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RESPONSES OF ROOT GROWTH AND PROTECTIVE ENZYMES TO COPPER STRESS IN TURFGRASS

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Root growth and protective enzymes of *Festuca arundinacea* L. and *Lolium perenne* L. under Cu stress were investigated in a hydroponic experiment. Cu stress significantly inhibited root growth (root elongation and dry biomass) of both turfgrasses. Malondialdehyde (MDA) content in roots of both turfgrasses markedly increased under copper stress. In *F. arundinacea* root, superoxide dismutase (SOD) activity increased greatly with increasing Cu concentration; peroxidase (POD) activity increased at low Cu level and decreased at high Cu level. Increased MDA content indicated the formation of free radicals under Cu stress, while increased SOD activity pointed to the operation of a scavenging mechanism. In roots of *L. perenne*, however, SOD and POD were not activated by copper. These results demonstrate that turfgrass cultivars clearly differ in tolerance to Cu stress, and that the tolerance depends largely on the enhanced activity of its antioxidant system.

Key words: Copper stress, *Festuca arundinacea* L., *Lolium perenne* L., MDA, antioxidant enzyme.

INTRODUCTION

With rapid industrialization and urbanization, heavy metal contamination, usually resulting from human activities, has become a serious environmental problem, limiting plant productivity and threatening human health (Demirevska-Kepova et al., 2004). Copper is an essential micronutrient for plants, playing an important role in maintaining plants' natural metabolism and growth, but in excess it is also a proven inhibitor of various physiological functions (Monnet et al., 2006). Copper induces toxicity at tissue concentrations slightly above its optimal levels (Fernandes and Henriques, 1991). A significant component of copper toxicity is oxidative stress, catalyzing the generation of harmful reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$), which can damage biological molecules and membranes by inducing lipid peroxidation (Weckx and Clijsters, 1996; Hall, 2002). To obviate copper toxicity, plants have developed various strategies employing secretion of organic acid, retention of copper in roots, and immobilization in the cell wall (Bradley et al., 1981; Hu et al., 2007; Wei et al., 2008). Plants have also evolved protective enzymatic mechanisms to scavenge ROS and alleviate their deleterious effects, such as peroxidases (POD), superoxide dismutase (SOD) and catalase (CAT) (Scandalios, 1993; Teisseire and Guy, 2000).

Many reports on Cu^{2+} stress have concentrated mainly on growth inhibition (including root inhibition), Cu accumulation in the organism, and antioxidant enzymes of plant leaves (Maksymiec and Krupa, 2007; Khatun et al., 2008; Wei et al., 2008), but there are far fewer on the responses of root antioxidant enzymes to copper stress (Panda, 2008; Kováčik et al., 2009; Madejón et al., 2009). *L. perenne* and *F. arundinacea* are common turfgrasses in north China. Their growing conditions directly affect lawn quality. The aim of this work was to investigate turfgrass root growth in hydroponic trials with Cu stress, and copper toxic effects on root protective enzymes. The two turfgrasses' root antioxidant enzyme responses to Cu stress should provide some insight into the exact mechanism of Cu inhibition in other plants.

MATERIALS AND METHODS

PLANT CULTIVATION, TREATMENT AND GROWTH ANALYSIS

Seeds of *Festuca arundinacea* L. and *Lolium perenne* L. were surface-sterilized with 50% NaClO (8% active Cl_2) for 10 min and then rinsed with

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TABLE 1. Root growth indices of turfgrass under Cu^{2+} stress

Turfgrass	Cu^{2+}concentration (mg/L)	Root growth indices		
		Root length (cm)	Root dry weight (g/plant)	Root/shoot ratio**
F. arundinacea	0	9.13±1.41a*	0.035±0.004a*	0.22±0.02ab*
	30	3.79±0.23b	0.024±0.004ab	0.22±0.01a
	60	4.39±0.73b	0.030±0.003ab	0.23±0.03a
	90	4.26±0.63b	0.027±0.002ab	0.20±0.01ab
	120	4.47±0.31b	0.022±0.004b	0.16±0.02b
L. perenne	0	10.00±1.93a	0.050±0.012a	0.25±0.02a
	30	5.98±0.97b	0.023±0.007bc	0.18±0.02b
	60	6.86±0.55b	0.028±0.006b	0.18±0.02b
	90	6.70±1.02b	0.016±0.004c	0.14±0.01bc
	120	6.66±1.22b	0.019±0.002bc	0.13±0.01c

* Mean values are presented±SE (n = 4). Values with different letters in the vertical column within the same plant differ significantly at $p<0.05$.
** Root/shoot ratio denotes the ratio of root biomass and shoot biomass.

deionized water. For germination, a 100 mL beaker was filled with 85 mL Hoagland nutrient solution (pH 5.5) and sealed with gauze. Then 100 seeds of turfgrass were germinated on the gauze, which was in contact with the Hoagland solution. The nutrient solution was replenished with distilled water twice daily and renewed once a week. The plants were grown in a controlled environmental growth chamber at 25°C and 50–60% relative humidity under white fluorescent light [photon flux density 135 μmol/(m^2s)] under a 16 h photoperiod. On day 33 after sowing the seedlings were treated with different concentrations of Cu supplied in $CuCl_2$ solution: 0 (control), 30, 60, 90, and 120 mg/L. Each treatment was made in four replicates. After two weeks of Cu treatment the plants were harvested. The roots were separated and their length determined. Then the roots and shoots were washed and oven-dried to constant weight for determination of dry biomass.

MDA DETERMINATION

MDA content was measured as described by Zhang and Qu (2003). Briefly, 0.1 g ground roots were homogenized in 5 ml 10% trichloroacetic acid (TCA) with a chilled mortar and pestle and then centrifuged at 4,000 rpm for 10 min. Then 2 ml supernatant was mixed with 2 ml solution containing 0.6% TBA in 10% TCA. The mixture was heated in a boiling bath for 15 min, quickly cooled and then centrifuged at 4,000 rpm for 10 min. Absorbance of the supernatant was determined at 532 nm and 600 nm. The MDA concentration was calculated after subtracting nonspecific absorbance at 600 nm using the extinction coefficient of 155 mM cm^{-1} (Monferrán et al., 2009), and expressed in μmol per gram fresh weight. The blank was 2 ml distilled water in 2 ml 0.6% TBA in 10% TCA without the extract.

ANTIOXIDANT ENZYME ACTIVITY

Roots (0.1 g FW) were homogenized with a mortar and pestle in 5 ml chilled sodium phosphate buffer (50 mM, pH 7.8). The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was stored at 4°C and used for POD and SOD assays. SOD activity was measured according to Zhang and Qu (2003) by its inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7.8), 0.1 mM EDTA, enzyme extract, 13 mM methionine, 2 μM riboflavin and 75 μM NBT. The reaction mixture was kept under white fluorescent light for 20 min and then stirred, and absorbance was recorded at 560 nm. One SOD unit was taken to be the amount of enzyme causing 50% inhibition of NBT reduction and expressed in units per gram fresh weight.

POD activity was determined using the guaiacol oxidation method (Zhang et al., 2005). The reaction mixture was prepared immediately in a total volume of 50 mL 100 mM sodium phosphate buffer (pH 6.0)

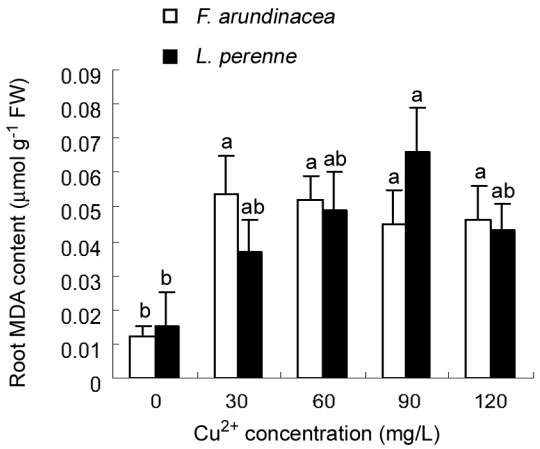

Fig. 1. Root MDA content under Cu^{2+} stress (means ±SE, n = 4). Values with different letters for the same plant differ significantly at p<0. 05.

containing 28 μL guaiacol (100%) and 9 μL H_2O_2 (30%). Then 1 mL enzyme extract was added to 3 mL reaction mixture. The reaction time was recorded immediately upon adding the enzyme extract. The increase in absorbance was recorded at 470 nm at 1 min intervals up to 3 min with a UV-vis spectrophotometer. One POD unit was expressed as a 0.01 change of ΔA_{470nm} per minute per g fresh weight.

STATISTICAL ANALYSIS

The experiments were performed in quadruplicate and the results were averaged (means ±SE). The significance of differences between the control and each treatment was analyzed using the SPSS statistical package (version 13.0 for Windows; SPSS, Chicago, IL, U.S.A.).

RESULTS AND DISCUSSION

EFFECT OF Cu^{2+} ON ROOT GROWTH

Cu treatments significantly inhibited root elongation of *F. arundinacea* and *L. perenne* (p<0.05). The decrease was more pronounced in *F. arundinacea* than in *L. perenne* (Tab. 1), but between Cu treatments the difference in root elongation was not significant. At higher Cu^{2+} concentration, turfgrass showed visible symptoms of copper toxicity. After 7d Cu treatment, growth was severely retarded, and short browning roots were observed. These observations accord with findings from other researchers (Liu and Sun, 1985; Groppa et al., 2008). By the end of the experiment, roots of *F. arundinacea* and *L. perenne* treated with 30 mg/L Cu^{2+} decreased 58.5% and 40.2% respectively versus the controls. The dry root biomass of the two turfgrasses also decreased greatly under copper stress, especially at the higher Cu concentration; in the 120 mg/L Cu treatment it decreased 37.1% and 62% respectively versus the controls for *F. arundinacea* and *L. perenne*. The root/shoot ratio in two turfgrass tended to decrease with increasing Cu concentration, indicating that root growth was more sensitive to copper stress than shoot growth. Studies have shown that heavy metals affect root cell elongation and decrease mitotic activity (Ouzounidou et al., 1995; Alaoui-Sossé et al., 2004; Maksymiec and Krupa, 2007), thus inhibiting root growth (Table 1).

EFFECT OF Cu^{2+} ON MDA CONTENT

MDA, a product of lipid peroxidation, has often been considered an indicator of oxidative damage (Shalata et al., 2001; Meloni et al., 2003). Lipid peroxidation has damaging effects on the cell membrane and ultrastructure (Wang, 2005). In the present study, MDA content in *F. arundinacea* roots increased significantly in the copper treatments (p<0.05). For *L. perenne*, though, there was no significant difference between Cu treatment and the control except at 90 mg/L, where there was a great increase in MDA content (Fig. 1). The increased MDA content indicates that Cu caused severe oxidative stress by stimulating ROS generation (Dietz et al., 1999). Similar observations have been reported in *Withania somnifera* (Khatun et al., 2008) and oat (Luna et al., 1994).

EFFECT OF Cu^{2+} ON SOD AND POD ACTIVITIES

Copper stress induces the formation of harmful reactive oxygen species (ROS), which causes irreversible damage to macromolecules (Lombardi and Sebastiani, 2005). To overcome this, cells have evolved enzymatic mechanisms to eliminate or reduce their damaging effects. Antioxidant enzymes such as SOD and POD are important for their ability to scavenge ROS and thereby prevent oxidative damages (Liang et al., 2003; Sundar et al., 2004). When ROS production exceeds the ability of the antioxidant system to counteract it, damage occurs (Choudhary et al., 2007). Plants with increased antioxidant enzyme activity have been shown to be tolerant to oxidative stress (Mittler et al., 2004). The SOD activity in *F. arundinacea* roots increased with increasing Cu concentration (Fig. 2a), suggesting that SOD activity was rapidly induced by copper. The higher SOD activity could increase the ability of the roots to scavenge O_2^- radicals, preventing oxidative damage to cells. In *L. perenne* roots, however,

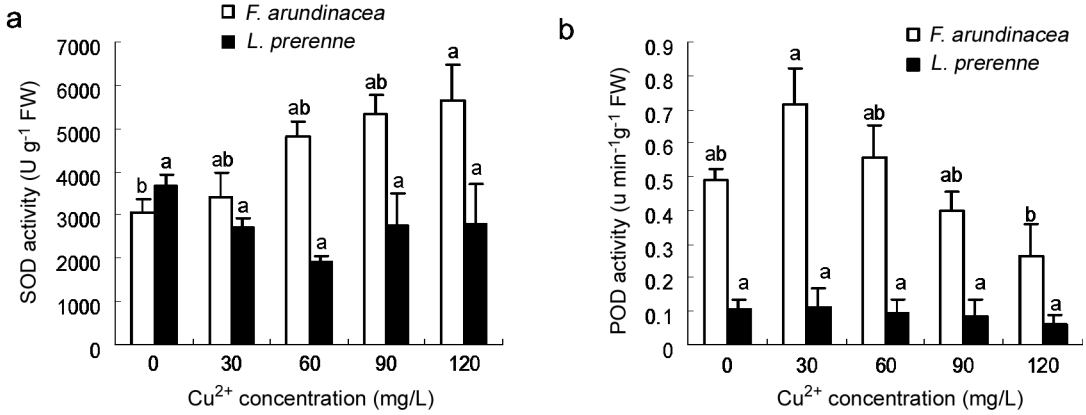

Fig. 2. Root SOD and POD activity under Cu^{2+} stress (means ±SE, n = 4). Values with different letters for the same plant differ significantly at $p<0.05$.

SOD activity in the copper treatments was lower than in the control, and there was no significant difference between treatments (Fig. 2a). A possible reason for this is that, unlike in resistant plants, *L. perenne* roots accumulated a much greater amount of copper ions, which damaged the mechanism of SOD production. Similar results were reported in other sensitive varieties with lower SOD and higher Cu concentration in leaves (Tanyolaç et al., 2007; Ke et al., 2007).

In *F. arundinacea* roots, POD activity increased first, reached maximum in the 30 mg/L treatment, and then decreased with increasing Cu concentration (Fig. 2b). In *L. perenne* roots, POD activity, in a way similar to SOD activity, tended to decrease under copper stress except in the 30 mg/L treatment; in the 120 mg/L treatment, POD activity decreased to 40.6% of the control (Fig. 2b). The increased POD activity in *F. arundinacea* roots at low Cu concentration was induced by Cu stress. With increasing Cu concentration, the decrease in POD activity might be due to inactivation of POD activity and a high level of oxidative stress (Ozden et al., 2009) caused by higher Cu content accumulated in roots (Liu and Xiong, 2005). This also might be the reason for lower POD activity in *L. perenne* roots under copper stress. High POD activity would enable the plants to scavenge H_2O_2 in cells, thus maintaining cellular membrane integrity and protecting against the oxidative stress induced by excess copper (Tanyolaç et al., 2007; Monferrán et al., 2009).

This work demonstrated strong inhibition of root growth in *F. arundinacea* and *L. perenne* under Cu exposure. MDA content in roots was enhanced, indicating lipid peroxidation under Cu stress. The higher SOD and POD activity boosted the resistance of *F. arundinacea* roots to copper stress. The lower SOD and POD activity in *L. perenne* roots under Cu exposure apparently was correlated with high Cu content in roots. Our results suggest that the turfgrass cultivars clearly differ in tolerance to Cu stress, and that the tolerance largely depends on the enhanced activity of their antioxidant systems.

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