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Synergistic utilization of arbuscular mycorrhizal fungi and *Festuca elata* for remediating cadmium-contaminated soils

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Abstract: A greenhouse pot experiment was conducted using tall fescue (*Festuca elata* 'Crossfire II') inoculated with or without the arbuscular mycorrhizal (AM) fungus *Funneliformis mosseae*. Plant biomass, defense enzyme activities, phosphorus (P) uptake, and available cadmium (Cd) content were determined in tall fescue plants grown in soil with Cd added at 0, 5, 15, and 30mg/kg. As the Cd concentration increased, the mycorrhizal colonization and mycorrhizal relative dependency of the tall fescue increased. The presence of AM fungi improved P transportation from roots to the shoots, which helped the plant accumulate more P in the shoots. In addition, the activities of antioxidant enzymes were largely induced by the AM fungi and Cd stress in the tall fescue plants. In particular, the catalase activity in AM fungus-inoculated plants increased significantly compared to the non-inoculated control. Malondialdehyde content decreased significantly in inoculated plants compared with control plants under Cd stress. Inoculating *F*. *mosseae* greatly enhanced the Cd enrichment capacity of the host plants and was conducive to fixing heavy metals within roots and reducing Cd content in the shoots compared to the non-inoculated control. This study shows that tall fescue inoculated with AM fungi has good potential as a Cd phytoremediator.

Key words: AM fungi, cadmium contamination, combined rehabilitation, *Festuca elata*

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丛枝菌根真菌与高羊茅协同修复镉污染土壤

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摘 要：本研究采用温室盆栽试验，利用丛枝菌根（AM）真菌摩西管柄囊霉 *Funneliformis mosseae* 进行接种试验，研究了在 Cd 胁迫下（0、5、15 和 30mg/kg）接种 AM 真菌对高羊茅 *Festuca elata* 'Crossfire II' 的生物量、防御酶活性、磷和镉（Cd）含量的影响。结果表明，随着 Cd 浓度的增加，高羊茅的菌根侵染率和菌根相对依赖性有所增加。接种 AM 真菌改善了磷从植株根系向地上部的转运，有助于植株在地上部积累更多的磷。此外，AM 真菌和 Cd 胁迫对高羊茅植株抗氧化酶活性都有显著影响，在镉胁迫下，与未接种植株相比，接种 AM 真菌显著提高了植株的过氧化氢酶活性，而显著降低了植株的丙二醛含量。与未接种植株相比，接种摩西管柄囊霉显著提高了寄主植物对 Cd 的富集能力，有利于重金属在根部的积累，同时降低了地上部的 Cd 含量。本研究表明，高羊茅-丛枝菌根共生体在 Cd 污染土壤的修复中具有潜在应用价值。

关键词：丛枝菌根真菌，镉污染，联合修复，高羊茅

Pollution with toxic metal ions is becoming increasingly serious with the increase in industrial and human activities. In particular, large amounts of heavy metals (HMs) are accumulating in soils as a result of irrigating agricultural land with sewage, sludge, and garbage. In addition, fertilizers and pesticides are often used unreasonably. The cultivated area affected by cadmium (Cd), mercury, arsenic, chromium, and lead has reached about 20 million km^2. Food loss is about 10 million tons due to HM pollution each year and 12 million tons of food is contaminated. The economic loss has reached 20 billion RMB (Wu *et al*. 2010; Huang *et al*. 2018). The wide existence of HMs, which are difficult to degrade and easy to accumulate, pose potential harm to humans (Yabanli *et al*. 2014).

Contaminated soil can be remediated by chemical, physical, or biological technologies. Some remediation technologies for HM-contaminated soils are costly or damaging to the environment, and do not make full use of existing resources. Bioremediation is a technique that uses natural biological processes to eliminate toxic pollutants. It can be any process that uses microorganisms, fungi, green plants, or their enzymes to restore the natural environment to its original state that has been altered by a pollutant (Mani & Kumar 2014). The potential role of bioremediation for metal-polluted sites has been a hot topic in recent years.

Arbuscular mycorrhizal (AM) fungi are soil microorganisms that establish a symbiotic relationship with most higher plants (Liu & Chen 2007) that is often used for bioremediation at metal-contaminated sites (Janeeshma & Puthur 2020). AM fungi are widely distributed in soils contaminated by mining and in soils treated with sewage or sludge (Regvar *et al*. 2010; Deram *et al*. 2011; Li *et al*. 2013). A large number of field investigations have isolated and identified a variety of AM fungi from soil contaminated with different HMs (Zhong 2012). These fungal symbionts contribute to the acquisition of mineral nutrients, particularly phosphorous (P), from soil to host plants in exchange for fixed carbon (C) from the plants (Plassard & Dell 2010; Smith *et al*. 2011; Hu *et al*. 2014; Sun *et al*. 2018). Furthermore, these fungal symbionts play important roles in alleviating various abiotic stressors in the soil (Evelin *et al*. 2009; Gamalero *et al*. 2009; Gianinazzi *et al*. 2010; Carrenho *et al*. 2018; Chaturvedi *et al*. 2018b). Previous studies have shown that some fungal symbionts mitigate HM stress in soil. AM fungi promote the absorption of Cd by *Lolium perenne* and reduce Cd stress on leaves (Liu 2011). *Rhizophagus intraradices* reduces the toxicity of Cd to *Salix viminalis* (Bissonnette *et al*. 2010), and *F. mosseae* and *Funneliformis caledonium* significantly increase the mycorrhizal colonization rate of the *Helianthus annuus* rhizosphere in severe Cd polluted soil (Zhang *et al*. 2018).

Plants and microorganisms absorb pollutants from contaminated soils and convert them into other chemicals (Dong *et al*. 2017). Tall fescue (*Festuca elata*) is a subtropical cool-season turfgrass that is an important forage resource widely used in lawn, stadium, and protective turf (Fraser *et al*. 2016). Tall fescue takes up HMs from the soil, such as Cd and zinc (Zn) (Wu *et al*. 2012; Huang *et al*. 2017). Inoculation with *F. mosseae* alleviates nickel (Ni)-induced stress by reducing Ni transport from tall fescue roots to shoots (Shabani *et al*. 2016). Inoculation with *Glomus caledoniun* increases the yield of tall fescue and polycyclic aromatic hydrocarbon accumulation in plants (Lu *et al*. 2015). *F. mosseae* and *R. irregularis* significantly increase root colonization, P content, compatible solutes (proline and glycine betaine), and antioxidant enzymes (superoxide dismutase and peroxidase) of tall fescue whether under greenhouse or field drought-stress conditions (Mahdavi *et al*. 2018). However, most previous studies focused on the effects of AM fungi on plants. No study has investigated the combined effects of AM fungi and tall fescue on mitigating Cd contamination in soil. The main goal of this study was to evaluate the combined effects of an AM fungus and tall fescue on mitigating Cd pollution in soil. We also determined the effects of AM fungi on plant growth, available P, and transportation of Cd in tall fescue. Our findings provide insight into utilizing a specific AM fungus and tall fescue to recover HM polluted soils.

1 MATERIALS AND METHODS

1.1 Experimental materials and plant set-up

The mycorrhizal inoculum was *F. mosseae* strain. *F. mosseae* inoculum consisted of a mixture of rhizospheric soil derived from cultures of *Trifolium repens* containing spores (5 000spores/kg), hyphae and mycorrhizal root fragments, which was provided by the Institute of Mycorrhizal Biotechnology of Qingdao Agricultural University.

The tall fescue (*F. elata* 'Crossfire II') seeds was purchased from Clover Group Co., Ltd., Beijing, China. The topsoil (5–20cm) was collected from the campus of Qingdao Agricultural University (total Cd<0.07mg/kg), with 0.75g/kg N, 0.43g/kg available P, 10.1g/kg available K, total C 1.35%, 8.85g/kg organic matter, and pH 7.1 (soil:water, 1:2.5, *W*/*V*), then sieved and mixed with washed sand (1:1). The mixture was sterilized twice in a steam autoclave (0.11MPa and 121°C, 1h), to eliminate the influence of indigenous AM fungi.

1.2 Experimental design

The experiment was conducted using a double factor completely randomized factorial design. The first factor was Cd added at four different levels of 0, 5, 15, and 30mg/kg (as solutions) to the root chamber as an analytical grade $CdCl_2$ solution mixed thoroughly with the soil. The second factor was inoculating with 0 and 150 *F. mosseae* spores. Therefore, there were eight treatments. Each treatment was replicated four times in a total of 32 pots.

Different portions of Cd were mixed with medium 2 weeks before planting. The medium (2kg/pot) was added to pots (17cm diameter and 20cm depth) sterilized with 75% alcohol. Each pot was inoculated with 30g of AM inoculum. An equivalent amount of sterilized inoculum and 15mL of culture filtrate were added to non-inoculated plants to provide the same medium conditions. Tall fescue seeds were soaked in a 5% (*V*/*V*) NaClO solution for 10min for surface sterilization, rinsed with sterilized distilled water, and germination was accelerated in a culture dish (28°C, in the dark). Then, 1g of seeds were sown in pots according to the standard of 30–50g/m^2. The experiment was carried out in a greenhouse under a controlled temperature of 25°C/22°C (day/night) and a 14h/10h day/night photoperiod. The plants were well watered but no fertilizer was provided. The plants were harvested 10 weeks after planting.

1.3 Estimation of root AMF colonization

1cm long fresh root systems were stained by 0.05% (*W*/*V*) of trypan blue according to the protocol described by Linderman (1981). Mycorrhizal infection rate = the number of root segments colonized/total number of observed root segments × 100. Photograph was taken with Olympus BX51 microscope.

1.4 Measurement of growth and physiological indices

Fresh and dry masses (after drying at 105°C for 5h) of the shoots and roots were recorded. Relative mycorrhizal dependency (RMD) = [(mycorrhizal plant dried weight – non-inoculated plant dried weight)/mycorrhizal plant dried weight] ×100%. Approximately 0.6g of dry shoots were burned in a muffle oven (480°C, 15h), cooled, the ashes were dissolved in HNO_3 (0.6mol/L), and filtered through Whatman® 145 ashless filter paper (Shanghai Huifen Electronics. Co., Ltd., Shanghai, China).

This acid extract was used to quantify the P content in the shoots by inductively coupled-plasma mass spectrometry (Thermo Electron Corp. Mod. IRIS Intrepid II XDL, Waltham, MA, USA).

Malondialdehyde (MDA) content was determined by the thiobarbituric acid method. The superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities were determined by nitroblue tetrazolium, the guaiacol, and the ultraviolet absorption methods, respectively.

The P and Cd concentrations, transportation coefficients, and Cd and P enrichment coefficients were measured by heat digestion with the perchloric acid-nitric acid method, and Cd content was determined by the atomic absorption method.

The contribution rate of mycorrhizae = [(Cd concentration of mycorrhizal plants – Cd concentration of non-inoculated plants)/Cd concentration of mycorrhizal plants]×100%. The enrichment coefficient (EC)=Cd content in the plants/soil Cd content. The transportation coefficient (TC)=shoot Cd content/root Cd content.

1.5 Statistical analysis

All data were analyzed using SPSS11.5 software package (SPSS, Chicago, IL). A one-way variance analysis (ANOVA) was carried out, followed by the Duncan's Multiple Range Test (P<0.05) to determine differences between means. All data are represented as means ± standard error (SE) of three independent experiments with three biological repeats per treatment.

2 RESULTS

2.1 AM colonization and relative mycorrhizal dependency

None of the non-inoculated control tall fescue seedlings were colonized with mycorrhiza. The mycorrhizal status was best manifested in the roots of plants inoculated with *F. mosseae*. The root mycorrhizal colonization rate varied between 28%–38% in different treatments, while RMD was 19%–23% (Table 1). The mycorrhizal colonization and RMD increased with the increase in the Cd concentration. The Cd (30mg/kg) plus treatment was a significant source of variation in mycorrhizal colonization. The highest colonization percentage of seedlings under the 30mg/kg treatment was 38%, while the colonization percentage of seedlings without Cd added was 28%. Inoculation with AM fungi significantly increased the root and root dry weight of tall fescue compared with the control without AM fungi, except at Cd concentrations of 5 and 10mg/kg. The shoot and root dry weights of tall fescue inoculated with AM fungi were significantly higher than those of non-inoculated plants under the same Cd concentration. The RMD of 30mg/kg Cd was 23%, while that of seedlings without Cd was 19%. Two-way analysis of variance (ANOVA) showed that the AM fungus had a significant effect on shoot P concentrations in tall fescue (P<0.05), while Cd and AM × Cd had no significant effect on shoot P concentrations in tall fescue. AM, Cd, and AM × Cd had no significant effect on root P concentration (Table 1).

Table 1 Mycorrhizal colonization as well as shoot and root dry weight of *Festuca elata* per pot at soils with different Cd concentrations

Treatments	Cd concentration (mg/kg)	Colonization (%)	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Mycorrhizal relative dependency (%)
Non-inoculated	Cd0	-	13.6±0.10c	4.4±0.07c	-
	Cd5	-	12.1±0.10f	3.6±0.07d	-
	Cd15	-	11.7±0.10g	3.4±0.04d	-
	Cd30	-	10.6±0.05h	2.6±0.08e	-
F. mosseae	Cd0	28±0.04b	15.9±0.06a	6.4±0.03a	19±0.30c
	Cd5	29±0.06b	13.0±0.09d	6.4±0.06a	19±0.50c
	Cd15	34±0.06ab	14.1±0.05b	5.3±0.03b	22±0.50b
	Cd30	38±0.03a	12.7±0.04e	4.7±0.06c	23±0.70a
AM	-	***	NS	***	-
Cd	-	NS	NS	***	-
AM×Cd	-	NS	NS	***	-

Note: Values represent the means±SE, n=4; the same letter within a column indicates no significant difference assessed by Duncan's multiple range test ($P≤0.05$) following analysis of variance. Two-way ANOVA output: NS, not significant; *$P<0.05$, **$P<0.01$, ***$P<0.001$. AM, arbuscular mycorrhizal fungus. -, not detected. The same below.

2.2 P absorption

The results showed that the shoot P concentration of tall fescue without *F. mosseae* decreased significantly with the increase in Cd concentration, while the shoot P concentration of tall fescue inoculated with AM fungus increased significantly with the increase in Cd concentration. Moreover, the shoot P concentration of tall fescue inoculated with AM fungus was significantly higher than that of tall fescue without *F. mosseae* under the same Cd concentration. These results indicate that inoculating with AM fungus increased the concentration of P in shoots, but reduced the concentration of P in roots. The contribution rate of mycorrhizae to P absorption increased by 6%, 8%, 13%, and 18% in the treatments at the four different Cd levels of 0, 5, 15, 30mg/kg with the increase in Cd concentration, respectively (Table 2). Under Cd stress, the AM fungi promoted the transport of P from roots to shoots, which helped the plant accumulate more P in shoots and maintain growth. However, regardless of AM fungal inoculation, the P concentration in roots of tall fescue with 30mg/kg Cd was significantly lower than that without Cd. Two-way ANOVA showed that AM fungi had a significant effect on shoot P concentration in tall fescue ($P<0.05$), while Cd and AM × Cd had no significant effect on shoot P concentrations in tall fescue. AM, Cd, and AM × Cd had no significant effect on root P concentration (Table 2).

Table 2 P concentrations in shoots and roots of *Festuca elata* grown in soils supplemented with varying Cd concentrations

Treatments	Cd concentration (mg/kg)	Shoots (mg/kg)	Roots (mg/kg)
Non-inoculated	Cd0	2 276.9±4.37a	1 544.1±3.62a
	Cd5	2 234.3±1.08b	1 456.6±5.01a
	Cd15	2 139.1±3.84c	1 390.4±3.44b
	Cd30	2 002.3±2.50d	1 362.9±5.87b
F. mosseae	Cd0	2 596.0±2.78d	1 463.7±2.93a
	Cd5	2 637.9±7.61c	1 407.8±2.21b
	Cd15	2 714.9±9.77b	1 369.8±49.3b
	Cd30	2 786.4±2.18a	1 337.5±5.43b
AM	-	*	NS
Cd	-	NS	NS
AM×Cd	-	NS	NS

2.3 MDA concentration and SOD, CAT, and POD activities

The Cd treatments resulted in significant increases in MDA concentrations in the inoculated and non-inoculated control plants. MDA content decreased significantly in inoculated plants compared with the control plants under Cd stress (Fig. 1A). SOD, POD, and CAT activities increased significantly with increasing Cd concentration in the mycorrhizal plants and the non-inoculated control. In addition, when the Cd concentration was< 30mg/kg, the POD activity of inoculated plants was significantly higher than that of non-inoculated controls (Fig. 1B), while when the Cd concentration was > 15mg/kg, the SOD activity of inoculated plants was significantly higher than that of non-inoculated controls (Fig. 1C). CAT activity of inoculated plants was significantly higher than that of the non-inoculated groups when the Cd concentration was 5–30mg/kg (Fig. 1D). SOD and CAT activities of plants inoculated with AM fungi significantly increased 38.41% and 35.88%, respectively, while POD activity of the inoculated plants was 28.82% higher than that of non-inoculated plants (Fig. 1).

2.4 Cd absorption and contribution rate of mycorrhizae

The Cd content in shoots and roots of tall fescue increased significantly with the increase in the Cd treatment concentration. When the concentration range of the Cd treatment was 0–15mg/kg, the shoot Cd concentration of inoculated tall fescue was significantly lower than that of non-inoculated plants. When the Cd concentration was <5mg/kg, the shoot Cd content of inoculated tall fescue was significantly lower than that of non-inoculated plants. However, the root Cd concentration and Cd content of inoculated tall fescue were higher than those of non-inoculated tall fescue under any of the Cd treatment levels. Two-way ANOVA showed that AM fungi had significant effects on aboveground Cd concentration (P<0.001) and root Cd content (P<0.01), but did not affect root Cd concentration or shoot Cd content. Cd had significant effects on the shoot and root Cd concentrations, root Cd content (P<0.001), and shoot Cd content (P<0.05). AM × Cd had a significant effect on Cd concentration in shoots and roots of tall fescue (P<0.001), but had no significant effects on Cd contents in shoots or roots.

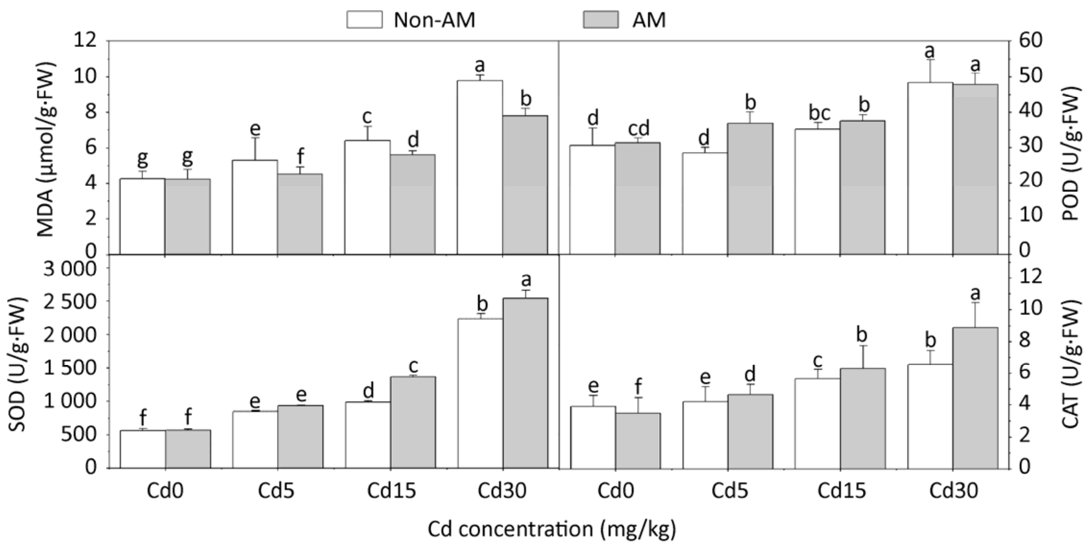

Fig. 1 Malondialdehyde concentration and defensive enzyme activity in *Festuca elata* with varying Cd concentrations. Values are means±SE, n=4; different letters in common within a graph indicate significant differences assessed by Fisher least significant difference test (P≤0.05) following analysis of variance. CAT, catalase; MDA, malondialdehyde; POD, peroxidase; SOD, superoxide dismutase.

Table 3 Effects of arbuscular mycorrhizae on the concentration and content of Cd in shoot and root of *Festuca elata*

Treatments	Cd concentration (mg/kg)	Shoot Cd concentration (mg/kg)	Root Cd concentration (mg/kg)	Shoot Cd content (μg/pot)	Root Cd content (μg/pot)
Non-inoculated	Cd0	0.35±0.08g	2.11±0.24h	4.80±0.29g	9.40±0.63h
	Cd5	3.92±0.11e	133.2±11.2f	47.3±1.23e	476.9±5.35f
	Cd15	5.69±0.13c	211.9±6.05d	66.6±1.33d	718.5±6.90e
	Cd30	7.92±2.88b	458.8±22.9b	87.0±1.52b	1192.9±5.32c
F. mosseae	Cd0	0.28±0.05h	2.59±0.32g	4.51±0.89g	16.50±1.23g
	Cd5	3.23±0.40f	160.7±13.2e	41.9±2.02f	1029.0±4.32d
	Cd15	5.28±0.63d	314.6±11.9c	74.7±1.37c	1664.6±9.26b
	Cd30	8.18±2.66a	514.6±31.8a	100.5±1.65a	2408.3±4.55a
AM	—	***	NS	NS	**
Cd	—	***	***	*	***
AM × Cd	—	***	*	NS	NS

2.5 Enrichment coefficient (EC) and transportation coefficient (TC)

The inoculated plants transferred less Cd from roots to shoots compared with non-inoculated plants, and the TC of Cd was significantly lower than that of the non-inoculated control. The EC of the inoculated plant shoots was relatively less than the control. In contrast, the EC of the inoculated plant roots was higher than the control (Table 4). The Cd TC decreased gradually in non-inoculated plants with the increase in Cd concentration but remained unchanged in inoculated plants. However, regardless of inoculation with AM fungi, the EC of tall fescue shoots and roots was negatively correlated with the Cd concentration. The EC of shoots and roots was the highest when the Cd concentration was 5mg/kg.

3 DISCUSSION

AM fungi are obligate symbiotic fungi that colonize the roots of over 80% of terrestrial vascular plants to form mutually beneficial symbiotic relationships. AM fungi promote plant growth and create a very high surface root area that helps plants absorb mineral nutrition from the soil while receiving photosynthates from the plant. AM fungi also play a central role in the natural attenuation of host metal toxicity (Lehmann *et al*. 2014; Eke *et al*. 2016). When soils are contaminated with excess HMs, they become toxic as the HMs damage physiological activities, leading to lower photosynthetic efficiency and inhibited plant growth and development (Maleva *et al*. 2012; Osório *et al*. 2012; Aslam *et al*. 2014; Mahardika *et al*. 2018). Our results indicate that *F*. *mosseae* improved tall fescue growth and P concentration while allowing the plants to accumulate high levels of Cd in root tissues and reduce the transfer of Cd to the shoots. The P concentration in mycorrhizal-inoculated tall fescue shoots increased with increasing Cd level. AM symbiosis may enhance soil phosphatase activity under HM polluted conditions, activate P in soil, or directly take up and carry P into plants. The transport of P across the symbiotic interface is regulated by a characteristic mycorrhizal plant phosphate

Table 4 Transportation coefficients (TC) and enrichment coefficients (EC) of Cd in soils supplemented with varying Cd concentrations

Treatments	Cd concentration (mg/kg)	TC of Cd	EC of shoots	EC of roots
Non-inoculated	Cd0	0.64±0.03	0.64±0.01	1.85±0.02
	Cd5	0.09±0.00	0.98±0.02	9.87±0.12
	Cd15	0.09±0.01	0.48±0.01	5.20±0.16
	Cd30	0.07±0.01	0.36±0.00	4.96±0.25
F. mosseae	Cd0	0.32±0.01	0.51±0.02	1.87±0.11
	Cd5	0.04±0.00	0.44±0.03	10.9±0.50
	Cd15	0.04±0.00	0.27±0.05	6.03±0.43
	Cd30	0.04±0.00	0.18±0.03	4.45±0.21

transporter (Karandashov & Bucher 2005; Wang *et al*. 2006), which is absent in non-mycorrhizal regions. P is involved in many plant physiological processes, such as energy storage and transfer, photosynthesis, enzyme regulation, and carbohydrate transport. Therefore, AM fungi play an important role in the phytoremediation of contaminated soils by elevating the uptake of P (Leung *et al*. 2010; Meier *et al*. 2012; Chaturvedi *et al*. 2018a). The highest AM fungal colonization and RMD values were seen in plants grown under the highest Cd level. The AM fungal colonization rate was 29% and the RMD was 19% when the soil Cd concentration was low, whereas the AM fungal colonization rate was 38% and the RMD was 23% at the highest Cd concentration. This finding is very consistent with that of Abdelhameed & Metwally (2019) who reported that AM fungi protect plant growth under high concentration Cd stress. However, no correlation has been observed between the degree of colonization and the effectiveness of the fungi in the symbiotic relationship.

Antioxidant capacity is used to characterize the nutritional status of plants and their bioactive components (Ayari *et al*. 2013). HM stress leads to the production of a large number of reactive oxygen species (ROS), which destroy biomolecules and cause membrane lipid peroxidation. The activities of the tall fescue antioxidant enzymes were mainly induced by the presence of the AM fungi and Cd stress. The low content of cellular MDA suggested that the ROS scavenging system was activated by the symbionts under the Cd stress condition. The Induction of antioxidant enzymes, which was attributed to excess production of ROS or overexpression of antioxidant enzyme coding genes (Chaturvedi *et al*. 2018), is a defense reaction that occurs during the early stage of a plant symbiotic relationship (Blilou *et al*. 2000; Hajiboland *et al*. 2010). Previous studies have shown that antioxidant enzyme activities are enhanced in inoculated plants compared to non-inoculated plants under different HM stressors (Janeeshma & Puthur 2020). Chaturvedi *et al*. (2018b) demonstrated that tomato (*Solanum lycopersicum*) inoculated with *Glomus mosseae* upregulates antioxidant enzyme (CAT, SOD, and APX) activities under Cd or Pb stress. Tan *et al*. (2015) reported that CAT, GPX, and APX activities in *Solanum photeinocarpum* leaves associated with *Glomus versiforme* improved in Cd-contaminated soils, which helped to reduce the damage caused by ROS. In contrast, Heydarian *et al*. (2018) determined that the lowest CAT activity was obtained when wheat was inoculated with *Glomus intraradices* under nickel stress and assumed that the ability of arbuscular mycorrhiza to produce low stress in plants under heavy stress depends on the ability of AM fungi to produce glomalin (Arriagada *et al*. 2005). The insoluble glycoproteins produced by the mycelia bind to potentially toxic elements, including HMs (Gonzalez-Chavez *et al*. 2004). These conclusions may depend on the type of HM, their concentration in the soil, and the symbiotic partners of the AM fungi. A single plant species inoculated with different AM fungi do not produce the same response, and different plants inoculated with the same AM fungi have different responses.

In natural and contaminated ecosystems, AM fungi form an extensive hyphal network in the soil, extending the plant roots by expanding the volume of accessible soil to absorb nutrients and pollutants (Göhre & Paszkowski 2006). The accumulation of Cd in the roots of inoculated plants was more than twice that of non-inoculated plants, and Cd content in roots was significantly higher than that in non-inoculated plants. Once the AM symbiosis forms, extensive extraradical mycelia develop, which directly absorb and transport Cd from a distance to the roots (Wu *et al*. 2016). Furthermore, this may be due to the observation that AM colonization changes the way plants absorb metals, such as the chelation of polyphosphate particles to metals in fungal vacuoles (Turnau *et al*. 1993).

Interestingly, the Cd content in shoots of mycorrhizal plants was lower than that of non-inoculated plants, except for the treatments with the highest soil Cd content, although their roots accumulated more Cd. One possible mechanism is that AM symbiosis, which promotes plant growth by promoting plant uptake of P, leads to the "growth dilution effect" on metals of plants (Chen *et al*. 2007), or Cd immobilization in mycorrhizal roots. The TC values of the mycorrhizal plants decreased compared to non-inoculated plants grown under the low and high Cd concentrations, indicating that mycorrhizal tall fescue reduced the transport of Cd from roots to shoots. This finding is consistent with that of Abdelhameed & Metwally (2019) who reported that *Trigonella* inoculated with AM fungi accumulate high concentrations of Cd in their roots, which is transferred to the shoots but in low concentrations. Wu *et al*. (2016) demonstrated that extraradical mycelia absorb Cd and transport it to mycorrhiza, but inhibit the transfer of Cd from roots to shoots, thus promoting the fixation of Cd in roots and reducing the phytotoxicity of Cd. It has been demonstrated that AM fungal structures (hyphae, arbuscules, and vesicles) can fix HMs (Janeeshma & Puthur 2020). HMs that reach the roots are mainly deposited in the root parenchyma cells, where most of the fungal structures are located. Moreover, the spores and extracellular mycelia of AM fungi limit the transport of HMs from the soil to the aboveground organs of the plant (Hildebrandt *et al*. 2007; Gonzalez-Guerrero *et al*. 2008). Gonzalez-Guerrero *et al*. (2010) reported that Cd toxicity results in the expression of the related transporter *GintABC1*. This may play a key role in the Cd immobilized in roots and reduced translocation to aboveground organs.

The most important finding of the present study is that AM fungi maintained the Cd in the roots of tall fescue plants, transported less Cd to the shoots, and alleviated the harmful effects of HM pollution. Mycorrhizal status was best in roots inoculated with *F. mosseae*. The ability of mycorrhizal plants to reduce metal migration from roots to shoots suggests that they are more suitable as plant stabilizers in metal-contaminated land. Phytoremediation is an environmentally friendly technology that uses plants to restore contaminated soil to a level that is available for both private and public use (Leung *et al*. 2013). Our technology deserves further consideration for potential application in related areas.

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