Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery

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ABSTRACT

The objective of this study was to investigate accumulation of reactive oxygen species, antioxidant enzyme activities, and gene expression patterns of antioxidant enzymes of Kentucky bluegrass (*Poa pratensis* L.) under drought stress and recovery. Grass (c.v. Midnight II) was subjected to soil drying for 5 d and then rewatered for 1 d in growth chambers. Drought stress increased superoxide (O_2^-) production of leaves and hydrogen peroxide (H_2O_2) content of the leaves and roots. Recovery enhanced leaf O_2^- production and root H_2O_2 content. Lipid peroxidation of the roots increased 4.5-fold and 2.4-fold under drought stress and recovery, respectively. Drought stress increased the leaf activities of ascorbate peroxidase (APX, EC 1.11.1.11), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and the root activities of catalase (CAT, EC 1.11.1.6), glutathione reductase (GR, EC 1.6.4.2) and MDHAR, while reducing the root activities of superoxide dismutase (SOD, EC 1.15.1.1) and DHAR, respectively. The increased leaf activities of APX, MDHAR, DHAR and the root activities of MDHAR and CAT were observed after rewatering, compared to the control. For the leaves, the expressions of iron SOD (*FeSOD*), cytosolic copper/zinc SOD (*Cu/ZnSOD*), chloroplastic *Cu/ZnSOD*, and *DHAR* were down-regulated by drought stress but recovered to control level after rewatering, while the expressions of *GR* and *MDHAR* were up-regulated and remained that levels after recovery. For the roots, the expressions of cytosolic *Cu/ZnSOD,* manganese SOD (*MnSOD*), cytosolic *APX, GR,* and *DHAR* were down-regulated under drought stress but recovered except for *GR* and *DHAR*, while *MDHAR* expression was up-regulated. No differences in *CAT* transcript abundance were noted among the treatments. Antioxidant enzymes and their gene expressions may be differentially or cooperatively involved in the defense mechanisms in the leaves and roots of Kentucky bluegrass exposed to drought stress and recovery.

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1. Introduction

The alteration of antioxidant metabolisms is one of the fundamental metabolic processes that may influence the drought tolerance of perennial grasses (Jiang and Huang, 2001; DaCosta and Huang, 2007). Drought stress promotes the production of reactive oxygen species (ROS), including superoxide (O_2^-), singlet oxygen ($^\bullet O_2$), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), which can be detrimental to proteins, lipids, carbohydrates, and nucleic acids (Smirnoff, 1993). Plants have evolved both enzymatic and non-enzymatic defense systems for scavenging and detoxifying ROS. In enzymatic systems, superoxide dismutase (SOD) scavenges O_2^- to H_2O_2 (Bowler et al., 1992). Peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), mono-dehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) decompose H_2O_2 to H_2O at different cellular locations (Asada, 1999; Mittler, 2002). The balance between ROS production and activities of antioxidative enzyme determines whether oxidative signaling and/or damage will occur (Møller et al., 2007).

Maintaining a high level of antioxidative enzyme activities may contribute to drought tolerance by increasing the capacity a better protection mechanisms against oxidative damage (Sharma and Dubey, 2005; Türkan et al., 2005). However, changes in activities of

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antioxidant enzyme under drought stress depend on plant species, cultivar and stress intensity and duration (Rensburg and Kruger, 1994; Zhang and Kirkham, 1996; DaCosta and Huang, 2007). The prolonged drought stress decreased SOD and CAT activities and increased lipid peroxidation in the leaves of three bentgrass species (DaCosta and Huang, 2007). Fu and Huang (2001) found that SOD activities 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 decreased with increasing stress period under full soil drying. Drought stress also had no effect on leaf SOD activity in Kentucky bluegrass (Zhang and Schmidt, 1999). Although these studies demonstrate the variable activities of CAT, POD and SOD in perennial grass species exposed to drought stress, responses of other important enzymes such as APX, GR, MDHAR, DHAR along with production of ROS to drought stress and recovery were not well-understood in perennial grasses, particularly in the roots.

The study of gene expression underlying the changes in antioxidant enzyme activities could provide insight into molecular adaptation of plant to water-deficit conditions. For example, drought stress up-regulated leaf cytosolic *GR* gene directly related to the intensity of the stress in both resistant and susceptible cowpeas (*Vigna unguiculata* L.) (Contour-Ansel et al., 2006). However, expression patterns of genes encoding antioxidant enzymes are complex and may not be consistent with changes in protein expression and enzyme activity under drought stress. The transcript abundance of cytosolic *APX* and cytosolic cupper/zinc *SOD* (*Cu/ZnSOD*) largely increased with increasing drought stress severity, but relatively small increases in APX protein and activity were observed in the leaves of peas (*Pisum sativum* L.) (Mittler and Zilinskas, 1994). Drought stress increased transcripts of *CAT* in horsegram (*Macrotyloma uniflorum* Lam.) seedlings (Reddy et al., 2008), but decreased the transcript abundance of *CAT1* and *CAT 2*, whereas H_2O_2 content and CAT activity increased in the leaves of wheat (*Triticum aestivum* L.) (Luna et al., 2005). The coordinative control and regulation of activity and gene expressions of antioxidant enzymes may be important to plant survival from drought stress.

Kentucky bluegrass is a cool-season species widely used as forage and turf grass. Drought stress is a major factor limiting grass quality, persistence and production. Antioxidant metabolisms of leaves and roots of Kentucky bluegrass may respond differently to drought stress and recovery following stress, and how ROS production, antioxidant enzyme activities and gene expression of antioxidant enzymes affected by pre-stress, stress and post-stress conditions are not well-known in perennial grass species. Exploring differential antioxidant responses of leaves and roots would provide valuable information for better understanding adaptation of perennial grasses to water-deficit conditions, thus enhancing the selection of drought-tolerant cultivars. Therefore, the objective of this study was to investigate the accumulation of ROS, antioxidant enzyme activities, and gene expression patterns of antioxidant enzymes in leaves and roots of Kentucky bluegrass under drought stress and recovery.

2. Materials and methods

2.1. Plant materials, growth, and stress treatments

Mature Kentucky bluegrass ('Midnight II') sod plugs were collected from the field in the William H. Daniel Research and Diagnostic Center at Purdue University in West Lafayette, IN. This cultivar has been widely used on various turfgrass sites due to its dark blue-green color and good summer performance. The 10-cm diameter cup cutter was used to obtain the plugs from the soil. The plugs were then cut to 3 cm thick (10-cm diameter) and grown in plastic pots (10 cm diameter by 14.5 cm deep) containing a mixture of topsoil (Seafield or Gilford fine sandy loam, Reynolds, IN, USA) and coarse river sand (1:1). The top soil had a pH of 6.9. The grasses were irrigated as necessary to prevent drought stress, mowed three times a week at 4 cm with electric hand-held clippers, and fertilized weekly with water soluble fertilizer of 20–20–20 ($N–P_2O_5–K_2O$) (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) to provide 244 kg N ha^{-1}, 107 kg P ha^{-1} and 202 kg K ha^{-1} per growing season. The grasses were maintained in a greenhouse for 60 d and then moved to growth chambers for 15 d under temperatures of $20 \pm 0.1\,^\circ C/15 \pm 0.1\,^\circ C$ (day/night), a relative humidity of 60%, a 14-h photoperiod, and a photosynthetically active radiation of 500 μmol m^{-2} s^{-1} (fluorescent lamps) prior to drought stress.

Grasses were subjected to the following treatments in the growth chambers, respectively: (1) control, well-watered; (2) drought stress, irrigation withheld until leaf wilting occurred at 5 d after stress (the leaves were no longer rehydrated at night or early morning); (3) recovery, drought-stressed plants were rewatered for 1 d after 5 d of stress.

2.2. Grass quality, water status and chlorophyll fluorescence

The grass quality was visually rated as an integral of color, uniformity, and density on a scale of 1 (brown leaves) to 9 (turgid, green leaves). Leaf relative water content (RWC) was determined according to the following equation: $RWC = (FW - DW)/(SW - DW) \times 100$, where FW is leaf fresh weight, DW is dry weight of leaves after drying at 85 °C for 3 d, and SW is the turgid weight of leaves after soaking in water for 4 h at room temperature (approximately 20 °C). A soil moisture probe (TDR 100, Spectrum Tech, Inc., Plainfield, IL, USA) was used to determine average soil moisture content from 0 to 10 cm deep. Leaf photochemical efficiency was determined by measuring chlorophyll fluorescence (Fv/Fm) on 5 randomly selected leaves in each pot using a fluorescent meter (OS-30P, OPTI-Sciences, Hudson, NH, USA).

2.3. Reactive oxygen species, enzyme assay and lipid peroxidation

The O_2^- production rate was measured as described previously (Jiang and Zhang, 2002) with some modifications. A 0.2-g powder of leaf or root tissue was homogenized in 1 mL of 50 mM Tris–HCl (pH 7.5) and centrifuged at $5000 \times g$ for 10 min at 4 °C. The reaction mixture (1 mL) contained 200 μL supernatant and 800 μL 0.5 mM 3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt (XTT sodium salt). The reduction of XTT was recorded at 470 nm for 5 min. The background absorbance was corrected in the presence of 50 units SOD. The O_2^- production rate was calculated using an extinction coefficient of 2.16×10^4 M^{-1} cm^{-1}.

The H_2O_2 content was determined using the methods of Bernt and Bergmeyer (1974) with some modifications. A 0.2-g powder of leaf or root tissue was homogenized in 0.8 mL of 100 mM sodium phosphate buffer (pH 6.8), and extractions were then centrifuged at $16,000 \times g$ for 15 min at 4 °C. The 0.17 mL of supernatant was added to 0.83 mL peroxidase reagent containing 83 mM sodium phosphate (pH 7.0), 0.005% (w/v) *o*-dianisidine, and 40 μg peroxidase/mL. The mixture was incubated at 30 °C for 10 min, and 0.17 mL of 1N perchloric acid was added to stop the reaction. The absorbance was read at 436 nm. The H_2O_2 concentration was calculated by using standard curve with known concentration.

For enzyme extraction, a 0.5 g leaf or 1.0 g root power was extracted with 4 mL of extraction buffer (50 mM potassium phosphate, 1 mM ethylenediaminetetraacetic acid [EDTA], 1% polyvinylpyrrolidone [PVP], 1 mM dithiothreitol [DTT], and 1 mM

phenylmethylsulfonyl [PMSF], pH 7.8). The extractions were centrifuged at $15,000 \times g$ for 30 min at 4 °C, and supernatant was collected for enzyme assay. The protein content was determined using Bradford's method (1976). The SOD activity was measured by recording the rate of p-nitro blue tetrazolium chloride (NBT) reduction in absorbance at 560 nm (Giannopolities and Rise, 1977). The activity of CAT, POD, APX, MDHAR, DHAR, and GR was determined by following changes in absorbance at 240, 470, 290, 340, 265, and 340 nm, respectively. Details of the method were described by Zhang and Kirkham (1996).

The lipid peroxidation was measured in terms of MDA content (Dhindsa et al., 1981) with some modifications. A 0.5-mL aliquot of supernatant was mixed with 2 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 95 °C for 30 min, quickly cooled, and then centrifuged at $10,000 \times g$ for 10 min. The absorbance was read at 532 and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Health and Packer, 1968).

2.4. Gene expression

Gene expression was performed using a reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA from the grass leaves or roots was isolated using TRI Reagent (Molecular Research Center, OH, USA) and was treated with DNase (TURBO DNA-Free Kit, Ambion, Inc., TX, USA) to remove contaminating genomic DNA. RNA (1.0 μg) was reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad, USA). The synthesized cDNA was subjected to PCR for 35 cycles. The conserved regions of gene sequence of *CAT*, *POD*, cytosolic *Cu/ZnSOD*, chloroplastic *Cu/ZnSOD*, *MnSOD*, *FeSOD*, cytosolic *APX*, *GR*, *DHAR* and *MDHAR* were obtained from other plant species and used to design primers for detecting gene expression in Kentucky bluegrass (Table 1). Aliquots of individual PCR products were resolved by agarose gel electrophoresis and the bands were determined with the Doc-it gel image system (UVP, LLC, Upland, CA, USA).

2.5. Experimental design and statistical analyses

The experiment was randomized complete block design with four replicates. The well-watered, drought-stressed, and recovered grass pots were arranged randomly within growth chambers. Data from 5 d control, 5 d drought, and 1 d recovery were used to analyze the significance of all treatments for a given measurement. Statistical Analysis System (SAS 9.1) (SAS Institute, Inc., Cary, NC, USA) was used for this significant analysis. The means of the treatments were separated using the least significant difference (LSD) at a 0.05 significant level.

3. Results

3.1. Water status and grass quality

Soil moisture measured at average from 0 to 10 cm deep decreased from 29% (v/v) (the control) to 7% under drought stress and recovered to 29% after 1 d of rewatering (Table 2). The leaf RWC dropped to 68% under drought stress and was back to 93% after recovery (Table 2). Drought stress decreased grass quality but had no effect on leaf chlorophyll fluorescence (Fv/Fm) (Table 2).

3.2. O_2^-, H_2O_2 and MDA content

The leaf O_2^- production increased 4.7-fold and 4.8-fold under drought stress and recovery, respectively; compared to the control (Fig. 1). The enhanced production of O_2^- in the roots was only observed after recovery. Leaf H_2O_2 content increased 67% under drought and returned to the control level after recovery (Fig. 1). The root H_2O_2 content increased 1.8-fold and 3.1-fold under drought stress and recovery, respectively, relative to the control. Drought stress increased root MDA content to 4.5-fold. After recovery, root MDA content reduced 49% relative to the level before re-watering but was still 2.4-fold higher than the control.

3.3. Antioxidant enzyme activities

The SOD activities remained unchanged in the leaves but significantly reduced to 32% in the roots under drought stress but were marginally affected by recovery (Fig. 2). No significant changes in leaf CAT activities or leaf and root POD activities were observed under all treatments (Fig. 2). However, root CAT activities significantly increased 3.1-fold and 2.1-fold under drought stress and recovery, respectively.

The leaf APX activities significantly increased 70 and 80% under drought stress and recovery, respectively; compared to the control (Fig. 3). There were no differences in root APX activities among all treatments. The leaf MDHAR activities were marginally affected under drought stress but increased 34% after recovery, while the root MDHAR activities increased 29 and 26% under drought and recovery, respectively (Fig. 3). Increases in DHAR activities up to 2.4-fold and 2.8-fold were found in the leaves under drought stress and recovery, respectively (Fig. 3). The root DHAR activities decreased 47% under drought stress. Recovery increased root DHAR activities 32% compared to the drought treatment but had a similar level to that of the control. Leaf GR activities were not

Table 1
The conserved region of gene sequence and annealing temperature (AT) for polymerase chain reaction (PCR) used for detecting gene expression in the leaves and roots of Kentucky bluegrass.

Gene	Sequence	AT (°C)
Cyt *Cu/ZnSOD*	F: 5′-GACACMACAAATGGHTGCAT-3′; R: 5′-TCATCBGGATCGGCATGGACAAC-3′	57
Chl *Cu/ZnSOD*	F: 5′-ATGGGTGCATATCDAYAG-3′; R: 5′-GCCAGTCTTCCACCAGCAT-3′	57
FeSOD	F: 5′-TTCAACAATGCWGCWCAGG-3′; R: 5′-CAAGGGGGTTCACAGCAT-3′	52
MnSOD	F: 5′-CAGRGBGCCATCAAGTTCAACG-3′; R: 5′-TACTGCAGGTAGTACGCATG-3′	58
CAT	F: 5′-CCTSTCATTGTGMGTTTCTC-3′; R: 5′-GTTAACTCCRAAVCCATCCATATG-3′	57
POD	F: 5′-AGGCCCAGTGCTHCAMCTTC-3′; R: 5′-TTGGTGTAGTAGGCGTTGTC-3′	57
Cyt *APX*	F: 5′-CTCMGAGGCTTCATCGCYGAGAAG-3′; R: 5′-AATCCAGAACGCTCCTTGTG-3′	57
MDHAR	F: 5′-CATGTGTGCGTHGGARGTGG-3′; R: 5′-GGGTAAACCATGCTGACATC-3″	55
DHAR	F: 5′-CTCGGCGACTGYCCNTT-3′; R: 5′-TGGAGATGGTAGAGCTTTGG-3′	56
GR	F: 5′-GCGATTTGCGAGMTCCCNTT-3′; R: 5′-ACAGCAATATATCCGCCACC-3′	56

Chl *Cu/ZnSOD*; chloroplastic *Cu/ZnSOD*; Cyt *Cu/ZnSOD*; cytosolic *Cu/ZnSOD*; Cyt *APX*; cytosolic *APX*. F and R represent forward and reverse.

Table 2
Effects of 5 d drought stress and 1 d recovery on soil moisture content (SMC), leaf relative water content (RWC), grass quality (GQ), and chlorophyll fluorescence (Fv/Fm) in Kentucky bluegrass.

Treatment	SMC (%)	RWC (%)	GQ	Fv/Fm
Control	29 ± 0.70 a	95 ± 1.1 a	8.3 ± 0.09 a	0.76 ± 0.01 a
Drought	7.0 ± 0.45 b	68 ± 4.5 b	6.0 ± 0.10 b	0.75 ± 0.01 a
Recovery	29 ± 0.25 a	93 ± 0.8 a	8.2 ± 0.03 a	0.76 ± 0.01 a

Range of grass quality: 9 = ideal, 6 = minimum acceptable. Means followed by the same letter within a column for a given measurement were not significantly different at $p < 0.05$. Values are mean ± S.E.
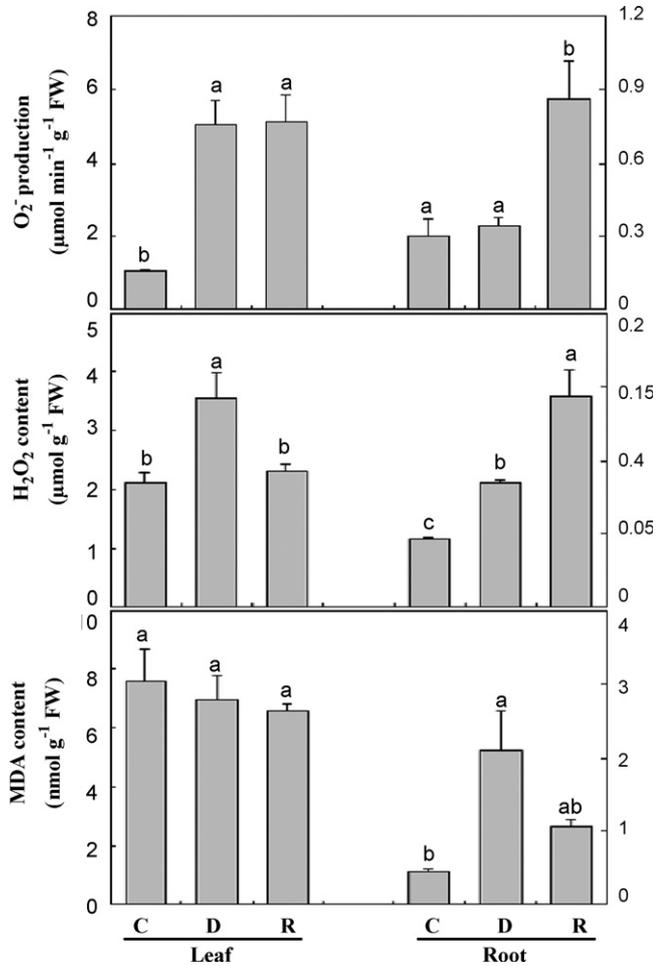
Fig. 1. Effects of 5 d drought stress and 1 d recovery from stress on superoxide (O_2^-) production, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in the leaves and roots of Kentucky bluegrass. Means followed by the same letter within the leaves or roots are not significantly different at $p < 0.05$. Vertical bars show $\pm$S.E.

Fig. 2. Effects of 5 d drought stress and 1 d recovery from stress on the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in the leaves and roots of Kentucky bluegrass. Means followed by the same letter the leaves or roots are not significantly different at $p < 0.05$. Vertical bars show $\pm$S.E.

affected by all treatments but root GR activities increased 38% under drought stress, compared to the control (Fig. 3). The GR activities of the roots returned back to the control level after recovery.

3.4. Gene expression

Drought stress inhibited expressions of *FeSOD*, chloroplastic *Cu/ZnSOD*, and cytosolic *Cu/ZnSOD* of the leaves, but gene expressions recovered back to the control level after 1 d of rewatering (Fig. 4). Expression of cytosolic *Cu/ZnSOD* was suppressed in the roots under drought stress and slightly recovered after rewatering (Fig. 4). No differences in the level of *MnSOD* expression were observed in the leaves under all treatments (Fig. 4). However, expression of *MnSOD* in the roots was inhibited under drought stress but recovered after rewatering.

There were no differences in the intensity of *CAT* expression among the treatments in both leaves and roots (Fig. 4). Expression of *POD* was only noted in the recovered leaves (Fig. 4). The patterns of cytosolic *APX* expression in both leaves and roots were similar to those of *MnSOD*, whereas a reduction in *APX* expression was observed in the roots under drought stress (Fig. 4). The expression of *GR* was not shown in the well-watered leaves but strongly induced by drought stress and remained at that level of intensity

after recovery (Fig. 4). In the roots, *GR* expression was slightly detected in the well-watered control but not under drought stress and recovery.

Expression of *MDHAR* was up-regulated in the leaves by drought stress and recovery. For the roots, *MDHAR* expression was only observed under drought stress but not identified after recovery. Unlike *MDHAR*, drought stress inhibited *DHAR* expression in the leaves, but the level of transcript abundance returned to that of control after rewatering (Fig. 4). Expression of *DHAR* was only found in well-watered roots, and it was inhibited by drought stress and not recovered.

4. Discussion

Grass quality was reduced to the minimum acceptable level at 5 d of drought stress, whereas leaf RWC dropped to about 68%. Under this stress intensity, the plants showed slightly wilting but Fv/Fm did not change. Zulini et al. (2007) reported that Fv/Fm remained unchanged when water potential was significantly different between drought-stressed and irrigated grapevine (*Vitis Binifera*), but Fv/Fm decreased with further increasing intensity of drought stress. The results indicate that leaf water content and visual quality are more sensitive to drought than Fv/Fm, depending on stress severity. The ratio of Fv/Fm represents photosynthetic
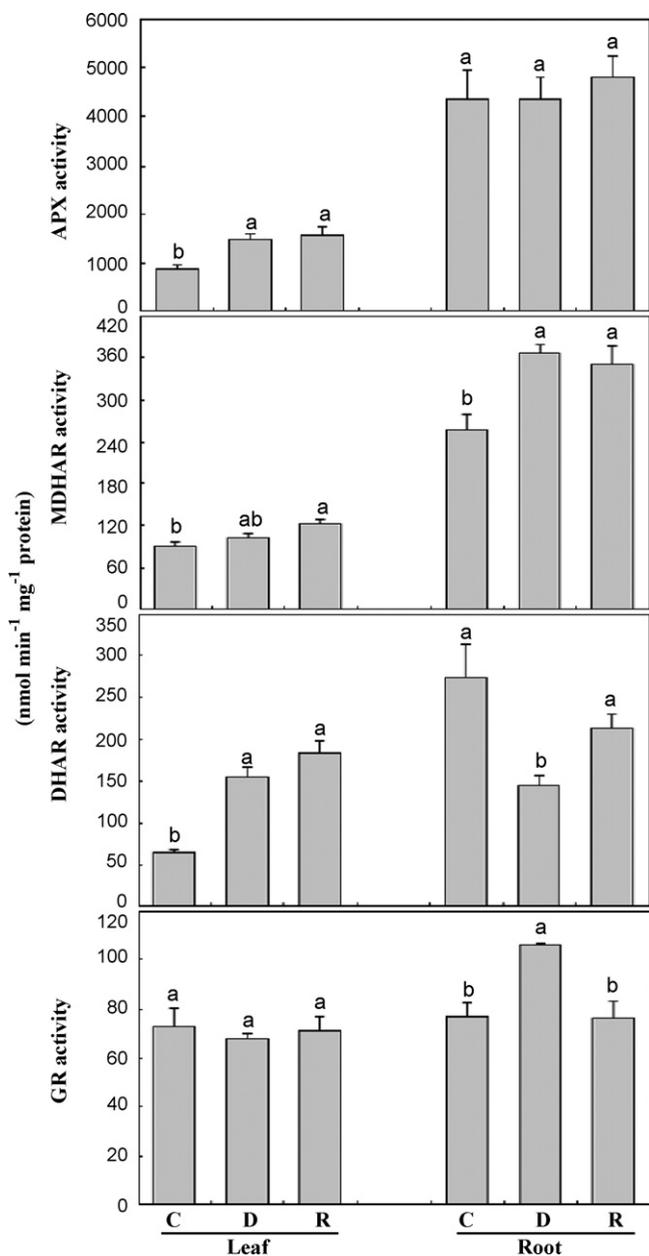
Fig. 3. Effects of 5 d drought stress and 1 d recovery from stress on the activities of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) in the leaves and roots of Kentucky bluegrass. Means followed by the same letter within the leaves or roots are not significantly different at $p < 0.05$. Vertical bars show ±S.E.

Fig. 4. Gene expressions of cytosolic copper/zinc superoxide dismutase (Cyt *Cu/ZnSOD*), chloroplastic copper/zinc superoxide dismutase (Chl *Cu/ZnSOD*), iron superoxide dismutase (*FeSOD*), manganese superoxide dismutase (*MnSOD*), catalase (*CAT*), peroxidase (*POD*), cytosolic ascorbate peroxidase (Cyt *APX*), monodehydroascorbate reductase (*MDHAR*), dehydroascorbate reductase (*DHAR*) and glutathione reductase (*GR*) in the leaves and roots of Kentucky bluegrass at 5 d of drought stress and 1 d of recovery. C, D, and R represent the well-watered control, drought stress, and recovery following stress, respectively. 18s was internal loading control.

efficiency of photosystem II, the unchanged Fv/Fm may indicate that photosynthetic efficiency is stable under the moderate level of drought stress in some plants including Kentucky bluegrass.

Lipid peroxidation is an indicator of the prevalence of free radical reaction in tissues (Halliwell and Gutterides, 1989). The lipid peroxidation increased with increasing drought stress intensity in the roots of rice (*Oryza sativa* L.) (Sharma and Dubey, 2005). Significant O_2^- and H_2O_2 accumulation in the leaves did not contribute to lipid peroxidation in Kentucky bluegrass, and dramatic increase in root lipid peroxidation suggested that more severe oxidative injury occurred under drought stress. The differential responses of lipid peroxidation and ROS accumulation between leaf and root may be associated with level of drought

stress intensity and different protective mechanisms. Unchanged lipid peroxidation was also observed in leaves of the drought-tolerant and drought-susceptible wheat (*Triticum durum* L.) cultivars when leaf water potentials decreased 3.1-fold to 3.6-fold under drought stress, compared to the well-watered control (Loggini et al., 1999). However, with the prolonged drought stress and severe dehydration (RWC < 40%), leaf lipid peroxidation increased in colonial bentgrass (*Agrostis capillaries* L.), creeping bentgrass (*Agrostis stolonifera* L.) and velvet bentgrass (*Agrostis canina* L.) species (DaCosta and Huang, 2007).

The ROS level during drought stress and recovery from drought stress may indicate the potential of oxidative stress or signaling in plants (Foyer and Noctor, 2005). The H_2O_2 content decreased in the leaves of *Prunus* hybrids and gas exchange of the plants recovered (Sofo et al., 2005), suggesting a low ROS production accompanied with recovered physiological activity upon rewatering. Upadhyaya et al. (2008) also found that lower ROS level and higher recovery antioxidant property of tea (*Camellia sinensis* L.) in response to rehydration after drought stress showed a better recovery potential. In this study, accumulations of O_2^- (in leaf and root) and H_2O_2 (in root) still occurred when grass quality and RWC were fully recovered. It is likely that the process of recovery after rewatering did not necessarily limit production of ROS in Kentucky bluegrass. Consistent with a higher level of root lipid peroxidation, the accumulation of O_2^- and H_2O_2 observed in the roots upon rewatering suggest that oxidative stress was potentially involved in root recovery from drought. The role of ROS in oxidative stress or oxidative signaling remains unclear when grasses are rehydrated.

The increased, decreased, and unchanged antioxidant enzyme activities in the leaves or roots of Kentucky bluegrass indicate a different antioxidant metabolism in response to drought stress and recovery. The SOD plays a central role in the enzymatic defense system in removing O_2^- (Bowler et al., 1992). The accumulation of O_2^- occurred in the leaves did not go along with the changes in total SOD activity. The unchanged O_2^- production in the roots under drought stress was also not correlated with the decreased

SOD activity. The results indicated that O_2^- accumulation had different sensitivity to drought stress in the leaves and roots of Kentucky bluegrass while SOD activity showed different patterns under the same stress level. Since enhanced information of ROS under stress conditions induces both protective responses and cellular damage (Blokhina et al., 2003), it is not known whether O_2^- accumulation in the leaves involves protective mechanism or causes cellular damage under drought stress and recovery in Kentucky bluegrass. There are three types of SODs, MnSOD, Cu/ZnSOD, and FeSOD, located in mitochondria and peroxisome, chloroplast and cytosolic, and chloroplast, respectively (Alscher et al., 2002). Mittler and Zilinskas (1994) found that transcript level of cytosolic *Cu/ZnSOD* increased in pea leaves under drought stress and was more enhanced during recovery, suggesting the role of cytosolic *Cu/ZnSOD* on drought tolerance. Although total SOD activity in the leaves of Kentucky bluegrass was not affected by drought stress, the suppressed expression of chloroplastic *Cu/ZnSOD*, cytosolic *Cu/ZnSOD* and *FeSOD*, and the consistent expression of *MnSOD* exhibited different sensitivities and responses of SOD genes to moderate drought stress. The recoverability of these inhibited gene expressions after rewatering indicates an important regulatory mechanism of SOD at transcript level, which could be beneficial to leaf rehydration against further oxidative injury. Inhibition of cytosolic *Cu/ZnSOD* and *MnSOD* expressions along with a reduced SOD activity in the drought-stressed roots suggest that the regulations of SOD can be at both transcriptional and protein levels in the roots of Kentucky bluegrass under moderate drought stress.

The ascorbate-glutathione cycle, an efficient antioxidant system in the detoxification of H_2O_2, involves four enzymes: APX, MDHAR, DHAR, and GR (Asada, 1999). The cycle maintains a ratio of a reduced per oxidized ascorbic acid and glutathione for proper scavenging ROS in plant cells (Mittler, 2002). The increased or stable activities of these four enzymes observed in both leaves and roots of Kentucky bluegrass under drought stress (except for a reduced DHAR activity in the roots) could maintain H_2O_2 detoxification. Compared to the well-watered control, the higher activities of APX, MDHAR and DHAR in the leaves and higher activities of MDHAR and DHAR in the roots after rewatering indicate the potential need for removal of ROS when plants were rehydrated (Fig. 3). The increased activities of APX, MDHAR, DHAR and GR were also found in the leaves of interspecific *Prunus* hybrids subjected to the prolonged drought stress, but enzyme activities were down-regulated during the rewatering phase (Sofo et al., 2005). The different responses of these enzyme activities to rewatering may depend on plant species, stress severity, and intensity of ROS production. Generally, the increased APX and DHAR activities in the leaves and increased GR and MDHAR activities and unchanged APX activities in the roots of Kentucky bluegrass under drought stress may help maintain levels of ASA and GSH, the two important antioxidants against ROS toxicity (Foyer, 1993). Responses of the ascorbate-glutathione cycle to drought stress and recovery indicate that a co-regulated anti-oxidant mechanism could develop in plant cells, which varied with leaves and roots in Kentucky bluegrass.

The pattern of cytosolic *APX* expression in the leaves and roots did not go along with the changes in APX activities when leaf RWC was 68% under drought stress and when soil moisture returned to the control level (Figs. 3 and 4). However, Mittler and Zilinskas (1994) reported that up-regulation of cytosolic *APX* occurred both at the level of steady-state transcript accumulation and by regulation of protein synthesis when pea leaf RWC was about 65% during stress and 85% after 10 h recovery, respectively. Similarly, the expression patterns of *DHAR* and *GR* in both leaves and roots under drought stress were not totally consistent with

enzyme activities. The results suggest that regulations of APX, DHAR and GR occurred at different levels in Kentucky bluegrass under drought stress and recovery. Expression of *GR* was strongly up-regulated in the leaves under drought stress and recovery, and the expression trend was similar to the trend in other studies in which moderate drought stress induced gene expression of cytosolic *GR* in the susceptible cultivar but remained stable in the tolerant cultivar of cowpea leaves (Torres-Franklin et al., 2008). Lunde et al. (2006) reported that expression of two *MDHAR* genes dramatically increased under osmotic stress in moss (*Physcomitrella patens* Hedw.). Our results also showed that the *MDHAR* gene expressions were up-regulated in both leaves and roots under drought stress. The enhanced gene expression along with increased or stable activities of MDHAR may be beneficial to antioxidative protections, although the relative importance of MDHAR and DHAR pathways is not well known in plants. Mittova et al. (2000) found that the specific activity of MDHAR was 10 times higher than DHAR in both mitochondria and chloroplast of tomatoes, suggesting a relatively important role of MDHAR in the antioxidative defense.

Catalase is found in peroxisomes, but is indispensable for decomposing H_2O_2 during stress (Willekens et al., 1997). The up-regulated *CAT* transcript was found in horsegram seedlings under drought stress (Reddy et al., 2008). In Kentucky bluegrass, the consistent and stable expressions of *CAT* and *APX* may facilitate leaf cells in scavenging H_2O_2 in an efficient way, although CAT and APX have different affinities for H_2O_2. The unchanged expression of *CAT* under drought stress and recovery could maintain the level of scavenging H_2O_2 in the root cells.

5. Conclusion

Drought stress and recovery following stress increased certain ROS production in leaves and roots and induced lipid peroxidation of roots in Kentucky bluegrass. The increased or unchanged activities of antioxidant enzymes were generally observed under drought and recovery, but decreased activities of certain enzymes were also noted. Up- or down-regulated gene expressions of antioxidative enzymes in leaves and roots were observed. The differential responses of ROS production, antioxidant enzyme activities and gene expression to drought stress and recovery between leaves and roots suggested different antioxidant metabolisms in Kentucky bluegrass exposed to water-deficit conditions.

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