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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.

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), monodehydroascorbate 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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Abbreviations: APX, ascorbate peroxidase; ASA, ascorbic acid; CAT, catalase; DHAR, dehydroascorbate reductase; Fv/Fm, chlorophyll fluorescence; GQ, grass quality; GR, glutathione reductase; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; O_2^-, superoxide; POD, peroxidase; RWC, relative water content; SOD, superoxide dismutase.

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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 ± 0.1 °C/15 ± 0.1 °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 × *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 × *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