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Full Length Research Paper

Manganese delays the senescence induced by drought in perennial ryegrass (*Lolium perenne* L.)

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Manganese (Mn) is an important micronutrient in plant development. In order to test its capability to delay the senescence related oxidative stress in perennial ryegrass (*Lolium perenne* L.); a pot experiment was conducted in China Agriculture University during 2009 and 2010. Plants were sprayed with Manganese at 51 days after sowing then leaves were harvested at both 53 and 55 days after sowing for analyzing superoxide anion production and antioxidation enzymes activity. Results indicated that Mn clearly enhanced the dry weight of individual plants by 34%, inhibited the lipid peroxidation through decreasing the malondialdehyde (MDA) by 26 and 24% in 53 and 55 days after sowing, respectively, prevented chlorophyll from decomposing and maintained the integrity of cell membrane. Meanwhile, the activity of superoxide dismutase (SOD) was increased in Mn treatment group by 13.8 and 11.9% in 53 and 55 days after sowing compared with control group, Mn effectively increased the proline content of treatment plants by 16.3 and 18.2% over the control plants in 53 and 55 days after sowing, respectively. So the results significantly proved that Mn can delay the senescence induced by drought in perennial ryegrass.

Key words: Manganese, senescence, oxidants, antioxidative enzymes.

INTRODUCTION

Manganese (Mn) is an essential micronutrient in the process of oxidoreduction of photosynthetic electronic transport system (Marschner, 1995). Mn is also a critical metallic ion of superoxide dismutase (SOD) in mitochondria. Being a powerful natural antioxidant, Mn enhances tolerance of the plants to many environmental stresses. MnSOD is the only form of SOD that has been identified to be essential for the survival of the aerobic life (Carlioz et al., 1986). The over-expression of MnSOD in tobacco significantly increased tolerance of oxidative stress (Bowler et al., 1991). The tobacco MnSOD gene expressed in the chloroplast of *Zea mays* has also been studied for chilling stress tolerance (Breusegem et al., 1995). Recently, Hua et al. (2009) demonstrated that Mn has dual effects on Medicago sativa depending on the dosage. Mn can stimulate the plant growth as an antioxidant at low concentrations, whereas at a higher concentration it reduces the yield as a pro-oxidant. All of these reports strongly suggest that increased expression of MnSOD in plants enhances tolerance of environmental stresses, and Manganese (Mn) is very important in the process of increasing the yield. However, little was known about the impact of Mn on delaying the senescence of plants induced by the environmental stresses.

Senescence, an integral part of plant development, may coincide with the production of free oxygen radicals and can be regulated by a variety of environmental and autonomous factors (Kar and Feirabend, 1984). Monocarpic senescence of plants has been earlier reported (Nooden 1984, 1988; Nooden et al., 1979; Nooden and Thompson, 1985), but the regulatory mechanisms of this process remain undescribed. Here, the effect of Mn on antioxidative systems was reported. Mn increase resist senescence induced by both drought and related oxidative stress in Perennial Ryegrass (*Lolium perenne* L.).

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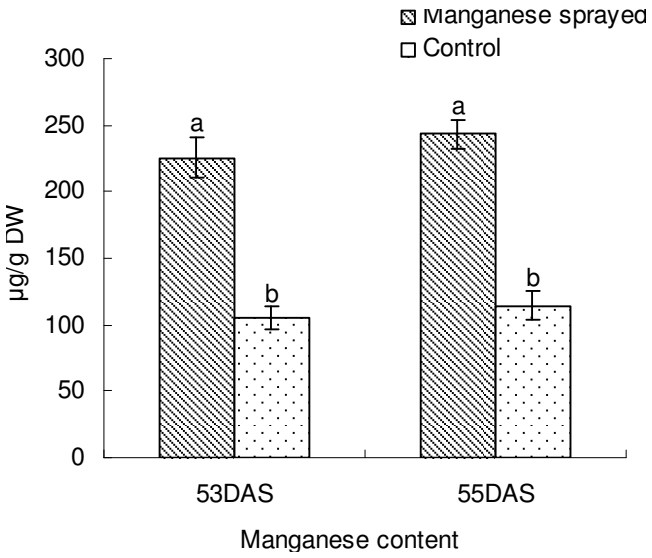

Figure 1. Effect of manganese spray on Manganese content in perennial ryegrass at 53 and 55 DAS. a, b=significant at P≤0.05.

MATERIALS AND METHODS

The seeds of Perennial Ryegrass (*Lolium perenne* L.) were sowed in pots in the greenhouse of China Agricultural University (300-400 µM m^{-2} s^{-1}) photosynthetic photo flux density, 65% relative humidity, 8 h dark and 16 h light with 18 to 30°C temperature ranges). The diameters of the pots were 79 cm with coarse textured soil (pH 7.8 in 1:2 soils: water suspension and 3.08% of organic carbon). Available nitrogen of the soil is 178.5 mg/kg ha^{-1}, available phosphorus is 20.4 mg/ kg and available potassium is 257.4 mg/kg. The total Mn content of the soil was 536.2 mg/kg, and the water soluble Mn was11.4 mg/kg.

The plants were watered using the deionized water every two days until 48 days after sowing (DAS).The Mn was sprayed on the plants on 51 DAS at the concentration of 0.091% (Mn as manganese chloride; the entire plant was completely drenched). Control plants were sprayed with deionized water. The leaves of plants in both Mn treatment and control groups at 53 DAS and 55 DAS, were harvested. The harvested leaves were washed and immediately frozen in liquid nitrogen and stored at -70°C for enzyme analysis. Each group (control and Mn treatment) has 6 replications.

Measurements of total dry matter production and Mn in the plants

Dry matter production as growth attribute was measured for 6 replicates with 2 treatments. The samples were dried in air oven at 60°C till constant weight and weighed. Mn content(µg g-1DW) was estimated in individual plants (n=10). The leaves collected from the plants were washed with distilled water and dried for 3 days in air oven at 80°C.

Then the samples were dry-ashed in a muffle furnace at 500°C for 8 h and digested with 2 M HCl. Mn was extracted as described by Sadzawaka et al. (2004), and the concentration was determined by atomic absorption spectrophotometer. For antioxidants, attention was placed on the Mn-induced changes in superoxide dismutase (SOD), catalase (CAT) and proline. Superoxide anion and lipid per-oxidation were analyzed for oxidant production. Besides the antioxidant and oxidant production, the contents of chlorophyll and the cell membrane penetrability were quantified as indicators of senescence processes.

Analysis of antioxidant enzymes and proline

In order to estimate the activity of SOD and catalase enzymes, the frozen tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.6) containing 1 mM EDTA, 1 mM dithiothreitol and 5 ml of 4% polyvinyl pyrrolidone per gram fresh weight. The homogenate was filtered and centrifuged at 20,000 X g at 4°C. The supernatant liquid was used for the enzyme activity measurement.

SOD was estimated using the method of Beauchamp and Fridovich (1971). CAT was determined on the basis of the method of samantary (2002). The activity of this enzyme was demonstrated as µM H_2O_2 reduced min/mg protein. Proline was extracted using 3% sulfosalicylic acid and estimated using acid ninhydrin reagent and measuring the toluene chromophore absorbency at 520 nm (Bates et al., 1973).

The analysis of Lipid peroxidation and superoxide anion

The content of malondialdehyde (MDA) was used to measure the lipid per-oxidation (Behra et al., 1999). Superoxide anion was estimated according to Chaitanya and Naithani (1994).

Analysis of chlorophyll contents and cell membrane penetrability

Chlorophyll was extracted in 80% acetone and estimated based on Arnon (1949). The analysis of chlorophyll contents consist of the chlorophyll a content, Chlorophyll b content and the total Chloro-phyll content. For measuring cell membrane integrity, the relative permeability of plasma was determined by placing 1.0 g leaves discs in a small Bunsen beaker with 20 ml distilled water. Oscillate for 30 min and use a vacuum suction pump to extract air for 5 times. Then lay the Bunsen beaker under ordinary temperature for 2 h. The cell membrane penetrability was checked using DDSJ2308A Conductivity Meter.

Statistical analysis

Statistical analysis was performed using SAS programme for PCs (SAS User's Guide version 6, 4th edn., SAS Institute, Cary, NC, 1990). The mean of three independent samples were taken to stand for the result of individual replicate. The significance between the control group and treatment group was estimated with Duncan's multiple range tests.

RESULTS AND DISCUSSION

Mn content and dry matter production of plants

Mn content was demonstrated in leaves of plants sprayed with Mn (Figure 1). Mn content of leaves in Mn treatment group was 4.0 and 4.1 times higher than that in control group at 53 and 55 DAS, respectively. Mn increased total dry matter production of perennial ryegrass (Figure 2).The dry matter (DM) of plants in Mn treatment group was significantly increased (34%) comparing with that in

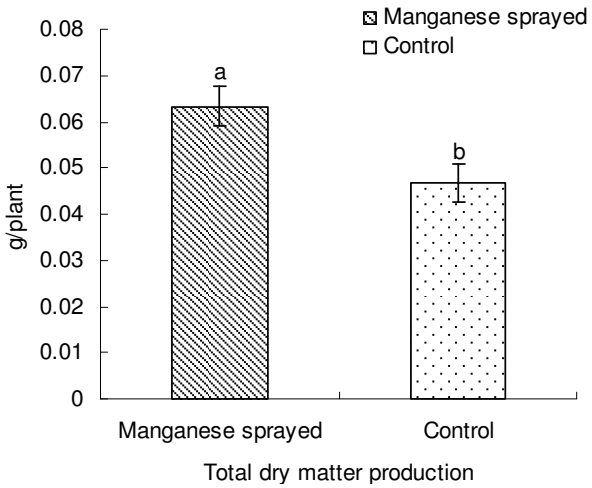

Figure 2. Effect of manganese spray on growth in perennial ryegrass at harvest. a, b=significant at P≤0.05.

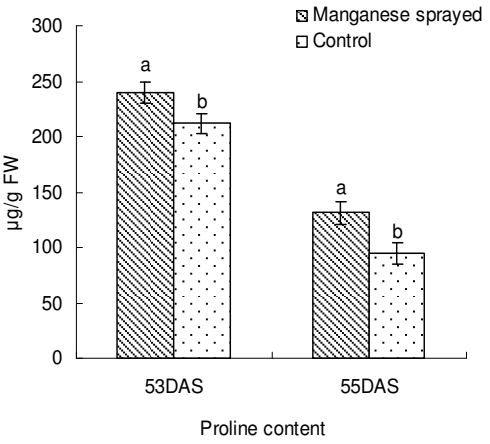

Figure 4. Effect of manganese spray on proline content in perennial ryegrass at 53 and 55 DAS. a,b=significant at P≤0.05.

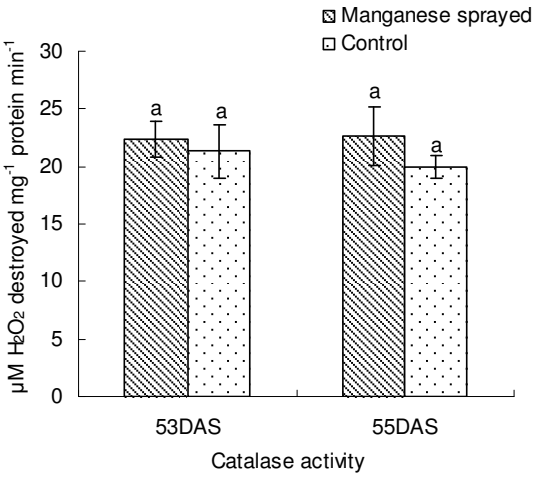

Figure 3. Effect of manganese spray on superoxide dismutase, catalase activity in perennial ryegrass. a, b=significant at P≤0.05.

control group (P<0.05).

Antioxidant enzymes and proline

The activity of SOD was significantly increased in Mn treatment plants compared with that in control group at both 53 and 55 DAS (Figure 3), whereas the activity of CAT did not show any significant difference (Figure 3). Mn effectively increased the proline content of treatment plants by 16.3 and 18.2% over the control plants in 53 and 55 DAS, respectively, (Figure 4).

The results shows that during the senescence process induced by drought, the plants in the control group produced more O^{2-} which is capable of causing oxidative damage (Shanker et al., 2004), while SOD was active in scavenging the superoxide as proved by the significant difference between the control group and Mn treatment group, this may be because Mn has altered the transcript levels of SOD enzymes thus altering SOD gene expression as description of Kwang-Hyun and Daniel (2006). It is known that SOD can act on superoxide anion and convert it to another reactive intermediate (H_2O_2), later, the catalase and glutathione peroxidase (GSH-Px) act on H_2O_2 and change it into water and oxygen (Mates, 2000). Although SOD was active in diminishing the superoxide anion at both 53 and 55 DAS, this was proved by significantly difference between the treatments. The reason for this is that CAT has low substrate affinity which needs simultaneous access of two molecules of H_2O_2 (Shanker et al., 2004). The study shows that, Mn enhanced the proline content by 34.4% at the 55 DAS. The mechanism of proline accumulation in Mn treatment group has not been previously reported. But it is shown here that proline is multifunctional under the condition of oxidative stress during senescence. The function includes cytosolic pH regulation, stabilization of proteins, and regulation of NAD/NADH ratio through singlet-oxygen quencher and scavenger of OH- radicals (Matysik et al., 2002).

Superoxide anion production and malondialdehyde (MDA) content

The superoxide anion production was observed significantly higher in the control group than in the Mn treatment group, there are 1.46 nmol/g FW and 4.03 nmol/g FW difference between control group and Mn treated at 53 and 55 DAS, respectively (Figure 5). MDA content in Mn treatment group was observed with 26 and 24% lower than that in the control group at both 53 and 55 DAS, respectively (Figure 5).

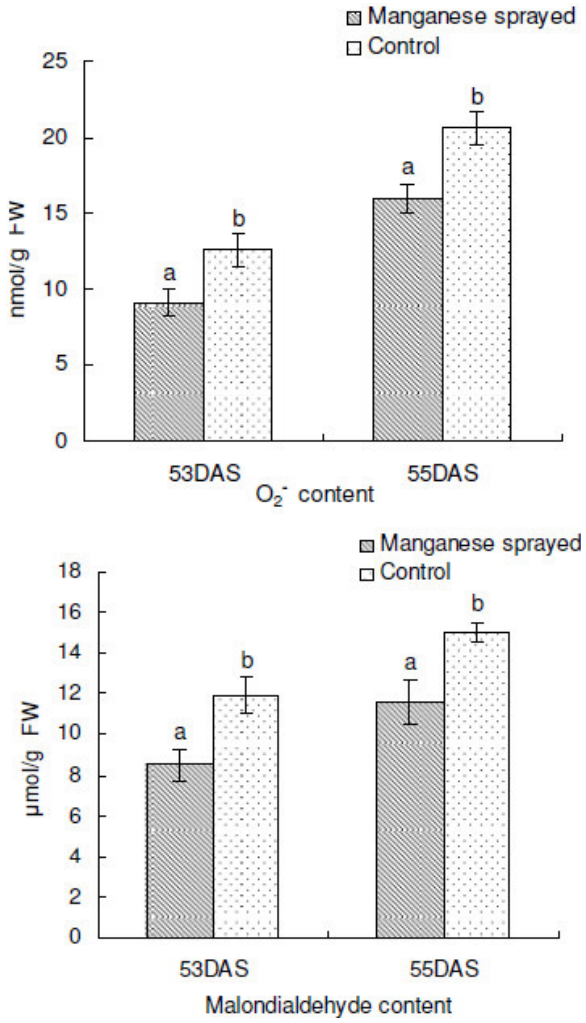

Figure 5. Effect of manganese spray on O_2- and Malondialdehyde content in perennial ryegrass at 53 and 55 DAS. a, b=significant at P≤0.05.

Superoxide anion O^{2-}.is one of the byproducts of cell oxygen metabolism (Berlett and Stadtman, 1997). The concentration of O^{2-} in the living cells of plants is the main cause of apoptosis, aging and cell cycle arrest (Epp et al., 1983; Stadtman, 1992). The direct reduction of superoxide anion radical by superoxide dismutase successfully maintained the balance of redox state in the cell. Mn has the growth stimulating function, because it may diminish the lipid per-oxidation and the production of superoxide radical (Chen et al., 2007).

Chlorophyll contents and cell membrane penetrability

Chlorophyll a, chlorophyll b, and total chlorophyll contents decreased in both control plants and Mn treatment plants during the period of 53 to 55 DAS. However, the reduced magnitude was lower in Mn treated plants (Figure 6). Relative permeability of plasma reflects the cell membrane integrity (Figure 6). Relative permeability of plasma in Mn treated plants was lower than that in control plants by 2 and 3%, respectively at 53 and 55 DAS. This means that Mn can help to maintain higher contents of chlorophyll a, chlorophyll b and total chlorophyll. These finding are in line with the find in capsicum (Zhou et al., 2009).

Panigrahi and Biswal (1979) reported that the total chlorophyll content decline after the leaf reaches full expansion. The decrease in chlorophyll content might be partly because of lipid peroxidation of chloroplast membranes (Heath and Packer, 1968) or the accumulation of hydroperoxides of fatty acids (Pieser and Yang, 1978). The increased chlorophyll a, b and total chlorophyll content in Mn treated plants were higher than control plants might be due to the efficient cleaning up the reactive oxygen species like O^{2-} by SOD and other antioxidant enzymes, or the chlorophyll pigment can be destroyed by reactive oxygen species (Thomas et al., 2001).

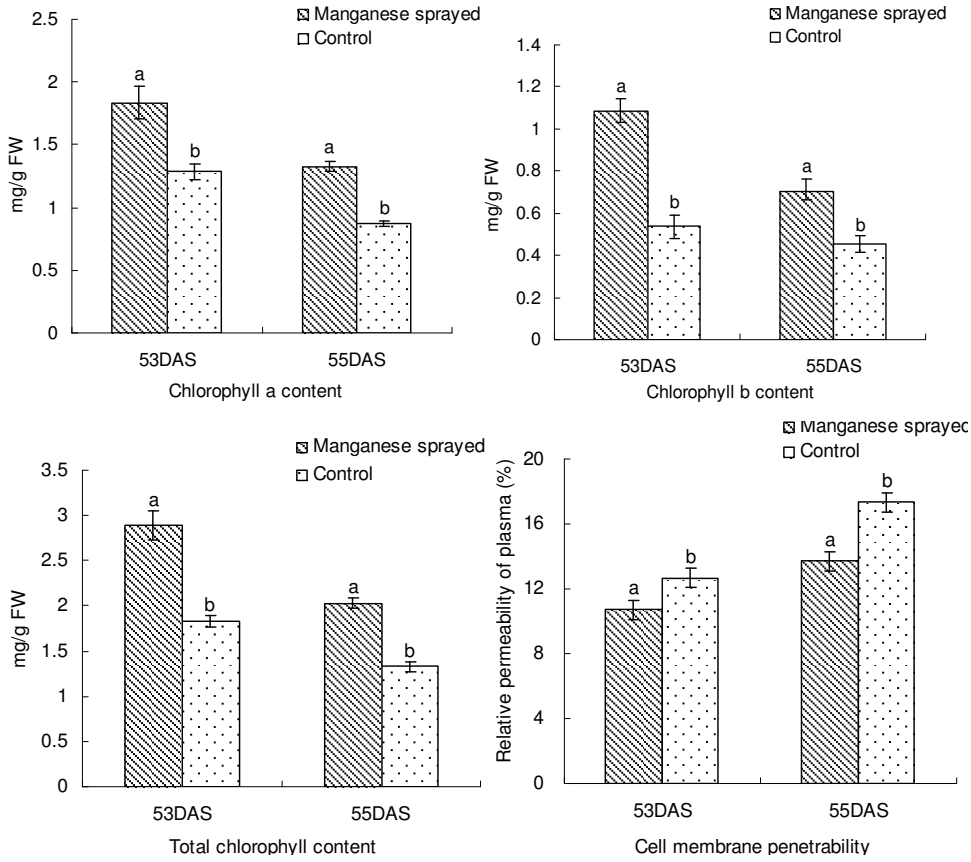

Figure 6. Effect of manganese spray on chlorophyll and cell membrane penetrability in perennial ryegrass at 53 and 55 DAS. a,b=significant at P≤0.05.

Conclusion

In summary, the present study shows that Mn spray in a proper concentration on the ryegrass increases production of some antioxidant enzymes. Also, delays the process of senescence that was induced by drought in perennial ryegrass (*Lolium perenne* L.).

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