4 °C for 3 hours (constant current of 26 mA, and voltage of 180V).

Staining of SOD isozymes was achieved according to Soltis and Soltis (Soltis and Soltis, 1990). Each experiment was repeated three times. Detected isoforms on each gel were designated numerically, with 1 given to the most anodally migrating isoform and so on.

Statistical Analysis

An image analysis program (MCID Analysis Evaluation 7.0) was used to quantify optical density× area (D×A) parameter for each isozymic band on gels. All the experiments were replicated three times. The significance of differences among the treatment means was determined by ANOVA using the Duncan test. Statistical analysis was performed in SPSS 16.0 software. To demonstrare significant interactions, the charts were drawn using EXCEL software.

Results

The results for banding pattern of SOD is illustrated in Fig. 1. Three unambigous isoforms for SOD, namely SOD_1, SOD_2 and SOD_3 were detected in barley seedling shoots.

Aanalysis of variance for activity of superoxide dismutase isozymes, SOD_1, SOD_2 and SOD_3, in barley is presented in Table 1. Significant differences were observed only for cultivar ($P < 0.01$) in all isozymes and salinity × cultivar interaction ($P < 0.05$) in SOD_2. Other factors and interactions did not showed significant effects.

The difference response of barley cultivars for enzymatic activity of SOD_1 and SOD_2 in our experiment conditions are indicated in Fig. 2. According to Fig. 2 A, the mean comparison of SOD_1 activity in studied cultivars showed that Karoon, Aras and Jonub showed the maximum enzymatic activity respectively and had significantly different activities from other cultivars. The lowest densitimetric

enzymatic activity, however, was detected in Sahra cultivar. Mean activity of cultivars for SOD_3, shown in Fig. 2 B, revealed that Nosrat and Torsh cultivars displayed maximum and minimum enzymatic activity, respectively.

Mean enzymatic activity of SOD_2 for combination of cultivars and salinity treatments is shown in Fig. 3. The observed cultivar × salinity interaction is a change-in-order type. According to Fig. 3 salinity of 100 mM led to a significantly reduction in SOD_2 enzyme activity in Bahman cultivar, and a significantly increase in enzyme activity in Torsh cultivar, compared to control (0 Mm). Salinity of 200 mM treatment in Bahman and Yusof cultivars caused a significantly decrease, but in Makoii, Kavir and Torsh cultivars a significantly increase in SOD_2 activity compared to normal and/or 100 mM salinity conditions. Torsh cultivar, however, showed highest densitometric enzymatic activity under salinity conditions.

Table 1. Analysis of variance for three barley seedling SOD isozymes activities in different levels of NaCl salinity and exogenous proline application.

		Mean squares of enzymatic activity (* 10^{-5})		
S.O.V	df	SOD_1	SOD_2	SOD_3
Cultivar (C)	9	2860.4725**	1.8866**	171.1767**
Salinity (S)	2	192.1637^{ns}	0.1761^{ns}	18.0159^{ns}
Proline (P)	2	44.0971^{ns}	0.2078^{ns}	19.9487^{ns}
C*S	18	66.1565^{ns}	0.5782*	21.8885^{ns}
C*P	18	22.7029^{ns}	0.1402^{ns}	16.0990^{ns}
S*P	4	17.9798^{ns}	0.1827^{ns}	4.2766^{ns}
S*C*P	36	33.7911^{ns}	0.1726^{ns}	15.1276^{ns}
error	180	205.7567	0.2875	23.6592

* and ** stand for significant at probability level of 0.5 and 0.1 present and non-significant, respectively.

Discussion

Exposure of plants to unfavorable environmental conditions, salinity stress among others, can increase the production of radical and non-radical oxygen intermediates known as reactive oxygen species (ROS). To protect themselves against ROS damage, plant cells and its organelles employ some indigenous enzymatic and non-enzymatic antioxidant defense systems (Manchania, 1999). For example, the impact of salinity stress on some wild plant species has been studied recently.

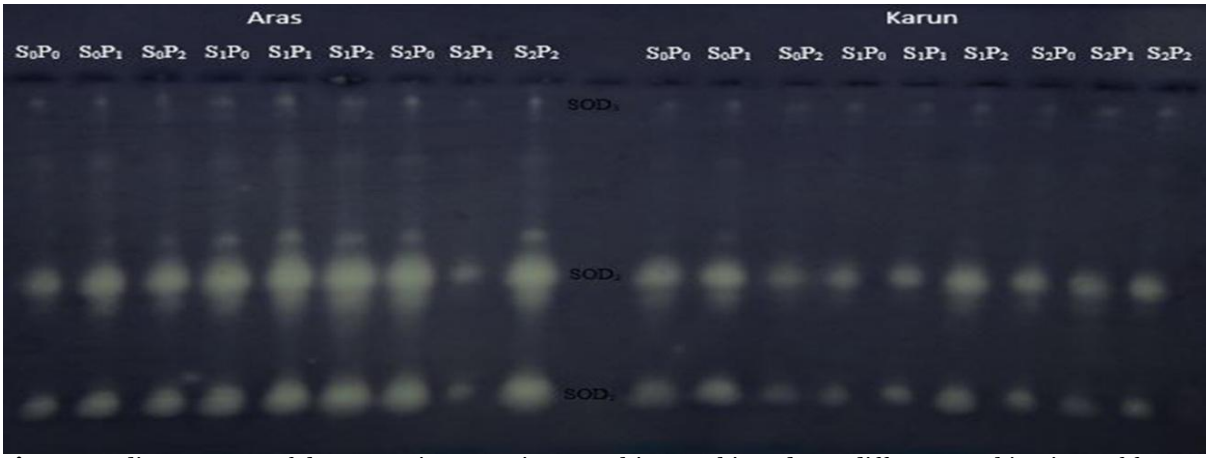

Fig. 1. Banding patterns of three SOD isozymes in two cultivars subjected to 9 different combinations of factors including three levels of salinity (0, 100, and 200 mM presented by S0, S1 and S2) and three levels of proline (0, 5 and 10 mM presented by P0, P1 and P2).

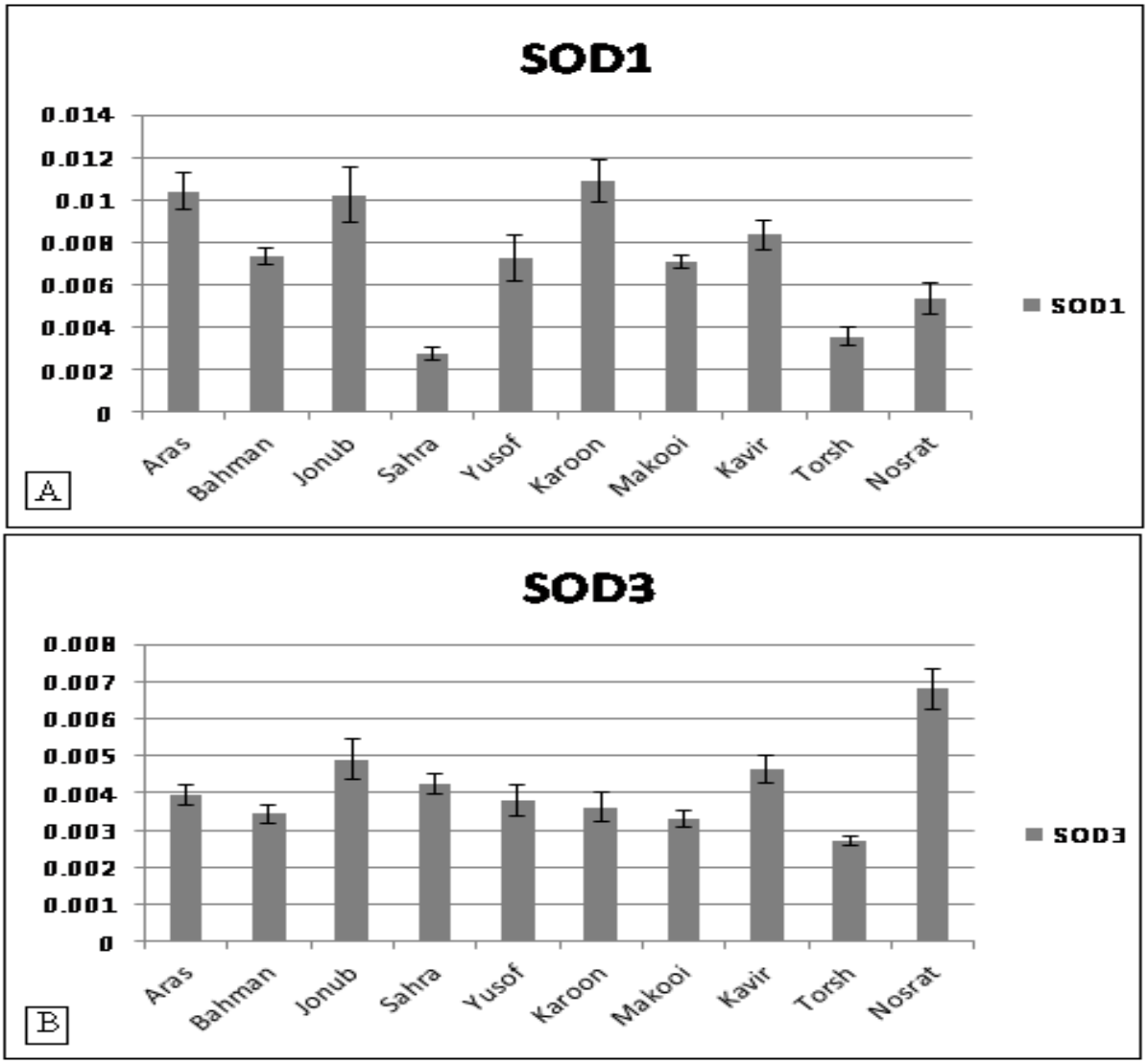

Fig. 2. (A) Mean of SOD1 activity in 10 barley cultivars at seedlind stage, (B) Mean of SOD3 activity in 10 barley cultivars.

The results revealed a significant negative correlation between level of SOD activity and the proline content in cell plant tissues, meaning plants that store a rather high proline have lower SOD activity (Kartashov *et al.*, 2008). More recently researchers reported that the SOD contents in *Thellungiella salsuginea* has dropped due to oxidative stress, in the presence of three levels (0.2, 2 and 5 mM) of proline treatment (Soshinkova *et al.*, 2013). It has also been suggested that antioxidant effect of proline appears only after 12 hours from the treatment, while antioxidant enzymes purge the plant cell of ROS in early stages of damage (Alscher *et al.*, 2002). The researchers postulated that the antioxidant effect of proline *via* ROS detoxification would be attributed to its pyrolic ring as well as much easier transportation inside the plant compared to non-enzyme antioxidants (including acid ascorbic, phenol and tocopherol). According to investigators reports SOD, commonly referred to as the basic defense against ROS, attacks the first radical generated from univalent reduction of oxygen, superoxide (O_2•-) (Alscher *et al.*, 2002). Therefore, lack of sever influence of stress factor on enzyme profile could be likely due to major accumulation of O_2•- during early stages of growth (Bakalova *et al.*, 2004).

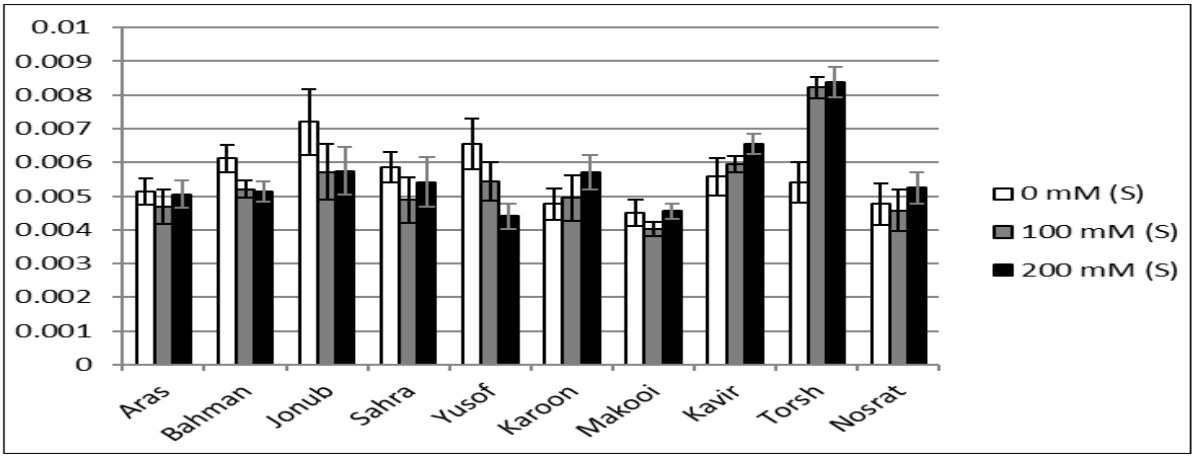

Fig. 3. Average densitometric activities of barley seedling SOD2 in different combination of cultivar and salinity levels.

Plants competency to neutralize oxidative stress-induced damages not only depends on antioxidant defense system, but it can also be a matter of plant genotype (Munns and Tester, 2008), with almost 150 genes involved in the biochemical mechanisms (Mittler *et al.*, 2004). Salt-tolerant barely cultivars show less SOD than POX activity compared to drought sensitive ones (Bakalova *et al.*, 2004); Contrary to tolerant plants, sensitive cultivars possess less effective mechanism to scavenge $O_2^{\bullet-}$ under stress condition (Salek jalali *et al.*, 2012). Ahemd *et al.*, 2013 reported that salinity stress could significantly increase SOD activity, whereas they found no significant change in POX activity. They concluded that higher tolerance to salt stress was closely related to antioxidant defence system in scavenging ROS (Ahmed *et al.*, 2013). In the present study, in consistence with above mentioned studies, the maximum densitometric enzyme activity under salinity stress was found in Torsh cultivar for SOD_2, thus, could possess the highest tolerance to salinity condition.

Reference

Ahmed IM, Dai H, Zheng W, Cao F, Zhang G, Sun D, Wu F. 2013. Genotypic differences in physiological characteristics in the tolerance to drought and salinity combined stress between Tibetan wild and cultivated barley. Plant Physiology and Biochemistry **63,** 49- 60.

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SOD) in controlling oxidative stress in plant. Journal of Experimental Botany **153,** 1331- 1341.

Apel K, Hirt H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review in Plant Biology **55,** 373- 399.

Bakalova S, Nikolova A, Nedeva D. 2004. Isoenzme profiles of peroxidase, catalase and superoxide dismutase affected by dehydration stress and ABA during germination of wheat seeds. Bulgarian Journal of Plant Physiology **30,** 64- 77.

Bartels D, Sunkar R. 2005. Drought and salttolerance in plants. Critical Reviewe in Plant Science **24,** 23- 58.

Choudhary NL, Sairam RK, Tyagi A. 2005. Expression of delta1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Indian Journal of Biochemistry and Biophysics **42,** 366– 370.

Del Rio LA, Sandalio LM, Corpas FJ, Palma JMBarroso JB. 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling, Plant Physiology **141,** 330- 335.

Garthwaite A, Bothmer R, Colmer, T. 2005. Salt tolerance inwild *Hordeum* species is associated with restricted entry of Na+ and Cl– into the shoots. Journal of Experimental Botany **56,** 2365– 2378.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry **48,** 909- 930.

Hasegawa PM, Bressnan RA, Zhu JK, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology **51,** 463– 499.

Hossain MA, Fujita M. 2010. Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. Physiology and Molecular Biology of Plants **16,** 19- 29.

Hossain MA, Hasanuzzaman M, Fujita M. 2011. Coordinate induction of antioxidant defense and glyoxalase system by exogenous proline and glycinebetaine is correlated with salt tolerance in mung bean. Frontiers of Agriculture in China **5,** 1– 14.

Kartashov AV, Radyukina NL, Ivanov Yu V, Pashkovskii PP, Shevyakova NI, Kuznetsov VV. 2008. Role of antioxidant systems in wild plant adaptation to salt stress. Russian Journal of Plant Physiology **55,** 463– 468.

Manchania AM, Banks SW, Gossett R, Bellaire BA, Lucas MC, Millhollon EP. 1999. The influence of alpha-amanitin on NaCl induced up-regulation of antioxidant enzyme activity in cotton callus tissue. Free Radical Research **30,** 429- 438.

Mittler R, Vanderauwera S, Gollery M, Breusegem F. 2004. Reactive oxygen gene network of plants. Trends in Plant Science **9,** 490– 498.

Mourato M, Reis R, Martins LL. 2012. Characterization of Plant Antioxidative System in Response to Abiotic Stresses: A Focus on Heavy Metal Toxicity. In: Dr. Montanaro G, Ed. Advances in Selected Plant Physiology Aspects.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review in Plant Biology **59,** 651- 681.

Nounjana N, Nghiab PT, Theerakulpisut P. 2012. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. Journal of Plant Physiology **169,** 596- 604.

Panda SK, Khan MH. 2004. Changes in growth and superoxide dismutase activity in *Hydrilla verticillata* L. under abiotic stress. Brazillian Journal of Plant Physiology **16,** 115- 118.

Pastori GM, Foyer CH. 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of "Redox" and abscisic acid-mediated controls. Plant Physiology **129,** 460– 468.

Poulik LMD. 1957. Starch gel electrophoresis in discontinuous system of buffers. Nature **180,** 1477- 1485.

Radyukina NL, Shashukova AV, Shevyakova NI, Kuznetsov VV. 2008. Proline involvement in the common sage antioxidant system in the presence of NaCl and paraquat. Russian Journal of Plant Physiology **5,** 649– 656.

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN. 2006. Mitochondrial reactive oxygen species: Contribution to oxidative stress and interorganellar signaling. Plant Physiology **141,** 357- 366.

Salek jalali M, Haddad R, Jafari B. 2012. Effect of soil water shortages on the activity of antioxidant enzymes and the contents of chlorophyllls and proteins in barley. Journal of Agricultural and Environmental Science **1,** 57- 63.

Scandalios JG. 2005. Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. Brazilian Journal of Medical and Biological Research **38,** 995- 1014.

Seckin B, Turkan I, Sekmen AH, Ozfidan C. 2010. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum* Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environmental and Experimental Botany **69,** 76– 85.

Shannon MC. 1997. Adaptation of plants to salinity. Advances in Agronomy **60,** 75– 120.

Smirnoff N, Cumbes QJ. 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry **28,** 1057- 1060.

Soltis DE, Soltis PS. 1990. Isozymes in Plant Biology. Chpman and Hall. London, P. 259.

Soshinkova TN, Radyukina NL, Korolkova DV, Nosov AV. 2013. Proline and functioning of the antioxidant system in *Thellungiella salsuginea* plants and cultured cells subjected to oxidative stress. Russian Journal of Plant Physiology **60,** 41- 54.

Szabados L, Savouré A. 2009. Proline: a multifunctional amino acid. Trends in Plant Science **15,** 89– 97.

Valizadeh M, Mohayeji M, Yasinzadeh N, Nasrullazadeh S, Moghaddam M. 2011. Genetic Diversity of Synthetic Alfalfa Generations and Cultivars Using Tetrasomic Inherited Allozyme Markers. Journal of Agriculture Science and Technology **13,** 425- 430.

Yoshiba Y, Kiyosue T, Katagiri T, Ueda H, Mizoguchi T, Yamaguchi-Shinozaki K, Wada K, Harada Y, Shinozaki K. 1995. Correlation between the induction of a gene for delta 1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. Plant Journal **7,** 751– 760.

Zhao GQ, Ma BL, Ren CZ. 2007. Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. Crop Science **47,** 123– 131.