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Do arbuscular mycorrhizal fungi affect cadmium uptake kinetics, subcellular distribution and chemical forms in rice?

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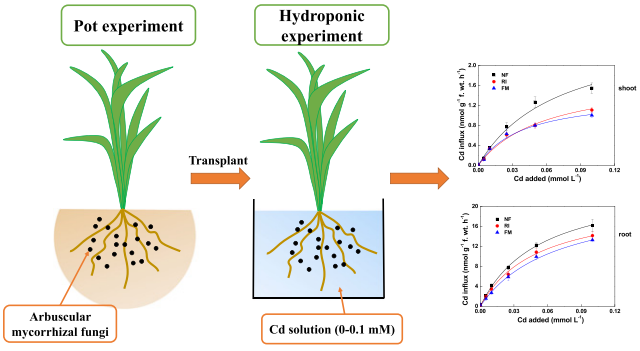
HIGHLIGHTS

- AMF significantly decreased the Cd concentrations both in shoots and roots of rice.
- AMF reduced the Cd concentrations markedly in the cell wall at high Cd substrate.
- Cd retention depends on cell wall at low substrate while vacuoles at high substrate.
- AMF can improve the tolerance to Cd of rice by converting Cd into inactive forms.

GRAPHICAL ABSTRACT



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ABSTRACT

Rice (*Oryza sativa* L.) plants were inoculated with two species of arbuscular mycorrhizal fungi (AMF) - *Rhizophagus intraradices* (RI) and *Funneliformis mosseae* (FM) and grown for 60 days to ensure strong colonization. Subsequently, a short-term hydroponic experiment was carried out to investigate the effects of AMF on cadmium (Cd) uptake kinetics, subcellular distribution and chemical forms in rice exposed to six Cd levels (0, 0.005, 0.01, 0.025, 0.05, 0.1 mM) for three days. The results showed that the uptake kinetics of Cd fitted the Michaelis-Menten model well ($R^2 > 0.89$). AMF significantly decreased the Cd concentrations both in shoots and roots in Cd solutions. Furthermore, the decrement of Cd concentrations by FM was significantly higher than RI treatment in roots. AMF reduced the Cd concentrations markedly in the cell wall fractions at high Cd substrate (≥0.025 mM). The main subcellular fraction contributed to Cd detoxification was cell wall at low Cd substrate (<0.05 mM), while vacuoles at high Cd substrate (≥0.05 mM). Moreover, the concentrations and proportions of Cd in inorganic and water-soluble form also reduced by AMF colonization at high Cd substrate (≥0.05 mM), both in shoots and roots. This suggested that AMF could convert Cd into inactive forms which were less toxic. Therefore, AMF could enhance rice resistance to Cd through altering subcellular distribution and chemical forms of Cd in rice.

1. Introduction

In recent years, cadmium (Cd) contamination in agricultural soils resulting from industrial and agricultural activities has become a

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significant environmental problem (Xin et al. 2013). Rice (*Oryza sativa* L.) is one of the most important cereal crops, feeding more than 50% population of the world. However, rice cultivation is facing a serious problem of heavy metal contamination, especially Cd (Xue et al. 2014). This non-essential element is highly mobile in the soil-plant system, which allows its easy entry into crops (He et al. 2014). Cadmium will not only hinder the yield of rice, but also accumulate through the food chain, which may cause severe adverse effects on human health, such as obstructive pulmonary disease, renal tubular damage, and lung, kidney and pancreatic cancer, under short and long-term exposure (Li et al. 2014; Sebastian and Prasad 2013). Therefore, there is an urgent need to develop effective techniques to reduce Cd accumulation in rice.

Various methods involved in the reduction of Cd influx into the soil system, such as site selection, and management practices, have been employed to decrease Cd concentrations in the soil solution and its uptake and translocation by plants (Wang et al. 2009). However, these methods are difficult to put into practice in farmland in many developing countries because of the time they take and/or the high costs of remediation, as well as the high demand for foodstuffs (Qiu et al. 2011; Wang et al. 2009). As an alternative, arbuscular mycorrhizal fungi (AMF) have been shown to reduce accumulation of certain heavy metals in rice, including copper (Cu) and arsenic (As) (Chan et al. 2013; Zhang et al. 2009). They are found naturally in top soil, forming a symbiotic association with most plant species (Chan et al., 2013; Garg and Aggarwal 2012; Watts-Williams et al. 2013). Plants associated with AMF usually have a better resistance to a variety of stresses, such as drought, nutrient deficiencies or imbalances, high levels of toxic elements or salinity (Abdel Latef 2011; Abdel Latef and He 2011; Abdel Latef and He 2014; de Andrade et al. 2015). A number of studies have evaluated the role of AMF on Cd uptake in plants (Abdel Latef 2013; de Andrade et al. 2008). Garg and Aggarwal (2012) found that inoculation with *Funneliformis mosseae* significantly restrained the Cd uptake into the root system and further translocation into the above-ground parts of *Cajanus cajan* (L.) Millsp. (pigeon pea). In contrast, Wang et al. (2012) found that *F. mosseae* increased the total Cd in the roots of *Medicago sativa* L. whereas it decreased Cd concentrations in the shoots. However, studies concerning the impacts of AMF on Cd uptake by rice are still scare. Only Zhang et al. (2005) investigated the uptake of heavy metals (Cu, Zn, Pb and Cd) by two rice cultivars inoculated with three species of AMF (*Glomus versiforme*, *F. mosseae* and *Rhizophagus diaphanus*). These authors found that mycorrhizae could exert some protective effects against the combined toxicity of Cu, Zn, Pb and Cd. However, there is a lack of information on how AMF would influence the Cd uptake by rice.

Compartmentalization plays a significant role in the detoxification of heavy metals in plants and may be associated with subcellular distribution and chemical forms of heavy metals within living cells (Lai 2015; Liu et al. 2014; Zhao et al. 2015). Cadmium will distribute in different chemical forms once taken up by plants. These include inorganic, water-soluble, pectate and protein-integrated, undissolved phosphate and oxalate forms (Lai 2015). Different distribution of Cd among tissue fractions can explain the differences in sensitivity to Cd between different plant species. The retention of Cd in root cell walls, compartmentation of Cd into vacuoles and suppressed transportation of Cd from roots to shoots are the most important mechanisms responsible for the detoxification of Cd in rice plants (Liu et al. 2014). Therefore, a critical step investigating the subcellular distribution and chemical forms of Cd in rice will certainly contribute to understanding the role of AMF in rice.

To the best of our knowledge, the behavior of Cd uptake by rice influenced by AMF is still largely unknown. Moreover, little information is available on Cd distribution patterns and chemical forms in response to Cd stress in rice inoculated with AMF. In our previous work (data not published), a six-month pot experiment had been carried out with eight rice cultivars inoculated with or without two species of AMF (*Rhizophagus intraradices* and *F. mosseae*) grown in Cd-contaminated soil, and it was found that Cd concentrations in Zhenghan 9 were reduced by mycorrhizal treatment. To further reveal the uptake mechanisms, a short-term experiment was conducted in the present study, in which the mycorrhizal rice was exposed to different concentrations of Cd solutions for three days. The aims of the present work were to: (i) determine the influences of two AMF (*R. intraradices* and *F. mosseae*) on Cd uptake kinetics of rice (Zhenghan 9), in which Michaelis-Menten functions could be applied; (ii) investigate the effects of two AMF on the Cd accumulation in shoots and roots of rice and (iii) investigate the subcellular distribution and chemical forms of Cd in rice at six different Cd treatment levels in order to explore the potential mechanisms regarding the uptake, translocation, accumulation and detoxification of Cd in the rice plant. The results of this study will provide new insights into the roles that AMF play in Cd uptake and distribution in rice.

2. Materials and methods

2.1. Plant cultivation

Seeds of rice (cv. Zhenghan 9) were obtained from Henan Academy of Agricultural Sciences, Zhengzhou, PR China. The seeds were sterilized with H_2O_2 for 30 s and washed thoroughly with deionized water. They were then germinated on moist filter papers and grown in 20% Hoagland-Arnon nutrient solution (Hoagland and Arnon 1938). After 2 weeks, uniform seedlings of rice were used for the pot trials.

2.2. Plant inoculation

Soil samples were collected from paddy fields at the campus of South China Agricultural University, Guangzhou, PR China. The soil contained 8.1% organic matter, 1.3 g kg^{-1} total N, 1.1 g kg^{-1} total P, and 0.18 mg kg^{-1} Cd, respectively, with a pH of 5.9 (Wang et al. 2011). After air-drying for 2 weeks, they were sieved through a 2-mm mesh sieve to remove stones, roots and rhizomes. Then soil and uncontaminated sands were autoclaved at 121 °C for 120 min for elimination of indigenous AMF. Seedlings were transferred to pots (diameter: 15 cm; height: 18 cm) with soil:sand (5:1) combination (including 30 g sterile or non-sterile AMF inoculants). Three mycorrhizal treatments included control (without AMF), *R. intraradices* (RI) and *F. mosseae* (FM). The two AMF were obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). The plants were watered with deionized water daily to maintain 80% water-holding capacity, and with 20% Hoagland-Arnon nutrient solution every week. After a growth period of 60 days all plants were washed thoroughly with deionized water to remove any soil/substrate particles attached to roots and used for subsequent experimental treatments. Three replicates of cleaned fresh roots for each treatment were stained with 0.05% Trypan Blue according to the method of Phillips and Hayman (1970) and Li et al. (2011). Arbuscular mycorrhizal fungi colonization was quantified on one hundred 1-cm-long root segments by the slide length method, expressing as a percentage of AM colonization (Giovannetti and Mosse 1980).

2.3. Plant Cd uptake

The washed plants were rinsed in Cd treatment solutions (0, 0.005, 0.01, 0.025, 0.05, 0.1 mM), supplied as $CdCl_2 \cdot 2H_2O$, with three replicates for each treatment. Hoagland-Arnon nutrient solution (20%) was supplied into the Cd solutions in order to maintain an adequate nutrient level. After 3 days, the plant roots were rinsed in 20 mM Na_2-EDTA for 15 min to remove Cd adhering to the root surfaces. The whole plants were then washed with deionized water, and separated into shoots and roots. All samples were frozen in liquid N_2 and divided into three portions for analysis of subcellular distribution and chemical forms of Cd, and total Cd concentrations.

2.4. Subcellular fractions

Cells were separated into three fractions (cell wall fraction, soluble fraction, and organelle-containing fraction) according to the method described by Wang et al. (2012). Frozen tissues were homogenized in cooled extraction buffer [50 mM Tris-HCl, 250 mM sucrose and 1.0 mM DTE ($C_4H_{10}O_2S_2$), pH 7.5] with a chilled mortar and a pestle. The homogenate was sieved through nylon cloth (80 μm) and the liquid squeezed from the residue. The residue was washed twice with homogenization buffer; as it contained mainly cell walls and cell wall debris it was designated as the 'cell wall fraction'. The filtrate was centrifuged at 20,000 × g for 45 min. The supernatant solution was referred to the 'soluble fraction' (including vacuoles), and the deposit the 'organelle fraction' (excluding vacuoles). All steps were performed at 4 °C. The subcellular fractions were dried at 70 °C to a constant weight.

2.5. Chemical forms

Chemical forms of Cd were extracted in the following order: (1) 80% ethanol, extracting inorganic Cd giving priority to nitrate/nitrite, chloride and aminophenol cadmium; (2) deionized water (d-H_2O), extracting water soluble Cd-organic acid complexes and $Cd(H_2PO_4)_2$; (3) 1 M NaCl, extracting pectates and protein integrated Cd; (4) 2% acetic acid (HAc), extracting undissolved cadmium phosphate including $CdHPO_4$ and $Cd_3(PO_4)_2$ and other Cd-phosphate complexes and (5) 0.6 M HCl, extracting cadmium oxalate (Fu et al. 2011).

Frozen tissues were homogenized in extraction solution with a mortar and a pestle, diluted at a ratio of 1:100 (w/v), and shaken for 22 h at 25 °C. The homogenate was then centrifuged at 5000 × *g* for 10 min, obtaining the first supernatant solution in a conical beaker. The sedimentation was re-suspended twice in extraction solution, shaken for 2 h at 25 °C, and centrifuged at 5000 × *g* for 10 min. The supernatants of the three suspensions were then pooled. The residue was extracted with the next extraction solution in the sequence using the same procedure described above. Each pooled solution was evaporated on an electric plate at 70 °C to a constant weight.

2.6. Analysis of Cd

The frozen tissues and centrifuged fractions were digested by acid mixture of HNO_3:$HClO_4$ (4:1, v/v) in a microwave digestion apparatus (MARS-X; CEM, USA), and the Cd concentrations determined by Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES, Optima 2000 DV, Perkin Elmer, USA). The blanks and standard material [GBW07602 (GSV-1)] (obtained from China Standard Materials Research Center, Beijing, PR China) were used to ensure the accuracy of metal determinations. The recovery rates of elements were within (90 ± 10)%. To verify the accuracy of the experimental results, the percentage recovery of Cd was calculated by dividing the sum of Cd concentrations of the three fractions by the total Cd concentrations of shoots and roots.

2.7. Statistical analysis

All results were tested by one-way ANOVA using the SPSS statistical package. All figures were drawn using the PC-based Origin program. Duncan tests at 5% probability were used for post hoc comparisons to test for treatment differences.

3. Results

3.1. Root colonization rates

For the rice grown without AMF, the root colonization rate was extremely small [(0.5 ± 0.3)%] whereas the root colonization rates of rice inoculated with RI and FM were (72 ± 3.7)% and (58 ± 6.2)%, respectively. Both of the AMF could significantly enhance the root colonization rates and the colonization rates of rice inoculated with RI were significantly higher than FM ($P < 0.05$).

3.2. Cd uptake kinetics

Before the hydroponic experiment, the fresh weight of shoots and roots in each plant were 11.2 ± 0.65 g and 2.74 ± 0.17 g, respectively, and this biomass did not change significantly after the three-day Cd treatments. The Cd fluxes into rice plants inoculated with and without AMF were fitted with non-linear and linear regressions. Table 1 shows that the data are better described by a Michaelis-Menten function ($R^2 > 0.89$ in all cases) than by linear regressions. It can also be seen from Table 1 that the V_{max} values for Cd uptake by roots were similar for non-mycorrhizal (NF), RI and FM treatments, which were in the range of 21–25 nmol g^{-1} f. wt. h^{-1}. The same trend could also be observed in the corresponding K_m values (0.05–0.07 mM). Conversely for shoots, the kinetic parameters differed greatly between non-mycorrhizal and mycorrhizal plants. The V_{max} value for the NF treatment (2.8 ± 1.34 nmol g^{-1} f. wt. h^{-1}) in shoots was 2-fold higher compared to that for the FM treatment (1.4 ± 0.27 nmol g^{-1} f. wt. h^{-1}).

3.3. Cd accumulation

Cadmium concentrations in shoots and roots of rice plants were measured and expressed as Cd influx into the plants with the changes of Cd addition (Fig. 1). According to Fig. 1, Cd influx of NF-, RI- and FM-treated plants increased with an increase in Cd concentrations in the solution. The Cd influx in both shoots and roots of non-mycorrhizal rice was significantly higher than in the mycorrhizal ones. Concentrations of Cd in shoots were 1.18, 2.84, 6.24, 10.21 and 12.47 mg kg^{-1} under 0.005, 0.01, 0.025, 0.05 and 0.1 mM Cd substrate without AMF inoculation; these values were 1.16, 1.04, 1.26, 1.58 and 1.39 times higher, respectively, than those in the RI-inoculated plants. With increases in the Cd addition, the difference in Cd influx between non-mycorrhizal and mycorrhizal plants showed an increasing trend, both for shoots and roots.

Table 1
Kinetic parameters for Cd influx into shoots and roots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM). Kinetic parameters were calculated from mean Cd influx using Michaelis-Menten function (nonlinear regression) and linear regression ($n = 3$).

Rice	AMF	Nonlinear regression			Linear regression		
		V_{max} (nmol g^{-1} f.wt. h^{-1})	K_m (mM)	R^2	a (slope)	b (intercept)	R^2
Shoot	NF	2.8 ± 1.34	0.07 ± 0.05	0.890	20 ± 3.5	0.01 ± 0.01	0.869
	RI	1.8 ± 0.64	0.06 ± 0.03	0.930	15 ± 3.3	0.01 ± 0.01	0.805
	FM	1.4 ± 0.27	0.04 ± 0.01	0.966	12 ± 2.4	0.02 ± 0.02	0.833
Root	NF	25 ± 5.28	0.05 ± 0.02	0.982	226 ± 29	0.52 ± 0.34	0.921
	RI	21 ± 5.36	0.05 ± 0.02	0.957	179 ± 32	0.32 ± 0.20	0.855
	FM	22 ± 7.13	0.07 ± 0.05	0.994	130 ± 3.6	0.30 ± 0.11	0.991

3.4. Subcellular distribution

Cadmium concentrations and subcellular distribution proportion in different fractions of root and shoot tissues varied with different Cd in the substrate (Figs. 2 and 3). Fig. 2 depicts the subcellular Cd in rice shoots exposed to different Cd stress. As shown in Figs. 2(b) and 3(b), the majority of Cd (82.2–99.4%) was present in the cell wall and soluble fractions, both for shoots and roots. For shoots, the total Cd concentrations in all subcellular fractions declined in mycorrhizal rice, compared with non-mycorrhizal ones (Fig. 2(a)). This decrement was significant at high Cd substrate concentrations (≥0.025 mM). Among the three subcellular fractions, the decrease of Cd concentration in cell wall fractions caused by AMF inoculation was the most prominent. At high Cd substrate concentrations (≥0.01 mM), the proportion of cell wall fractions decreased with AMF inoculation, whereas the proportion of soluble fractions increased (Fig. 2(b)). An increase of Cd in the substrate led to a decrease in the proportion in cell wall fractions, but an increase in the proportion in the soluble fractions. For the roots of rice (Fig. 3(a)), the total Cd concentrations in all subcellular fractions declined with RI or FM inoculation, which was similar with the results found in shoots. The greater Cd decrement was in the cell wall and soluble fractions. For mycorrhizal roots, as the Cd substrate concentration increased, the proportion of Cd in the cell wall fraction showed a decreasing trend, whilst the proportion of Cd in the soluble fraction showed an increasing trend (Fig. 3(b)).

3.5. Chemical forms

Cadmium in six chemical forms in rice shoots and roots was enhanced with increasing Cd substrate concentrations (Figs. 4(a) and 5(a)). In the shoots, undissolved Cd phosphate (extracted with HAc) was predominant in both non-mycorrhizal and mycorrhizal plants (Fig. 4(b)). In the roots, undissolved Cd phosphate and pectate- and protein- integrated Cd were the predominant forms (Fig. 5(b)). The decline in HAc-extractable Cd concentrations in the shoots after inoculation with AMF was the most significant among all chemical forms (Fig. 4(a)). As shown in Figs. 4(b) and 5(b), at high Cd substrate concentrations (0.05 and 0.1 mM), the proportions of Cd extracted by ethanol and d-H_2O declined significantly in the RI and FM treatments, both in shoots and roots ($P < 0.05$). With an increase of Cd substrate concentrations, the proportion of Cd extracted by NaCl tended to reduce, whereas the HAc-extractable Cd proportion tended to rise. This trend was similar in both the shoots and the roots of rice.

4. Discussion

4.1. The effects of mycorrhizal colonization on Cd uptake by rice

In soil without any Cd contamination, the root colonization rates of RI and FM were over 50%, implying that both AMF types could be

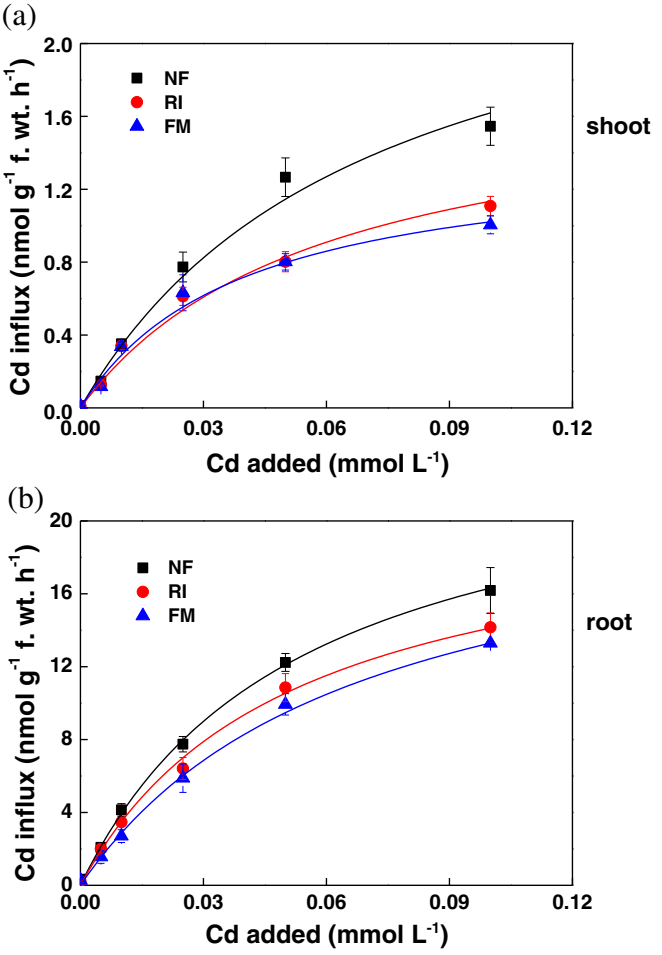

Fig. 1. Concentration-dependent kinetics for Cd influx into shoots and roots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM). Each point is an average of three replicates. Error bars are ±S.D. of the replicates.

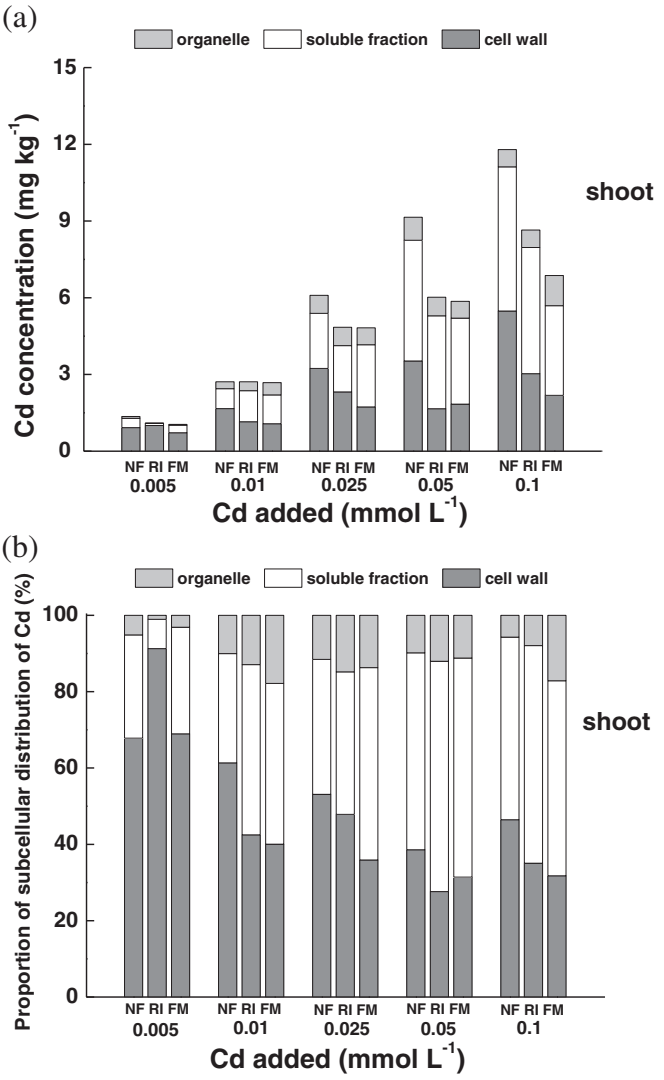

Fig. 2. Different subcellular distribution and its proportion of Cd in shoots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM) grown in Cd solutions for 3 days.

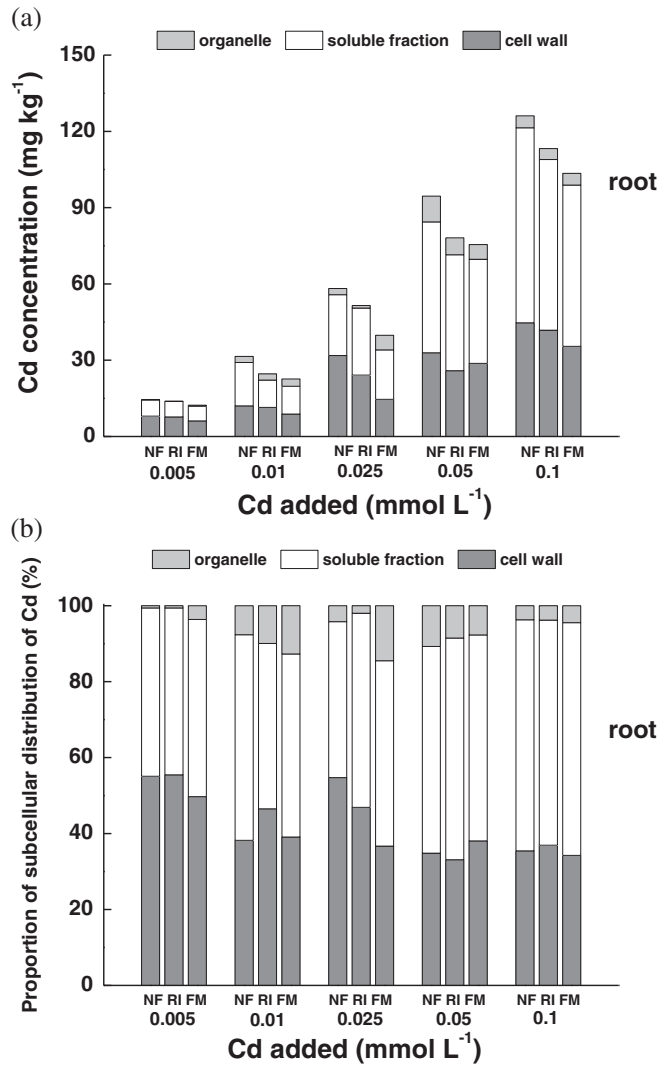

Fig. 3. Different subcellular distribution of Cd and its proportion in roots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM) grown in Cd solutions for 3 days.

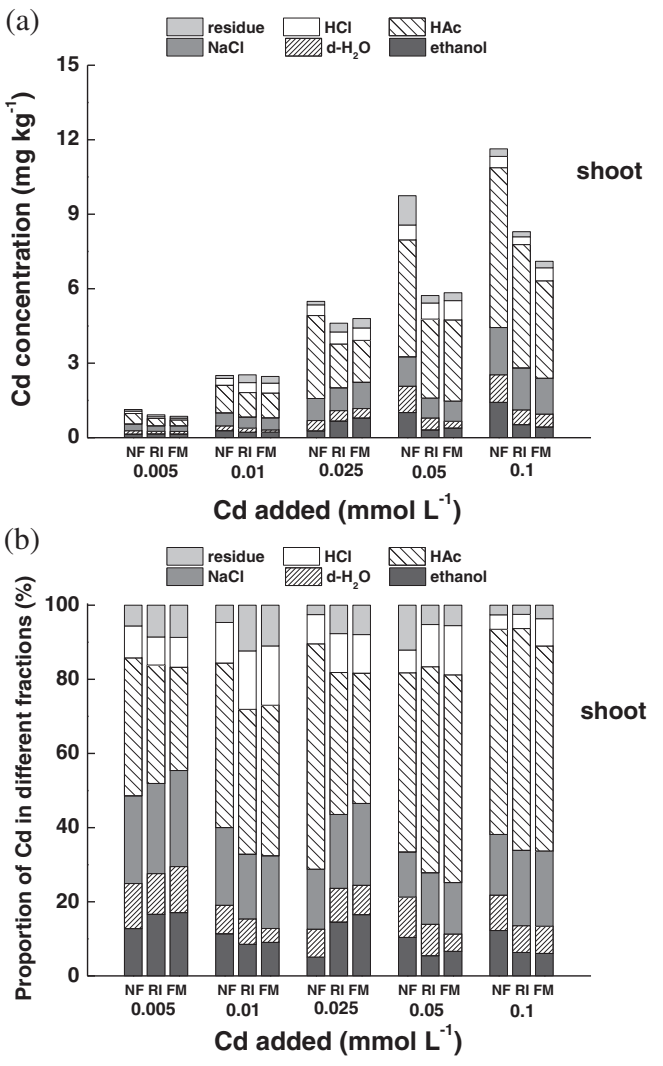

Fig. 4. Different chemical forms of Cd and its proportion in shoots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM) grown in Cd solutions for 3 days.

regarded as powerful colonizers of rice roots. These results were in good agreement with a previous study (Duan et al. 2015) where it was reported that the root colonization rates of winter wheat by RI and FM in soil without Cd contamination were 84.9% and 56.8%, respectively.

In our previous study (data not published), a six-month pot experiment had been carried out with eight rice cultivars inoculated with or without RI and FM grown in Cd-contaminated soil, and the results indicated that the two species of AMF both decreased the Cd concentrations in Zhenghan 9. Therefore, a short-term experiment was conducted to further reveal the Cd uptake and detoxify mechanisms under the influence of AMF. The Cd uptake kinetics by mycorrhizal and non-mycorrhizal roots were fitted better by a Michaelis-Menten model than a linear regression (Table 1), implying that the transport of Cd by mycorrhizal and non-mycorrhizal rice roots was an active process, which requires an energy supply as the driving force and selective binding sites (Abedin et al., 2002; Li et al. 2011). The characteristics of the uptake kinetics can be considered as one of the important criterion for selecting a plant species cultivated in areas irrigated by Cd-contaminated irrigation water.

Our study suggested that both RI and FM mycorrhizal types could restrict Cd accumulation in rice shoots and roots (Fig. 1), which may further result in lower Cd concentrations in rice grains. These results are consistent with those from the study by Liu et al. (2011), who found that the Cd concentrations and accumulation in marigold plants decreased with three AMF (RI, *F. constrictum* and FM) inoculations, both in shoots and roots. There are several possible mechanisms responsible for the Cd reduction in rice inoculated with AMF. One possible explanation is a biomass dilution effect due to the positive growth response to AM symbiosis (Liu et al. 2011). Guo et al. (2013) indicated that FM and *Glomus versiforme* could promote plant growth by significantly increasing the nutrient uptake, such as nitrogen (N), phosphorus (P), and potassium (K), resulting in increased root and shoot dry weight and thus a decrease in Cd concentrations in AMF-associated plants. The ability to bind heavy metals in the rhizosphere by releasing an insoluble glycoprotein (glomalin) may also account for the reduction of Cd concentrations in shoots and roots (Shahabivand et al. 2012). Moreover, plants inoculated with AMF under heavy metal stress may result in the expression of specific genes, which are responsible for the production of proteins (including metallothioneins) that increase the resistance of plants to stress. Metallothioneins are metal-binding proteins produced in many different organisms when exposed to high levels of heavy metals such as Cu, Zn and Cd, and AM symbiosis can regulate the transcription of such genes and improve the plant resistance to heavy metals (Guo et al. 2013; Miransari 2010). In addition, heavy metal toxicity can cause unfavorable oxidative damage to biomolecules and adversely influence plant growth. Arbuscular mycorrhizal fungi may also

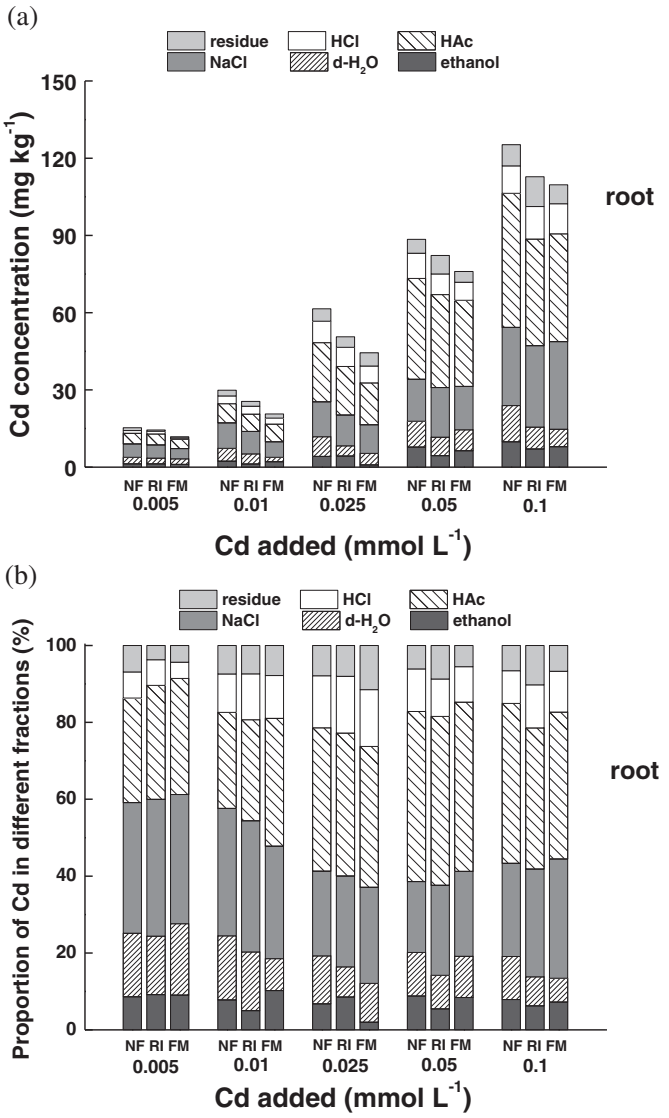

Fig. 5. Different chemical forms of Cd and its proportion in roots of rice uninoculated/inoculated with *R. intraradices* (RI) or *F. mosseae* (FM) grown in Cd solutions for 3 days.

enhance the production of antioxidant enzymes to alleviate the stress of heavy metals (Garg and Aggarwal 2012; Liu et al. 2011; Miransari 2010).

Different results for the effects of AMF on Cd uptake by plants have been observed, which may be due to the different plant species, plant tissues, and Cd stresses. For example, in sunflower plants, Hassan et al. (2013) found that the shoot Cd concentrations inoculated with RI were higher than in non-mycorrhizal plants, whereas the shoot Cd concentrations in plants inoculated with FM were lower. Wang et al. (2012) observed a decreasing Cd concentration in shoots while an increasing Cd concentration in roots in *Medicago sativa* L. inoculated with RI, compared to non-mycorrhizal plants. The results of Aghababaei et al. (2014) indicated that the shoot Cd concentrations of maize colonized by RI and FM were reduced in Cd contaminated soils (10 and 20 mg kg^{-1}), whilst root Cd uptake was decreased at low Cd soil concentrations (10 mg kg^{-1}) but increased at high Cd (20 mg kg^{-1}) when roots were colonized by RI and FM.

The Cd concentrations in the mycorrhizal and non-mycorrhizal roots of rice were >10 times higher than those in the shoots, indicating the restriction of Cd transport from roots to shoots (Wang et al. 2008). These results suggest that by retaining the greater proportion of toxic metals (Cd) in the roots protects the above-ground parts is one of the strategies employed by plants to survive in heavy metal stressed environments (Yang et al. 2015). Chelation of Cd inside the fungal biomass or adsorption of Cd on chitin in the fungal cell walls caused immobilization of Cd in roots has been shown to limit Cd translocation from roots to shoots (Shahabivand et al. 2012).

4.2. Subcellular distribution

In our study, a large proportion of Cd was present in the soluble and cell wall fractions, with very little in the organelle fraction (Figs. 2 and 3). The results were similar to those found in ramie (Wang et al. 2008), pokeweed (Fu et al. 2011), and edible seaweed (Zhao et al. 2015). Under low Cd substrate concentrations (0.005–0.025 mM), cell wall fractions constituted the largest proportion of Cd in shoots and roots of non-mycorrhizal rice (38.2%–67.8%), followed by the soluble and organelle fractions. Therefore, the protective role of cell wall is notable under low Cd stress. The cell wall is considered as the first barrier to protect protoplast from the toxic effects of heavy metals (Weng et al. 2012). Cadmium compartmentation in the root cell wall is one of the mechanisms inhibiting Cd transport by plants (Qiu et al. 2011). Plant cell walls are mainly composed of polyose (including cellulose, hemicellulose and pectin) and protein, providing a number of potential ligands such as carboxyl, hydroxyl, amino groups and aldehyde groups (Haynes 1980; Qiu et al. 2011; Wang et al. 2008). These ligands can participate in a variety of reactions including ion exchange, adsorption, complexation, precipitation and crystallization, and thus can bind Cd cations and restrict their transport across the cytomembrane (Wang et al. 2009). It has been reported that AMF can influence the subcellular distribution of Cd in plants. Wang et al. (2012) suggested that AMF decreased the shoot Cd concentrations and also decreased the proportion of Cd in shoot cell wall fractions of *Medicago sativa*, compared with non-mycorrhizal plants, which is consistent with the results of our study. A high proportion of Cd (35.9%–91.3%) was bound to the cell wall fraction at low Cd substrate (<0.05 mM), both in shoots and roots of the mycorrhizal and non-mycorrhizal rice in our study (Figs. 2 and 3), suggesting that the cell wall is indeed a large buffer which can accumulate heavy metals and therefore plays a role in metal resistance at low Cd stress (Fu et al. 2011; Zhao et al. 2015). In contrast, the proportion of the soluble fraction was higher than the cell wall fraction at high Cd substrate concentrations (≥0.05 mM) both for the non-mycorrhizal and mycorrhizal rice, probably due to the acute toxicity caused by high Cd stress to the plants, leading to severe damage to cell wall resulting in increased Cd transfer to the soluble fraction. Despite the fact that AMF enhanced the soluble fraction Cd proportions in shoots, a decreasing Cd concentration in the cell wall and soluble fraction of shoots was found under high Cd substrate concentrations (≥0.05 mM), implying that AMF can also alleviate the toxic influences of Cd. The soluble fraction consists mainly of vacuoles and acts as the subdominant site of preferential Cd binding in both shoots and roots. This strategy could further decrease the amount of Cd interfering with the organelles (Wang et al. 2008; Zhao et al. 2015). It can be speculated that in shoots and roots of rice plants, the cell wall plays an important role in Cd retention at low Cd substrate concentrations (<0.05 mM), whilst the main mechanisms of Cd detoxification are located inside vacuoles at high Cd substrate concentrations (≥0.05 mM). Furthermore, our results also suggest that the reduction in total Cd concentrations by FM was significantly higher by RI treatment in roots, which suggests that the fungal species of the AMF might be an important factor affecting the ability to alleviate heavy metal stress. Therefore, the choice of a suitable AMF is crucial in bioremediation practice and can influence the plant response to heavy metals.

4.3. Chemical forms

Chemical speciation of heavy metals is closely related to their biological function. Different chemical forms of Cd are extracted by different

designated extraction solutions, and have distinct toxicity degree and migration of Cd (Fu et al. 2011). Cadmium in inorganic form (extracted with 80% ethanol) and in the water-soluble form (extracted with d-H_2O) have a higher migratory capacity and are more toxic to plant cells than pectate- and protein-integrated Cd (extracted with 1 M NaCl), insoluble Cd phosphates (extracted with 2% HAc) and the residues (Qiu et al. 2011; Wang et al. 2012). In the present study, the largest proportion of Cd was accounted for by the pectate- and protein-integrated and insoluble phosphates forms, both in the shoots and roots of rice (Figs. 4 and 5). Similar results were also observed by Zhao et al. (2015). Both the concentrations and percentages of Cd in inorganic form and the water-soluble form in shoots and roots were notably lower in mycorrhizal plants than in the non-mycorrhizal under high Cd stress (≥0.05 mM), whereas the proportion of inactive forms of Cd (pectate- and protein-integrated, insoluble and residual Cd) was higher in mycorrhizal plants, implying that the mobility and toxicity of Cd cations in mycorrhizal plants were much lower than those in the non-mycorrhizal plants. The proportion of Cd extracted by 2% HAC increased, suggesting AMF inoculation increased the plant's ability to combine Cd with phosphates which are insoluble leading to lower mobility and toxicity in vivo (Qiu et al. 2011; Zhao et al. 2015). These results were in good agreement with the previous study conducted by Wang et al. (2012). It was hypothesized that Cd is chelated with some specific polar material, such as hydroxyl or carboxyl, to form a non-toxic complex, leading to a reduction in its toxicity (Fu et al. 2011). AMF may thus also improve the resistance to Cd of rice plants by converting Cd into inactive forms which are less toxic.

5. Conclusions

This study is the first to report the effects of mycorrhizal inoculation on Cd uptake kinetics, subcellular distribution and chemical forms in rice. Cadmium influx in both shoots and roots of mycorrhizal rice were lower than non-mycorrhizal ones. At low Cd substrate concentrations (<0.05 mM), the cell wall appears to play an important role in Cd retention, whilst the main subcellular fraction that contributed to Cd detoxification is the vacuoles at high Cd substrate concentrations (≥0.05 mM). Furthermore, the concentrations and proportions of Cd extracted by ethanol and d-H_2O were also lower in shoots and roots of mycorrhizal rice compared with the non-mycorrhizal at high substrate concentrations (≥0.05 mM), indicating that AMF can convert Cd into inactive forms which are less toxic. In conclusion, our results demonstrated that AMF could improve the resistance to Cd of rice by altering the subcellular distribution and chemical forms of Cd. These findings provide a new insight into the role of AMF on Cd uptake by rice plants and highlight the need for further research on the contribution of AMF in a full field study.

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