Growth and Enzymatic Activity of Four Warm-season Turfgrass Species Exposed to Waterlogging

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ABSTRACT. Waterlogging (WL) negatively affects plant growth and development, but the physiological responses of turfgrass species to WL are not well understood. The objective of this study was to examine growth and physiological mechanisms of WL tolerance in warm-season turfgrass species. Knotgrass (*Paspalum paspaloides*), spiny mudgrass (*Pseudoraphis spinescens*), seashore paspalum (*Paspalum vaginatum*), and centipedegrass (*Eremochloa ophiuroides*) were subjected to 30 days of WL. At the end of the treatment, knotgrass and spiny mudgrass maintained the shoot and root biomass while seashore paspalum and centipedegrass showed reductions in biomass under WL. Root oxidase activity (ROA) was unaffected until after 12 or 18 days of WL but decreased by 14.3%, 17.8%, 32.0%, and 68.7% at 30 days of WL for knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass, respectively. Waterlogging increased root activities of lactate dehydrogenase and alcohol dehydrogenase, but generally to a lesser extent in knotgrass and spiny mudgrass. The leaf and root activities of superoxide dismutase (SOD) and peroxidase (POD) were induced after 6 or 12 days of WL, but to a greater extent for knotgrass and spiny mudgrass. At 30 days of WL, the increased leaf and root activities of SOD and POD were higher in knotgrass and spiny mudgrass than that of seashore paspalum and centipedegrass; while centipedegrass showed 37.8% reduction in root SOD activity. The total soluble protein (TSP) concentration remained unchanged in both leaves and roots during the entire WL treatment for knotgrass, while a decreased leaf TSP was found in the other three species after 12 or 24 days of WL as well as in the roots of seashore paspalum and centipedegrass. More reductions in leaf or root TSP were observed in seashore paspalum and centipedegrass than in knotgrass and spiny mudgrass at 30 days of WL. The results indicated that higher ROA, activities of antioxidant enzymes and TSP contributed to WL tolerance of warm-season turfgrass species.

Waterlogging is a common environmental stress that limits plant growth and development. Under waterlogging conditions, soil pores are filled with water and hypoxia often occurs, thereby hindering the exchange of O₂ and other gases between roots and the atmospheric environment (Armstrong, 1979). Oxygen deficiency is one of the primary root stresses in the waterlogged or flooded soils (Kozłowski, 1984). As a result, plant metabolism at various growth stages can be significantly affected due to lack of oxygen supply in the environment (Colmer and Voesenek, 2009).

In a water excess environment, one of the fundamental physiological alterations is waterlogged or flood-induced anaerobic respiratory pathways. In the absence of oxygen, roots rely on anaerobic respiration pathways to produce limited energy to maintain metabolic activity (Bailey-Serres and Voesenek, 2008). Thus, adaptation of roots to flooding stress

is critical to whole-plant survival. Compared with flood sensitive species, flood tolerant species are better able to regulate their processes of glycolysis and fermentation to ethanol (Drew, 1997). However, depending on the species and duration of stress, alterations of anaerobic enzyme activity are not always consistent with waterlogging tolerance. The increased alcohol dehydrogenase (ADH) or lactate dehydrogenase (LDH) under waterlogging can be found in the tolerant and sensitive cultivars of a plant species (Kato-Noguchi and Morokuma, 2007; Wei et al., 2013; Yin et al., 2009, 2010). ADH and LDH activities were enhanced in the roots of a flood tolerant cultivar of sorghum (*Sorghum bicolor*) during 72 h of flooding but a transient increase in the activities was found in the roots of a flood sensitive cultivar up to 24 h flooding followed by a decline in activities of these enzymes (Jain et al., 2010). In addition, plant species vary in their selection of anaerobic respiration pathways for survival of flood stress. Flood tolerant *Dendranthema zawadskii* showed higher root ADH activities in favor of ethanol fermentation, whereas flood sensitive *Dendranthema nankin-gense* preferred lactic acid fermentation as a main way of anaerobic respiration (Yin et al., 2010). The ethanol produced by anaerobic respiration is composed of neutral molecules

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that can diffuse and may have a smaller negative effect on plants (Kato, 2000). Moreover, lactic acid fermentation produces high amounts of lactic acid, leading to cytoplasmic acidification, which may not be a long-term survival strategy of plants under stress. Collectively, responses of anaerobic enzymes to waterlogging stress can be influenced by the plant species, duration, and intensity of the stress. Their roles in waterlogging tolerance are not fully understood, especially for perennial grass species exposed to a relatively longer period of stress.

Waterlogging stress can block the chloroplast and mitochondrial electron transport chain inside the plant cell and cause decreases in cell energy charge and increases in the reducing power. As a result, production of active oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen is enhanced, which can break cell homeostasis and is detrimental to the plant cell (Mittler, 2002). Plants have evolved defense systems to protect cells against oxidative injury by removing, decomposing or scavenging ROS. Antioxidant metabolisms play an important role in detoxification of ROS (Mittler, 2002). By de novo sequencing, assembly, and analysis of the roots and shoots transcriptome in response to short-term waterlogging, Qi et al. (2014) concluded that ROS detoxification and energy maintenance were the primary coping mechanisms of 'Zhongshansa', a hybrid of baldcypress (*Taxodium distichum*) and montezuma cypress (*Taxodium mucronatum*) in surviving waterlogging. In enzymatic defense systems, SOD plays a central role in catalyzing the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2 (Bowler et al., 1992). In creeping bentgrass (*Agrostis stolonifera*), SOD activities in the roots increased 83% and 44% in the waterlogging tolerant 'PennG-6' and increased 32% and 26% for intolerant 'Penncross' when waterlogging occurred at 15 and 1 cm below soil level, respectively (Wang and Jiang, 2007). The decreased and unchanged SOD activities as well as differential responses of other antioxidant enzymes such as catalase (CAT), POD, and ascorbate peroxidase (APX) to waterlogging or submergence have also been found in different plant species (Ahmed et al., 2002; Arbona et al., 2008; Lin et al., 2004; Tan et al., 2010; Wang and Jiang, 2007). In addition, significant correlations between SOD and APX and between SOD and CAT were observed in the

waterlogging tolerant citrus genotype 'Carrizo' citrange (*Poncirus trifoliata* × *Citrus sinensis*), supporting the idea of synergistic action in the positive antioxidant response (Arbona et al., 2008). Similar to anaerobic metabolism, differential responses of antioxidant enzymes to waterlogging vary with species and stress duration and intensity. The enhanced activity of antioxidant enzymes during waterlogging stress could contribute to waterlogging tolerance in the tolerant cultivar; however, responses of antioxidant enzymes to waterlogging tolerance are not fully understood, especially for warm-season turfgrasses.

Turfgrasses are often subjected to water excess environments due to frequent, heavy rainfall or over-irrigation followed by slow drainage. Although growth and physiological responses to waterlogging or submergence have been investigated in cool-season turfgrass species (Jiang and Wang, 2006; Wang and Jiang, 2007; Yu et al., 2012), alterations in growth and anaerobic and antioxidant enzymatic activities are

Fig. 1. Shoot and root biomass as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) for each species. Values for each species for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

not well understood in perennial grasses under waterlogging stress, especially for warm-season turf species under an extended period of waterlogging. Therefore, the specific objectives of this study were to determine growth response of four warm-season turfgrass species exposed to waterlogging stress and to examine anaerobic and antioxidant metabolism in relation to waterlogging tolerance. The outcome of this study would provide a basis for selecting appropriate

species for turfgrass sites adjacent to flood plains or for vegetation restoration.

Materials and Methods

PLANT MATERIALS AND GROWING CONDITIONS. The experiment was conducted in a greenhouse in the Grass Research Center at the Institute of Botany, Jiangsu Province and Chinese Academy

Fig. 2. Root oxidase activity as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively.

Table 1. The percentage reduction or increase in root oxidase activity (ROA), activities of root lactate dehydrogenase (LDH), root alcohol dehydrogenase (ADH), leaf superoxide dismutase (LSOD), root superoxide dismutase (RSOD), leaf peroxidase (LPOD), root peroxidase (RPOD), leaf total soluble protein concentration (LTSP), and root total soluble protein concentration (RTSP) for four turfgrass species under 30 d of waterlogging compared with grasses under control conditions.

Species	Percentage reduction (–) or increase (+)								
	ROA (–)	LDH (+)	ADH (+)	LSOD (+)	RSOD (+)	LPOD (+)	RPOD (+)	LTSP (–)	RTSP (–)
Knotgrass	14.3 d	8.5 a	26.0 c	140.0 a	46.8 a	153 a	76.3 b	4.7 d	7.8 b
Spiny mudgrass	17.8 c	14.3 a	15.7 c	87.0 b	39.9 b	91.5 b	118 a	25.0 c	4.1 b
Seashore paspalum	32.0 b	30.8 b	99.0 b	56.8 c	17.7 c	58.8 c	45.7 c	32.8 b	32.7 a
Centipedegrass	68.7 a	16.5 c (–)	207 a	11.1 d	37.8 d (–)	84.7 d	14.3 d	36.5 a	29.7 a

of Sciences. Four species of knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass, varying with submergence tolerance (Gao et al., 2014), were used in this study. On 18 May 2013, 30 stolon segments of each species at the same growth stages were planted in plastic pots (12 cm height, 14 cm diameter) containing sand and soil (1:4 mix). Forty-two pots of each species were grown for 2 months. During the growth period, samples were cut once every 10 d. Based on the growth characteristics of the different grass species, cutting heights were 5 cm for seashore paspalum and centipedegrass and 10 cm for knotgrass and spiny mudgrass. Trimming was stopped when the waterlogging treatment started. During the experiment, the average day air temperatures was 28 °C and maximum photosynthetic photon flux density was 1700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the greenhouse, with 14 h of natural light.

WATERLOGGING TREATMENT. Waterlogging treatment started when plant tissue coverage was more than 80% for all species. The waterlogging treatment began on 20 July 2013 and lasted for 30 d. The pots were placed into barrels (24 cm tall, 29 cm diameter) containing distilled water. Since the pots had drainage holes, the distilled water got into the pots with soil and the roots were exposed to the distilled water. The distilled water was a stress to the plants (Pearson and Kirkham, 1981), which

could affect the responses of plants to waterlogging. The water level was 2–3 cm higher than the soil surface of the pots. To compensate for water loss caused by evapotranspiration, water was added every 3 d to maintain the water level in the barrels. The water was replaced every 5 d, and algae were removed if accumulated to maintain transparency of the water. The control plants were well-watered without soil saturation.

SAMPLING AND MEASUREMENTS. Samplings for leaves and roots were made at 0, 2, 6, 12, 18, 24, and 30 d of waterlogging for enzyme assay. At each day, three pots of the control and three waterlogged pots were removed from their respective containers. The fully developed leaves were harvested and roots were washed free of soil. The fresh leaves and roots were immediately used for enzyme assay. At the end of treatment (30 d), shoots and roots were harvested and the dry weight was determined.

Root oxidase activity was used to indicate vigor of the roots. The measurement of ROA was conducted with 1 g fresh roots using the α -naphthylamine method (Li, 2000). For extraction of LDH and ADH, ≈ 0.5 g roots were placed into a precooled mortar and ground in an ice bath with a precooled enzyme extract containing 1.6 mL of 5 mM MgCl_2 , 5 mM β -mercaptoethanol, 15% glycerol, 1 mM EDTA, 1 mM EGTA, with 0.1 mM

Fig. 3. Root activity of lactate dehydrogenase (LDH) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

phenylmethylsulfonyl fluoride added. The mixture was centrifuged at 12000 g_n for 20 min at 4 °C and the supernatant was collected. Activities of both LDH and ADH were measured with the kit (A020-1 and A083; Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) at wavelengths of 440 nm for LDH and 340 nm for ADH. The rate of 1 μ mol substrate reduced per minute was obtained as a unit of enzyme activity (U), and the enzyme activity was expressed in U per gram protein. The protein content was determined using Bradford's method (Bradford, 1976).

For extraction of the antioxidant enzyme, up to 0.2 g leaves and 0.5 g roots were ground with 4 mL of precooled phosphate buffer solution (pH 7.8). The mixture was centrifuged for 20 min at 4000 g_n , and the supernatant was collected. 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 3-mL reaction contained 1.5 mL of 50 mM phosphate buffer solution, 0.3 mL of 130 μ M methionine solution, 0.3 mL of 750 μ M NBT solution, 0.3 mL of 100 μ M EDTA- Na_2 solution, 0.3 mL of 20 μ M riboflavin solution, 0.05 mL of enzyme, and 0.25 mL of distilled water. The mixture was illuminated under 60–70 μ mol·m⁻²·s⁻¹ for 20 min. The reaction mixture lacking the enzyme developed maximum color as maximum reduction of NBT. The additional reaction mixture serving as the control was placed in the dark. One unit of SOD activity was defined as the amount of enzyme that

caused 50% inhibition in the rate of NBT reduction. POD activity was measured by the guaiacol method (Li, 2000). A 0.1-mL aliquot of the supernatant was added to the reaction mixture containing 2.9 mL of 50 mM phosphate buffer (pH 5.5), 1.0 mL of 2% hydrogen peroxide, and 1.0 mL of 50 mM guaiacol, and compared with phosphate buffer without enzyme. The absorbance was read every 30 s at the wavelength of 470 nm for 3 min. The enzyme activity was calculated based on unit change per minute.

DATA ANALYSIS. The experiment was arranged in a completely randomized design with three replicates for the control and waterlogging treatments for each species. Data were analyzed using SAS (version 9.1; SAS Institute, Cary, NC). The means of the treatments were separated using least significant difference (LSD) at a 0.05 significance level.

Results and Discussion

Analysis of variance indicated that treatment and accession all had significant effects on all parameters. Significant WL by species interactions also were shown in all parameters (data not shown).

EFFECTS OF WATERLOGGING ON BIOMASS. Compared with the control, WL at 30 d significantly increased shoot dry weight (SDW) of knotgrass and did not change SDW of spiny

Fig. 4. Root activity of alcohol dehydrogenase (ADH) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

mudgrass but decreased SDW of seashore paspalum and centipedegrass (Fig. 1). The root dry weight (RDW) was unaffected by 30 d of WL for knotgrass and spiny mudgrass but RDW was reduced in seashore paspalum and centipedegrass (Fig. 1). The SDW and RDW was 112% and 96% for knotgrass, 107% and 92% for spiny mudgrass, 86% and 83% for seashore paspalum, 65% and 75% for centipedegrass, respectively. Decreased RDW were also found in cool-season kentucky bluegrass (*Poa pratensis*) and creeping bentgrass exposed to waterlogging stress with more reductions in RDW observed in the intolerant cultivar (Jiang and Wang, 2006; Wang and Jiang, 2007). Our findings in the current study of four warm-season turfgrass species were consistent with the results in cool-season turfgrass study. The increased biomass in knotgrass under WL conditions indicated that WL simulated growth of this species. The simulated growth was also found in some accessions of perennial ryegrass exposed to submergence (Yu et al., 2012). Hormones such as ethylene and gibberellic acid could play a role in regulating plant growth under waterlogged soil (Jackson, 1985). The differential responses of growth to waterlogging could be also associated with other metabolic changes of the plants such as anaerobic and antioxidant metabolism (Wang and Jiang, 2007; Wang et al., 2009).

EFFECTS OF WATERLOGGING ON ROOT ACTIVITY. The ROA activity significantly decreased after 18 d of WL for knotgrass and spiny budgrass and after 24 d of WL for seashore paspalum (Fig. 2). A decline in ROA was observed at 12 d of WL for centipedegrass. At 30 d of WL, four species differed significantly in reduction ROA, with 14.3% for knotgrass, 17.8% for spiny mudgrass, 32.0% for seashore paspalum, and 68.7% for centipedegrass, respectively (Table 1).

Waterlogging-induced ROA were also found in other plant species (Wang and Jiang, 2007; Ye et al., 2003). The oxidase activity (oxidation of α -naphthylamine) indicates oxygen diffusion to the immediate vicinity of the roots and the power of oxidation in the root tissue (Ota, 1970). The declines in ROA under prolonged waterlogging could impair metabolic activities of the roots in the plants. The smaller reductions in ROA in knotgrass and spiny budgrass suggested that these two species maintained root metabolic activity, which could contribute to the growth of these two species (Fig. 1).

ACTIVITIES OF ROOT ANAEROBIC RESPIRATORY ENZYME. The LDH activities of knotgrass and spiny budgrass significantly increased after 2 d of WL and lasted until 18 d of WL, compared with the control, while significantly increased LDH activities were observed after 6 d and lasted until 24 d of WL for both

Fig. 5. Leaf activity of superoxide dismutase (SOD) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

seashore paspalum and centipedegrass (Fig. 3). The maximum increased LDH activities during WL treatment were 57.3%, 54.0%, 1.5- and 1.1-fold higher than the control for knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass, respectively (Fig. 3). At 30 d, LDH activities under WL conditions returned to the control level for all species; however, four species differed significantly in percentage reduction or increase in root LDH activities (Fig. 3, Table 1).

Enhanced root activities of ADH were also noted in the four species (Fig. 4). Compared with the control, ADH activities increased after 2 d of WL for all species except for spiny mudgrass, whereas its ADH activity increased after 6 d of WL (Fig. 4). The increased ADH activities lasted for 10 d for knotgrass, 18 d for spiny mudgrass, and 28 d for both seashore paspalum and centipedegrass. The maximum increased ADH activities during WL were 81.9% for knotgrass, 95.2% for spiny mudgrass, and 2.4-fold for both seashore paspalum and centipedegrass, compared with the control. Unlike knotgrass and spiny mudgrass, ADH activities were still higher than the control in both seashore paspalum and centipedegrass at the end of the WL treatment. Compared with the control, the percentage increases in ADH under 30 d of WL were significantly higher in centipedegrass and

seashore paspalum than in knotgrass and spiny mudgrass (Table 1).

Root anaerobic metabolism plays an important role in producing energy for the short-term survival of plants in anaerobic environments through fermentative metabolism (Drew, 1997; Richard et al., 2006), which involves several key enzymes including ADH and LDH. Compared with flood sensitive species, flood tolerant species are better able to regulate their processes of glycolysis and fermentation to ethanol (Drew, 1997). Waterlogging stress or submergence induces ADH and LDH activities in plant species (Chen and Qualls, 2003; Wang et al., 2009; Yin et al., 2009); however, the results are often inconsistent when comparing the anaerobic responses of the tolerant and sensitive species and cultivars to stress. The activities or expressions of these anaerobic enzymes exhibit different patterns under various flooding stresses. In rice (*Oryza sativa*), the short-term anoxic stress did not change LDH activity or lactate concentration but increased ADH and pyruvate decarboxylase (PDC) activities as well as ethanol concentration in the coleoptiles of four cultivars, to a greater extent in the two cultivars with more coleoptile elongation (Kato-Noguchi and Morokuma, 2007). The results of that study suggest that the ability to increase ethanolic fermentation may be one of the determinants in

Fig. 6. Root activity of superoxide dismutase (SOD) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

anoxia tolerance of rice coleoptiles under short-term stress. In another study, activities of ADH and LDH increased in the roots of sesame (*Sesamum indicum*), but ADH activity was higher in the tolerant cultivar and LDH activity was higher in the intolerant cultivar (Wei et al., 2013). Enhanced ADH and LDH activities were also found in chrysanthemum (*Dendranthema grandiflorum*), but to a greater extent in the waterlogging sensitive cultivar (Yin et al., 2009). Our results supported these observations found in chrysanthemum, with more pronounced ADH and LDH activities noted in the intolerant seashore paspalum and centipedegrass. The results indicate a stronger root ethanol fermentation in the waterlogging sensitive grass species exposed to a longer period of stress. The enhanced anaerobic respiration could result in accumulation of anaerobic respiration products (e.g., lactic acid and ethanol), which may cause a metabolism disturbance of cells in the roots and influence the function of normal cells (Drew, 1983). This might be one of the factors causing the reduced growth and poor waterlogging tolerance of seashore paspalum and centipedegrass in this study. Furthermore, maize (*Zea mays*) and barley (*Hordeum vulgare*) mutants lacking the ADH1 gene became more sensitive to flooding injury than the wild type plants (Roberts et al., 1989), suggesting a role of ADH in flooding tolerance.

However, strong over-expression of LDH and ADH had no effect on improving survival under low oxygen stress in *Arabidopsis thaliana*, whereas over-expression of PDC enhanced survival (Ismond et al., 2003). All the results suggest that species variation and duration of stress influence anaerobic metabolism and waterlogging tolerance. The anaerobic responses in the four warm-season turfgrass species found in this study indicate that low sensitivity of fermentation pathway to waterlogging may be associated with waterlogging tolerance in some perennial grass species.

ACTIVITIES OF ANTIOXIDANT ENZYME. The activities of antioxidant enzymes in response to waterlogging conditions have been investigated in plant species; however, the results are often inconsistent. In this study, WL increased SOD activities in both leaves and roots, varying with stress duration and species. In the leaves, the increased SOD activities were shown at 12 d of WL for knotgrass and centipedegrass and 6 d for spiny mudgrass and seashore paspalum (Fig. 5). At 30 d of WL, species differed significantly in increased SOD activities, with 1.4-fold, 87.0%, 56.8%, and 11.1% for knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass, respectively, compared with the control (Fig. 6, Table 1). In roots, the increased SOD activities were observed starting at 12 d of WL for knotgrass and at 6 d for spiny mudgrass (Fig. 6). For

Fig. 7. Leaf activity of peroxidase (POD) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

seashore paspalum, the increased SOD activities occurred at 6 d of WL and lasted for 18 d (Fig. 6). For *E. ophiuroides*, the decreased SOD activities were found as early as 2 d of WL, and SOD activities were then unchanged at 6 d, increased at 12 d, and finally decreased by the end of the stress treatment. At 30 d, the root activities of SOD increased 46.8% for knotgrass, 39.9% for spiny mudgrass, 17.7% for seashore paspalum and were reduced by 37.8% for centipedegrass (Table 1).

Waterlogging increased POD activities, starting at 6 d of treatment in the leaves and roots for all species except for roots in seashore paspalum, whereas its POD activity increased at 2 d of WL (Figs. 7 and 8). The increased POD activities were continuously noted in the leaves till the end of the stress. At 30 d, leaf POD activities were 1.5-fold, 91.5%, 58.8%, and 83.7% higher than the control for knotgrass, spiny mudgrass, seashore paspalum and centipedegrass, respectively (Table 1, Fig. 7). At 30 d, root POD activities were 76.3%, 1.2-fold, and 45.7% higher than the control for knotgrass, spiny mudgrass, and seashore paspalum, respectively (Table 1, Fig. 8). There were no changes in POD activities in roots between the control and WL for centipedegrass at 30 d of treatment (Fig. 8).

The primary reaction of hypoxic-treated roots has been associated with activation of the antioxidative defense system

to prevent cells experiencing ROS poisoning (Qi et al., 2014). In the enzymatic defense system, SOD constitutes the first line of defense against ROS by dismutating O_2^- to H_2O_2 (Bowler et al., 1992). Then H_2O_2 can be decomposed by POD and/or catalase. The increased, decreased, and unchanged SOD activities were found in different plant species subjected to waterlogging stress (Ahmed et al., 2002; Arbona et al., 2008; Lin et al., 2004; Tan et al., 2010; Wang and Jiang, 2007). In creeping bentgrass, SOD activities in the roots increased under WL in both tolerant and sensitive cultivars, but to a greater extent in the tolerant cultivar (Wang and Jiang, 2007). Similar results were found in the waterlogging tolerant chrysanthemum and sesame compared with the intolerant plants (Wei et al., 2013; Yin et al., 2009). Our results for the four different warm-season grasses in this study were consistent with those findings. Particularly, the higher SOD activities in the roots of the tolerant knotgrass along with the lower SOD activities in the intolerant centipedegrass under a longer period of stress could indicate the importance of SOD in root tolerance to WL. Similarly, the results demonstrated that maintenance of POD activities in the roots were associated with WL tolerance, especially under an extended period of stress. The increased POD activities were also observed in bermudagrass (*Cynodon dactylon*) under different levels of

Fig. 8. Root activity of peroxidase (POD) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

submergence stress (Tan et al., 2010). However, activities of POD remained unchanged in the roots in response to 21 d of different depths of WL in both tolerant and intolerant creeping bentgrass cultivars (Wang and Jiang, 2007). It appears that the patterns of antioxidant enzymes under waterlogging may depend on particular species or cultivars and the duration and intensity of stress.

TOTAL SOLUBLE PROTEIN. Waterlogging did not alter TSP concentrations in the leaves of knotgrass throughout the treatment but decreased TSP at 18, 24, and 12 d of WL for spiny mudgrass, seashore paspalum, and centipedegrass, respectively (Fig. 9). The more reductions in leaf and root TSP were found in seashore paspalum and centipedegrass than in knotgrass and spiny mudgrass by the end of WL treatment (Table 1). At 30 d of WL, leaf TSP was reduced by 25.0% for spiny mudgrass, 32.8% for seashore paspalum, and 36.5% for centipedegrass, compared with their respective controls (Table 1). In the roots, TSP was unaffected in knotgrass and spiny mudgrass throughout the treatment but was reduced by 32.7% and 29.7% at the end of the treatment for seashore paspalum and centipedegrass, respectively (Fig. 10, Table 1).

The responses of TSP to flooding stress vary with plant species. The increased, decreased and unchanged TSPs have been reported in plants under WL or submergence (Chen and Qualls, 2003; Kreuzwieser et al., 2002; Mohanty and Ong, 2003). In creeping bentgrass, Jiang and Wang (2006) found that WL reduced root TSP; however, no significant reductions in TSP were observed among three different depths of WL. Reduced TSP was also observed in both leaves and roots in two *Dendranthema* species subjected to waterlogging (Yin et al., 2010). When red clover (*Trifolium repens*) was exposed to 14 d of WL, the sensitive red clover had reductions in leaf soluble protein and Rubisco revealed by protein gel, along with more increased protease activity (Stoychev et al., 2013). The stable TSP in spiny mudgrass and decreased TSP in centipedegrass found in this study provided additional evidence for the role of TSP in WL tolerance in warm-season turfgrass species.

In conclusion, responses of shoots and roots to WL stress varied among four warm-season turfgrass species. Of them, knotgrass showed better WL tolerance, followed by spiny mudgrass and seashore paspalum, while centipedegrass had poor tolerance. Shoot and root biomass was unaffected by WL

Fig. 9. Leaf total soluble protein concentration (TSP) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

Fig. 10. Root total soluble protein concentration (TSP) as affected by 30 d of waterlogging in knotgrass, spiny mudgrass, seashore paspalum, and centipedegrass. Comparisons are made between the control (CK) and waterlogging (WL) under each day of treatment for a given species. Values for each date for a given treatment averaged by three replications; * and ** indicate significant differences at $P < 0.05$ and < 0.01 , respectively.

in knotgrass and spiny mudgrass and decreased in seashore paspalum and centipedegrass. The higher ROA, the less-increased root LDH and ADH activities as well as maintenance of antioxidant enzyme activity and TSP concentration contributed to WL tolerance of warm-season turfgrass species.

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