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Growth and Some Physiological Activities of Pepper (*Capsicum annuum* L.) in Response to Cadmium Stress and Mycorrhizal Symbiosis

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ABSTRACT

A greenhouse experiment was conducted to investigate the effects of mycorrhial fungus (*Glomus mosseae*) on cadmium (Cd) toxicity in pepper (*Capsicum annuum* L. cv. Zhongjiao 105) plants. Half of plants were inoculated with arbuscular mycorrhizal fungi (AMF). Cd was supplied in the form of cadmium chloride at 0.0, 0.1 and 0.5 mM through irrigation water in the soil. Mycorrhizal colonization was higher in the control than in cadmium-treated soil. Dry weights of root and shoot of mycorrhizal (M) plants were higher than non mycorrhizal (NM) ones in both control and cadmium treatments. Measurements of Cd concentration indicated that M plants immobilized more Cd in the root and partitioned less Cd to the shoots. Cd decreased the leaf chlorophyll content, total sugar and total protein contents, and the concentrations of phosphorous and magnesium. M plants had greater contents of chlorophyll, total sugar, total protein and P and Mg concentrations than NM plants. Moreover, increasing the Cd concentration caused an increase in malondialdehyde (MDA) content in leaves of pepper plants; however, M plants showed a lower MDA content than NM plants. Cd decreased the activity of superoxide dismutase (SOD) in leaves of NM and M plants, on the other hand, it increased the activity of peroxidase (POD) and ascorbate peroxidase (APX) in leaves of NM and M plants. APX was stimulated more than POD in M plants versus NM plants, suggesting that APX is a major player in H_2O_2-scavenging in M plants. The study suggests that mycorrhization with G. *mosseae* can be a suitable way to induce Cd-stress resistance in pepper plants.

Keywords: Ascorbate peroxidase, Chlorophyll, *Glomus mosseae*, Heavy metals, Root colonization.

INTRODUCTION

Cadmium (Cd) pollution of soil is a serious problem due to the heavy use of mineral fertilizers, sewage sludge, and pesticides (Yang *et al*., 2011). Cd is highly toxic to plants, animals, and humans because it has been ranked No.7 among the top 20 toxicants (Gill *et al*., 2012). Although Cd is not essential for plant growth, in several species, Cd is easily taken up by roots and readily transported to shoot (Scebba *et al*., 2006; Lux *et al*., 2011) and enters into the food chain and can create risk for human and environmental health (Vassilev 2002; Meng *et al*., 2009). Regarding its risk to human health, International Agency for Research on Cancer (IARC, 1993) classified Cd as a human carcinogen and it has also been reported that plants are the most important source of non-occupational exposure to Cd for humans (Gill *et al*., 2012). Most plants are sensitive to low Cd concentrations, which can result in symptoms of phytotoxicities such as inhibition of plant growth, chlorosis, pigments degradation, imbalance in the uptake and distribution of macronutrients and

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micronutrients and final death (Jain *et al*., 2007; Yang *et al*., 2011, Gill *et al*., 2012). In addition, Cd causes disturbances in the plant antioxidant defenses, producing an oxidative stress through increased lipid peroxidation (Benavides *et al*., 2005). Concerning the mechanism of reactive oxygen species (ROS) production, Cd does not participate in Fenton-type reactions (Gzyl *et al*., 2009) but can indirectly favor the production of different ROS giving rise to an oxidative burst (Hana *et al*., 2008; Gill and Tuteja, 2010). To protect themselves against these toxic ROS, plants have evolved efficient systems that include ROS-scavenging antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX). SOD catalyzes the dismutation of two molecules of superoxide into oxygen and H_2O_2. POD is implicated in removal of H_2O_2. APX reduces H_2O_2 to water, with ascorbate as electron donor (Abdel Latef, 2011).

Under natural conditions, 70–90% of plants are colonized by arbuscular mycorrhizal fungi (AMF) leading to mutualistic associations (Abdel Latef, 2011). Mycorrhizae provide a stable environment for plants to survive by colonizing the root system (Rivera-Becerril *et al*., 2002; Al-Ghamdi *et al*., 2012). The host plant supplies soluble carbon source to the fungus, while the fungus enhances the ability of plant to absorb water and nutrients from the soil (Al-Ghamdi *et al*., 2012). Furthermore, AMF are recognized as biological agents that potentially increase the tolerance of plants to heavy metal toxicity. Heavy metals (HM) are taken via the fungal hyphae and can be transported to the plant. Thus, in some cases, mycorrhizal plants can show enhanced HM uptake and root-to-shoot transport (phytoextraction) while in other cases, AMF contribute to HM immobilization within the soil (phytostabilization) (Göhre and Paszkowski, 2006).

Pepper (*Capsicum annuum* L.) is one of the main crops for greenhouse cultivation, and high-quality yield is an essential prerequisite for pepper as a food crop. Recently, Turkmen *et al*. (2008) indicated that AMF inoculated pepper could benefit from its association with AMF.

The hypothesis of this investigation was that arbuscular mycorrhizal fungi have a protective action against heavy metals stress, increasing the tolerance of pepper (*Capsicum annuum* L.) to cadmium. The objective of this study was to test this hypothesis by determining the effect of different concentrations of Cd on pepper (*Capsicum annuum* L.) dry weight and biochemical parameters (chlorophyll, total sugar and total protein contents, Cd, P, and Mg concentrations, MDA content and the activity of SOD, POD and APX) in non-inoculated and inoculated plants with the AM fungus *Glomus mosseae*.

MATERIALS AND METHODS

Experimental Material and Plant Growth

Glomus mosseae was selected on the basis of literature data reporting its presence in heavy metal contaminated soils (Citterio *et al*., 2005), and its ability to enhance heavy metal uptake in other plant species (Li *et al*., 2009).

Seeds of pepper (*Capsicum annuum* L. cv. Zhongjiao 105) and mycorrhizal fungus inoculums of *Glomus mosseae* were obtained from Institute of Vegetables and Flowers, CAAS, Beijing, China. Seeds were surface-sterilized with 1:3 (v/v) of 2.5% sodium hypochlorite solution for 10 minutes, rinsed four times with distilled water, and kept for germination on wet filter paper in Petri dishes at 28°C. Five pre-germinated seeds were sown in separate pots (five replicates) containing 2 kg of an autoclaved mixture of black soil and sand (1:1.5, v/v). The soil mix was collected from greenhouse of Institute of Vegetables and Flowers and sterilized (160°C, 4 hours). Soil characteristics were: pH 7.26, 11.1% organic matter, 150 mg kg^{-1} available phosphorus, 451 mg kg^{-1} available nitrogen, and 518 mg kg^{-1} available

potassium. Half of the pots were inoculated with *G. mosseae*. Mycorrhizal inoculums were placed below the pepper seeds at sowing time. Each pot received approximately 2,500 spores at the time of sowing. The non-mycorrhizal treatments received washings of the soil-inoculum mixture filtered through Whatman no 42 filter paper. Seedlings were thinned to two seedlings per pot one week after emergence. This experiment was performed under greenhouse conditions including 30/22 °C day/night temperature, and a relative humidity of 60-80%. Cd was supplied as cadmium chloride at 0.0, 0.1, and 0.5 mM in irrigation water. The plants were supplemented with a nutrient solution recommended by Epstein (1972) once per week in the following composition: KNO_3 5 mM, KH_2PO_4 1 mM, $Ca(NO_3)_2$ 5 mM, $MgSO_4$ 2 mM, H_3BO_3 50 μM, $MnCl_2$ 10 μM, $ZnSO_4$ 1 μM, $CuSO_4$ 0.4 μM, H_2MoO_4 0.1 μM, and Fe-EDTA 20 μM. Distilled water was supplied on alternate days. Plants were harvested 8 weeks after transplantation.

Measurements and Analysis

Root Colonization

A fraction of the roots were carefully washed, cut into 1 cm long segments, cleared in 10% KOH at 90°C for 20 minutes, acidified in 2% HCl for 5 minutes, and stained with 0.01% acid fuchsin (Kormanik *et al*., 1980). Mycorrhizal colonization rate was measured using the gridline intersect method described by Giovannetti and Mosse (1980).

Dry Weight Determination

At harvest, root and shoot were separately washed with tap water to remove any adhering debris. The root and shoot dry weights were determined after oven-drying at 70°C for 48 hours.

Chlorophyll Content

The chlorophyll content of the youngest fully-expanded leaf 1 week before harvest was assayed according to Arnon (1949). The extraction was made from a 200 mg fresh sample in 20 ml ethanol, acetone and water (4.5: 4.5: 1, v/v/v) mixture and measured at 645 and 663 nm with a UV/VIS spectrophotometer.

Total Sugar and Total Protein Content

Total sugar was determined by the anthrone sulfuric acid method described by Badour (1959). The dried tissue of shoot was extracted by HCl. 1 ml of the carbohydrate extract was mixed with 9 ml of anthrone sulfuric acid reagent in a test tube and heated for 7 minutes at 100°C. The absorbance was measured spectrophotometrically at 620 nm against blank containing only distilled water and anthrone reagent.

Total protein of shoot was determined according to the method described by Bradford (1976), in which 5 ml of the protein reagent were added to 0.1 ml of the extract and the contents were mixed on a vortex mixer. The absorbance was measured at 595 nm after 1 h. The concentration of protein was calculated from a previously constructed standard curve for bovine serum albumin.

Mineral Analysis

Dried samples were ground, digested in concentrated acid (HNO_3: $HClO_4$, 2:3v/v) at 140–160°C. After cooling, the extracts were diluted with 1M HCl and made up to 25 ml (Allen, 1989). Reagent blanks were prepared by carrying out the whole extraction procedure but in the absence of sample.

Cadmium concentration of root and shoot was determined with atomic absorption spectrometry with flameless atomization (Salim *et al.,* 1992). Phosphorus concentration in shoot was determined by the ammonium molybdate blue method (Allen, 1989). The same digest was also used to determine the concentration of Mg in shoot by atomic absorption spectrometry.

Lipid Peroxidation

Malondialdehyde (MDA) was measured according to the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Leaf samples were homogenized with 5% trichloroacetic acid and centrifuged at 4,000 g for 10 minutes. Two milliliters of the extract was added to 2 ml 0.6% TBA placed in a boiling water bath for 10 minutes, and absorbance was read at 532, 600, and 452 nm. The MDA concentration was calculated according to the formula: $6.45\times(A_{532}-A_{600})-0.56\times A_{450}$.

Extraction of Antioxidant Enzymes

For enzyme extracts and assays, 500 mg fresh leaves were frozen in liquid nitrogen and then ground in 4 ml solution containing 50 mM phosphate buffer (pH 7.0), 1% (w/v) polyvinylpolypyrrolidone, and 0.2 mM ascorbic acid. The homogenate was centrifuged at 15,000 g for 30 minutes, and the supernatant was collected for enzyme assays.

Assays for Antioxidant Enzymes Activities

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to Stewart and Bewley (1980). The reaction mixture (3 ml) contained 13 mM methionine, 75 mM NBT, 100 mM EDTA and 50 µl of enzyme extract within 50 mM phosphate buffer (pH 7.8). The reaction was started with 2 mM riboflavin by exposing the cuvette to a 15-W fluorescent tube for 10 minutes. The absorbance of each reaction mixture was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of the photochemical reduction of NBT.

Peroxidase (POD, EC 1.11.1.7) activity was measured by following the change of absorption at 470 nm due to guaiacol oxidation. The activity was assayed for 1 min in a reaction solution (3 ml final volume) composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM H_2O_2 and 0.15 ml enzyme extract (Polle *et al.,* 1994).

The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured as a decrease in absorbance at 290 nm for 1 minute (Nakano and Asada, 1981). The assay mixture consisted of 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.0), and 0.15 ml enzyme extract.

Statistical Analysis

The experimental data were subjected to analysis and two-way analysis of variance using ANOVA. Means were compared by Duncan's test at the 5% level using SPSS program.

RESULTS

No mycorrhization was observed in roots of non-inoculated pepper plants, whereas all the plants roots inoculated with *Glomus mosseae* were mycorrhized. Percentages of mycorrhizal colonization with *Glomus mosseae* ranged from 29 to 65%. Cd addition negatively influenced mycorrhizal root colonization, which decreased with increasing cadmium concentration in soil (Table 1).

In comparison to the control, the concentration 0.1 mM $CdCl_2$ had no effect on dry weight of root and shoot of non mycorrhizal (NM) and mycorrhizal (M) pepper plants (Table 1), however, the concentration 0.5 mM $CdCl_2$ drastically reduced the growth of NM and M root and shoot. This reduction was more pronounced in NM than M. The dry weight of pepper root and shoot was generally higher for M than NM plants (Table 1). The positive effect of mycorrhization on root and shoot dry weight was recorded at the level 0.5 mM $CdCl_2$ compared to NM plants (Table 1). At this level, the increase in root and shoot dry weight due to AMF inoculation was 61 and 34%, respectively, compared to NM plants (Table 1).

Chlorophyll content showed a reduction under cadmium treatment in both NM and M plants (Table 1). Chlorophyll content negatively correlated with cadmium concentration in irrigation water. However, in M plants higher chlorophyll content was observed versus NM plants. At high Cd level, M plants exhibited 44% increase in chlorophyll content when compared to NM plants (Table. 2). On the other hand, AMF inoculation had no significant effects on chlorophyll content at the control level of Cd versus NM plants (Table 1).

The effect of Cd on the total sugar and total protein contents of shoot of NM and M plants showed a similar pattern as the effect on chlorophyll content (Table 2). However, M plants compared to NM plants had greater amount of sugar and protein, especially at high level of Cd. The enhancement in total sugar and total protein due to AMF inoculation was 57 and 61%, respectively, under high Cd level compared to NM plants (Table 2).

Cd concentration in root and shoot of both NM and M plants increased as the metal concentration increased in soil. M root of pepper, at both Cd levels accumulated more Cd as compared to NM root (Table 2). However, The M pepper plants reduced Cd accumulation in shoot compared to NM plants grown in the corresponding Cd treatment (Table 2), but the Cd concentrations in root and shoot of NM and M plants were not significantly different than the control treatment (Table 2).

The phosphorous status of shoot showed that P uptake was stimulated by AMF inoculation. Shoot P concentration in M was higher than NM plants especially at the high level of Cd (Table 2). Cd addition to soil reduced the P concentration in shoot of both NM and M plants, but no significant differences were noted between NM or M plants for shoot P concentrations up to 0.1 mM $CdCl_2$ (Table 2). Shoot concentrations of Mg were apparently higher for M than NM plants regardless of Cd treatments. Shoot Mg concentration in both NM and M plants decreased with increasing Cd in the

Table 1. Arbuscular mycorrhizal fungi (AMF) colonization (%), root and shoot dry weight (g $plant^{-1}$) and chlorophyll content (mg g^{-1}) of non mycorrhizal (NM) and mycorrhizal (M) pepper (*Capsicum annuum* L.) plants grown in soil with increasing Cd concentrations. Mean pairs followed by different letters are significantly different ($P< 0.05$) by Duncan's test.

Treatments $CdCl_2$ (mM)	AMF	AMF colonization	Root dry weight	Shoot dry weight	Chlorophyll content
0	NM		0.45b	1.37c	2.87ab
	M	65a	0.65a	2.05a	3.23a
0.1	NM		0.42b	1.34c	2.05bc
	M	44b	0.53ab	1.76ab	2.75ab
0.5	NM		0.18de	0.68e	1.36de
	M	29c	0.29c	0.91d	1.96c

Table 2. Shoot total sugar and total protein contents (mg g^{-1}), root and shoot Cd concentration (µg g^{-1}), shoot P and Mg concentrations (mg g^{-1}), leaves malondialdehyde (MDA) content (nmol g^{-1}) of non mycorrhizal (NM) and mycorrhizal (M) pepper (*Capsicum annuum* L.) plants grown in soil with increasing Cd concentrations. Mean pairs followed by different letters are significantly different (P< 0.05) by Duncan's test.

Treatments $CdCl_2$ (mM)	AMF	Total sugar	Total protein	Cd root	Cd shoot	P	Mg	MDA
0	NM	87.24ab	49.52b	0.21g	0.11g	3.9ab	5.61a	51d
	M	99.08a	60.34a	0.23g	0.09g	4.7a	5.9a	46de
0.1	NM	68.34bc	39.34c	0.51f	0.53c	2.91bc	4.42b	72c
	M	84.35ab	55.51a	0.82e	0.4d	3.12bc	5.22a	60d
0.5	NM	36.12e	22.09d	2b	0.89a	1.11e	3.23d	97a
	M	56.67d	35.66c	2.6a	0.69b	1.83d	4.14c	77b

soil, but no significant differences were noted for shoot Mg concentrations compared to the control treatment (Table 2).

MDA content was measured to determine whether application of excessive Cd caused oxidative stress in leaves of pepper. $CdCl_2$ treatment resulted in a marked increase in MDA content, as indicator of lipid peroxidation in both NM and M plants, and was lower in M than NM plants at all treatments. At the high level of Cd, the increase in MDA content was 90 and 51% in NM and M plants, respectively, compared to the control plants (Table 2).

The analysis of SOD activity in leaves showed a reduction by cadmium treatment. Under no-Cd treatment, AMF inoculation significantly increased the activity of SOD versus NM plants, while under both 0.1 and 0.5 mM $CdCl_2$ treatments, AMF inoculation did not exhibit significant variation in SOD activity as compared to non-inoculated plants (Figure 1-a).

Cd addition caused a marked increase in POD activity of NM and M leaves. This increase was more obvious in NM than M plants only at the level of 0.5 mM $CdCl_2$, where the increase in POD activity in NM plants was 39% versus M plants (Figure 1-b). POD activity of NM and M leaves was similar in the absence of Cd addition and at the level of 0.1 mM $CdCl_2$. The activity of APX of NM plants clearly enhanced under Cd stress, and the M plants had higher APX at the same Cd addition level. The variation in APX between M and NM plants were enhanced with increasing addition levels of Cd (Figure 1-c). Mycorrhization enhanced APX activity by 43% at high level of Cd in comparison to NM plants.

DISCUSSION

The association of plant roots with AMF may promote plant growth and increase heavy metal tolerance (Abdel Latef, 2011). Plant tolerance to heavy metals stress may increase by mycorrhizal fungi inoculation through one or more mechanisms such as: (*i*) immobilization of heavy metals by compounds secreted by the fungus; (*ii*) precipitation in polyphosphate granules in the soil; (*iii*) HM adsorption to chitin in the cell wall; (*iv*) chelation of metals inside the fungus, (*v*) changes in rhizophere pH, (*vi*) the regulation of gene expression under stress conditions (Göhre and Paszkowski 2006; Malekzadeh *et al*., 2011).

The mycorrhizal colonization on pepper root significantly decreased with increasing Cd concentration added to the soil. Reduction or even inhibition of mycorrhizal colonization by cadmium has been reported by some authors (Weissenhorn and Leyval, 1995; Liu *et al*., 2011). This reduction in mycorrhizal colonization may be due to (1) deleterious effects of Cd on fungal spore

germination leading to a loss in the fungal infective capacity, (2) the high Cd accumulation in roots that, probably, has distributed AMF hyphal growth inside the cell.

The decrease in dry weight production of pepper root and shoot was observed as Cd addition to soil increased. This inhibition of growth can be attributed in part to the inhibition of mitosis, the reduction of cell wall components, damage to the Golgi apparatus and changes in the polysaccharide metabolism (Sai Kachout *et al*., 2010). In the absence of Cd, the results showed that the higher growth was determined in plants inoculated with *G. mosseae*. Cd toxicity was also lower in the mycorrhizal- than in the non mycorrhizal plants, which strongly suggests that AM fungi could protect plants against HM toxicity by immobilizing HM in the soil. Mycorrhizal colonization promoted plant growth as compared to non mycorrhizal plants. This positive effect was likely attributed to the mycorrhizal-mediated enhancement of host mineral nutrient uptake, especially of immobile soil nutrients such as P (Abdel Latef, 2011).

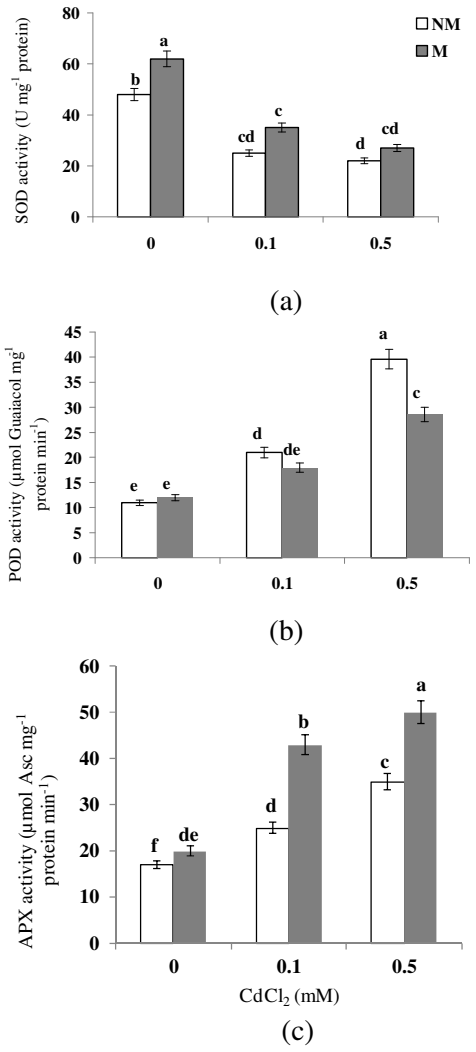

Figure 1. The activity of superoxide dismutase (SOD) (a); peroxidase (POD) (b) and ascorbate peroxidase (APX) (c) in leaves of non mycorrhizal (NM) and mycorrhizal (M) pepper (*Capsicum annuum* L.) plants in response to increasing Cd concentrations in the soil. Mean pairs followed by different letters are significantly different (P< 0.05) by Duncan's test.

Reduction of chlorophyll content of pepper in cadmium treatments could be due to the following effects of cadmium:(1) Cd reduced the uptake of Mg (see the results) needed for chlorophyll biosynthesis (Kapoor and Bhatnagar, 2007); (2) Cd replaced the central Mg^{2+} of chlorophyll in plants to prevent light harvesting (Stobert *et al.,* 1985); (3) Cd reduced the uptake of P (see the results), which contributes to pigment biosynthesis in its role as an energy carrier; (4) Cd induced lipid peroxidation, which causes degradation of photosynthetic pigments (Somashekaraiah *et al.,* 1992); (5) Cd decreased the synthesis of chlorophyll enzyme, consequently, reducing the photosynthesis and inhibiting the growth of plants (Liu *et al.,* 2011). In this study, M plants had higher chlorophyll content than NM ones. This enhancement in chlorophyll content may be due to improved uptake of Mg by AMF (see the results), which can support a higher chlorophyll content and AM fungi facilitate the uptake of P (see the results) from the soil by the plants (Gianinazzi-Pearson and Gianinazzi, 1983; Giri *et al.,* 2003; Andrade *et al.,* 2008).

The reduction in total sugar of NM plants may be due to a decrease in chlorophyll content, which leads to low photosynthesis

efficiency. Mycorrhizal inoculation of plants alleviate the drastic effect of Cd by changing the translocation of cadmium and sequestering it in their hypha, so that the toxic effects of Cd on photosynthesis and carbohydrates metabolism might decrease.

The total protein content in AMF colonized were higher than in non-colonized ones, confirming the results of Liu *et al.* (2011) and indicating that M plants could increase the ability to withstand adversity by delaying protein degradation and maintaining normal metabolism of proteins.

In this study, the results showed that Cd concentration was lower in shoot of M than in NM plants. *Glomus mosseae* decreased translocation of Cd to the shoot. Göhre and Paszkowski (2006) reported that AMF may immobilize metals in several ways including (a) secretion of special compounds and the precipitation of heavy metals in polyphosphate granules in the soil. For example, Glomalin is an insoluble glycoprotein that is released and which immobilizes heavy metals by binding them in the soil; (b) fungal vesicles may be involved in storing toxic metals and thereby avoiding their translocation to shoot; (c) the binding of heavy metals to chitin in the fungal cell walls causes a reduction in the translocation of heavy metals to the shoot of the plants. Several studies have reported lower translocation of heavy metals to shoot by mycorrhization (Rivera-Becerril *et al.,* 2002; Hutchinson *et al.*, 2004; Medina *et al.,* 2005). Therefore, it is suggested that AM plants with larger biomass could be used as phytoremediators of cadmium stress in contaminated soils.

Mycorrhization can increase the absorption of P since AMF are specialists in uptake and transport of this element (Bucher, 2006) to the plant cells by specialized fungal structure called arbusculaes (Parniske, 2008). As expected, mycorrhization increased the uptake of P, besides, it was reflected in enhanced growth. These results are also in agreement with previous studies reported by Kapoor and Bhatnagar (2007) who stated that the phytotoxicity caused by Cd may be alleviated due to the effect of increased absorption of major nutrients such as P (Sieverding, 1991).

Under Cd stress, lipid peroxidation is initiated as a result of oxidative stress. The high accumulation of malondialdehyde (MDA) indicates severe lipid peroxidation. In the present study, the increase in MDA content was significant even after an exposure to the low Cd treatment, indicating that Cd caused an oxidative stress in NM plants. Cadmium-induced loss of membrane permeability, coupled with increased MDA production, has also been observed by Garg and Aggarwal (2012). In this study, the reduction of MDA in M plants in comparison with NM plants may be a result of AMF reducing oxidative stress and ROS production in heavy metal toxicity. As the results of cadmium stress, changes occur in the lipid peroxidation composition and the membrane becomes rigid, thus, resulting in changes in the activity of enzymes bound with membranes (Sai Kachout *et al.,* 2010). Plants synthesize numerous antioxidant enzymes as a defense against oxidative stress. The antioxidant enzymes such as SOD, POD, and APX are important components in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Abdel Latef, 2010). SOD is a key enzyme in protecting cells against oxidative stress (Alscher *et al.,* 2002), however, in this work, no relationship could be observed between the tolerance to cadmium and the activity of SOD, since the activity of SOD in NM and M plants decreased with increasing the concentration of cadmium ions. Cadmium-induced reduction of SOD activity has also been observed in pea plants (Sandalio *et al.,* 2001), rice roots (Guo *et al.,* 2007), and cucumber cells (Gzyl *et al.,* 2009). Thus, the inactivation of SOD observed in this work might be caused by an excess of H_2O_2 produced in different cell compartments as a result of Cd toxicity (Sandalio *et al.,* 2001;

Gzyl *et al.,* 2009). The decline of SOD activity was accompanied by a significant increase in the activity of H_2O_2-scavanging enzymes (POD and APX) in NM and M plants. The peroxidase activity has been used to evaluate contaminant exposure to terrestrial and aquatic plants. Increased peroxidase levels are thought to protect plant cell from free radical oxidation, allowing the plant to adapt to the stressor (Sai Kachout, 2010). APX can also be involved in H_2O_2 removal by the activation of ascorbate-glutathione cycle. APX can be found in different cells compartments, such as the cytosol and plastids, possibly participating in the fine modulation of ROS for signaling (Abdel Latef, 2011). It is worthy to mention that the activity of APX was higher in M plants than NM ones and the activity of APX was stimulated more than that of POD in M plants versus NM plants. This suggests that APX is a major player in H_2O_2-scavenging in M plants and might be responsible for the fine modulation in detoxification of H_2O_2.

CONCLUSIONS

Glomus mosseae inoculation enhanced the plant growth in the presence of Cd. *Glomus mosseae* displayed a higher capacity for immobilizing Cd in roots and increased chlorophyll content, total sugar and total protein, the concentrations of P and Mg, and the activity of APX, but it reduced lipid peroxidation. Thus, this mycorrhizal fungus alleviated the toxic effects of cadmium in pepper and confirmed the hypothesis proposed.

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رشد و برخی فعالیتهای فیزیولوژیکی فلفل(*Capsicum annuum* L.) در پاسخ به تنش کادمیوم و همزیستی با میکوریز

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چکیده

هدف این پژوهش گلخانه ای بررسی اثر قارچ های ریشه ای(میکوریز) روی مسمومیت کادمیوم در گیاه فلفل (Capsicum annuum L. cv. Zhongjiao 105) بود. نیمی از گیاهان مطالعه شده با قارچ های ریشه ای(AMF)تلقیح شدند. کادمیوم به صورت کلرید کادمیوم با غلظت های ۰.۰، ۰.۱، و۰.۵ میلی مول در آب آبیاری به خاک افزوده شد.مشاهدات نشان داد که استقرار قارچ ها در تیمار شاهد بیشتر از تیماری بود که به آن کادمیوم داده شده بود. در هر دو تیمار شاهد و تیمار شده با کادمیوم، وزن خشک ریشه و ساقه گیاهان تلقیح شده از گیاهان تلقیح نشده بیشتر بود.اندازه گیری غلظت کادمیوم چنین اشاره داشت که گیاهان تلقیح شده با قارچ کادمیوم بیشتری در ریشه تثبیت کرده بودند و مقدار کمتری به ساقه فرستاده بودند. از سوی دیگر، کادمیوم منجر به کاهش مقدار کلروفیل برگ ، قند کل و پروتئین کل، و کم شدن غلظت فسفر ومنیزیم در ساقه شد. مقدار کلروفیل ، قند کل و پروتئین کل، و غلظت فسفر ومنیزیم در گیاهان تلقیح شده بیشتر از گیاهان بدون تلقیح بود. افزون بر این، افزایش غلظت کادمیوم باعث افزایش مالون دی الدئید(MDA) در برگ گیاه فلفل شد ولی مقدار این ماده در گیاهان تلقیح شده کمتر از مقدار آن در گیاهان تلقیح نشده بود.همچنین، کادمیوم موجب کاهش فعالیت(SOD) superoxide dismutase در برگ گیاهان تلقیح شده و بدون تلقیح شد، ولی باعث افزایش فعالیت پراکسیداز(POD)و اسکوربیت پراکسیداز (APX) در برگ همان گیاهان شد. القای فعالیتAPX در گیاهان تلقیح شده بیشتر از گیاهان بدون تلقیح بود که نشانگر آن است که این آنزیم نقش بزرگی در خنثی کردن H_2O_2 دارد. نتایج این مطالعه حاکی از آن است که تلقیح گیاه با قارچهای *G. mosseae* می تواند روش مناسبی برای بالا بردن مقاومت گیاهان باغی(سبزیجات) در برابر تنش ناشی از کادمیوم باشد.