ARBUSCULAR MYCORRHIZAL FUNGI ALLEVIATE THE TOXICITY OF CADMIUM INTERACTION AFFECTING THE GROWTH OF RYEGRASS (*LOLIUM PERENNE* L.)

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Abstract. A pot experiment was conducted to examine the effects of *Glomus mosseae* on some growth and physiological parameters, and cadmium (Cd) amounts in ryegrass (*Lolium perenne* L.) plants under the toxic levels of Cd. The experiment was carried out with two treatments (*G. mosseae* inoculation and non-inoculation), each having four Cd concentrations (0, 30, 90 and 180 mg Cd kg^{-1} soil). The results showed that mycorrhizal infection rate of *G. mosseae* was still as high as 43% under 180 mg kg^{-1} Cd concentration, indicating that *G. mosseae* colonized plants were tolerant to Cd stress. In non-inoculated and inoculated plants, by increasing Cd concentration in the soil, growth parameters, root activity, growth index, biomass, N and P content in plant and photosynthetic characteristic were reduced. Root activity, growth index, biomass, N and P content, photosynthetic physiology, superoxide dismutase (SOD) and catalase (CAT) contents of *G. mosseae* inoculated plants were increased compared to non-inoculated plants, whereas malondialdehyde (MDA) content decreased to a certain extent. In conclusion, inoculation of *G. mosseae* enhanced the resistance and tolerance of ryegrass to Cd stress, reduced the damage of harmful substances to cells, and promoted its growth.
Keywords: *Glomus mosseae, cadmium stress, plant growth, nutrition accumulation, photosynthetic characteristics*

Introduction

In the natural ecosystem, 80% of all terrestrial plant species form symbiotic associations between arbuscular mycorrhiza fungi (AMF) and their roots (Smith and Read, 1997). The AMF obtain carbohydrates from the host plants. In return, the fungi benefit their host plants principally by enriching nutrition uptake, enhancing resistance to pathogens (Pozo et al., 2002), improving tolerance to drought stress (Augé et al., 1994) and improving soil structure (Rillig and Steinberg, 2002). In addition, AM symbiosis has been shown to take an active part in plant resistance to heavy metal contamination including Zn, Pb, Cu, Cr and Cd (Shetty et al., 1995; Díaz et al., 1996; Davies et al., 2001; Chen et al., 2007; Shahabivand et al., 2012). For example, Vodnik (2008) demonstrated that AM symbiosis can prevent plant from absorbing heavy metal by secreting some organic compounds in soils to chelate metal ions. Gonzalez-Chavez (2002) have investigated that AM fungi colonized in *H. lanatus* carried out their role by aiding the host to fix toxic metals within the rhizospheric zone, thereby preventing the uptake of toxic metals into the plant (Gonzalez-Chavez et al., 2010).

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In recent years, heavy metals released to soils bring about irreversible soil degradation and hence severely limit vegetation establishment, which mainly resulted from human activities (Shukurov et al., 2005; Rajkumar et al., 2012). Cadmium (Cd) is non-essential heavy metal element for plant growth with phytotoxicity even at very low concentration-0.5 mg kg^{-1} (Yong et al., 2009). Cd enters the aquatic environment from natural (weathering of rocks) as well as anthropogenic sources (industrial effluents, agricultural run offs) (Schützendübel et al., 2002). The presence of an excessive amount of Cd causes an inhibition of enzyme activities, water imbalance and alterations of membrane permeability in plants (Yılmaz and Parlak, 2011). It exerts an adverse effect on seed germination (Peralta et al., 2001), morphology, growth and the photosynthetic processes in plants, even leading to plant death (Zorrig et al., 2013; Hassan and Mansoor, 2014; Asgher et al., 2015). Improper discharge of Cd into environment has resulted in severe contaminations, and subsequently threatened the environmental quality and human health over the long-term (Kirkham, 2006; Ali et al., 2013).

Mycorrhizal fungi have been found to influence Cd uptake and accumulation in plants with changes in its immobilization and mobilization in Cd-polluted soils (Chen et al., 2004b). Investigations indicated that many plant species, such as *Fragaria vesca*, *Viola calaminaria*, *Veronica rechingeri*, *Thymus polytrichus* and *Lolium perenne*, growing well at natural heavy metal polluted sites and all of them were colonized by AMF (Turnau et al., 2001; Whitfield et al., 2004; Zarei et al., 2008a; Alguacil et al., 2011). Ryegrass (*Lolium perenne* L.) was used as a model plant because of its rapid growth, tolerance to various environments, soil types and its common use in phytoremediation studies (Arienzo et al., 2004; Meng et al., 2011). The objectives of our experiment were to investigate the efficiency of AMF application into different Cd-added soils (0, 30, 90 and 180 mg Cd kg^{-1} soil) for alleviating the toxicity of Cd to *L. perenne*. Mainly focused on the effects of AMF on plant growth, nutrition accumulation, photosynthetic characteristics, and antioxidant activities of *L. perenne* grown in different Cd-added soils. The results of this study will provide new insights into the mechanism of Cd phytotoxicity alleviation in associate with AM inoculation.

Methods and materials

Experimental design

The experiment was designed as a 4×2 factorial treatments of Cd level and AMF inoculation organized in a completely randomized design with three replications. Treatments consisted of full factorial combinations of four Cd levels (0, 30, 90 and 180 mg kg^{-1}) applied as $3CdSO_4 \cdot 8H_2O$, two AMF treatments (inoculated and non-inoculated) in soils planted with ryegrass under greenhouse conditions. The average day and night temperatures were maintained at 25℃ and 23℃, respectively, and a 12 h photoperiod with a light intensity 400 μmol m^{-2} s^{-1}. The relative humidity was 75%.

Soil preparation

A sandy loam soil from the 20-30 cm layer was collected from in forestry station, Northeast Forestry University of Heilongjiang province (latitude, 126°63′N; longitude, 45°72′E, northeast China). It was air-dried, sieved (2 mm mesh) and stored at 25℃ before use. The soil was steam-sterilized (121℃ for 2 h) by autoclaving to eliminate indigenous mycorrhizal propagules and pathogens, and more specifically to eliminate the influence of

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the AM fungal species used. The soils were artificially contaminated with $3CdSO_4 \cdot 8H_2O$ at the following rates: 0 (control), 30, 90 and 180 mg Cd kg^{-1}. Deionized water was added to the soils to achieve moisture content of 60-70% of field capacity. To ensure even distribution of Cd, soils were thoroughly mixed while adding $3CdSO_4 \cdot 8H_2O$ and water. The soils were incubated at room temperature (about 20±5℃) for one month to achieve an equilibrium condition, and to lower the effects of soil preparation and disturbance.

Mycorrhiza inoculum and host plants

The AM fungal inoculum (*G. mosseae*) was propagated using *Trifolium pretense* L. as host plants, and the inoculum was the mixture of spores, hyphae, colonized root fragments and substrates. Approximately 50 g of each AM fungal inoculum was added to 1 kg sterilized soils. Seeds of ryegrass (*Lolium perenne* L.) were surface sterilized in a 10% v/v solution of H_2O_2 for 10 min, washed in sterilized distilled water and were germinated on wet filter paper in Petri dishes. Two days later, the ryegrass seedlings were selected for uniformity and transplanted into plastic pots. 20 seedlings were transplanted into each pot (12 cm×15 cm). Seedlings in pots were irrigated every second day until water drained from the bottom of the pot. After 2 weeks, the cultivated seedlings reached a height of 10 cm, they were thinned a density of 10 plants pot^{-1} and supplied with 1/2 Hoagland's solution every week.

Estimation of mycorrhizal infection rate

The mycorrhizal infection rate was determined by trypanblue staining (McGonigle et al., 1999). After dyeing, the mycorrhizal infection rate was determined by the modified cross method under the microscope.

$$\text{Mycorrhizal infection rate (\%)} = \frac{\text{the number of root segments forming clumps}}{\text{the number of measured root segments}} \times 100 \quad \text{(Eq.1)}$$

Estimation of root vigor

Roots were sampled from each treatment (three replicates) were taken. The mixture of roots and soil was placed in a polythene bag and washed with tap water. The roots were carefully refrigerated for further testing. The root vigor was determined using the TTC method (Zhang, 1990).

Estimation of gas exchange

Net photosynthesis (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) were measured on expanded leaves. Photosynthetic rate measured at 400 μmol m^{-2} s^{-1} CO2 under 800 μmol m^{-2} s^{-1} PFD was used to calculate photorespiration with a portable photosynthetic system (CIRAS-1, PP systems, UK).

Antioxidant enzymes assays

SOD activity was measured according to the method of Wang et al. (1990). Fresh tissues (0.2 g) were ground to a fine powder in liquid N_2 then homogenized in 2 mL of phosphate buffer (pH 7.8). The reaction mixture was comprised of 50 mM phosphate buffer (pH 7.8), 130 mM methionine, 750 μM nitro-bluetetrazolium, 100 μM EDTA-

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Na_2, 100 μM EDTA, 100 μM riboflavin, and 0.1 ml of enzyme extract in a final volume of 4 ml. There action mixture was incubated for 30 min under 4000 Lx, and absorbance was determined at 560 nm. Catalase (CAT) activity was measured at 25°C previously according to the method of Paglia and Valentine (1987) that used hydrogen peroxide as substrate and one unit of catalase was defined as the rate constant of the first order reaction (k).

Measurements of lipid peroxidation

MDA content was measured following the method of Zhang et al. (1990). Fresh tissues (0.2 g) were ground to a fine powder in liquid N_2 then homogenized in 2 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 10,000 r/min for 10 min. For every 2 mL of the supernatant, 2 mL of 0.6% 2-thiobarbituric acid (TBA) was mixed. The mixture was incubated at 100℃ for 15 min and then transferred into an ice bath to stop the reaction. The tubes were centrifuged at 10,000 r/min for 10 min and the absorbance of the resulting supernatant was measured at 450, 532 and 600 nm. The concentration of MDA (lM) in the solution was estimated according to the following formula:

$$MDA \text{ concentration}=6.45(A_{532}-A_{600})-0.56A_{450} \quad \text{(Eq.2)}$$

where A_{450}, A_{532} and A_{600} were represent wavelength measured at 450, 532 and 600 nm.

Measurements of plant growth and biomass

The middle growth stage was 45 days after sowing. Fresh plants were collected from each pot after plant establishment at the middle growth stage. The 10 plants were collected and rinsed twice with distilled water, and subsequently, the growth parameters (plant height, root length, root/shoot ratio, aboveground, underground, and total biomass per plant) were measured. Plant height was determined with meter rule (cm) from the base of the plant (above the ground level) to the apical region of the leaf. The root length (the longest root) was also obtained using meter rule (cm). Aboveground, underground, and total biomass were weighted after drying the plant samples in a hot air oven at 60℃ using a constant weight. Root/shoot ratio=Underground biomass/aboveground biomass.

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 program. The data were analyzed by analysis of variance (ANOVA), where means and standard derivations were calculated for the three replicates. To detect the statistical significance of differences (P<0.05) among Cd levels, the Tukey test was performed. T-test was performed to detect the statistical significance of differences between non-inoculated and inoculated with AM fungi treatments.

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Results

Mycorrhizal infection rates

Mycorrhizal infection rate of *G. mosseae* to the root of ryegrass was observed in different Cd-contaminated soils (*Fig. 1*). The rates of mycorrhiza infection were generally reduced (from 69% to 43%) in the presence of Cd (P<0.05). The rates of mycorrhiza infection in 30, 90 and 180 mg Cd kg^{-1} soils were significantly decreased by 16.14%, 24.93% and 58.45%, respectively, compared with Cd-uncontaminated soil.

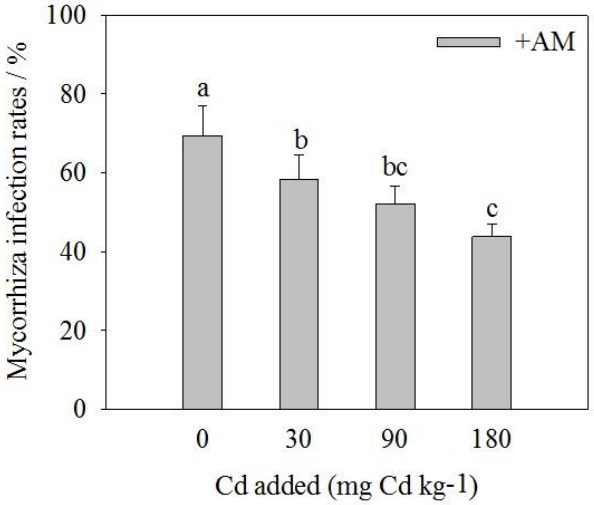

Figure 1. *Mycorrhiza infection rates of L. perenne inoculated with G. mosseae with increasing Cd level. Abbreviations: +AM, inoculated with AMF. Values shown are averages, calculated using 3 replicates for each treatment (± s.d. of the mean). Different letters indicate significant differences at P<0.05*

Root vigor

The two-way interaction between Cd and AMF factors was significant for root vigor *(P*<0.05) (*Fig. 2*). There were significant decreases in root vigor for the plants exposed to Cd compared with Cd-uncontaminated plants. Root vigor was negatively affected by Cd, decreasing with rising the Cd level in both *G. mosseae*-inoculated and without inoculated plants, but without significant when added at 30 and 90 mg Cd kg^{-1} in *G. mosseae*-inoculated soils and at 90 and 180 mg Cd kg^{-1} in without inoculated soils, respectively. *G. mosseae* inoculation significantly increased root vigor compared with non-inoculated soil at 0, 90 and 180 Cd kg^{-1} levels. Compared to non-inoculated plants, root vigor increased by 43.27%, 70.56% and 55.33% for *G. mosseae*-inoculated plants added with 0, 90 and 180 mg Cd kg^{-1}, respectively.

Photosynthetic characteristic

Net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) were presented in *Table 1*. The *G. mosseae* inoculation had higher T_r and P_n than non-inoculated treatments, indicating AMF alleviated Cd-inducted photosynthetic inhibition of ryegrass leaves. At the highest Cd level, P_n, G_s, and T_r of *G. mosseae* inoculated plants were increased by 54.91% (P<0.05), 37.79% (P<0.05) and 10.06% (P>0.05), respectively, in comparison to non-inoculated

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plants. Photosynthetic characteristics of ryegrass were sensitive to Cd addition in non-inoculated plants. In general, P_n decreased with increasing Cd levels in non-inoculated plants. The decrease of G_s in Cd-contaminated plants was accompanied with those of T_r, but at different extents among treatments. On the contrary, the increase of C_i in 180 mg Cd kg^{-1} treatment was of 8.98% (P>0.05) if compared to the 90 mg Cd kg^{-1} treatment.

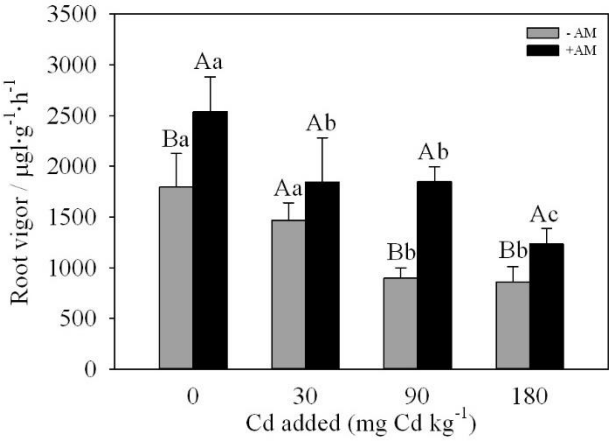

Figure 2. *Root vigor of L. perenne inoculated with G. mosseae with increasing Cd level. Abbreviations: –AM, non-inoculated with arbuscular mycorrhizal fungi (AMF); +AM, inoculated with AMF. Values shown are averages, calculated using 3 replicates for each treatment (±s.d. of the mean). Different lowercase letters are significantly difference (P<0.05) among Cd levels by the Tukey test. Different uppercase letters are significantly difference (P<0.05) between non-inoculated and inoculated with AM fungi treatments by the t-test*

Table 1. *Gas exchange parameters of ryegrass leaves inoculated with G. mosseae with increasing Cd level*

Treatment	AM fungi		Mean (Cd)	AM fungi		Mean (Cd)
Cd added (mg kg-1)	-AM	+AM		-AM	+AM	
	Net photosynthetic rate (μmol m^{-2} s^{-1})			**Transpiration rate (μmol m^{-2} s^{-1})**		
0	3.01A	3.44A	3.13 A	0.88A	1.17A	1.05 A
30	2.62AB	2.91A	2.67 A	0.87A	1.04AB	0.95 A
90	2.21AB	2.83A	2.42 A	0.80A	0.87B	0.82 A
180	1.73 bB	2.68 aA	2.11 A	0.74A	0.89B	0.76 A
Mean (AM fungi)	2.39 b	2.97 a		0.82 b	0.99 a	
	Stomatal conductance (μmol m^{-2} s^{-1})			**Intercellular CO_2 concentration (μmol m^{-2} s^{-1})**		
0	49.81 A	56.90A	55.35 A	280.7A	301.3A	291.00 A
30	40.12A	47.5AB	45.80 AB	262.8A	291.9A	277.35 A
90	36.39AB	38.12BC	39.26 AB	247.8A	281.8A	264.80 A
180	23.10 B	36.21C	30.15 B	265.9A	249.3A	257.60 A
Mean (AM fungi)	37.35 a	44.68 a		264.30 a	281.08 a	

Which each row, mean values (n=3) with the different lowercase letter are significantly difference (P<0.05) among AM fungi treatments by the Tukey test. Within each column, mean values (n=3) with the different uppercase letter are significantly difference (P<0.05) among Cd levels by the Tukey test

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Antioxidant activity

The enhanced SOD and CAT activities were observed for the plants exposed to Cd compared with the control (*Fig. 3*). SOD activity increased linearly with Cd addition levels, and no significant difference was found between Cd addition levels of 90 and 180 mg kg^{-1} (P>0.05). The maximum SOD activity was recorded at 180 mg Cd kg^{-1} in both *G. mosseae*-inoculated and without inoculated plants (0.69 and 0.67 U mg^{-1} FW^{-1}, respectively). There was no significant difference in SOD activity between *G. mosseae*-inoculated and without inoculated plants under the same Cd concentration (P>0.05). CAT activity also increased linearly with increasing Cd levels, whereas, a decline in CAT activity with an increase in 180 mg Cd kg^{-1} level was observed.

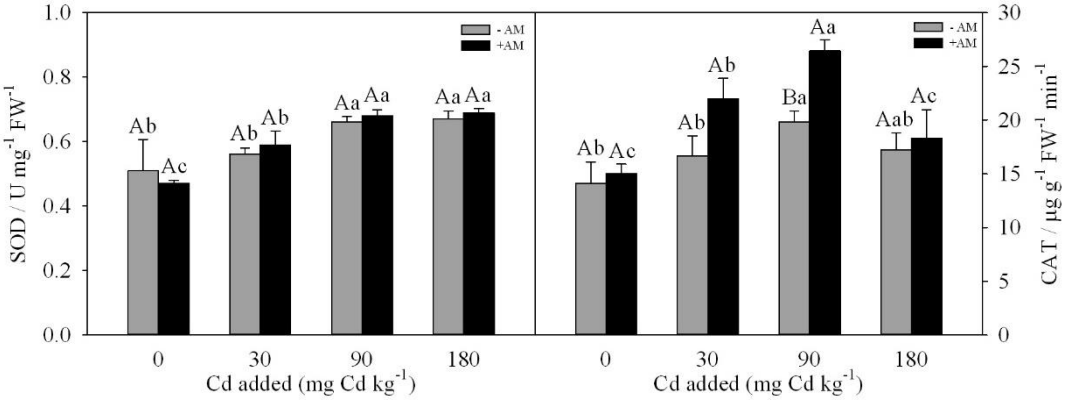

Figure 3. *Antioxidative enzyme activities of L. perenne L. inoculated with G. mosseae with increasing Cd level. Abbreviations: –AM, non-inoculated with arbuscular mycorrhizal fungi (AMF); +AM, inoculated with AMF. Values shown are averages, calculated using 3 replicates for each treatment (±s.d. of the mean). Different lowercase letters are significantly difference (P<0.05) among Cd levels by the Tukey test. Different uppercase letters are significantly difference (P<0.05) between non-inoculated and inoculated with AM fungi treatments by the t-test*

CAT activity of *G. mosseae*-inoculated and non-inoculated plants showed the same change trend, first increased and then decreased. But the increase range of inoculated was higher than that of non-inoculated plants. The CAT activity was different among different Cd pollution concentrations in both *G. mosseae*-inoculated and non-inoculated plants. There was no significant difference in CAT activity between inoculated and non-inoculated plants under 0 and 30 Cd kg^{-1} concentration. CAT activity was the highest in both inoculated and non-inoculated plants under 90 Cd kg^{-1} concentration, and the CAT activity of inoculated plants increased by 29.6% compared with that of non-inoculated plants at this concentration ($P<0.05$). While the CAT activity of the inoculated and non-inoculated plants decreased under 120 Cd kg^{-1} concentrations, but it was slightly higher than that of the control. There was no significant difference in CAT activity between *G. mosseae*-inoculated and without inoculated plants under 120 Cd kg^{-1} concentration (P>0.05).

Lipid peroxidation

The effect of Cd and AMF on MDA content is shown in *Fig. 4*. There were significant increases in MDA content for the plants exposed to Cd compared with Cd-

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uncontaminated plants (P<0.05). Whether inoculated or not, MDA content increased linearly with increasing Cd levels. No-inoculated plants had significantly higher MDA content than plants inoculated with *G. mosseae* at all Cd levels (P<0.05). Thus, decreased MDA indicated that AMF relieved the damage of Cd to cell membrane.

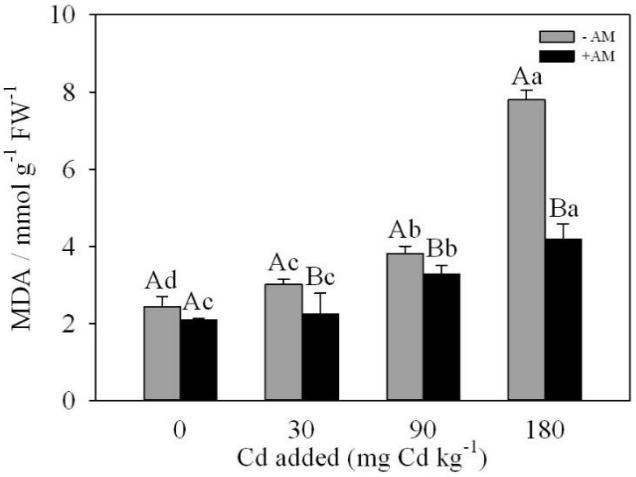

Figure 4. *MDA content of L. perenne L. inoculated with G. mosseae with increasing Cd level. Abbreviations: –AM, non-inoculated with arbuscular mycorrhizal fungi (AMF); +AM, inoculated with AMF. Values shown are averages, calculated using 3 replicates for each treatment (±s.d. of the mean). Different lowercase letters are significantly difference (P<0.05) among Cd levels by the Tukey test. Different uppercase letters are significantly difference (P<0.05) between non-inoculated and inoculated with AM fungi treatments by the t-test*

Plant growth and biomass

Results describing plant height, root length, root/shoot ratio, aboveground biomass, underground biomass and total biomass for different treatments are presented in *Table 2*. Plant growth was influenced by Cd level. Whether *G. mosseae* inoculated or not, decreasing plant height, root length, and biomass (underground and aboveground) were observed with in high Cd levels (*Table 2*). Obviously, high Cd levels inhibited ryegrass growth. When compared to Cd-uncontaminated plants, Cd addition decreased plant height, root length, aboveground biomass and underground biomass by 30.67%, 36.51%, 19.31% and 30.46% for *G. mosseae* inoculated treatments and 18.72%, 45.58%, 18.23% and 29.98% for non-inoculated treatments at Cd addition levels of 180 mg kg^{-1}, respectively. At the low Cd level (30 mg kg^{-1}), Cd stress had no significant effect on aboveground traits (plant height and aboveground biomass), but significant decrease underground traits (root length and underground biomass). Therefore, low soil Cd level increased the root/shoot ratio of ryegrass. The application of AMF alleviated the inhibitory effect of Cd on ryegrass growth traits (*Table 2*). At the highest Cd level, plant height, root length, aboveground, underground and total biomasses of *G. mosseae* inoculated plants were increased by 16.46% (P>0.05), 18.60% (P<0.05), 23.33% (P<0.05), 22.86% (P<0.05) and 24% (P<0.05), respectively as compared to the control plants. In generally, inoculation with *G. mosseae* had no effect on root/shoot ratio in different Cd levels.

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Table 2. *Growth characteristics of L. perenne inoculated with G. mosseae with increasing Cd level*

Treatment	AM fungi		Mean (Cd)	AM fungi		Mean (Cd)
Cd added (mg kg-1)	-AM	+AM		-AM	+AM	
	Plant height (cm)			**Root length (cm)**		
0	20.89 AB	25.76 A	23.33	14.67 A	16.87 A	15.77
30	21.67 A	27.11 A	24.39	12.37 B	13.89 B	13.13
90	20.03 AB	24.23 A	22.13	12.02 B	12.55 C	12.29
180	16.98 B	17.86 B	17.42	7.69 C	10.71 D	9.2
Mean (AM fungi)	19.89b	23.74a		11.69	13.51	
	Root /shoot ratio			**Aboveground biomass (g)**		
0	0.817 B	0.832 B	0.825	0.0417 A	0.0523 A	0.0470
30	0.868 A	0.908 A	0.888	0.0382 AB	0.0522 A	0.0452
90	0.820 B	0.841 B	0.831	0.0376 AB	0.0461 B	0.0419
180	0.720 C	0.725 C	0.723	0.0341 B	0.0422 B	0.0382
Mean (AM fungi)	0.806	0.827		0.0379	0.0482	
	Underground biomass (g)			**Total biomass (g)**		
0	0.0348 A	0.0447 B	0.0398	0.0740 A	0.0970 AB	0.0855
30	0.0342 A	0.0510 A	0.0426	0.0724 A	0.1032 A	0.0878
90	0.031 A	0.0388 C	0.0349	0.0686 AB	0.0849 BC	0.0768
180	0.0242 B	0.0313 D	0.0278	0.0600 B	0.0735 C	0.0668
Mean (AM fungi)	0.0311	0.0414		0.0688	0.0897	

Which each row, mean values (n=10) with the different lowercase letter are significantly difference ($P<0.05$) among AM fungi treatments by the Tukey test. Within each column, mean values (n=3) with the different uppercase letter are significantly difference ($P<0.05$) among Cd levels by the Tukey test

Plant N and P uptake

Plant N and P uptake in ryegrass plants decreased with Cd addition, especially in non-inoculated plants (*Table 3*). In soil added with 30 mg Cd kg^{-1}, non-inoculated controls had the highest P content in leaves. However, Cd tress had no significant influence on P content in leaves colonized by *G. mosseae*. In all Cd treatments, the *G. mosseae* inoculation had profound effects on N and P accumulation in leave and root. Overall, *G. mosseae* inoculation increased N and P contents in leaves and roots. In soil added with 30 and 180 mg Cd kg^{-1}, N content of roots colonized by *G. mosseae* was significantly higher than that of without inoculation ($P<0.05$). However, N content of leaves colonized by *G. mosseae* were significantly higher than that of without inoculation in low Cd level (30 and 90 mg Cd kg^{-1}) ($P<0.05$). P content in leaves and roots of *G. mosseae*-inoculated plants was significantly higher than the non-inoculated plants at the same Cd addition level except 90 mg Cd kg^{-1} level treatment ($P<0.05$). Therefore, not all the N and P accumulation in both leave and root of *G. mosseae* inoculated plants were significantly higher than those of non-inoculated controls in Cd-contaminated soil.

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Table 3. *N and P concentrations of ryegrass leave and root inoculated with G. mosseae with increasing Cd level*

Treatment	AM fungi		Mean (Cd)	AM fungi		Mean (Cd)
Cd added (mg kg-1)	-AM	+AM		-AM	+AM	
	Root N content (g kg^{-1})			**Leave N content (g kg^{-1})**		
0	0.60 aA	0.63 aA	0.62 A	1.15 aA	1.26 aA	1.21 A
30	0.57 bA	0.69 aA	0.63 A	0.96 bAB	1.22 aA	1.09 A
90	0.51 aB	0.52 aB	0.52 B	0.81 bB	0.88 aC	0.85 B
180	0.32 bC	0.49 aB	0.41 C	0.80 aB	0.76 aD	0.78 C
Mean (AM fungi)	0.50 b	0.58 a		0.93 b	1.03 a	
	Root P content (g kg^{-1})			**Leave P content (g kg^{-1})**		
0	3.29 bA	4.11 aA	3.70 A	3.22 bB	4.31 aA	3.77 B
30	2.48 aB	2.45 aB	2.47 B	4.25 aA	4.20 aA	4.23 A
90	1.80 bC	2.31 aB	2.06 C	3.11 bBC	4.22 aA	3.67 B
180	1.75 bC	2.12 aBC	1.94 C	3.26 bB	4.26 aA	3.76 B
Mean (AM fungi)	2.33 b	2.75 a		3.46 b	4.25 a	

Which each row, mean values (n=3) with the different lowercase letter are significantly difference ($P<0.05$) among AM fungi treatments by the Tukey test. Within each column, mean values (n=3) with the different uppercase letter are significantly difference ($P<0.05$) among Cd levels by the Tukey test. – AM=non-mycorrhizal, +AM= with mycorrhizal

Discussion

The AM fungal specie (*G. mosseae*) used in our research successfully colonized ryegrass plants grown in Cd-contaminated soils, indicating that *G. mosseae* is Cd tolerant and able to maintain an efficient symbiosis with ryegrass root systems even under high Cd stress. However, Cd contamination decreased ryegrass colonization by *G. mosseae*. Similar findings were found for maize (Chen et al., 2004a), sunflower (Hassan et al., 2013) and other plants (Li et al., 2009). The reduction in root colonization rate with Cd addition indicates the toxicity of Cd for AM fungal species.

In our study, we found that the rate of mycorrhizal infection inoculated with *G. mosseae* decreased with the increase of Cd concentration, but the rate was still as high as 43% under high concentration of Cd (180 mg Cd kg^{-1}). Secondly, compared with the plants of non-inoculated with *G. mosseae*, the inoculated plants improved the root activity at the same Cd concentration level, indicating that AM fungal has strong tolerance and resistance to Cd contamination. Gao (2017) showed that low concentration Cd could promote plant growth, whereas high concentration can inhibit plant growth, which are consistent with our results.

By Cd stress, net photosynthetic rate (*P*n), stomatal conductance (*G*s) and transpiration rate (*T*r) and intercellular CO_2 concentration (*C*i) of ryegrass leaf are lower, which indicated photosynthetic capacity under Cd stress were more obvious inhibitory effect. This is related to the effect of Cd stress on photosynthesis by affecting the absorption, reduction and assimilation of N, P, Fe and Mg related to photosynthesis, reducing pigment content, inhibiting stomatal opening, destroying photosynthetic apparatus, affecting water balance and electron transport (Bishnoi et al., 1993).

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However, the increase in *C*i index of non-inoculated *G. mosseae* at high Cd concentration (180 mg Cd kg^{-1}) may be due to the decrease in net photosynthetic rate, which reduces the consumption of intercellular CO_2 and increases the concentration of intercellular CO_2 (Anjum et al., 2016). *P*n, *G*s, *T*r and *C*i of ryegrass inoculated with *G. mosseae* alleviated the negative effects of photosynthesis to some extent. Chen (2017) found that under the stress of Cd, AM fungi inoculation restored the photosynthesis of female poplar to a certain extent. This is related to the promotion of host plant nutrient balance and antioxidant capacity by inoculation of AM fungi as well as the increase of host photosynthetic capacity and photochemical efficiency (Li et al., 2016).

MDA has been widely used as an indicator of membrane lipid peroxidation damage. Under the stress of Cd, the balance between the production and clearance of reactive oxygen species is broken, which is likely to cause oxidative damage to cells and excessive accumulation of ROS and inhibit plant growth (Maiti et al., 2012). In this experiment, with the increase of Cd pollution concentration, MDA content in both inoculated and non-inoculated with *G. mosseae* plants showed an increased trend, indicating that Cd stress caused the increase of reactive oxygen species in leaves and promoted the degree of membrane lipid peroxidation in leaves. Under the same concentration of Cd, MDA content in the leaves of the inoculated with *G. mosseae* was lower than that of the non-inoculation leaves, which alleviated the Cd toxicity to a certain extent, cleared the excess reactive oxygen species, and reduced the damage to the cell membrane. SOD and CAT can effectively remove harmful substances produced under the stress of Cd, such as superoxide anions, and help to keep ROS at controlled level and reduce stress state (Ning and Yan, 2019). In this study, the SOD concentration of plants in the inoculated and non-inoculated with *G. mosseae* increased with the increase of Cd concentration, while the content of CAT first increased and then decreased. The results showed that with the increase of Cd concentration, the accumulation of reactive oxygen radicals led to the increase of CAT activity. The CAT activity of inoculated plants increased more, and the ability of scavenging active oxygen free radical was stronger, which improved the resistance of ryegrass to Cd pollution. The increase in SOD and CAT activity could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via an increase in levels of superoxide radicals (Chongpraditnun et al., 1992), which could alleviate cell membrane damage. However, the stress of high concentration of Cd (180 mg kg^{-1}) may cause the inactivation of CAT protein, indicating that antioxidant enzymes of plants have certain limits on the clearance of reactive oxygen species. Compared with non-inoculated with *G. mosseae*, inoculated plants reduced the damage of harmful substances to cells, which could be confirmed by increasing the biomass, plant height, root length and MDA content of ryegrass by inoculated *G. mossea*.

The plant height and root/shoot ratio of the inoculated and non-inoculated AM fungal plants, and the aboveground, underground and total biomass of the inoculated AM fungal plants first increased and then decreased under low Cd concentration. Compared with non-inoculated with *G. mosseae*, inoculated plants alleviated Cd pollution and promoted the growth of ryegrass. On the one hand, after mycorrhizal fungi infect the host, the mycorrhizal fungi secrete mucus, polyphosphoric acid, organic acid and the metal chelating peptide formed by plants can adsorb heavy metals in the root, inhibit the Cd transport to the aboveground part of ryegrass, and reduce the toxicity of Cd stress to ryegrass (Wang et al., 2010). On the other hand, inoculation of AM fungi can expand the absorption area of roots, improve the absorption ability of ryegrass to nutrients and

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mineral elements, and promote the growth of plants. Meanwhile, AM fungi can produce various growth hormones to promote the growth and development of plant roots. However, with the increase of Cd concentration, the inoculated AM fungi plants were all poisoned by Cd. Zarei (2008b) proved that AM fungi diversity decreased in the soil contaminated by high concentration of zinc. Inoculation of AM fungi improved the tolerance of ryegrass to Cd stress to some extent, but it was still affected by high concentration of Cd.

Heavy metal pollution tends to adversely affect the nutritional status of plants, mainly due to the inhibition of nutrient uptake. Tanaka and Yano (2010) found that increase in the N uptake in plants of AM fungi inoculated. Our research showed that plants of inoculated with *G. mosseae* increased the N and P contents in roots and leaves compared with the non-inoculated plants under the stress of Cd, which indicated that AM fungi promoted the nutrient uptake of ryegrass. This may be related to the fact that the plants infected by AM fungi changes the composition of soil microbial community, leading to the increase of saprophytes, the acquisition of nitrogen in organic matter, and the promotion of nitrogen uptake by plants (Cuéllar et al., 2015). Cruz (2017) found that plant roots of AM fungi infected changed the configuration of roots and increased the absorption capacity of active root areas, which promoting the absorption of N, P and mineral elements. Moreover, in the same Cd level, the content of N and P in leaves of inoculated with *G. mosseae* was higher than that of root. Cui (2019) found that Cd tolerance genotypes reduced Cd transport to leaves and reduced the toxicity of Cd to plant leaves by reducing GmHMA18 expression.

Conclusion

In summary, compared with uncontaminated ryegrass, the growth of inoculated and non-inoculated AM fungi ryegrass was inhibited with the increase of Cd pollution concentration. Compared with non-inoculation of AM fungi ryegrass, inoculation of AM fungi could improve the resistance and tolerance of ryegrass to Cd stress, and to a certain extent, restored mycorrhizal activity, plant growth and biomass, photosynthetic physiology, and N and P content in roots and leaves. Moreover, the content of SOD and CAT was increased, MDA was decreased, which could alleviate the damage of harmful substances on cells and promote the growth of ryegrass. We propose that AM fungi might potentially be important not only in protecting plants against Cd toxicity, but also in its bioaccumulation and phytoremediation techniques in these polluted soils.

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