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Effects of Arbuscular Mycorrhizal Colonization on the Growth of Upland Rice (*Oryzal Sativa* L.) in Soil Experimentally Contaminated with Cu and Pb

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Abstract

A pot culture experiment was carried out to investigate the roles of *Glomus mosseae* in plant Cu and Pb acquisition by *Oryzal sativa* L., and Cu-Pb interactions. Cu was applied to the soil with different Cu levels including 0 (Cu0), 100 (Cu100), 200 (Cu200) mg kg^{-1} and Pb addition including 0, 300, 600 mg kg^{-1}. All the treatments were designed with (+M) or without (-M) *Glomus mosseae* as randomized block. The results indicated that Cu addition and Pb addition decreased root colonization significantly. AM (arbuscular mycorrhizal) inoculation increased the root biomass under all the Cu and Pb treatments and increased the shoot biomass under Cu200 with Pb600 treatments observably compared with -M treatments. +M treatments enhanced plant Cu acquisition by *Oryzal sativa* L. compared with -M treatments except for shoot Cu uptake of Cu200-Pb300 treatments. However, plant Cu concentrations were decreased markedly by the root colonization under all the Cu-Pb interactions except for shoot Cu concentrations of Cu200-Pb600 treatments compared with –M treatments. Irrespective of Cu and Pb addition levels, +M plant had higher Pb uptake than –M plants. On the contrary, the roots of +M plants had significantly lower Pb concentrations than those of –M plant.

Keywords: Arbuscular mycorrhizal fungi; Colonization; *Oryzal Sativa* L.; Cu uptake; Pb uptake

Introduction

Arbuscular mycorrhizal fungi (AMF), a special microorganism can connect soil and a plant directly, plays an important role in environmental remediation [1]. When the plant is in the heavy metal adversity, mycorrhizal association can enhance the resistance to the metals of plants [2]. Chen *et al.* [3], reported Cu uptake by four kind of mycorrhizal plants including two native plant species, *Coreopsis drummondii* and *Pteris vittata*, together with a turf grass, *Lolium perenne* and a leguminous plant *Trifolium repens*. As a comprehensive consideration, the species of plants can affect the uptake by mycorrhizas.

Rice is an important crop in Asia, especially in China. However, contamination with a mixture of heavy metals of paddy soils is not uncommon. There are several studies about the effects of mycorrhizal association on the uptake of heavy metals by upland rice [4]. Based on evidence with other heavy metals, mycorrhizal associations with rice could potentially reduce Cu and Pb concentrations in the upland rice compared with non mycorrhizal plants under a mixture of Cu, Zn, Pb and Cd [5]. No matter what the results are, the factors can affect the uptake by plants are comprehensive, since these metals may interfere with each other, the results cannot precisely illuminate the role of AM fungi in Cu and Pb uptake. To our knowledge, considering the above aspects, our objectives for the present study were to test the effects of inoculation with *Glomus mosseae* on the growth and Cu and Pb uptake of upland rice in soils with different Cu and Pb levels.

Materials and Methods

Soil mixture

The soil (paddy soil) was collected from Huzhou, Zhe-jiang province, China. Soil was sampled from the surface layer (0-20 cm) of cultivated fields which were free of contamination by Cu and Pb. The soil was sieved to pass a 2 mm mesh, autoclaved (121°C, 2 h) to eliminate indigenous AMF, and then air-dried. Soil pH (soil: water 1: 2.5) were 6.3 before sterilization. The soil contained 11.75 mg Olsen P kg^{-1} soil. Soil Cu and Pb concentrations were 40.0 and 45.0 mg kg^{-1} by digesting the soil samples with aqua regia perchloric acid and monitoring with ICP-AES.

Experiment procedure

Three Pb addition levels (0, 300 and 600 mg kg^{-1}) were applied as analytical grade Pb $(NO_3)_2$ solutions mixed thoroughly with the soil and balanced for two weeks. Three Cu application levels of 0, 100 and 200 mg kg^{-1} (Cu0, Cu100 and Cu200) were designed and achieved by adding analytical grade Cu $(NO_3)_2$ solutions and then balanced for two weeks. The solutions were prepared in deionized water and mixed with the soil manually. Upland rice plants were either inoculated with (+M) the AM fungus *Glomus mosseae* or uninoculated controls (–M) under all Cu addition and Pb application levels. Thus, there were 18 treatments in total with 3 replicates, giving a total of 54 pots in a randomized block design.

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Host plants

Seeds of upland rice (*Oryza sativa* cv. 91 B 3) were surface sterilized with 10% (v/v) peroxide (H_2O_2) for 10 min and immersed in deionized water for 24 h. They were then germinated on moist filter paper until radicals appeared and were selected for uniformity before sowing.

Mycorrhizal inoculum and plant growth conditions

The rice was inoculated with *Glomus mosseae* (BGC, XJ01) isolates or left uninoculated control. Inoculum of *Glomus mosseae* contained dried roots of grain sorghum, hyphae and spores.

Plants were grown in round plastic pots (diameter: 4 cm; height: 8 cm) containing 420 g soil plus 80 g inoculum for the mycorrhizal treatments or 420 g soil plus 80 g inoculum which had been sterilized before hand to eliminate the fungus for the –M treatments. Three pre-germinated seeds were sown into each pot. Pots were regularly watered to weight with ddH_2O to maintain soil moisture at 70% of field water holding capacity. Plants were harvested after six weeks of growth.

Root colonization

The clean roots were cut into segments about 1 cm long. A randomly selected subsample of fresh root was taken for assessment of root colonization. Roots were assessed, the root colonization according to Phillips and Hayman [6], with some modifications. Roots were cleared in 10% KOH at 90ºC for 30 min in a heat water bath before KOH was removed from the root samples. The roots were then stained with acid fuchsin at 90ºC for 30 min in a heat water bath before acid fuchsin was removed from root samples. Percent colonization determined by the grid intersection method [7].

Plant biomass

Shoots and roots of harvested plants were washed free of soil with tap water and dried at 70ºC or 72 h. The dry weight of shoots and roots was then determined and samples were ground to pass a 0.5-mm sieve.

Plant Analysis

Plants were harvested after six weeks of growth. Shoots were first cut off, and roots were carefully washed free of soil with tap water, and then washed with 1mM $CaCl_2$ for 30 min and rinsed in deionized water. Subsamples of dried roots and shoots were ground to pass a 0.5 mm sieve and digested at 160°C using concentrated nitric acid (HNO_3) for elemental analysis (such as Cu, Pb) by ICP-AES.

Data analysis

Results are presented as the mean. Data were calculated and analyzed statistically using the analytical tools of Microsoft Excel 2003. All data were subjected to multi-comparisons by the least significant difference (LSD) at the 5% level using SAS software.

Results

Mycorrhizal colonization

The root colonization was not detected in the non-inoculated plants while the inoculated plants had relatively high colonization rates (Table 1). Mycorrhizal colonization exceeded 60% when no Cu and Pb was added, however, Cu and Pb addition remarkably inhibited the root colonization as the root colonization rate decreased to around 16%. There was significant difference in root colonization between two levels of Cu and Pb, and significant interaction effects of Cu and Pb on the root colonization was detected.

Plant growth

Neither shoot or root dry matter yield was inhibited by 300 mg kg^{-1} Pb additions, while 600 mg kg^{-1} Pb additions significantly decreased the growth of upland rice. Under all Pb addition levels, plant dry weight was inhibited by Cu application rates except for shoot's being increased significantly by 100 mg kg^{-1} Cu application rate under 300 mg kg^{-1} Pb treatments (Figure 1A,B).

Plant growth was generally enhanced by mycorrhizal colonization, especially the mycorrhizal root showed the larger dry weights significantly than uninoculated roots did under 300 and 600 mg kg^{-1} Pb treatments (Figure 1A,B). However, with a few specific exceptions, there were differences in shoot dry weight between mycorrhizal and non-mycorrhizal treatments.

Cu concentration in the shoots and roots of upland rice with combined Cu and Pb levels (mg kg^{-1})

Cu concentration in the shoot increased with increasing soil Cu concentrations (Figure 2A). Compared with no Pb addition level, 300 mg kg^{-1}Pb addition level did not affect the shoot Cu concentrations under Cu100 levels, while increased the shoot Cu concentrations under Cu200 levels. Under 600 mg kg^{-1}Pb application rates, the shoot Cu concentrations were enhanced significantly. In the roots (Figure 2B), Pb treatments decreased the shoot Cu concentrations markedly.

Compared with –M treatments, mycorrhizal colonization showed different effects on Cu concentrations of upland rice plants depending on the experimental treatments (Figure 2 A,B). Mycorrhizal plants showed the higher shoot and root Cu concentrations under 200 mg kg^{-1} application levels when no Pb was added. Under 300 Pb levels, mycorrhizal colonization increased the shoot Cu concentration under Cu 100 while decreased shoot and root Cu concentrations under Cu 200 levels significantly. Under 600 Pb levels, the shoot and root Cu concentrations were decreased by mycorrhizal colonization under Cu 100 addition, the shoot Cu concentrations in the mycorrhizal plants

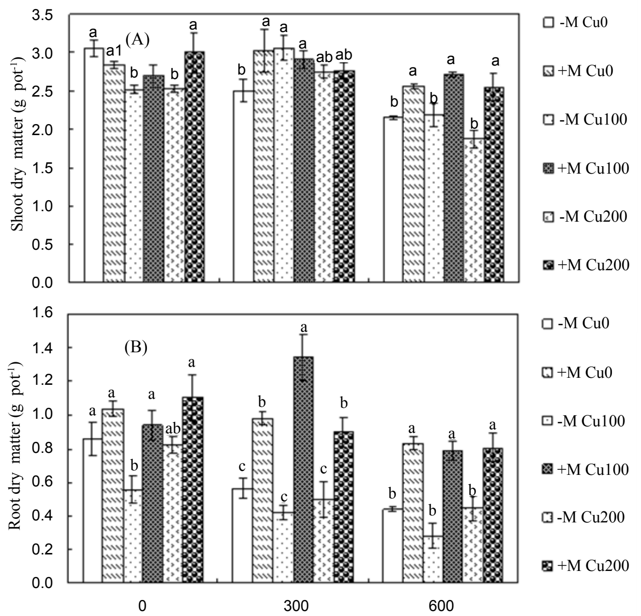

Figure 1: Shoot (A) and root (B) dry matter yield of upland rice under different treatments. Different letters above the columns indicate significant difference between means by LSD at the 5% level.

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was increased by the Cu 200 addition while the root Cu concentrations markedly in the mycorrhizal plants was decreased by the Cu 200 addition.

Pb concentration in the shoots and roots of upland rice with combined Cu and Pb levels (mg kg^{-1})

Compared with no Pb treatments, Pb concentration in the shoots and roots increased with Pb addition in the soils under all the Cu

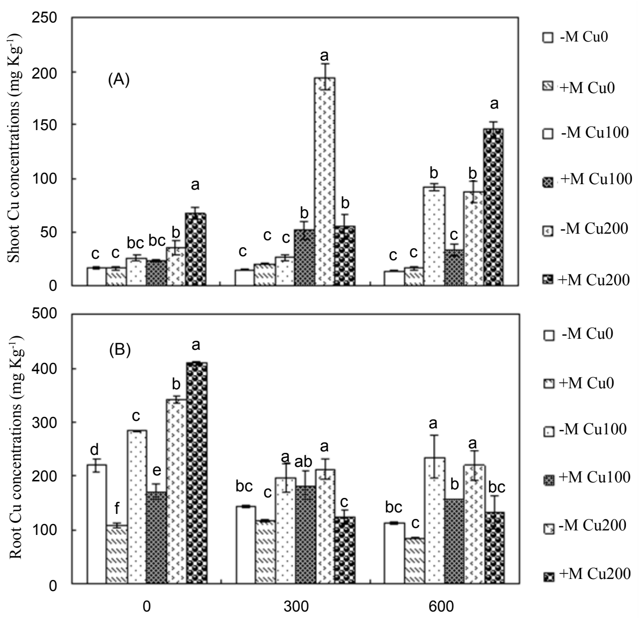

Figure 2: The shoot and root Cu concentration (A), (B) of upland rice under different treatments. Different letters above the columns indicate significant difference between means by LSD at the 5% level.

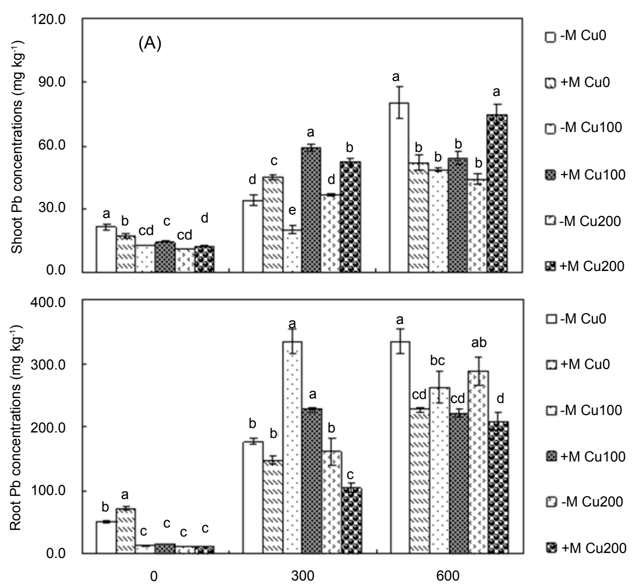

Figure 3: The shoot and root Pb uptake (A), (B) of upland rice under different treatments. Different letters above the columns indicate significant difference between means by LSD at the 5% level.

Pb treatments (mg kg^{-1})	Inoculation treatments	Root colonization of plants		
		Cu0[1]	Cu100	Cu200
0	-M[2]	0.0 d[3]	0.0 d	0.0 d
	+M	69.0 a	54.9a	35.5a
300	-M	0.0d	0.0 d	0.0 d
	+M	44.6 b	26.0 c	29.0 b
600	-M	0.0d	0.0d	0.0d
	+M	24.6c	35.1b	15.9 c
Significance[4] of:				
Pb addition level:			***	
Inoculation:			***	
Cu addition level:			***	
Pb×inoculation:			***	
Pb×Cu:			**	
Cu×inoculation:			***	
Pb×Cu×inoculation:			**	

[1]Cu0, Cu100, Cu200: Cu application rate of 0, 100 and 200 mg kg^{-1}.
[2]–M, +M: uninoculated control, inoculation with the mycorrhizal fungus *G.mosseae*.
[3]In each column, means followed by the same letter are not significantly different by LSD at the 5% level.
[4]By analysis of variance; *** $p<0.001$; ** $p<0.01$

Table 1: The root colonization of plants with different combined Cu and Pb levels (%).

levels (Figure 3 A,B). Cu addition in the soil obviously increased Pb concentration in the roots (B). However, Pb concentration in the shoots and roots under all the Cu addition levels was decreased compared with that of Pb treatments singlely. Mycorrhizal colonization generally enhanced shoot Pb concentrations, while reduced root Pb concentrations under all the Pb levels compared with –M treatments.

Discussion

Root colonization

It has been suggested that mycorrhiza status is concerned with environmental elements, especially with contamination levels in soil [1,8]. Cu and Pb addition decreased the percent of root colonization in the present study and root colonization decreased with increasing of soil Cu and Pb concentrations. These results demonstrated that high levels of soil Cu and Pb inhibited the growth of arbuscular mycorrhizal fungi and then inhibited the formation of mycorrhizas. Andrade et al. [9], reported that increasing soil Pb levels decreased the root colonization and the spore numbers, which is in accord with the present study. However, Andrade et al. (2010) [10], also reported that Cu addition unaltered the growth of *Glomus etunicatum*, which is contrary to the present study. The difference in the tolerance to the Cu addition of fungus may contribute to the contrary results. Leyval [11], Leyval and Joner [12], demonstrated that the tolerance of the fungus separated from contaminated environment was less affected compared with that that from uncontaminated soils. In the present study, under 200 mg kg^{-1} Cu treatment, the root colonization still maintained 16%-30%, which indicated that *Glomus mosseae* had certain tolerance to Cu.

Plant biomass

It is well known that the association of plants with arbuscular mycorrhizal fungi is beneficial to metal-induced stress, increasing tolerance in metal-contaminated soils [4,5,13-15]. In the present study, Plant growth was inhibited by Cu treatments, on the contrary, enhanced by mycorrhizal colonization, especially the mycorrhizal root showed the larger dry weights significantly than uninoculated roots did under 300 and 600 mg kg^{-1} Pb treatments. It is demonstrated that the formation of mycorrhizal alleviated the Cu toxicity to the plants. Andrade et al. [10] reported that mycorrhizal increased the jack bean biomass under 450 mg kg^{-1} Cu treatments. Chen et al. [3] also reported that mycorrhizal promoted the biomass of *Trifolium repens*, *Pteris vittata* and *Coreopsis drummondii* planted in the Cu mine tailings. A significant decrease in plant biomass was observed as Pb concentration increased in soil, suggesting that metabolic processes might be impaired by high Pb concentrations in soil. Mycorrhizal promoted the plant biomass accumulation, which also indicated that the formation of mycorrhizal between plants and fungus was a beneficial symbiosis. The promotion of mycorrhizal plants roots was significantly several-fold larger than that of without inoculating fungus under combined Cu and Pb treatments in the present study. Many other studies showed the similar results [5,13,3,16,17]. The function of mycorrhizas may attribute to the Cu, Pb uptake and P uptake.

Cu, Pb uptake

In the adversity condition, the supplying of nutrients plays an important role on the growth of plants. Better mineral nutrition such as P uptake and lower heavy metal uptake and transport may be the possible mechanisms for success of mycorrhizal plants growing in soils with excessive levels of metals [18-20,5,21]. In the present study, under low Cu and Pb treatments, a sharp increase of P uptake in mycorrhizal plants was observed and then increased the biomass suggested that mycorrhiza was beneficial for upland rice to take up P nutrition and then enhanced the capability against the Cu and Pb toxicity. However, under high Cu and Pb treatments, P uptake in the mycorrhizal shoots showed no difference with that of the nonmycorrhizal shoots. It is related with the dilution effect because mycorrhizal enhanced biomass while decreased the P concentrations in the shoots.

The mechanisms of mycorrhizal plants against heavy metals are related to the uptake of heavy metals [17,3,22,23]. In the present study, Under Pb and Cu additions, the concentrations of Pb in mycorrhizal shoot was increased obviously compared with that of –M treatments. One of the part causes may be attributed to the dilution effect because mycorrhizas enhanced the root biomass, the other cause may be the selective uptake of elements by mycorrhizas. Reports have demonstrated that mycorrhiza can change the bioavailability of heavy metals in the soil [12,24,25] and then change the heavy metal supply to the plants. Li et al. [26], suggested that mycorrhizas increased Cu uptake of clover under low Cu addition to the soil while inhibited under high Cu addition treatments. Zhang et al. [13], indicated that *Glomus mosseae* decreased Pb uptake of *Vicia faba* in the soil contaminated by multi-metals. Transportation of the metals from root to shoot affected the uptake of plants. Irrespective of mycorrhizas, Pb showed a trend to fix more Pb in the roots with increasing Pb treatments. On the contrary, Cu showed a trend to transport more Cu in the shoots with increasing Cu treatments. The difference in Pb and Cu resulted in the diversity uptake by upland rice.

The hyphae of arbuscular mycorrhizal fungi possessed higher ability of adsorbing multi-metals [27]. Therefore, heavy metals can be immobilized in the structure of external hyphae or internal hyphae and then alleviated the metal toxicity to the plants [21,13]. However, the formation of mycorrhizas increased the Pb transportation from root to shoot, which may be attributed that Pb treatments destroyed the internal mycorrhizal structure and thus inhibited the growth of *Glomus mosseae*. Andrade et al. [9], reported that the arbuscular structure decreased obviously under 600 mg kg^{-1}Pb addition. On the other hand, the partitioning of metals in the plants is the results affected by multi-factors, such as the species of fungus, the variety of plants, the plants ability of metal transportation, the concentration of metals in the soil systems. There is no doubt that there is an urgent need to conduct further investigations to understand the involvement of AMF in plant interactions and encouraging concentrations of metals and to reveal the underlying mechanisms.

Conclusion

1. Mycorrhizal colonization exceeded 60% when no Cu and Pb was added, however, Cu and Pb addition remarkably inhibited the root colonization as the root colonization rate decreased to around 16%.

2. Mycorrhizal colonization improved the root growth markedly compared with –M treatments under all the treatments. The roots showed more mycorrhizal dependency than the shoots under all the treatments almost.

3. Cu and Pb showed different effects on the plant growth, under all the treatments, compared with –M treatments, mycorrhizal colonization possessed different effects on Cu concentration, Pb concentration, Cu uptake and Pb uptake.

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