Arbuscular mycorrhizal colonisation increases copper binding capacity of root cell walls of *Oryza sativa* L. and reduces copper uptake

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ARTICLE INFO

Article history:

Received 2 March 2008

Received in revised form 9 August 2008

Accepted 11 August 2008

Available online 5 September 2008

Keywords:

Arbuscular mycorrhizae

Copper uptake

Cell wall

Hemicellulose

Pectin

Rice

ABSTRACT

There is evidence that colonisation by mycorrhizal fungi can protect host plants from toxic concentrations of heavy metals. The mechanism by which protection is provided by the fungus for any particular metal is poorly understood. Rice (*Oryza sativa* L.) plants were inoculated with *Glomus mosseae* and grown for 4 weeks to ensure strong colonisation. The plants were then exposed to low to toxic concentrations of copper (Cu) and the uptake and distribution were examined. The effect of mycorrhizal colonisation on the cell wall composition and Cu binding capacity of roots was also investigated. Mycorrhizal plants showed moderate reductions in Cu concentrations in roots but large reductions in shoots. In roots, mycorrhizal plants accumulated more Cu in cell walls but much less in the symplast compared to non-mycorrhizal plants. The differences in cell wall binding of Cu could be partly explained by changes in the composition of the cell wall. The mechanistic basis for the reduced Cu accumulation and the potential beneficial consequences of mycorrhizal associations on plant growth in Cu toxic soil are discussed.

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1. Introduction

Plant tolerance to heavy metals can be achieved through a range of physiological and morphological changes (Sanità di Toppi and Gabbriellini, 1999; Hall, 2002). Metal retention by roots is often cited as a beneficial strategy (Wagner, 1993), although in few cases has the site of increased metal storage in roots been identified. Similarly, some mycorrhizal fungi have been shown to reduce accumulation of certain heavy metals in shoots and it is presumed that this is due to increased retention within the root/mycorrhizal structures (Brunner and Frey, 2000; Chen et al., 2004; Zhang et al., 2005). There are a number of possible explanations for the observed reductions in heavy metal concentrations in shoots of mycorrhizal plants. These include (1) a greater selectivity of metal transporters in the cell membranes of mycorrhizae, (2) compartmentation of metals in fungal vacuoles, thereby reducing transfer of the metals to the plant, and (3) increased capacity of mycorrhizal roots to immobilise metals within their cell walls. The binding of heavy metal ions to plant root most probably involves charged groups on cell wall polysaccharides binding metal efficiently (Cohen-Shoel et al., 2002). Of these the acidic side groups of

galacturonic acid in pectin are likely to be most important. (Eticha et al., 2005). Pectins have been implicated as the main cell wall binding site for Al^{3+} (Eticha et al., 2005).

Evidence for intracellular sequestration of metals in ectomycorrhizal fungi is largely indirect and metal-specific (Blaudez et al., 2000; Frey et al., 2000). However, there appear to be no data on relative metal selectivity between plants and endomycorrhizal fungi; the obligate nature of the endomycorrhizal association makes it difficult to separate binding to plant root from binding to the fungus. Adsorption on or precipitation of heavy metals in plant cell walls has been reasonably well described (Lignell et al., 1982; Pellegrini et al., 1993; Amado Filho et al., 1999; Andrade et al., 2002), but there is limited information on metal binding by mycorrhizal cell walls. Joner and Leyval (1997) reported that the arbuscular mycorrhizal fungus *Glomus mosseae* had a high capacity for Cd uptake but they did not distinguish between immobilization on cell walls or in vacuoles. Tam (1995) demonstrated that in the ectomycorrhizal fungus *Pisolithus tinctorius*, Cu and Zn were retained in extrahyphal slime, while Bradley et al. (1981, 1982) showed that in the dwarf shrub *Calluna vulgaris* (L.), Cu accumulated in the associated ericoid mycorrhizal fungus *Pezizella ericae*. Despite the apparent versatility of different types of mycorrhizae to accumulate heavy metals, how this is achieved largely remains a mystery.

Rice is an important crop in Asia, especially in China. However, contamination with a mixture of heavy metals of paddy soils is not

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uncommon (Chen et al., 2005; Lin et al., 2007). Based on evidence with other heavy metals (Brunner and Frey, 2000) mycorrhizal associations with rice could potentially reduce the uptake of Cu. In this study we have focused on the role of cell walls in binding of Cu in the association between rice and the arbuscular mycorrhizal fungus *G. mosseae*. The principal aim was to try to establish whether reduced uptake of copper could be explained by compositional changes in cell walls of roots as a result of mycorrhizal colonisation.

2. Materials and methods

2.1. Cu toxicity trial

Seeds of rice (*Oryza sativa* L.) cultivars, Jiahua 1, were surface-sterilized with 10% (v/v) H_2O_2 for 10 min and immersed in deionized water for 24 h. They were then germinated on moist filter paper until the radicles appeared. For each treatment, 20 uniform seedlings were placed in 20 ml of treatment solution (KNO_3 0.2 mM, $\text{Ca}(\text{NO}_3)_2$ 0.2 mM, MgSO_4 0.1 mM) with varying concentrations of Cu supplied as $\text{Cu}(\text{NO}_3)_2$. The solutions were changed every 12 h for 7 days then the root lengths were measured.

2.2. Production of mycorrhizal and non-mycorrhizal plants

Since the presence of different concentrations of Cu in the medium during the inoculation and colonisation of plants by the mycorrhiza could potentially lead to variations in the degree of colonisation that would complicate interpretation of the results, plants were pre-cultured without Cu for 4 weeks until strong and uniform colonisation was established. Plants were then exposed to Cu over the following 2 weeks to examine the uptake and distribution of Cu.

Rice seeds were surface-sterilized with 10% (v/v) H_2O_2 for 10 min and immersed in deionized water for 24 h. They were then germinated on moist filter paper until the radicles appeared. The seedlings were selected for uniformity before planting. *O. sativa* L. was inoculated with *G. mosseae* isolate (Gm) (BGC.XJ01, isolated from a non-contaminated soil, provided by YS Wang, Beijing Academy of Agriculture and Forestry) or left uninoculated (NM). The inoculum contained dried roots of sorghum, hyphae, spores and soil. Plants were grown in round plastic pots containing 200 g perlite plus 40 g inoculum for the mycorrhizal treatment, or 200 g perlite plus 40 g sterilized inoculum for the NM treatment. The inoculum was mixed with the perlite. Three germinated seedlings were sown per pot. Containers were watered and fertilized as needed with a 1/5 strength nutrient solution (Feigin et al., 1987). Pots were regularly weighed to maintain appropriate water content. After 4 weeks of preculture, some plants were removed gently from perlite, and were washed carefully to remove adhering particles on the root surface. Plants were then transferred to hydroponic treatment solutions containing varying concentrations of Cu for 2 weeks; other plants were grown for a further 4 weeks in perlite without Cu to provide material from which to extract cell walls for the Cu binding studies. For the hydroponic experiment, inoculated roots showing good colonisation, along with NM roots, were transferred into 1/4 strength modified Hoagland's nutrient solution with or without Cu. Fe-EDTA was omitted from uptake solutions to avoid complexation of Cu. The pH of uptake solutions was maintained at 5.5. During the growth period, the containers were aerated with air pumps and arranged in an environment-controlled growth chamber with a 14-h photoperiod at a photon flux $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the range 400–700 nm.

2.3. Root colonisation by *G. mosseae*

To determine the degree of colonisation after 4-week and 8-week preculture, a portion of the roots (about 1 g fresh weight)

was washed free of perlite with tap water, and then fully rinsed in deionized water. The clean roots were cut into segments around 1 cm long, cleared by soaking in 10% KOH and stained according to Phillips and Hayman (1970) but without using phenol. Percentage colonisation was determined by the grid intersect method (Giovannetti and Mosse, 1980).

2.4. Distribution of Cu between root apoplast and symplast

In order to distinguish between Cu bound to cell walls and Cu taken up into cells, roots were desorbed using a modified desorption procedure as described in Reid and Liu (2004). Briefly, after being treated with different concentrations of Cu in hydroponic solution, roots were excised. The entire root system from each treatment was then desorbed in 5 mM CaCl_2 , which was changed every 5 min. After 20 min the roots were rapidly frozen in liquid nitrogen to disrupt cell membranes and desorption was continued for 40 min. The Cu released in the 20 min desorption plus the Cu remaining in the root after the freeze-thaw plus rinse was considered to be apoplastic. The Cu released following the freeze-thaw was considered symplastic. The Cu concentration in the desorbed solution and in the solution after freeze-thaw was analyzed directly by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

2.5. Measurement of tissue Cu content

Plants were harvested at the end of the treatments and the roots rinsed with Milli-Q water and blotted gently. The roots and shoots were oven-dried at 70 °C for 72 h and the dry weights determined. The dried samples were ground with a stainless steel mill and digested in concentrated nitric acid (HNO_3) at 160 °C. Cu in the extracts was measured by ICP-AES.

2.6. Preparation of cell walls

Root cell walls of 8 weeks mycorrhizal and non-mycorrhizal plants were extracted according to Zhong and Lauchli (1993). Roots were frozen in liquid N_2 and homogenized to a powder with a mortar and pestle. The root powder was washed into a 50-ml centrifuge tube with three aliquots of 15 ml ice-cold 75% ethanol, mixed well and left undisturbed for 20 min in an ice bath. The homogenates were then centrifuged for 10 min at 1000 g and the pellets were washed in three steps, each repeated three times, as follows: 1) ice-cold acetone (1:7, w/v), 2) ice-cold methanol-chloroform mixture (1:1, v/v) and 3) ice-cold methanol. The supernatant of each wash was discarded and the final pellet was freeze-dried overnight. The dried pellets were further ground to powder with a mortar and pestle and considered as crude cell walls. The powder was stored at 4 °C until required.

2.7. Fractionation of cell walls

Individual cell wall components were extracted according to Zhong and Lauchli (1993). The crude cell walls of mycorrhizal and non-mycorrhizal roots (0.1 g) were incubated in 4 ml 0.5% ammonium oxalate buffer (containing 0.1% NaHB_4), and washed twice in boiling water for 2 h. After centrifugation the supernatants were collected, considered as pectin, and the volume was recorded. The precipitate was washed with deionized water twice and freeze-dried. The dried precipitate was washed with 4 ml 4% NaOH (containing 0.1% NaHB_4) three times for 24 h at room temperature. After centrifugation the supernatants were collected and the volume was recorded. After being neutralized with cold acetic acid, the supernatant was freeze-dried and designated hemicellulose 1 (HC1). In the same way the precipitate was washed and freeze-

dried, then washed in 4 ml 24% NaOH (containing 0.1% NaHB₄) three times for 24 h at room temperature and the supernatant was collected. This supernatant was designated hemicellulose 2 (HC2). The extracted solution was kept at 4 °C for further use. The concentration of uronic acids in different parts of the cell walls was determined according to Taylor and Buchanan-Smith (1992).

2.8. Cu binding assays

Binding of Cu was measured by monitoring the depletion from solution following addition of cell walls or cell wall fractions. Cu concentrations in solution were determined using a Cu²⁺ ion-selective electrode (Orion 9629B, Thermo Orion, USA) (Cu-ISE). Calibration curves were prepared using Cu(NO₃)₂ in deionized water. The ionic strength of the solutions was matched using KNO₃. The pH was adjusted to 5.5 with HNO₃ or KOH. The activity of Cu²⁺ in the standards was calculated using Geochem-PC (ver. 2) (Parker et al., 1993) which estimated that about 90% of the Cu was free Cu²⁺ at pH 5.5. The calibration curve typically had a slope around $-28.2 \text{ mV pCu}^{-1}$ and was linear over the range pCu 2–7 ($R^2 = 0.9947$). The various root fractions (0.1 g DW) were incubated in 50 ml Cu solutions and uptake monitored over 1 h.

2.9. Statistics

Results are presented as mean $\pm$ standard error of the mean. Data were tested by two-way analysis of variance by SAS software 6.0 and means were compared by LSD at the 5% level.

3. Results

3.1. Cu toxicity

A preliminary experiment was conducted to assess the toxicity of Cu to the growth of rice in hydroponic culture. Root elongation was measured from germination to 7 days at different Cu concentrations. Cu concentrations up to 1 μM had weakly positive or no effects on root growth compared to no Cu addition, while 5 μM Cu reduced root elongation by 90%, 10 μM and higher Cu concentrations reduced growth by >95%.

3.2. Plant growth under different Cu addition levels

Mycorrhizal colonisation was approximately 50% at the beginning of the Cu treatments (4 weeks) but was reduced to approximately 40% at the end of the experiment (8 weeks) (Fig. 1).

3.3. Cu accumulation in mycorrhizal and non-mycorrhizal plants

During the 2-week incubation period in Cu-containing growth solutions, approximately 90% of plant Cu was retained in the roots (Fig. 2). The differences in root Cu concentration between non-toxic (1 μM) and toxic (5 μM) were relatively small. However, with increasing toxic concentrations, there was a noticeable trend in which the Gm plants accumulated significantly less Cu than the NM plants; this difference was more striking in the shoots at 5 and 100 μM Cu where the shoot Cu concentration in the Gm plants was less than 10% and 48% of those in the NM plants, respectively (Fig. 2a).

3.4. Cu distribution between symplast and apoplast in roots

The overall concentration of Cu in roots is comprised of both intracellular and extracellular fractions. The use of combined desorption and freeze-thaw techniques to separate the two fractions revealed that Gm plants accumulated more Cu in their cell

Fig. 1. Dry matter yield of *O. Sativa* L. under different Cu addition levels. Plants were pre-cultured without Cu for 4 weeks then Cu treatments were applied for a further 2 weeks before harvest. The horizontal line indicates the mean root and shoot biomass at 4 weeks. Data are means $\pm$ standard error of the mean. $n = 4$ for dry matter yield of *O. Sativa* L. Asterisks indicate significant differences ($P < 0.05$).

walls than NM plants, and conversely, that NM plants absorbed more Cu into the root cells compared to the Gm plants (Fig. 3).

3.5. Binding on cell walls

Purified cell walls were found to have a high capacity for binding Cu, with saturation only being achieved at around 1.5 mM Cu at pH 5.5 (Fig. 4). Nevertheless, for the same weight of cell wall material, Gm plants were able to bind significantly more Cu than NM plants across the entire concentration range tested in these experiments (Fig. 4). Fig. 5a shows a typical time course of binding of Cu in cell walls. Adsorption of Cu was rapid with saturation of binding sites being achieved within 10 min. There were no large differences in the rate of Cu binding in cell walls from NM and Gm plants, but the steady-state concentration of Cu was significantly higher in Gm plants. Treatment of the isolated cell walls to remove pectin and some of the hemicellulose resulted in a moderate reduction in the binding capacity of roots from the NM plants, but a much greater reduction in binding in the Gm plants (Fig. 5b).

3.6. Cell wall content and composition of mycorrhizal and non-mycorrhizal plants

Cell walls were extracted from NM and Gm plants that had been grown for 8 weeks without Cu. At this stage the average colonisation of inoculated roots was more than 40%, with no colonisation in the control plants. There was a small but significant increase in the proportion of cell walls in Gm plants compared to NM plants (i.e. for the same fresh weight, for Gm plants the combined cell wall production from plant plus fungus was higher) (Table 1).

Fig. 2. Effect of mycorrhizal colonisation on the concentrations of Cu in roots and shoots of rice. Each point is the mean $\pm$ standard error of 4 replicates. Asterisks indicate significant difference between mycorrhizal treatment (Gm) and the non-mycorrhizal (NM) control ($P < 0.05$).

Measurement of the total uronic acid concentration showed that these acidic groups were significantly more abundant in the cell walls from roots of Gm plants compared to NM plants (Table 1).

Binding studies with the various cell wall fractions confirmed that Gm roots were able to bind a greater amount of Cu in the pectin plus hemicellulose 1 (HC1) fraction than the same fraction from NM plants (Table 2). In both mycorrhizal and non-mycorrhizal plants there remained a significant amount of bound Cu that could not be attributed to either of the pectin + hemicellulose fractions. This fraction was significantly higher in the Gm plants (Table 2).

4. Discussion

4.1. The effects of mycorrhizal colonisation on Cu uptake by rice

It has been well documented that mycorrhizal colonisation can have significant impacts on metal uptake by host plants (Weisenborn and Leyval, 1995; Chen et al., 2007). The mechanisms to explain the altered uptake have mainly focused on metal immobilization in the root and/or mycorrhizosphere (Marschner, 1995). The results of the current experiments show that mycorrhizal colonisation improves the ability of rice plants to resist Cu toxicity. Mycorrhizal colonisation decreased Cu concentrations in the roots and shoots under 3, 5 and 100 µM Cu treatments, with a concomitant increase in root and shoot biomass. It is possible that the reduced Cu concentrations were partially due to the improved growth as a result of mycorrhizal colonisation, i.e. growth dilution effect, but the results presented here suggest that Cu immobilization on roots could be a major factor. Extensive binding of Cu to roots and ectomycorrhizae has been shown in other studies (Turnau, 1998; Kaldorf et al., 1999).

Fig. 3. Effect of mycorrhizal colonisation on the distribution of Cu in roots of rice. Each point is the mean $\pm$ standard error of 4 replicates. Asterisks indicate significant difference between mycorrhizal treatment (Gm) and the non-mycorrhizal (NM) control ($P < 0.05$).

In agreement with the literature (Li et al., 1991; Lin et al., 2007), a substantial reduction in Cu accumulation in shoots of the mycorrhizal plants was observed, and the reduction became more noticeable under Cu-toxic conditions (Fig. 2a). However, the corresponding differences in Cu accumulation in roots were relatively small, and the cause of the reduced root to shoot transfer in mycorrhizal plants was not immediately obvious from measurements of total root Cu content. The 'roots' of mycorrhizal plants are a composite structure of two organisms whose abilities to adsorb or absorb heavy metals are not necessarily the same. The distribution

Fig. 4. Concentration dependence of Cu binding in cell walls isolated from mycorrhizal (Gm) and non-mycorrhizal (NM) rice roots. Each point is the mean $\pm$ standard error of 4 determinations.

Fig. 5. Time course of Cu binding to isolated root cell walls extracted from of mycorrhizal (Gm) and non-mycorrhizal (NM) rice plants. Cu concentration = 100 μM . (a) Crude cell wall fraction, (b) crude cell wall fraction after removal of pectin and HC1. Each point is a single measurement.

of Cu between the apoplast and symplast may play a key role in Cu toxicity, and mycorrhizal colonisation may change the Cu distribution between these two components. Separation of the root Cu into symplastic and apoplastic fractions revealed that the mycorrhizal plants had substantially less Cu inside the cells, and more bound in the plant and fungal cell walls. The reduced symplastic Cu content correlates well with the magnitude of the reductions in shoot Cu. The lower symplastic content of mycorrhizal plants was associated with an increase in the Cu content of the cell walls of mycorrhizal plant roots. We hypothesize that there is a causal relationship between retention of Cu in the cell walls and uptake across the plasma membrane into the root cells, leading to a lower extent of transfer to the shoots.

4.2. Characteristics of Cu binding in cell walls

The range of Cu adsorbed by the cell wall was between 2.6 and 3.0 mg g^{-1} cell wall. This range is in accordance with Cu-sorption studies using soil fungi, e.g. *Aspergillus niger* (2–10 mg Cu g^{-1}) and

Table 2

The Cu binding capacity of different components of cell walls extracted from mycorrhizal and non-mycorrhizal roots equilibrated with 100 μM Cu.

Inoculation	Cu bound ($\mu\text{mol g}^{-1}\text{DW}$)			
	Cell wall total	P + HC1	HC2	Remainder
NM	41.6 $\pm$ 0.2 b	3.4 $\pm$ 0.4 b	23.3 $\pm$ 0.3 a	14.9 $\pm$ 0.1 b
Gm	47.0 $\pm$ 0.8 a	19.0 $\pm$ 1.6 a	6.8 $\pm$ 1.6 b	21.3 $\pm$ 1.0 a

Note: The binding capacities of the various fractions were calculated from the amount removed by successive treatments. P + HC1 = pectin plus hemicellulose 1 fraction; HC2 = hemicellulose 2 fraction; remainder = Cu remaining bound after removal of pectin, HC1 and HC2 fractions. Data are expressed as mean $\pm$ standard error of the mean, $n = 3$, $P < 0.05$.

Mucor rouxii (1–3 mg Cu g^{-1}) (Mullen et al., 1992; Kapoor and Viraraghavan, 1995), *Rhizopus arrhizus* (1.5 mg Cu g^{-1}), *Trichoderma viride* (2.2 mg Cu g^{-1}) (Morley and Gadd, 1995) and *G. mosseae* (3–14 mg Cu g^{-1}) (Gonzalez-Chavez et al., 2002). A higher Cu-sorption capacity was observed in the cell walls of mycorrhizal roots compared to non-mycorrhizal roots, which may reflect differences in cell wall chemistry (Gadd, 1993; Kapoor and Viraraghavan, 1995). It was noted that mycorrhizal plants had a slightly greater percentage (10%) of total root cell wall material which by itself would not account for the increase in binding described in Fig. 3b. Metal binding capacity is correlated with the density of acidic polysaccharides whose dissociation exposes anionic side groups capable of complexing cations, or simply reducing their mobility via electrostatic attraction (Kinraide, 1994). Various studies have identified uronic and galacturonic groups as the dominant binding moieties for Cu and other cations in cell walls (Zheng et al., 2004; Konno et al., 2005). In this study there was a significant increase (23%) in uronic acids in the mycorrhizal roots which would be expected to enhance the adsorption of Cu ($P < 0.05$). Fractionation of the cell walls from the mycorrhizal and non-mycorrhizal plants indicated quite large shifts in the binding of the various types of polysaccharide. Mycorrhizal roots showed a 5-fold increase in Cu in the pectin plus hemicellulose 1 fraction, which mirrored a reduction in the binding in the hemicellulose 2 fraction (Table 2).

In general, the absorption of Cu by fungi and other microorganisms is a process which consists of two phases (Kapoor and Viraraghavan, 1995; Morley and Gadd, 1995; Tsezos et al., 1997; Blaudez et al., 2000; Gonzalez-Chavez et al., 2002): (a) a rapid phase, which represents the binding of Cu to accessible exchange sites on the surfaces of cell walls or (b) a slower phase where Cu diffuses into the porous cell wall structure and is sequestered or removed by other processes until an equilibrium is reached. In this study, Cu-sorption was a rapid process not only in the cell walls of intact roots but also in the purified cell wall fractions (Fig. 5). Gardea-Torresdey et al. (1997) noted that rapid Cu sorption was correlated with metabolism-independent sorption and most likely represented surface Cu-binding in mycelium of *Mucor rouxii*. Similar fast sorption processes were recorded by Joner et al. (2000) for Cd when the external hyphae of four isolates of arbuscular mycorrhizal fungi were incubated in solution, and by Blaudez et al. (2000) for Cd in the ectomycorrhizal fungus *Paxillus involutus*.

4.3. Cu binding in fungal versus plant cell walls

It is likely that most of the binding sites would be non-specific for Cu and that in complex nutrient solutions, the actual amount of Cu would be considerably less due to competition with other cations. It is not clear from the results presented whether the changes in partitioning of Cu in roots are due solely to the fact that in mycorrhizal plants there are two types of cell wall, one of which has a superior capacity for immobilising Cu (Gadd, 1993; Leyval

Table 1

Differences in the proportion of cell wall and uronic acid content of roots of mycorrhizal (Gm) and non-mycorrhizal (NM) rice seedlings.

Inoculation	Cell wall (% root FW)	Uronic acid (mg g^{-1} cell wall DW)
NM	8.04 $\pm$ 0.06 a	1.14 $\pm$ 0.003 a
Gm	8.81 $\pm$ 0.03 b	1.40 $\pm$ 0.04 b

Data are means $\pm$ standard error of the mean. $n = 4$ for cell wall %, and $n = 3$ for uronic acid content.

Different letters in each column indicate significant differences ($P < 0.05$).

et al., 1997), or whether the fungus induces changes in the composition of the cell wall of the host. Our results demonstrate that the cell walls of mycorrhizal plants are different from those of non-mycorrhizal plants, and that this difference is a causal factor in reduced Cu uptake into the symplasm, at least partially.

4.4. Conclusions

The focus of this study was to evaluate the compositional difference in cell walls between mycorrhizal roots and non-mycorrhizal roots, and the binding capacity of cell walls of mycorrhizal roots to Cu. Overall, our results demonstrated that: 1) the reduced accumulation of Cu in shoots was largely due to the ability of mycorrhizal roots to limit uptake into the symplasm; 2) the reduced accumulation in the symplasm correlates well with the ability of mycorrhizal roots to bind Cu in the apoplasm; and 3) compositional difference in the mycorrhizal roots that explained the enhanced binding capacity of mycorrhizal roots to Cu. All of these points provide novel insights into the mechanism of reduced Cu accumulation and give a sound basis for investigation of similar mechanisms for other heavy metals. Biosorption on cell walls and extracellular slime by mycorrhizal fungi and other microorganisms clearly has the capacity to alter the bioavailability of metals in the rhizosphere, thereby altering the uptake into the plant.

Acknowledgements

This study was supported by the Natural Science Foundation of China and the Ministry of Science and Technology, China. We thank Prof. Wang YS for providing arbuscular mycorrhizal fungal isolates.

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