Pak. J. Bot., 42(5): 3517-3524, 2010.

EFFECT OF DROUGHT STRESS ON WATER STATUS, ELECROLYTE LEAKAGE AND ENZYMATIC ANTIOXIDANTS OF KOCHIA (*KOCHIA SCOPARIA*) UNDER SALINE CONDITION

ALI MASOUMI[1], MOHAMMAD KAFI[2], HAMIDREZA KHAZAEI[2] AND KAMRAN DAVARI[2]

[1]*Ph.D. student, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran*
[2]*Faculty member, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.*
Email: m.kafi@um.ac.ir

Abstract

Drought stress is considered as the main factor of yield limitations in arid and semi-arid areas, where drought and salinity stresses are usually combined. *Kochia* species have recently attracted the attention of researchers as forage and fodder crop in marginal lands worldwide due to its drought and salt tolerant characters. This field experiment was performed at the Salinity Research Station (36°15´N, 59°28´E) of Ferdowsi University, Mashhad, Iran in 2008, in a split plot based on randomized complete block design with three replications. Three levels of drought stress (control, no irrigation in vegetative stage (recovery treatment) and no irrigation at reproductive stage for one month (stress treatment)), and two *Kochia* ecotypes (Birjand and Borujerd) were allocated as main and sub-plots, respectively. Relative water content (RWC), membrane permeability and antioxidant enzymes were assayed at the beginning of anthesis. Stress treatment caused a significant decrease in the leaf RWC and increase in electrolyte leakage compared with control and recovered conditions. Furthermore, stress treatment caused a significant increase in antioxidant enzyme activities except of superoxide dismutase (SOD) and peroxidase (POX). The Birjand ecotype was significantly more tolerant to drought than Borujerd ecotype. According to the results, there were no difference between recovered plants and control treatment, therefore, *Kochia* can recover quickly after removing drought stress. *Kochia* showed high tolerance against drought and salinity stresses and different antioxidant enzymes had different behavior under stress conditions.

Introduction

Drought stress is one of the major constrains of crop production systems in many parts of the world including Iran. Water shortages and soil water losses due to environmental and land use changes are challenges to crop production (Xia *et al*., 2005).

Kochia is a hardy, drought and salt resistant plant widely used as emergency forage for livestock. *Kochia* can establish on saline soils, not only produce protective short-lived vegetation coverage, but also is being used as an alternative fodder crop, especially in regions faced with fodder shortage (Jami Al-Ahmadi & Kafi 2006; Kafi *et al*., 2010). Several researchers showed that *Kochia* produces high biomass in saline-sodic soils (Kafi *et al*., 2010). Damage in leaves as a consequence of applied drought stress was indicated as extension of membrane lipid peroxidation, decrease in levels of antioxidant enzyme activity such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and ascorbate peroxidase (APX) activities, and apparent leakage of electrolytes from cell membranes (Blokhina *et al*., 2003; Valentovic *et al*., 2006; Ashraf, 2009). Reactive oxygen species (ROS) are highly reactive and can seriously disturb normal metabolism through oxidative damage to pigments, lipids, proteins and nucleic acids in the absence of any protective mechanism (Pessarakli, 1999; Ashraf, 2009). Peroxidation of lipids, commonly taken as an indicator of oxidative stress, disrupts the membrane integrity of the plant cell (Blokhina *et al*., 2003; Raza *et al*., 2007; Ashraf, 2009; Noreen *et al*., 2010).

Water stress causes water loss within the plant and therefore a reduction in its relative water content. In this sense, one of the most reliable and widely used indicators for defining both the sensitivity and the tolerance to water stress in plants is leaf relative water content (Rampino *et al*., 2006; Sanchez-Rodriguez *et al*., 2010). Sanchez-Rodriguez *et al*., (2010) reported that RWC was one of the best indicators in tomato plant for separating tolerant and sensitive cultivars.

In general, the damage caused by water stress has two primary causes: first, the formation of reactive oxygen species (ROS) and second the alteration of water relationships within the plant. The extent to which plants can avoid or buffer these physiological processes determines the degree of resistance to water stress. Therefore, the study of the metabolic and biochemical responses to water deficit is vital in order to select plants with high yield and stability under this type of stress (Yordanov *et al*., 2000).

The objective of this work was to examine the effects of drought stress on leaf water status and membrane permeability as well as the activities of antioxidant enzymes in the leaves of *Kochia* under prolonged drought stress and natural field conditions irrigated with saline water.

Material and Methods

Field studies were conducted during the summer of 2008 at the Salinity Research Station (36°15´N, 59°28´E) of Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. This experiment was performed in a split plot based on randomized complete block design with three replications. Water treatment, including three levels: control, no irrigation at vegetative stage (30^{th} Jun. to 21^{th} Jul.) then irrigated up to ripening, and non irrigated at reproductive stage (21^{st} Jul. to 17^{th} Aug.) then irrigated up to ripening, by using saline irrigation water with 5.2 dS m^{-1} and two Kochia ecotypes (Birjand and Borujerd) were allocated as main and sub-plots, respectively. RWC measured every two weeks for monitoring drought stress. Two Kochia ecotypes were selected from different parts of Iran. Borujerd city has cold weather, without salinity in the soil and water and Birjand has arid climate and salty soil. This experiment was designed to study stress and recovery treatment, therefore, relative water content (RWC), electrolyte leakage (%EL) and antioxidants were assayed at the end of reproductive stress (15^{th} August).

The main chemical properties of the soil and irrigation water are presented in Table1. Volume of water application for irrigation was controlled by counter at each plot.

Relative water content (RWC) and electrolyte leakage (EL): Leaf relative water content measured by soaking leaf sample (0.5 g) in 100 ml of distilled water at 4°C in the dark for 24 h. The turgid leaves were quickly blotted dry prior to the turgid weightmeasurement. Dry weight of leaves were determined after oven-drying at 70°C for 48 h. RWC was calculated according to Smart & Bingham (1974), using the following equation:

$$RWC = [\text{fresh weight- dry weight/ turgid weight} - \text{dry weight}] \times 100$$

Table 1. Main chemical properties of the water and soil at the study site.

	Na	Ca	Mg	K	SO_4 (meq.l^{-1})	CO_3	HCO_3	Cl	EC $dS.m^{-1}$
Water	32.50	8.60	9.20	0.23	15.00	0.40	2.40	34.40	5.20
Soil	31.1	10.6	10.2	0.75	31.3	0	1.8	26.8	5.15

Leaf membrane damage was determined by recording of electrolyte leakage (EL) as described by Valentovic *et al*., (2006) with a few modifications. Plant material (0.5 g) washed with deionized water was placed in tubes with 20 ml of deionized water and incubated for 24 h at 25°C. Subsequently, the electrical conductivity of the solution (L1) was measured. Samples were then autoclaved at 120°C for 20 min and the final conductivity (L2) was measured after equilibration at 25°C. The EL was defined as follows:

$$EL (\%) = (L1/L2) \times 100$$

Enzyme assays: Leaf fresh materials (0.1 g) were homogenized in liquid nitrogen into microtubes by electrical homogenizer, after that, 1 ml of extracting reaction including 0.1 *M* Sodium phosphate buffer (pH 7.8) and 1 mM ethylenediaminetetraacetic acid (EDTA) was added to homogenized leaf material. Insoluble materials were removed by Beckman refrigerated centrifuge at 15000 g for 20 min at 4 °C and supernatant used as the source of enzymes assays. An aliquot of 100 µl was used for assaying the activities of ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POX), and superoxide dismutase (SOD) and stored at -80°C until assay enzyme activities.

Ascorbate peroxidase activity was measured according to Yamaguchi *et al*., (1995) and catalase activity was assayed by measuring the initial rate of hydrogen peroxide disappearance according to Velikova *et al*., (2000).

Superoxide dismutase activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium, based on the method of Yu & Rengel (1999).

Peroxidase activity was based upon the method as described by Srinivas *et al*., (1999) and glutathione reductase activity was determined according to Rao *et al*., (1996) which depended on the rate of decrease in the absorbance of oxidized NADPH at 340 nm with the extinction coefficient of 6.2 mM cm^{-1}. The data compiled were submitted to an analysis of variance (ANOVA) and means were compared by LSD test ($p \leq 0.05$).

Results and Discussion

Leaf water status and electrolyte leakage: The RWC of the leaves declined by 20.5%, while the EL increased by 50% at stress treatment compared with control, but there were no significant differences in RWC and EL between control and recovered plants (Fig. 1). Birjand ecotype had more RWC (64%) and least EL (53%) compared with Borujerd with RWC (59%) and EL (66%) on stress situation, respectively. Difference between ecotypes could return back to their original habitats. Birjand area has harsher, dryer climate than Borujerd. Therefore, *Kochia* in Birjand area could adapt with hard situation and in this experiment it also showed a better performance than Borujerd. Other reports also pointed out this trend. For example, water stress decreased the RWC in maize seedlings of a drought sensitive cultivar. Valentovic *et al*., (2006) reported that RWC in the leaves grown last 24 h at low water potential (–1.4 MPa) decreased significantly in both drought-sensitive and drought resistant maize cultivars compared to their respective controls. Khanna-Chopra & Selote (2007) also reported that, during mild and severe water stress, the drought-resistant wheat plants maintained better leaf water relations in terms of turgor potential and RWC as compared to susceptible cultivar under both acclimated as well as non-acclimated conditions.

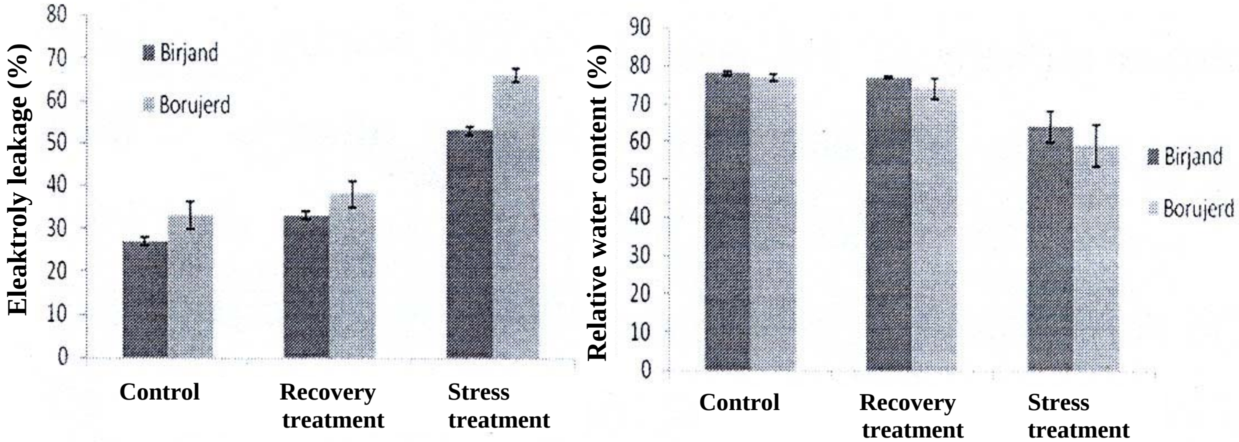

Fig. 1. Difference of EL and RWC between kochia ecotypes in control, drought stressed plants and after recovery.

Plant membranes are subject to changes often associated with the increases in permeability and loss of integrity under environmental stresses (Blokhina *et al*., 2003). Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues. Valentovic *et al*., (2006) reported that the electrolyte leakage of the sensitive maize cultivar increased from 11 to 54%, but the increase in ion leakage of tolerant cultivar was not so high. Sreenivasulu *et al*., (2000) observed positive correlations between salt sensitivity and membrane damage in foxtail millet (*Setaria italica*) seedlings. Quan *et al*., (2004) also found higher electrolyte leakage in drought stressed maize (*Zea mays* L.) plants than in plants grown under well watered conditions. Our data indicate a water stress-induced membrane injury, especially in Borujerd *Kochia* ecotype.

Antioxidant enzymes activities: Result showed significant difference ($p<0.05$) in CAT activity between control and stress treatments but there were no significant differences in CAT activity between control and recovered plants. CAT activity in stressed plants, increased by 97% compared with control (Fig. 2). Birjand ecotype had more CAT activity (34%) compared with Borujerd. Recovered plants after drought stress statistically had CAT activity similar to control.

Drought stress significantly ($p\leq0.01$) increased APX activity in kochia leaves at flowering stage. APX activity increased 91% in stress treatment compared with control (Table 2). Recovered plant did not show any significant effect in APX activity compared with control. APX activity in Birjand ecotype was higher (59%) than that in Borujerd ecotype at stress treatment.

The GR activity in the leaves of *Kochia* at stress treatment was 40% higher than that in control (Table 2). There was no difference in GR activity between the ecotypes. However, GR activity in Birjand ecotype was 31% higher than that in Borujerd under stress conditions (Fig. 2). POX activity didn't show any significant difference between treatment and ecotypes (Table 2).

SOD activity showed different patterns as compared to the other enzymes and recovered plants had the most SOD activity (Fig. 2).

Table 2. Effect of drought stress and ecotype on antioxidants activity, Electrolyte Leakage (EL) and relative water content (RWC) of Kochia ecotypes.

		Asp	CAT	SOD	POX	GR	EL	RWC
Treatment	Control	46	286	22	3814	21	30	78
	Recovered plants	50	353	72	3886	20	36	76
	Stress treatment	88	564	46	4166	30	60	62
LSD(0.05)		13.6	195.6	15.6	812.0	13.0	12.8	9.3
Ecotype	Birjand	75[a]	460[a]	47[a]	3947[b]	25[a]	38[b]	73[a]
	Borujerd	47[b]	343[a]	46[a]	3963[a]	22[a]	46[a]	70[a]

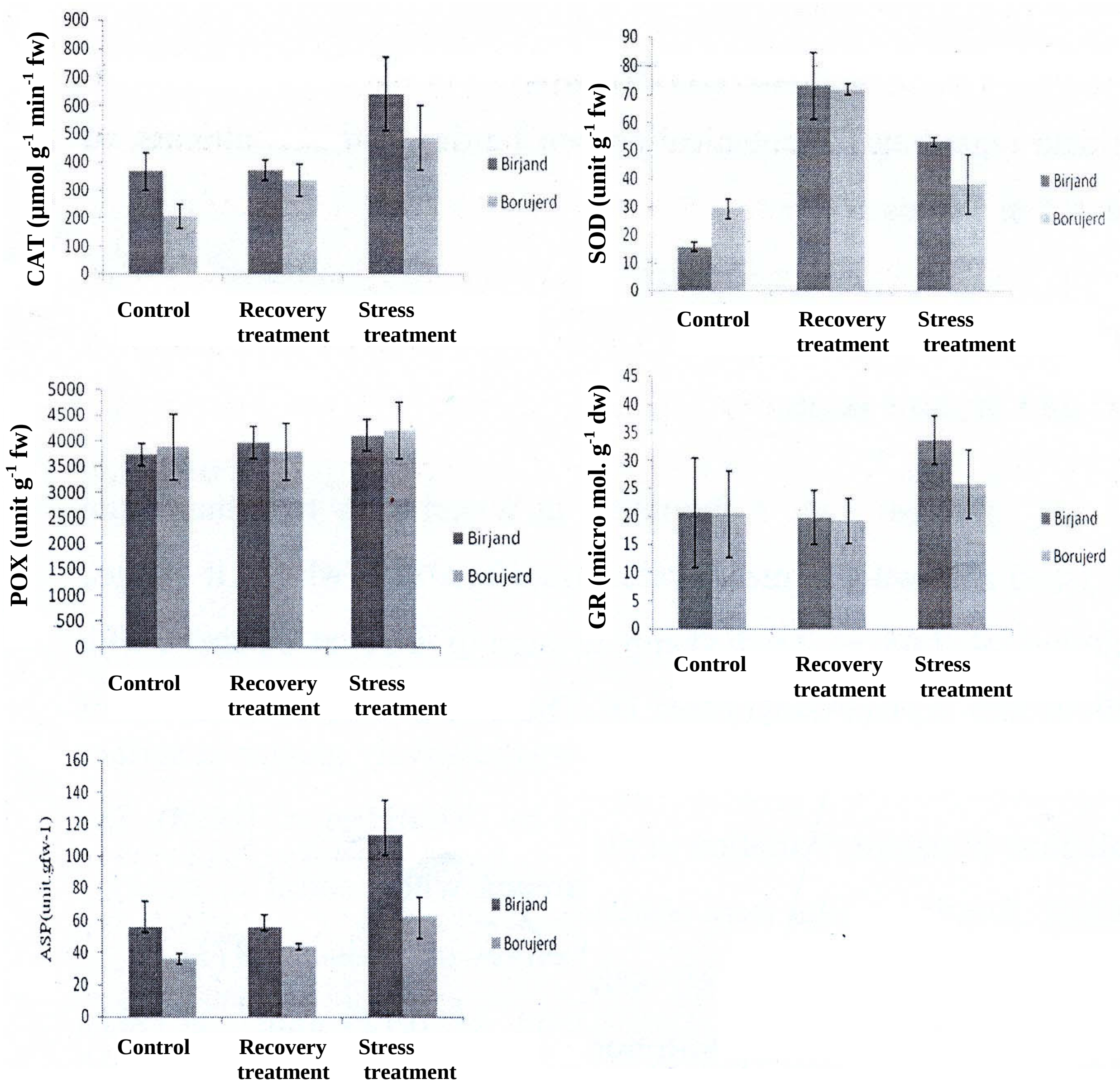

Fig. 2. Effect of drought stress on antioxidant enzymes activity of *Kochia* ecotypes in control, drought stressed plants and after recovery.

Many antioxidant enzymes showed maximum enzymatic activity in stress treatment but there were no significant differences between control and recovered plants. Birjand had more enzyme activity compared with Borujerd especially under stress conditions. Thus, *Kochia* in Birjand area could adapt with hard situation and this experiment also showed its better performance than that of Borujerd. Fazeli *et al*., (2007) studied the behavior of antioxidant enzymes under water stress in two sesame cultivars, observing that the SOD and CAT activities were higher in the most tolerant cultivar. Gossett *et al*., (1994) reported that salt-tolerant cotton cultivars had higher constitutive levels of catalase, tocopherol, ascorbate peroxidase, and glutathione reductase than sensitive cultivars when grown under salt stress. The changes in antioxidant metabolism associated with water deficits in this experiment are in agreement with the findings of Sgherri *et al*., (2000), who reported increased activity of some antioxidants such as ascorbate peroxidase activity in the leaves of wheat exposed to a cycle of water deficit and rewatering. At the same time, their findings showed that the activity of superoxide dismutase was diminished indicating that different antioxidant components can respond differently to water deficits. In our experiment also, superoxide dismutase had different behavior as compared to the other enzymes and recovery treatment had the highest amount of superoxide dismutase. Our results do not agree with those of Iturbe-Ormaetxe *et al*., (1998), who found that both ascorbate peroxidase activity and ascorbate concentration declined in peas exposed to water deficits or paraquat. Sgherri & Navari-Izzo (1995) found increases in glutathione and glutathione reductase activity in the leaves of sunflower following exposure to water stress in the field.

In conclusion, *Kochia* is tolerant against drought stress and can recover very fast after removing stress. This plant could improve membrane integrity, RWC and antioxidant level after releasing the stress.

Acknowledgments

This work was funded by a research grant from the Ferdowsi University of Mashhad, Ministry of Science, Research, and Technology, Iran. We are also grateful to Astan-e-Ghods Razavi Model Farm, Mashhad, for permitting the use of the farm for this study.

References

Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv*., 27: 84-93.

Blokhina, O., E. Virolainen and K.V. Fagerstedt. 2003. Antioxidants, oxidative damage and oxygen deprivation stress. *Ann. Bot*., 91: 179-194.

Fazeli, F., M. Ghorbani and V. Niknam. 2007. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biol. Plant*, 51(1): 98-103.

Gossett, D.R., E.P. Millhollon and M.C. Lucas. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci*., 34: 706-714.

Iturbe-Ormaetxe, I., P.R. Escuredo, C. Arrese-Igor and M. Becana. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiol*., 116: 173-181.

Jami Al-Ahmadi, M. and M. Kafi. 2006. Salinity effects on germination properties of *Kochia scoparia*. *Asian J. Plant Sci*., 5(1): 71-75.

Kafi, M., H. Asadi and A. Ganjeali. 2010. Possible utilization of high-salinity waters and application of low amounts of water for production of the halophyte *Kochia scoparia* as alternative fodder in saline agroecosystems. *Agric. Water Manage*., 97: 139-147.

Khanna-Chopra, R. and D.S. Selote. 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than susceptible wheat cultivar under field conditions. *Environ. Exp. Bot*., 60: 276-283.

Noreen, Z., M. Ashraf and N.A. Akram. 2010. Salt-induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). *J. Agron Crop Sci*. (In Press).

Pessarakli, M. 1999. *Hand book of Plant and Crop Stress*, Marcel-Dekker Inc, New York, pp. 231-270.

Qadir, M. and J.D. Oster. 2004. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. *Sci. Total Environ*., 323(1-3): 1-19.

Quan, R., M. Shang, H. Zhang, Y. Zhao and J. Zhang. 2004. Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. *Plant Biotechnol. J*., 2(6): 477-486.

Rampino, P., S. Pataleo, C. Gerardi, G. Mita and C. Perrotta. 2006. Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ*., 29(12): 2143-2152.

Rao, M.V., G. Paliyath and D.P. Ormrod. 1996. Ultraviolet-B- and ozoneinduced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol*., 110: 125-136.

Raza, S.H., H.R. Athar, M. Ashraf and A. Hameed. 2007. Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environ. Exp. Bot*., 60: 368-376.

Sanchez-Rodriguez, E., M. Rubio-Wilhelmi, L.M. Cervilla, B. Blasco, J.J. Rios, M.A. Rosales, L. Romero and J.M. Ruiz. 2010. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant Sci*., 178: 30-40.

Sgherri, C. and F. Navari-Izzo. 1995. Sunflower seedlings subjected to increasing water deficit stress: Oxidative stress and defense mechanisms. *Physiol. Plant*., 93(1): 25-30.

Sgherri, C., L.M. Maffei and F. Navari-Izzo. 2000. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. *J. Plant Physiol*., 157: 273-279.

Smart, R.E. and G.E. Bingham.1974. Rapid estimates of relative water content. *Plant Physiol*., 53: 258-260.

Sreenivasulu, N., B. Grimm, U. Wobus and W. Weschke. 2000. Differential response of antioxidant compounds to salinity stress in salt-tolerant and saltsensitive seedlings of foxtail millet (*Setaria italica*). *Physiol. Plant*., 109: 435-442.

Srinivas, N.D., K.R. Rashmi and K.S.M.S. Raghavarao. 1999. Extraction and purification of a plant peroxidase by aqueous two phase extraction coupled with gel filtration. *Process Biochem*., 35: 43-48.

Valentovic, P., M. Luxova, L. Kolarovic and O. Gasparikova. 2006. Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. *Plant Soil Environ*., 52(4): 186-191.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. *Plant Sci*., 151: 59-66.

Xia, J., M.Y. Liu and S.F. Jia. 2005. Water security problem in North China: Research and perspective. *Pedosphere J*., 15: 563-575.

Yamaguchi, K., H. Mori and M. Nishimora. 1995. A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal memberanes in pumpkin. *Plant Cell Physiol*. 36(6): 1157-1162.

Yordanov, I., V. Velikova and T. Tsonev. 2000. Plant responses to drought, acclimation and stress tolerance. *Photosynthetica J*., 38(2): 171-186.

Yu, Q. and Z. Rengel. 1999. Micronutrient deficiency influences plant growth and activities of superoxide dismutase in narrow-leafed lupines. *Ann. Bot*., 83: 175.

(Received for publication 1 Janaury 2010)