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ANALYSIS OF ANTIOXIDANT ENZYMES ACTIVITY, LIPID PEROXIDATION AND PROLINE CONTENT OF *Agropyron desertorum* UNDER DROUGHT STRESS

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Abstract. *Agropyron desertorum Fischer is one of the most important drought-tolerant grasses. This grass has been recently proposed as ground cover in landscapes. The objective of this investigation was evaluation of morphological and physiological responses of A. desertorum subjected to drought stress conditions in semiarid regions of Iran. A. desertorum was grown in six replicates and exposed to outdoors by withholding irrigation for 20d until leaf wilting of most plants reach 90% then rewatered for 8d. During the stress period, all traits such as relative water content (RWC), electrolyte leakage (EL), leaf wilting (LW), grass quality (GQ), chlorophyll content (Chl), superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), malondialdehyde (MDA) and proline were measured 5d intervals in this experiment. The results showed that drought stress declined grass quality and chlorophyll content, while electrolyte leakage and leaf wilting increased with prolonged stress treatment. Leaf wilting decreased during rewatering and it was similar to control plants at 8d. Drought stress had no effect on RWC, whereas induced oxidative injury due to increase in lipid peroxidation. Superoxide dismutase (SOD) and catalase (CAT) initially increased and then decreased at 20d, while ascorbate peroxidase (APX) remained unchanged for 10 days and then increased. The higher activities of catalase (CAT), glutathione peroxidase (GPX) and proline content were observed during exposure to drought stress in comparison with control plants. The physiological indicators under drought stress were significantly correlated each other except CAT and SOD. These results suggested that drought tolerance could be related to increase in antioxidant activities and proline content. However, prolonged drought stress suppressed SOD and CAT activities and induced lipid peroxidation.*

Key words*: Agropyron desertorum, drought, antioxidant, lipid peroxidation, proline*

INTRODUCTION

Water stress is one of the main environmental factors that adversely affect plant growth, productivity and survival. This stress usually induces the accumulation of reactive oxygen species (ROS), which cause oxidative damage to plants (Papadakis & Roubelakis-Angelakis 2005; Apel & Hirt 2004). If not effectively and rapidly removed from plants, ROS can damage a wide range of cellular macromolecules such as lipids, enzymes and DNA (Foyer & Noctor 2002). Plants can protect themselves against oxidative damage by antioxidant system including antioxidative enzymes and nonenzymatic compounds (Mittler 2002). Plants possess many antioxidative enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (POD; EC 1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), glutathione peroxidase (GPX; EC 1.11.1.9) (Wang et al. 2009b) and non-enzymatic antioxidants such as reduced glutathione (GSH) and ascorbate (AsA) (Liu et al. 2009). SOD converts superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), POD reduces H_2O_2 to water using various substrates as electron donors, APX uses ascorbate as an electron donor to reduce H_2O_2 to water, CAT dismutates H_2O_2 into water and oxygen and GPX reduces peroxides to alcohols using glutathione thus preventing the formation of free radicals (Wang et al. 2009a).

The accumulation of ROS leads to lipid peroxidation and the death of plant cells (Imlay 2003). ROS increased under stress conditions induces both protective responses and cellular damage (Blokhina et al. 2003). It is well documented that a critical component of the dehydration tolerance for grasses is cell membrane stability (Volaire & Lelievre 2001). In fact, the resistant cultivars exhibited better membrane stability than susceptible ones under simultaneous drought, as demonstrated by the lower EL (Jinrong et al. 2008). Proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Reddy et al. 2004).

The prolonged drought stress decreased SOD and CAT activities and increased lipid peroxidation in the leaves of three bentgrass species (DaCosta & Huang 2007). Fu & Huang (2001) found that SOD activity increased and CAT and POD activities remained unchanged in the leaves of Kentucky bluegrass and Tall fescue (*Festuca arundinacea* Schreb.) under prolonged surface soil drying, while SOD, POD and CAT activities decreased with increasing stress period under full soil drying. Drought stress also had no effect on leaf SOD activity in Kentucky bluegrass (Zhang & Schmidt 1999). Significant increase in APX activity was noted under water stress in three cultivars of *Phaleolus vulgaris* and *Picea asperata*. Trans-

genic tobacco seedlings overexpressing GPX showed enhanced seedling growth under stressed environment. It was noted that GPX activity in transgenic *Gossypium hirsutum* seedlings was 30-60% higher than under normal conditions (Gill & Tuteja 2010). Drought stress is a major factor limiting grass quality, persistence and production. Wheat grasses are an important group of stress tolerant perennial species. Among wheat grasses, *Agropyron desertorum* Fischer is more drought-tolerant than many other grasses. Recently, some topics have been discussed about using *A. desertorum* as turf in areas without cover, surrounding roads and regions with soil erosion. The regulation of antioxidant mechanism in *A. desertorum* is still unknown. Therefore, assay of the antioxidant enzymes is also important for elucidating antioxidant mechanism of *A. desertorum* under water stress.

MATERIALS AND METHODS

Materials

The seeds of native grass *Agropyron desertorum* were collected from semiarid regions of South Esfahan province, Iran. Seeds of *A. desertorum* were disinfected and sown into split polyvinylchloride (PVC) tubes (60cm deep×15cm diameter), filled with the field silt-clay-loam soil (16.5% sand, 44% silt, 39.5% clay). The grass was irrigated as necessary to prevent drought stress, fertilized weekly with water soluble fertilizer of 20-20-20 (Cristalon fertilizer) and hand clipped weekly at 4 cm. The clippings after each mowing were removed. Plants were exposed to outdoors drought stress by withholding irrigation for 20 days until leaf wilting of 90% of most plants. Control plants were irrigated daily until drainage occurred. During the stress period, all measurements were made 5d intervals in the experiment. The first sampling was carried out one day before stress treatment. Drought stressed plants were rewatered for 8d after 20d of stress and recovery percent were recorded.

Methods

Abbreviations

RWC- relative water content
EL- electrolyte leakage
LW- leaf wilting
GQ- grass quality
Chl- chlorophyll content
SOD- superoxide dismutase
CAT- catalase
POD- peroxidase
APX- ascorbate peroxidase
GPX- glutathione peroxidase
MDA- malondialdehyde

GQ, LW, RWC, EL and Chl content

The grass quality was visually rated on 0 to 9 scales, where 0 brown leaves, dead grass; 9 turgid green leaves, optimum density and uniformity (Turgeon 2002). LW% was determined by visually estimating the total percentage of wilting leaf area. LW was evaluated on 0 to 100 scales, where 0 no observable leaf wilting and 100 completely wilted. RWC % was calculated according to Barrs & Weatherley (1962). Leaf samples were detached from the plants and immediately weighed to determine fresh weight (FW). Samples were placed into covered petri dishes filled with water for leaves to reach full turgid. After approximately 24h at 4°C, leaf samples were blotted dry with paper towels and weighted to determined turgid weight (TW). Leaf tissue was then dried in an oven at 80°C for 48h to determined dry weight (DW). Leaf RWC was calculated as (FW-DW)/(TW-DW)×100. Cell membrane stability was determined as electrolyte leakage (EL). For EL analysis, whole fully-expanded leaves (0.1g from each pot) were incubated in 15ml distilled water on a shaker for 24h. The conductance of the incubation solution was measured as the initial level of EL (Ci) using a conductance meter (YSI-3100, Guangzhu, China). Leaf tissue in the incubation solution was killed in an autoclave at 120°C for 30min. The conductance of the incubation solution with killed tissues (Cmax) was determined following 24h incubation on a shaker. Relative EL was calculated as (Ci/Cmax)×100 (Blum & Ebercon 1981). In order to test chlorophyll content, 0.1gr fresh leaf were placed into 10 ml of dimethylsulfoxide (DMSO) and pigments were allowed to extract in the dark at 65°C for 30min. Absorbance of the extracts was measured using spectrophotometer (T80 UV/Visible) at 645 and 663 nm. Chlorophyll content was calculated using Arnon's (1949) equations (Hiscox & Israelstam 1979).

Enzyme assay

The SOD activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). One unit of SOD was defined as the amount of enzyme necessary to cause 50% inhibition of the rate of NBT reduction at 560nm. The reaction mixture contained 75µM NBT, 2 µM riboflavin, 13mM methionine, 0.1mM EDTA, 50mM phosphate buffer (pH 7.0) and 50 µl of enzyme extraction. The test cells containing the mixture were placed on a shaker under light at 78 µmol photons s^{-1} m^{-1} for 15min and absorbance at 560 nm was recorded. A nonirradiated reaction mixture that did not develop color served as the control and its absorbance was subtracted from A_{560} of the reaction solution (Giannopolitis & Ries 1977). For Guaiacol POD, the oxidation of guaiacol was measured by the increase in absorbance at 470nm for 1min. The reaction solution contained 450 µl of 225mM guaiacol, 100 µl of 50mM phosphate buffer (pH 7.0) and 100 µl enzymes extract. The reaction was started with 450 µl of 45mM H_2O_2 (Zhang 1992). Activity of CAT was measured using the method of Aebi (1984). For CAT, the decomposition of H_2O_2 was measured by the decline in absorbance at 240nm for 70s. The reaction mixture contained 50mM potassium buffer (pH 7.0), 10mM H_2O_2 and 100µl enzyme extract, which initiated reaction. APX activity was measured by monitoring the decrease in absorbance at 290nm for 70s. The assay mixture contained 50mM potassium buffer (pH 7.0), 0.1mM EDTA, 1.25mM H_2O_2, 0.5mM AsA and 100µl en-

zyme extract. The reaction was initiated by adding H_2O_2 (Nakano & Asada 1981). The determination of GPX activity was based on the method as described by Nickel & Cunningham (1969). Activity was measured by the increase in absorbance at 470nm for 70s. Reaction mixture contained 50mM potassium buffer (pH 7.0), 25mM guaiacol, 10mM H_2O_2 and 100µl enzyme extract.

MDA and Proline measurement

For measurement of MDA content, 3ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to a 1ml aliquot of the supernatant. The mixture was heated at 95°C for 30min and then quickly cooled in an ice bath. The tube was centrifuged at 10,000×g for 10min, and then absorbance of the supernatant was read at 532nm. The value for the nonspecific absorption at 600nm was subtracted from the 532nm reading. The concentration of MDA was calculated using MDA's extinction coefficient of $155mM^{-1}$ cm^{-1} (Heath & Parcker 1968). The proline content was determined by the method given by Bates et al. (1973). For this, 10 ml of 3% (w/v) sulphosalylic acid was added to 0.4gr leaf materials. The homogenate was filtered through filter paper. Two ml acetic acid and 2ml acidic ninhydrin regent were added to a 2ml aliquot. The mixture was thoroughly stirred and incubated in a boiling water bath for 1h and then the reaction was stopped by using an ice bath and warmed to room temperature. The mixture was extracted with toluene and the absorbance of the fraction with toluene extracted from the liquid phase was read at 518nm. The concentration of proline in the sample was computed from a standard curve of proline.

Experimental design and statistical analysis

The split plot in time experiment was designed in randomized complete block design with six replicates in February 2011. Analysis of variance was carried out on the collected data and the mean value of data was compared at 5% level of probability using least significant design (LSD).

RESULTS

GQ, LW, RWC, EL and Chl content

Grass quality declined gradually due to drought stress at 5d of stress and the least GQ was observed at 20d (Fig. 1A). Leaf wilting rate began to increase at 5d of drought stress and was dramatically increased at 20d. After 8d of drought stress, recovery percent was 100% (Fig. 1B). Drought stress had no effect on RWC. Leaf Chl content in plants exposed to drought stress were significantly lower than the control plants. Although leaf Chl was decreased between different days of sampling under drought treatment, but this decrease was low (Fig. 1C). EL increased in stress treatment (Fig. 1D), so that EL at 15d and especially 20d under drought stress was significantly higher than that at 5d and 10d of treatment.

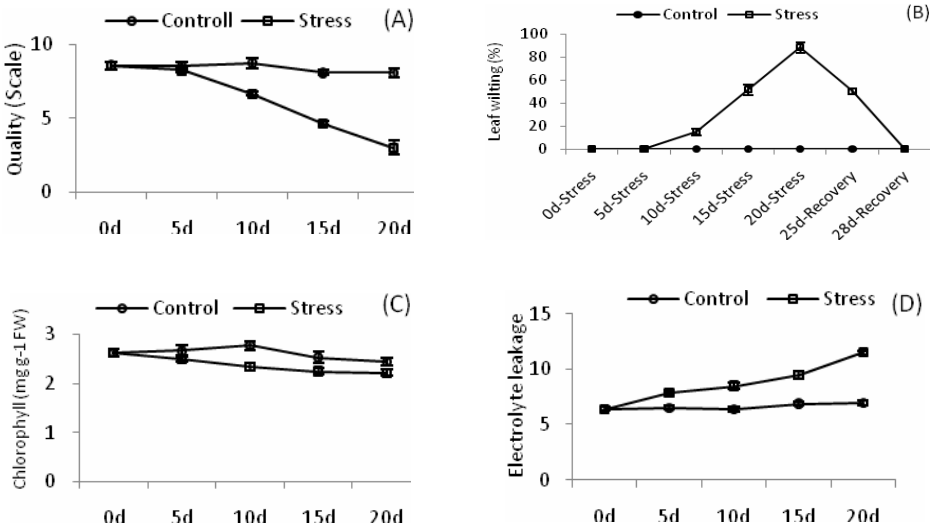

Figure 1. Effects of 20d drought stress on grass quality (A), chlorophyll (C), electrolyte leakage (D) and effects of 20d drought stress and 8d recovery from stress on leaf wilting (B)

Enzyme assay

The activity of CAT had no significant increase under normal conditions. Under stress condition, CAT activity significantly increased from 5d to 15d of stress treatment, then it decreased at 20d; so that, CAT activity at 20d of drought stress was almost similar to that at 20d of control plants (Fig. 2A). The SOD activity gradually increased from 5d to 15d of stress, but a slightly decline was observed at 20d of stress treatment. The activity of SOD at 10d of drought stress was similar to that at 20d of treatment. Activity pattern of SOD at 0d, 5d and 20d of stress was almost similar to that of control plants. The activity of this enzyme didn't exhibit significant change in normal plants (Fig. 2B). A continuous increase in POD activity occurred after 5d of stress. The POD activity remained unchanged in the control plants. Activity of POD at 20d of stress was 6.2-fold than control plants (Fig. 2C). The activity of APX increased rapidly from 10 to 20d of stress. In addition APX activity significantly increased 2.5-fold in the second 10 days of stress relative to the first 10 days of treatment and control plants (Fig. 2D). Activity of APX at the first 10 days of drought stress was similar to control plants. Increase in GPX activity up to 1.93-fold at 20d of stress was found, as compared with one day before treatment (Fig. 2E). No significant change in GPX activity

was observed at 15 and 20d of treatment. Activity of GPX was unchanged in control plants during the entire experimental period.

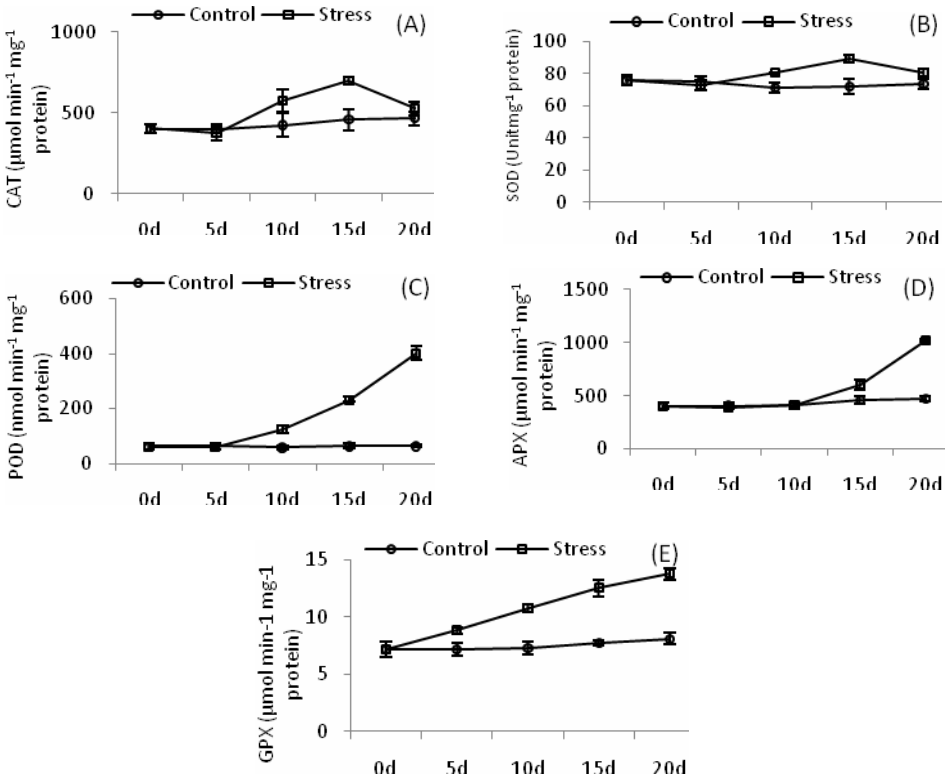

Figure 2. Effects of 20d drought stress on catalase (A), superoxide dismutase (B), peroxidase (C), ascorbate peroxidase (D) and glutathione peroxidase (E).

MDA and Proline measurement

Drought stress induced lipid peroxidation. Malondealdehyde content exhibited significant increase during the stress period (Fig. 3A). Malondealdehyde content was significantly higher than control plants. The proline content increased but the extent of increase was smaller value until 10d of stress and then increased rapidly with the progress in drought conditions. Drought stress increased proline content to 4-fold than normal plants (Fig. 3B).

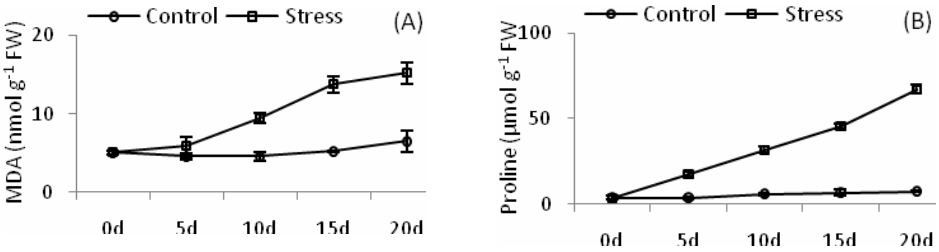

Figure 3. Effects of 20d drought stress on malondialdehyde (A) and proline (B).

Through correlation analysis, was observed that GQ and Chl had significant negative correlation with EL, CAT, APX, GPX, POD, SOD, MDA and Proline, However GQ had significant positive correlation with LW and Chl at the 0.01 level. There was no significant correlation between EL and CAT, EL and SOD, LW and CAT, LW and SOD, CAT and APX, CAT and POD, APX and SOD, POD and SOD. A significant positive correlation was observed between other traits (Table1).

DISCUSSION

When subjected to drought stress, plants responded through alternation in physiological and biochemical processes. Physiological parameters like turf quality, RWC and EL (an indicator of cell membrane stability) have been widely used as physiological indicators for the selection of drought tolerant plant materials in turfgrasses and other species (Jiang & Huang 2001). Relative water content of *A. desertorum* was not affected by drought stress. This may be suggested that *A. desertorum* was capable of surviving soil drying and maintaining favorable water status by most of its roots was exposed to drought stress. GQ decreased and LW increased in *A. desertorum* from 5 to 20d under drought stress. It can be suggested that the drought stress period is the main factor that affects on the GQ and LW as morphological traits (Fig.1A,B). This study also revealed that there was positive correlation between GQ with Chl and negative correlation between EL with GQ and Chl, Therefore the higher Chl, the lower EL and the better GQ were highly associated each other. This finding suggested that EL can be used as an important physiological indicator for the evaluation of adaptability to drought stress. The lower EL correlated with the higher cell membrane stability in turfgrasses and hence the better GQ observed normally. Drought stress has been reported to damage cell membrane stability (Ban-

Table1. Correlation analysis to determine relationship among measured traits.

Proline	MDA	SOD	POD	GPX	APX	CAT	EL	Chl	LW	GQ	
										1	GQ
									1	-0.963**	LW
								1	-0.776*	0.883**	Chl
							1	-0.871**	0.956**	-0.972**	EL
						1	0.659ns	-0.812*	0.702ns	-0.776*	CAT
					1	0.589ns	0.877**	-0.661ns	0.944**	-0.865**	APX
				1	0.808*	0.795*	0.961**	-0.918**	0.922**	-0.981**	GPX
			1	0.918**	0.956**	0.681ns	0.961**	-0.775*	0.997**	-0.963**	POD
		1	0.665ns	0.771*	0.452ns	0.768*	0.701ns	-0.770*	0.703ns	-0.769*	SOD
	1	0.809*	0.916**	0.950**	0.828*	0.880**	0.925**	-0.928**	0.925**	-0.953**	MDA
1	0.934**	0.743*	0.921**	0.983**	0.814*	0.713*	0.983**	-0.902**	0.925**	-0.962**	Proline

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.
ns Correlation is not significant

durska & Gniazdowska 1995). Cell membrane stability was shown to be affected by lipid peroxidation caused by active oxygen species under various stress conditions (Dhindsa et al. 1981). By withholding irrigation for 21d until complete leaf wilting in Kentucky bluegrass, turf quality degreased and EL increased (Jirong et al. 2008). GQ was reduced to the minimum acceptable level at 20d drought stress, whereas RWC remained unchanged. Under this stress intensity, Chl content was slightly declined, this suggested that *A. desertorum* was capable of maintaining some photosynthetic capacity by preserving photosynthetic pigments. Zulini et al. (2007) reported that Chl content decreased with further increasing rate of drought stress. Drought-induced decreases in photosynthetic electron transfer and chlorophyll content have been reported previously in various species (Moran et al. 1994). The loss of Chl during drought stress could also be related to photooxidation resulting from oxidative stress (Kato & Shimizu 1985). The results indicated that Chl content, LW and visual quality were more sensitive to drought stress than RWC. Decrease in LW during recovery indicated the recovery potential of *A. desertorum* after 90% leaf wilting. No change in RWC, could be related to the increase in SOD activity to scavenge active oxygen. The increase in activity of SOD during initial periods of drought stress might protect plants from oxidative injury (Fu & Huang 2001). The increased, decreased and unchanged antioxidant enzyme activities in the various times of sampling of *A. desertorum* indicate a different antioxidant metabolism in response to drought stress. CAT catalyzes the oxidation of substrates by H_2O_2, breaks down and detoxifies H_2O_2 (Asada 1992). Thus, decrease in CAT activity would result in H_2O_2 accumulation, which can react with (O_2^-) to produce hydroxyle-free radicals via the Herbert-Weiss reaction (Bowler et al. 1992). The hydroxyle-free radicals can directly damage the membrane by attacking unsaturated fatty acids of lipid to induce lipid peroxidation (Okuda et al. 1991). In this study, activity of CAT declined with prolonged stress period (Fig. 2A), then higher MDA and EL could be the result (Fig. 3A, 1D). These results indicated that the ability of CAT to destroy active oxygen was increased during initial stress but was decreased during a prolonged period of drought stress; however, plants were able to maintain some CAT activity even when water was available only in deep soil. Decline in CAT activity in response to prolonged drought have been reported for Kentucky bluegrass and Tall fescue (Fu & Huang 2001). There was significant positive correlation between CAT and MDA. SOD is the key enzyme in the antioxygen scavenger system because it catalyzes superoxide free radical dismutation into H_2O_2 and O_2^- (Bowler et al. 1992). It is possible that the accumulation of O_2^- occur in the leaves did go along with the change in SOD activity (Blokhina et al. 2003). In this study the SOD activity

increased at 5-15d and then slightly declined at 20d of stress (Fig. 2B). Some researchers also found the increase in SOD activity during the first days of environmental stresses and decline in SOD activity after 17-21d of these stresses (Liu & Huang 2000, Huang et al. 2001). Previous studies have shown that responses of SOD activity to water deficit have varied with drought severity, duration and species. Zhang & Kirkham (1996) suggested that water stress did not influence SOD activity under moderate stress in sorghum (*Sorghum bicolor* L.). Jagtap & Bhargava (1995) reported that SOD activity increased in drought-tolerant cultivars of maize (*Zea mays* L.). In wheat (*Triticum aestivum* L.), SOD activity increased or remained unchanged in the early phase of drought and then decreased with further water stress (Zhang et al. 1995). Reddy & Vajranabhaiah (1993) observed that SOD activity in upland rice (*Oryza sativa* L.) decreased with osmotic stress. The decline in SOD and CAT activity after prolonged stress indicated that the scavenging function of both enzymes were impaired with prolonged, severe drought stress. The decrease in SOD activity would favor accumulation of O_2^-. This result indicated that under severe drought stress, the balance between active oxygen formation and the scavenging system could be disturbed (Breusegem et al. 1998).

Peroxidase catalyzes H_2O_2-dependent oxidation of substrate. *A. desertorum* was able to increase POD activity for detoxifying active oxygen in response to drought stress. Other studies have reported increase (Zhang et al. 1995), decrease (Zhang & Kirkham 1996) and no change (Fangmeier et al. 1994) in POD activity in response to drought stress. APX has a higher affinity for H_2O_2 than CAT and POD and it may have a more crucial role in the management of ROS during stress (Gill & Tuteja 2010). The higher activity of APX from 15 to 20d of treatment indicated the potential need for removal of ROS (Fig. 2D). Increased activity of APX was also found in the leaves of Kentucky bluegrass (*Poa pratensis* L.) subjected to the prolonged drought stress (Bian & Jirong 2009). The highest GPX activity was found in 20d of drought stress treatment. Tohidi-Moghaddam et al. (2009) reported that leaves of canola (*Brassica napus* L.) under drought stress showed a significant increase in GPX activity. Results of the study carried out by Sayfzadeh & Rashidi (2011) showed that drought stress increased the activity of GPX in sugar beet leaves. These results are in agreement with our findings. Increasing of recovery potential in *A. desertorum* may be due to increase in POD, APX and GPX activities. The increased or stable activities of these five enzymes observed in *A. desertorum* under drought stress could decrease H_2O_2 detoxification. The different responses of these enzyme activities to drought treatment may depend on plant species, stress severity and intensity of ROS production.

All the physiological indexes under drought stress were significantly correlated each other, except SOD and CAT.

This result indicated that when *A. desertorum* was stressed by drought, increased of SOD activity can't inhibit formation and accumulation of free radicals, but many delay accumulation of free radicals to a certain degree to alleviate active oxygen damage to cell membrane. Accumulated H_2O_2 can be detoxified by increase in APX, GPX and POD activities. Lipid peroxidation indicates the prevalence of free radical reactions in tissues (Smirnoff 1993). Malondialdehyde is the final product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage; this assay has often been used as an indicator of the level of lipid peroxidation (Haliiwell & Gutteridge 1989, Scandalios 1993). The result also indicated that EL value and MDA content always went together and increased under drought stress, performing significantly positive correlation between increasing rate of EL and MDA content. After 20 days of drought stress, MDA increased up to twofold (Fig. 3A), suggesting that prolonged full drying caused membrane lipid peroxidation, which could be attributing to the decrease SOD and CAT activities. Decreased activities induced by severe drought stress favor accumulation of O_2^- and H_2O_2, which can result in lipid peroxidation. The increase in MDA content after a prolonged period treatment (20 days) was in agreement with results of other studies (Price & Hendry 1991, Zhang et al. 1995). With the prolonged drought stress and severe dehydration, leaf lipid peroxidation increased in colonial Bentgrass (*Agrostis capillaries* L.), Creeping bentgrass (*Agrostis stolonifera* L.) and Velvet bentgrass *(Agrostis canina* L.) species (DaCosta & Huang 2007). One of the important factors controlling cell tolerance to desiccation or dehydration is the cell's capability to maintain adequate turgor pressure during drought stress. Generally, this is done by increasing the concentration of compatible solutes within the cell. One of the compatible solutes is proline. That is a nonprotein amino acid (Pessarakli 2008). It has been reported that the proline accumulation is only a response to drought (Jia et al. 2002). Our research showed that the proline content increased under drought related to control plants. Wang et al. (2007) suggested that the proline accumulation was not positively correlated with the tolerance to the stress, but responded to injury of drought.

CONCLUSION

The increased or unchanged activities of antioxidant enzymes were generally observed in different intensities of drought stress, but decreased activi-

ties of certain enzymes were also noted in some times of sampling. In summary, the results suggested that long drought stress was detrimental for wheat grass. Drought stress induced oxidative injury, as demonstrated by the change in antioxidant enzymes and increase in lipid peroxidation. *A. desertorum* exhibited a defensive mechanism to protect against free radicals in the early periods of stress treatment, as showed in CAT and SOD activities, and in the end periods of stress, as showed in APX, GPX and POD activities. Clearly, decline of GQ and increase of EL in drought stress associated closely with change in antioxidant enzymes activities and an increase in lipid peroxidation. Proline content increased with prolonged drought stress. These physiological parameters could be used to select *A. desertorum* for the improvement of drought stress survival. This grass is recommended for use as cover ground in semi arid areas without cover.

REFERENCES

Aebi, H. (1984): Catalase *in vitro*. Methods in Enzymology 105: 121-126.

Apel, K., Hirt, H. (2004): Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annul Review of Plant Biology 55: 373-399.

Arnon, D.I. (1949): Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiology 24: 1-15.

Asada, K. (1992): Ascorbate peroxidase-a hydrogen peroxide scavenging enzyme in plants. Physiologia Plantarum 85: 235-241.

Bandurska, H., Gniazdowska, S.H. (1995): Cell membrane stability in two barley genotypes under water stress conditions. Acta Societatis Botanicorum Poloniae 64: 29-32.

Barrs, H.D., Weatherley, P.E. (1962): A reexamination of the relative turgidity technique for the estimating of water deficits in leaves. Australian Journal of Biological Sciences 15: 413-428.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973): Rapid determination of free proline for water stress studies. Plant and Soil 39: 205-207.

Bian, S., Jiang, Y. (2009): Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Scientia Horticulturae 120: 264-270.

Blokhina, O., Virolainen, E., Fagerstedt, K.V. (2003): Antioxidants, oxidative damage and oxygen deprivation stress: a review. Annales Botanici Fennici 91: 179-194.

Blum, A., Ebercon, A. (1981): Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21: 43-47.

Bowler, C., Montagu, M.V., Inze, D. (1992): Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 43: 83-116.

Breusegem, F., Van Montagu, M., Van Inze, D., Van Breusegem, F., Van Montagu, M. (1998): Engineering stress tolerance in maize. Outlook on Agriculture 27: 115-124.

DaCosta, M., Huang, B. (2007): Changes in antioxidant enzyme activities and lipid peroxidation for bentgrass species in responses to drought stress. Journal of the American Society Horticultural Science 132: 319-326.

Dhindsa, R.S., Dhindsa, P.P., Thorpe, T.A. (1981): Leaf senescence: correlated with

increased level of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany 32: 93-101.
Fangmeier, A., Brunschon, S., Jager, H.J. (1994): Time course of oxidant stress biomarkers in flag leaves of wheat exposed to ozone and drought stress. New Phytologist 126: 63-69.
Foyer, C.H., Noctor, G. (2002): Oxygen processing in photosynthesis: regulation and signaling. New Phytologist 146: 359-388.
Fu, J., Huang, B. (2001): Involvement of antioxidants and lipid peroxidant in the adaptation of two cool-season grasses to localized drought stress. Environmental and Experimental Botany 45: 105-114.
Giannopolities, C.N., Rise, S.K. (1977): Superoxide dismutases. I. Occurrence in higher plants. Plant Physiology 59: 304-309.
Gill, S.S., Tuteja, N. (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930.
Halliwell, B., Gutterides, J.M.C. (1989): Lipid peroxidation: a radical chain reaction. pp. 188-260. In Halliwell, B., Gutteridge, J.M.C. (eds) Free Radicals in Biology and Medicine. Clarendon Press, Oxford, UK.
Heath, R.L., Parker, L. (1968): Photoperoxidation in isolated chloroplasts: I. Kinetics and stiochiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125: 189-198.
Hiscox, J.D., Israelstam, G.F. (1979): A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57: 1332-1334.
Huang, B., Liu, X., Xu, Q. (2001): Supraoptimal soil temperature fluctuainduced oxidative stress in leaves of creeping bentgrass cultivars differing in heat tolerance. Crop Science 41: 430-435.
Imlay, J.A. (2003): Pathways of oxidative damage. Annual Review Microbiology 57: 395-418.
Jagtap, V., Bhargava, S. (1995): Variation in the antioxidant metabolism of drought tolerant and drought susceptible varieties of *Sorghum bicolor* (L.) Moench. exposed to high light, low water and high temperature stress. Journal of Plant Physiology 145: 195-197.
Jia, W.S., Wang, Y.Q., Zhang, S.Q., Zhang, J.H. (2002): Salt-stress-induced ABA accumulation is more sensitively triggered in roots than in shoots. Journal of Experimental Botany 53: 2201-2206.
Jiang, Y., Huang, B. (2001): Physiological responses to heat stress alone or in combination with drought: a combination between tall fescue and perennial ryegrass. Horticultural Science 36: 682-686.
Jinrong, L., Xiaorong, X.D., Jixiong, S., Xiaomin, B. (2008): Effects of simultaneous drought and heat stress on Kentucky bluegrass. Scientia Horticulturae 115: 190-195.
Kato, M., Shimizu, S. (1985): Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degradation. Plant Cell Physiology 26: 1291-1301.
Liu, X., Huang, B. (2000): Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Science 40: 503-510.
Liu, Z.J., Zhang, X.L., Bai, J.G., Suo, B.X., Xu, P.L., Wang, L. (2009): Exogenous paraquat changes antioxidant enzyme activities and lipid peroxidation in drought-stressed cucumber leaves. Scientia Horticulturae 121: 138-143.
Mittler, R. (2002): Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7: 405-410.
Moran, J.F., Becana, M., Iturbe-Ormaetxe, I., Frechilla, S., Klucas, R.V., Aparicio-Tejo, P. (1994): Drought induces oxidative stress in pea plants. Planta 194: 346-352.
Nakano, Y., Asada, K. (1981): Hydrogen peroxide is scavenged by ascorbate-specific

peroxidase in spinach chloroplasts. Plant Cell Physiology 22: 867-880.
Nickel, R.S., Cunningham, B.A. (1969): Improved peroxidase assay method using leuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. Analytical Biochemistry 27: 292–299.
Okuda, T., Matsuda, Y., Yamanaka, A. (1991): Abrupt increases in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. Plant Physiology 97: 1265-1267.
Papadakis, A.K., Roubelakis-Angelakis, K.A., (2005): Polyamines inhibit NADPH oxidasemediated superoxides generation and putrescine prevents programmed cell death syndrome induced by the polyamine oxidase generated hydrogen peroxide. Planta 220: 826-837.
Pessarakli, M. (2008): Hand book of turfgrass management and physiology. pp. 431-442. In McCann, S.E., Huang, B. (eds) Turfgrass drought physiology and irrigation management. University of Arizona, CRC Press.
Price, A.H., Hendry, G.A.F. (1991): Iron-catalysed oxygen radical formation and its possible contribution to drought in nine native grasses and three cereals. Plant, Cell and Environment 14: 477-484.
Reddy, A. R., Chaitanya, K. V., Vivekanandan, M. (2004): Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology 161: 1189-1202
Reddy, P.C., Vajranabhaiah, S.N. (1993): Drought induced lipid peroxidation: defensive mechanism in upland rice (*Oryza sativa* L.) seeds during germination. Current Advances in Plant Science 6: 229-236.
Sayfzadeh, S., Rashidi, M. (2011): Response of antioxidant enzymes activities of sugar beet to drought stress. ARPN Journal of Agricultural and Biological Science 6: 1-4.
Scandalios, J.G. (1993): Oxygen stress and superoxide dismutases. Plant Physiology 101: 7-12.
Smirnoff, N. (1993): The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125: 27-58.
Tohidi-Moghaddam, H.R., Shirani-Rad, A.R., Noormohammadi, G., Habibi, D., Boojar, M.M. A. (2009): Effect of super absorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. American Journal of Agriculture and Biological Science 4: 215-223.
Turgeon, A.J. (2002): Turfgrass Management. 6th Edition. Prentice Hall, Upper Saddle Brook, N.J.
Volaire, F., Lelievre, F. (2001): Drought survival in *Dactylis glol. merata* and *Festuca arundinacea* under similar rooting conditions in tubes. Plant Soil 229: 225-234.
Wang, S.X., Xia, S.T., Peng, K.Q., Kuang, F.C., Cao, Y., Xiao, L.T. (2007): Effects of Formulated Fertilizer Synergist on Abscisic Acid Accumulation, Proline Content and Photosynthetic Characteristics of Rice under Drought. Rice Science 14: 42-48.
Wang, W.B., Kim, Y.H., Lee, H.S., Kim, K.Y., Deng, X.P., Kwak, S.S. (2009a): Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiology and Biochemistry 47: 570-577.
Wang, Y., Yang, Z.M. Zhang, Q.F., Li, J.L. (2009b): Enhanced chilling tolerance in *Zoysia matrella* by pre-treatment with salicylic acid, calcium chloride, hydrogen peroxide or 6-benzylaminopurine. Biologia Plantarum 53: 179-182.
Zhang, J., Kirkham, M.B. (1996): Antioxidant responses to drought in sunflower and sorghum seedings. New Phytologist 132: 361-373.
Zhang, J., Cui, S., Li, J., Kirkham, M.B. (1995): Protoplasmic factors, antioxidant responses and chilling resistance in maize. Plant Physiology and Biochemistry 33: 567-575.

Zhang, X., Schmidt, R.E. (1999): Antioxidant response to hormone-containing product in Kentucky bluegrass subjected to drought. Crop Science 39: 545-551.
Zhang, X.Z. (1992): The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. pp. 208-211. In Zhang, X. Z. (eds.) Research methodology of crop physiology. Agriculture Press, Beijing.
Zulini, L., Rubinigg, M., Zorer, R., Bertamini, M. (2007): Effects of drought stress on chlorophyll fluorescence and photosynthetic pigments in grapevine leaves (*Vitis vinifera* cv. 'White Riesling'). Acta Horticulturae 754: 289-294.