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Antioxidant Defenses of Mycorrhizal Fungus Infection Against SO_2-Induced Oxidative Stress in *Avena nuda* Seedlings

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Abstract Colonization of arbuscular mycorrhizal fungi *Glomus mosseae* increased *Avena nuda* seedling tolerance to SO_2 exposure, as indicated by elevated total plant biomass and ameliorative photosynthetic rate, when compared to the non-mycorrhizal plants. This is associated with an improved antioxidant capacity as shown by enhanced superoxide dismutase and catalase activity, increased ascorbic acid and glutathione content, and reduced malondialdehyde and hydrogen peroxide level in the mycorrhizal plants relative to the non-mycorrhizal plants under SO_2 exposure. The mycorrhizal fungi colonization had no effect on the stomatal conductance. To our knowledge, this is the first finding of this sort.

Keywords *Avena nuda* · Sulfur dioxide · Arbuscular mycorrhizal symbiosis · Antioxidant defense

Sulfur dioxide (SO_2) is one of the first air pollutants recognized as harmful to humans and ecosystems. Inhibition of photosynthesis was generally considered to be one of the first effects of SO_2 on plants (Lüttge et al. 1972; Pfanz et al. 1987; Miszalski and Niewiadomska 1993; Li et al. 2007). Sulfite (SO_3^{2-}) and bisulfite (HSO_3^-), which have been shown to be toxic to many biochemical and physiological processes in plant cell, are formed when SO_2 dissolves in cellular fluid (Pfanz and Heber 1986). The (bi)sulfite ions lead to an increase of the reactive oxygen species (ROS) including superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) by free radical chain oxidation (Asada 1980; Alscher et al. 1997; Bowler et al. 1992; Peiser and Yang 1985; Li et al. 2007; Surówka et al. 2007). Some evidence has indicated the existence of a correlation between antioxidant enzyme such as superoxide dismutase (SOD) activity and plant sensitivity to SO_2 (Asada and Kiso 1973; Tanka and Sugahara 1980; Madamanchi and Alscher 1991).

Arbuscular mycorrhizal symbiosis is an almost ubiquitous rhizospheric interaction (Smith and Read 1997). The typical benefit of arbuscular mycorrhizal fungi (AMF) to the host is phosphate uptake. Nevertheless, non-nutritional effects of AMF on host plants have attracted increasing attention. For example, AMF can induce plant tolerances to abiotic and biotic stresses including water deficiency (Smith and Read 1997; Wu et al. 2006; Marulanda et al. 2007), heavy metals (Hetrick et al. 1994; Schützendübel and Polle 2002), salinity (Rosendahl and Rosendahl 1991; Feng et al. 2002; Giri et al. 2007), and phytopathogens (Azcón-Aguilar and Barea 1996; Smith and Read 1997). Although the ameliorative effect of AMF on the host response to detrimental circumstances has been attributed to a wide variety of mechanisms, the antioxidant defense role of arbuscular mycorrhizas is generally considered (Ruiz-Lozano et al. 2001a, b; Ruiz-Lozano 2003; Schützendübel and Polle 2002; Lambais et al. 2003; Porcel et al. 2003; Wu et al. 2006). Oat is an important and popular food crop in terms of its high quantity of protein, vegetable fat, and essential amino acids in its seeds compared to other

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cereal grain crops. Also, it is a healthy food with medical attributes for some human diseases. In our lab, excellent in vivo and in vitro experimental systems related to this plant have been established (Hao et al. 2006; Xu et al. 2008). Herein, we use oat plants to evaluate the possible role of AMF colonization in plant response to elevated SO_2.

Materials and Methods

The experiment was conducted three times using two treatments: non-mycorrhizal controls, and inoculated with AMF *G. mosseae* [Thaxter and Gerd. (Gerd. and Trappe)]. Ten replications were used for each treatment totalling 20 pots. Twenty seeds of naked oat (*A. nuda* cv. North China No. 1), obtained from Wulanchabu City Academy of Agricultural Science, Inner Mongolia, China, were sown in 18 cm height and 10 cm diameter sterile pots containing a 3:2 mixture of sterilized silica sand (diameter <1.2 mm) and soil. Seedlings were thinned to 10 per pot after emergence and grown in growth chambers at 23/18°C (day/night) temperature, 70 ± 5% relative humidity, and a light intensity of 500 μM m^{-2} s^{-1} with a 12 h photoperiod provided by cool-white fluorescent light. They were watered every 3 days with distilled water and received a weekly application of 50 mL 1 × Hoagland's nutrient solution. For the preparation of the AMF inoculum, *G. mosseae* was multiplied with fine sand as substrate and *Trifolium subterraneum* as host and cultured for 3 months. The inoculum consisted of colonized *T. subterraneum* root fragments, sand, hyphae, and spores. A 20-g portion of inoculum was added to each pot at sowing time just below the seeds. The non-mycorrhizal controls received sterilized inoculum.

For each treatment, half of the plants (10 pots) were subjected to SO_2 fumigation while the other half were treated using charcoal filtered air. For SO_2 fumigation, 10 pots of 4-week-old seedlings (five pots of non-mycorrhizal plants and five pots of mycorrhizal plants) were transferred to glass chambers (0.8 m width, 0.8 m depth, and 0.8 m height) for SO_2 exposure. The SO_2 gas was supplied directly from cylinders, into a dilution reservoir into which charcoal filtered air was drawn, and delivered to plants through a 1 cm diameter PVC ducts joined to the individual chamber at the top air inlet to each chamber and distributed homogeneously via an internal fan. Chamber SO_2 concentration was monitored during treatment by measurement every 30 min using pararosaniline hydrochloride spectrophotometry (Goyal 2001). For the control group (five pots of non-mycorrhizal plants and five pots of mycorrhizal plants), filtered air alone was supplied. The concentration of SO_2 used was derived from a pre-experiment. Briefly, oat plants were exposed to SO_2 concentrations of 0, 0.1, 0.5, 1.0, and 2.0 μL L^{-1}, respectively. The result showed that under the treatment of 1.0 μL L^{-1} or higher SO_2, all indices determined in this paper had changed significantly related to controls. Thus, the mean concentration of 1.0 μL L^{-1} was used in the present study. Plants were fumigated during the light period for 3 h per day for 5 days per week for 2 weeks.

Biomass and root colonization determination: At harvest (immediate end of the fumigation), the roots were washed immediately and the shoots and roots were separated, and then shoots were oven-dried at 65°C for 2 days and dry mass recorded. Fresh roots were used to determine fungal colonization. The percentage of mycorrhizal root infection was estimated by visual observation of fungal colonization according to Koske and Gemma (1989). The extent of mycorrhizal colonization was calculated using the gridline intersect method (Giovannetti and Mosse 1980). One hundred intersections were observed out of the root fragments of one plant and the presence of at least one mycorrhizal hypha was counted as infection.

Photosynthesis rate and stomatal conductance were measured in situ on the flag leaf using a portable photosynthesis system (LI-6200, LI-COR, Inc., Lincoln, NE, USA) at ambient climatic conditions. During the measurement the PAR over the waveband 400–700 nm ranged between 500 and 600 μM m^{-2} s^{-1}, at 25°C. Total SOD (EC 1.15.1.1) and catalase (CAT) (EC 1.11.1.6) activity was assayed as described by Beyer and Fridovich (1987) and Aebi (1983), respectively. Protein concentration was estimated by the method of Bradford (1976) using bovine albumin as standard. Ascorbic acid content was assayed based on the method of Keller and Schwager (1977). The reduced glutathione content was determined as described by Griffith and Meister (1979). Hydrogen peroxide level was determined according to the method described by Mukherjee and Choudhuri (1983). The level of lipid peroxidation was determined by measuring the level of thiobarbituric acid reactive substance following Shalata and Tal (1998) and expressed as nM of malondialdehyde (MDA) g^{-1} fresh weight. Determinations of all indices were performed in at least five independent experiments. Data from multiple experiments were pooled and analyzed using Duncan's multiple range test.

Results and Discussion

The changes of total plant biomass, root/shoot ratio and extent of AMF colonization of plants were shown in Table 1. The SO_2 fumigation reduced the total plant biomass by 31.1% related to control. The AMF colonization not only increased the total plant biomass by 18.8% under

Table 1 Effect of SO_2 on total plant biomass (g DW $plant^{-1}$), root/shoot (g g^{-1}), and the extent of AMF colonization (percentage of root length infected)

Parameters	Control		SO_2	
	Non-AMF	AMF	Non-AMF	AMF
Total plant biomass	0.16 ± 0.01b	0.19 ± 0.02a	0.11 ± 0.01c	0.15 ± 0.01b
Root/shoot	0.42 ± 0.03c	0.44 ± 0.03c	0.53 ± 0.05b	0.62 ± 0.05a
Root colonization (%)	0.00 ± 0.00c	23.45 ± 1.96a	0.00 ± 0.00c	16.28 ± 1.32b

The different letters (a, b, c) in the same line indicate a significant difference at $p = 0.05$

control conditions, but significantly reduced the SO_2-induced growth inhibition where the total plant biomass only decreased 6.3% related to the control. The root/shoot ratio was markedly increased at SO_2 exposure, and AMF colonization further elevated the ratio whereas it had no effect on the ratio under control condition. The SO_2 fumigation decreased AMF root colonization by 30.1% related to control. No AMF colonization was observed in the roots of the non-mycorrhizal control.

The SO_2 fumigation significantly reduced the SOD and CAT activity, ascorbic acid and reduced glutathione content and stomatal conductance, suppressed the photosynthetic rate, and markedly elevated H_2O_2 level and MDA content (Fig. 1A–H). The AMF colonization effectively counteracted the SO_2-induced changes, elevating the photosynthetic rate, maintaining SOD activity near the control level, and increasing CAT compared to the control. Likewise the ascorbic acid content was higher than the control and reduced glutathione level was increased compared to the non-mycorrhizal plants. The H_2O_2 and MDA levels decreased in comparison with the non-mycorrhizal plant determinations. However, the AMF colonization had no effect on the stomatal conductance in both the SO_2 fumigation and control.

In this experiment, SO_2 fumigation caused plant oxidative stress, as shown by an increase of MDA, which is consistent with other observations in different plant species (Wingsle et al. 1991; Hao et al. 2005). Under SO_2 fumigation, the plant antioxidant level was reduced (Fig. 1A–D), which correlated well with the increase of H_2O_2 and MDA

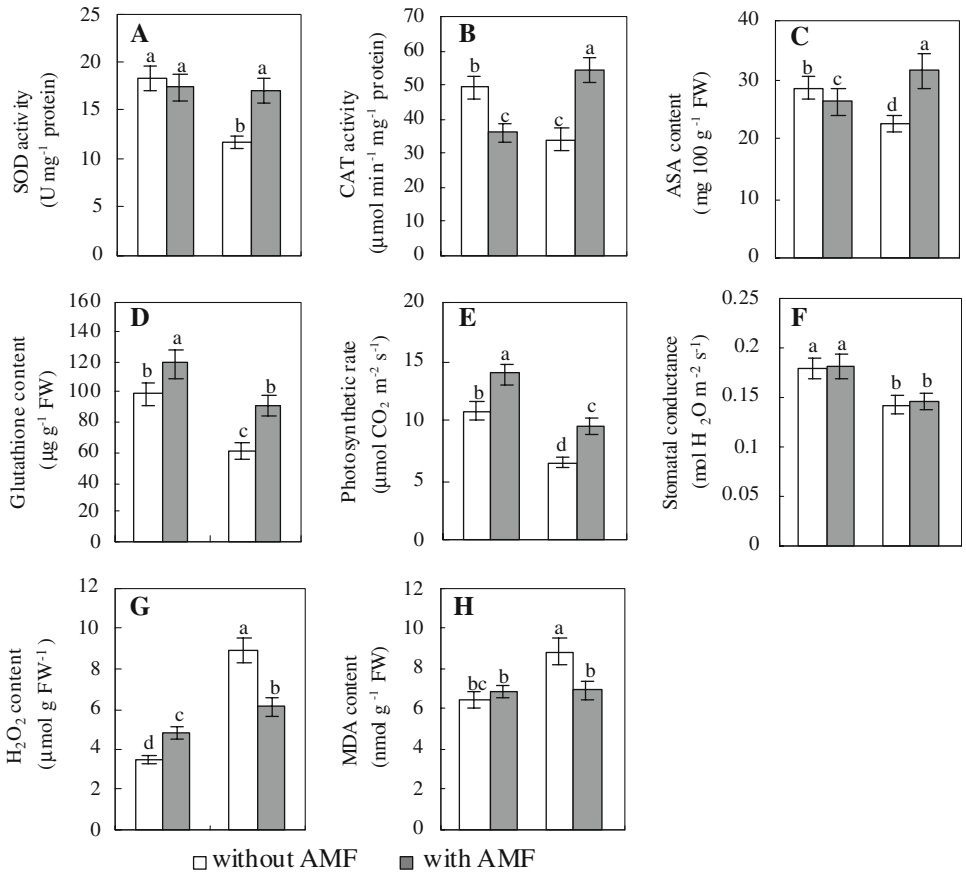

Fig. 1 Effect of inoculation of *Glomus mosseae* on superoxide dismutase (**A**), catalase (**B**), ascorbic acid (**C**), glutathione (**D**), photosynthetic acid (**E**), stomatal conductance (**F**), hydrogen peroxide (**G**), and malondialdehyde (**H**) in *Avena nuda* plants exposed to sulfur dioxide. Left group of columns: control (exposed to charcoal filtered air); Right group of columns: sulfur dioxide fumigation (exposed to 1.0 μL L^{-1} of SO_2). The different letters (a, b, etc.) indicate a significant difference at $p = 0.05$

content (Fig. 1G, H), and the decrease of photosynthetic rate (Fig. 1E) and plant biomass (Table 1), suggesting ROS formed in the presence of SO_2 and its oxidation products resulted from a chain reaction of free radicals may be a major cause of the decline of plant biomass or yield.

Inoculation of AMF *G. mosseae* significantly enhanced the plant antioxidant level, which correlated with the amelioration of photosynthetic rate and plant biomass, indicating the *G. mosseae/A. nuda* symbiosis observed in this experiment increased the plant tolerance to SO_2, at least in part through elevating the antioxidant level, which agree with the general observation in other plant species/AMF symbiosis under other stressful factors (Ruiz-Lozano et al. 2001a, b, Ruiz-Lozano 2003; Schützendübel and Polle 2002; Lambais et al. 2003; Porcel et al. 2003; Wu et al. 2006). In view of no response of the stomatal conductance to the fungi colonization (Fig. 1F), the improved photosynthetic rate due to the *G. mosseae* inoculation was not associated to the stomatal limitation which is generally believed to be one of the major factors causing photosynthesis decline (Dubey 2005). To our knowledge, this is the first finding that AMF colonization counteracts the SO_2-induced plant growth inhibition.

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