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Growth and arsenic uptake by Chinese brake fern inoculated with an arbuscular mycorrhizal fungus

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ABSTRACT

A split-root experiment investigated the effects of inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae* and arsenic (As) addition on As uptake by *Pteris vittata* L. Either part or all of the root system was inoculated with *G. mosseae* or exposed to As addition (50 ml 1000 μmol L^{-1} As 1 week before harvest). Mycorrhizal colonization substantially increased frond and root dry weight and P and As contents irrespective of As addition. Frond As contents in mycorrhizal plants were highest when the whole root system was exposed to As. Frond As concentrations and contents were higher when inoculation and As addition were in the same parts of the root system than when spatially separate. There were positive effects of arbuscular mycorrhiza inoculation on plant growth and As uptake, and inoculation of part of the roots seemed to be as effective as inoculation of the whole root system.

1. Introduction

Arsenic contamination is widespread in soils and waters due to anthropogenic activities or from geogenic sources. In recent years there has been increasing contamination of water, soil and crops by this metalloid in many regions of the world (Fitz and Wenzel, 2002; Tripathi et al., 2007), particularly in some countries of southern Asia (Abedin et al., 2002; Meharg, 2004). The carcinogenicity and mutagenicity of As pose a great threat to human health and thus there is an urgent need to remediate As-contaminated environments. Some arsenic hyperaccumulator plants have been found to be potentially useful in clean-up of arsenic as they can remove significant amounts of As from contaminated soils or waters. In addition, phytoextraction using arsenic hyperaccumulators may be a cost-effective and environmentally friendly remediation technique (Salt et al., 1998).

Pteris vittata L. (Chinese brake fern) was the first reported of the eight As hyperaccumulator plant species identified so far (Ma et al., 2001). This species has been found to accumulate As in its fronds with extraordinary efficiency, primarily due to high translocation from roots to shoots and to effective detoxification mechanisms within the plant (Lombi et al., 2002; Webb et al., 2003; Singh and Ma, 2006). Frond As concentrations reached 22,630 mg kg^{-1} in a soil spiked with 1500 mg kg^{-1} arsenic (Ma et al., 2001) and 4200 mg kg^{-1} when grown in a soil containing 131 mg As kg^{-1} (Fayiga et al., 2004). In addition, this species also has a relatively large biomass, fast growth rate, perennial habit, ease of reproduction and resistance to adverse soil conditions (Fayiga and Ma, 2006; Santos et al., 2008). It is therefore an excellent candidate for phytoextraction of As from contaminated soil and the uptake and metabolism of As in *P. vittata* have been intensively studied. However, interactions between the plant and arbuscular mycorrhizal fungi (AMF) have received less attention, perhaps because of an early assumption that hyperaccumulators generally belong to typically non-mycorrhizal plant families (Leyval et al., 1997; Pawlowska et al., 2000).

Arbuscular mycorrhizal fungi are indigenous soil-borne microorganisms that live in mutualistic association with the roots of about 80% of all terrestrial land plants (Smith and Read, 1997). The fungi assist the host plant in the uptake of nutrients (especially relatively immobile nutrients such as P) in exchange for carbon substrates from host plant photosynthesis. Arbuscular mycorrhizal fungi can also increase plant resistance to diverse adverse abiotic factors such as drought and saline conditions or biotic stresses such as attack by pathogens or pests. AMF can also substantially depress the uptake of heavy metals by plants and this is regarded as one of the mechanisms by which metallophytes thrive on sites polluted

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with heavy metals (Leyval et al., 1997; Ouziad et al., 2005). There is evidence that some hyperaccumulator species are associated with AMF and the AMF may have co-evolved with the plants to adapt to the harsh soil conditions in metal-contaminated sites. For instance, zinc violets were strongly colonized by AMF (Hildebrandt et al., 1999) and the indigenous AM fungal isolate *Glomus intraradices* Br1 was found to confer heavy metal tolerance on a range of plants cultivated in soils contaminated with heavy metals (Hildebrandt et al., 1999, 2007). In ultramafic soils in South Africa naturally occurring Ni-hyperaccumulating plants of the Asteraceae were heavily colonized by AMF (Turnau and Mesjasz-Przybylowicz, 2003).

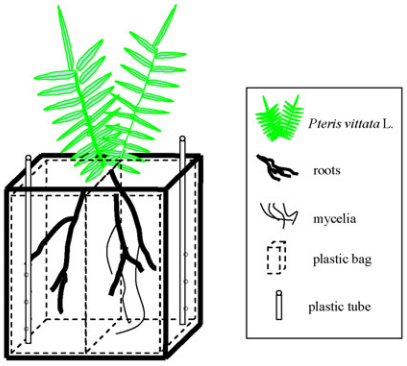
Although it has been recognized that higher plants adapted to As-polluted soils generally form mycorrhizal associations (Cairney and Meharg, 1999; Gonzalez-Chavez et al., 2004), Wu et al. (2007) were the first to conduct a field survey of AM fungal status in the rhizosphere of *P. vittata*. They found that AMF colonization was low to moderate (4.2–12.8%). Nevertheless, another field survey reported that AMF colonization rates were up to 73% (Leung et al., 2007). AMF may have evolved As tolerance and may therefore play an important role in the bioremediation of contaminated sites. Studies have shown that AMF decreased As concentrations but increased As accumulation in shoots of *P. vittata* inoculated with *Glomus mosseae* and grown in soil under greenhouse conditions (Liu et al., 2005). Indigeneous mycorrhizas enhanced As accumulation in As mine populations of *P. vittata* and also sustained plant growth by aiding P absorption (Leung et al., 2006). In contrast, Trotta et al. (2006) reported that As concentrations were significantly lower in roots of *P. vittata* grown in sand but As translocation factors were significantly increased by AMF. Our information on AMF–As interactions in *P. vittata* is still very limited and more studies are required, particularly on the role of plant–AMF interactions in increasing the efficiency of phytoremediation of As-contaminated soils.

Some studies have indicated that metal hyperaccumulators can actively forage for metals in the substrate. For example, roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens* actively foraged for metals in metal-enriched soil patches (Schwartz et al., 1999; Whiting et al., 2000). The present study was carried out to investigate effects of interactions between AMF inoculation and As application on growth, uptake and accumulation of As in *P. vittata*. A split-root device was used to compare the effects of (1) inoculation of part of the root system with AMF or growth of part of the root system in As-amended substrate and (2) inoculation of the whole root system with AMF or growth of the whole root system in As-amended substrate. Thus, in addition to the inoculation treatment, inoculation and As addition pattern were also incorporated in the design of the experiment. Roots of *P. vittata* were inoculated with *G. mosseae* with inoculation either restricted to one part of the split-root system (partial inoculation) or in both root compartments (whole inoculation). One week prior to harvest, As solution was added either to one root compartment (partial As addition) or to both root compartments (whole As addition).

2. Materials and methods

2.1. Growth medium and container

Two plastic bags of equal volume (500 ml) were glued together using adhesive tape to produce two adjacent root compartments and placed in a rectangular 1-L plastic container 15 cm high × 10 cm deep × 10 cm wide (Fig. 1). Each bag had a plastic tube for the application of nutrient solution and deionized water. The tube was included to minimize accumulation of salts on the surface of the substrate due to evaporation of water.



Inoculation treatment		As addition levels (μmol L^{-1})	
Left	Right	Left	Right
-M	-M	0	0
-M	-M	0	1000
-M	-M	1000	1000
-M	+M	0	0
-M	+M	0	1000
-M	+M	1000	0
-M	+M	1000	1000
+M	+M	0	0
+M	+M	0	1000
+M	+M	1000	1000

Fig. 1. The split-root growth system and experimental treatments. Roots of *P. vittata* remained un-inoculated or were inoculated with *G. mosseae* in one root compartment or in both root compartments for 16 weeks. Plants were irrigated with 50 ml water or 50 ml 1000 μmol L^{-1} As solution applied to one root compartment or to both root compartments for 1 week before harvest.

Perlite (diameter 2–3 cm) was used as the plant growth substrate. Prior to use the Perlite was thoroughly washed with tap water, rinsed twice with deionized water, air-dried, sieved (<1 cm) and autoclaved at 120 °C for 2 h. Each bag contained about 450 ml Perlite.

2.2. Plants and AM fungus

Seedlings of *P. vittata* L. were propagated from spores and cultivated in sterilized vermiculite in 3-L plastic pots. When seedlings were about 1 cm long they were transplanted into seed trays filled with sterilized Perlite and were irrigated twice a week with Hoagland nutrient solution (Hoagland and Arnon, 1938) with 1/10 P concentration. The pH value of the nutrient solution was adjusted to 6.5 using 1 mol L^{-1} HCl and 0.5 mol L^{-1} NaOH. Deionized water was used daily to maintain the substrate moisture content at about 60% (w/w). When the third or fourth leaves emerged and the fronds were about 6 cm long the seedlings were carefully transplanted into split-root bags. Roots were divided equally and carefully placed in the two root growth compartments. A black plastic film was used to cover the surface of the substrate in order to minimize water evaporation and algal growth.

Spores and mycelium of the arbuscular mycorrhizal fungus *G. mosseae* BEG167 were propagated on tomato plants (*Lycopersicon esculentum* L.) grown in root bags (4 cm wide × 12 cm long) containing a small amount of Perlite. Root bags were made of 30 μm nylon net which allowed hyphae but not roots to penetrate. Root bags were inserted vertically into the middle of a plastic cup (250 ml) with pinholes on the bottom and surrounded