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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).

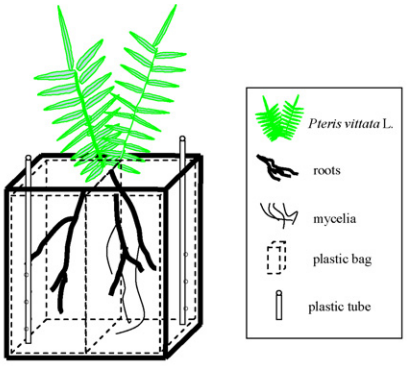
Although it has been recognized that higher plants adapted to As-polluted soils generally form mycorrhizal associations (Cairney and Meharg, 1999; Gonzalez-Chavez et al., 2004), Wu et al. (2007) were the first to conduct a field survey of AM fungal status in the rhizosphere of *P. vittata*. They found 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

by glass beads (0.8 mm diameter). The plastic cups were then placed on a saucer containing up to 2–3 cm of Hoagland 1/10 P nutrient solution. Plants were grown for 10 weeks and then harvested. The glass beads were poured out into water and fresh spores and mycelia which floated on the water surface were collected and used as inoculum. The inoculum contained approximately 70 spores per milligram mycelium as determined under the microscope.

About 100 mg inoculum were placed near the plant roots to produce the mycorrhizal treatments and 100 ml sterilized inoculum plus 5 ml mycorrhizal fungus-free filtrate from washings of the inoculum were added to produce the non-mycorrhizal treatments in order to provide a similar microflora except for the mycorrhizal fungus.

2.3. Experimental design and plant growth conditions

The experiment consisted of split-root systems with treatments consisting of all combinations of mycorrhizal inoculation and As addition (Fig. 1). Plants remained un-inoculated in both root compartments (un-inoculated treatment) or the roots in one compartment were inoculated with *G. mosseae* (partial inoculation) or the roots in both compartments were inoculated (whole inoculation) for 16 weeks. At week 15 the roots in one compartment (partial addition) or roots in both compartments (whole addition) were irrigated or not with 50 ml 1000 μmol L^{-1} As solution in the form of $Na_3AsO_4{\cdot}12H_2O$ (pH 7.0) for 1 week. Plants were grown for a further week and then harvested. Thus, there were 10 treatments (Fig. 1) in triplicate, giving a total of 30 pots.

The experiment was conducted from April to September in a growth chamber with a temperature regime of 20–25 °C and a photoperiod of 14 h using supplementary lighting (230–280 μE m^{-2} s^{-1}, reflector sunlight dysprosium lamps, DDF 400, Nanjing, China). Plants were irrigated with a 1/10 P Hoagland nutrient solution (pH 6.5) twice a week. Deionized water was used daily to maintain the moisture of the Perlite at about 60% (w/w).

2.4. Harvest and chemical analysis

Fronds and roots in the split-root compartments were harvested separately. Samples were rinsed carefully with deionized water. Portions of fresh roots were collected for determination of the percentage of root length colonized by the mycorrhizal fungus. Roots were cut into 1-cm long bundles, cleared in 10% (w/v) KOH at 90 °C for 12–15 min in a water bath, rinsed three times and then stained with Trypan blue at 90 °C for 3–5 min, stored in glycerin and measured by the gridline-intersect method (Giovannetti and Mosse, 1980). The dry weights of fronds and the remaining roots were determined after oven drying at 70 °C for 48 h. Ground sub-samples were digested in a microwave accelerated reaction system (CEM Corporation). A sub-sample of about 200 mg was weighed into a PTFE pressure vessel and 8 ml of concentrated HNO_3 were added to digest the samples. The microwave digestion program was as follows: power 1200 W, temperature 165 °C, pressure 350 × 105 Pa, and holding time 20 min. After cooling, the sample solution was transferred with deionized water and filtered into a 25-mL acid-washed plastic bottle. Arsenic was determined using an atomic fluorescence spectrometer (Model AFS-920A, Beijing Jitian Analytical Instrument Co., China) (Vilanó and Rubio, 2001) and P was determined by colorimetry using the standard vanado-molybdate method. Certified reference material (bush twigs and leaves, catalogue number GBW07603) was used to test the accuracy of the digestion and analysis procedures.

2.5. Statistical analysis

The data were subjected to analysis of variance using the SAS software package (Version 9.0; SAS Institute, Cary, NC). Two-way ANOVA was used for analysis of frond parameters with inoculation and As addition as the variables. With regard to root parameters the split-root treatment was added as the third variable. Mean values were compared using Duncan's multiple range test at the 5% level. Student's *t*-test was used to compare root parameters between the left and right root compartments.

3. Results

The percentage of root length colonized by *G. mosseae* was about 25% on average. Neither As addition nor mycorrhizal inoculation had any significant influence on root colonization rate. No AMF colonization was observed in the un-inoculated or partially inoculated treatments. In the latter treatment this indicates that spores and/or hyphae of *G. mosseae* remained in the inoculated root compartment and were not dispersed to the roots in the other compartment.

Total dry weights of fronds and roots were significantly increased by mycorrhizal colonization (Fig. 2). Frond and root dry weights of mycorrhizal plants were 1–2 times higher than those of non-mycorrhizal plants. In general, As addition had no significant effect on total dry weights of fronds or roots of mycorrhizal plants irrespective of the inoculation mode (Fig. 2a and b). In contrast, frond and root dry weights of non-mycorrhizal plants decreased by 49% and 36%, respectively when As was added to both root compartments. As additions to one compartment decreased dry weights of roots grown in the compartment when the roots were un-inoculated or only partially inoculated.

Frond and root P concentrations were not significantly affected by mycorrhizal inoculation (Fig. 3a and b). As addition generally did not affect P concentrations in fronds or roots except when As was added to both root compartments where root P concentrations of un-inoculated plants were significantly higher and those of partially inoculated plants were lower.

The effect of mycorrhizal inoculation and As addition on total P uptake of fronds and roots followed a similar pattern to their

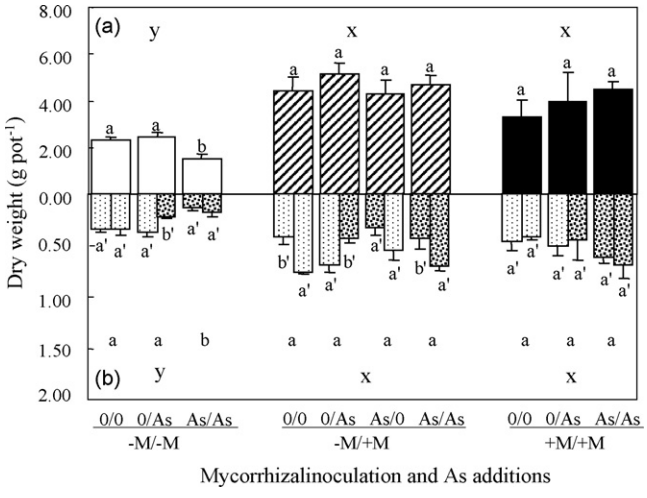


Fig. 2. Dry weight of fronds (a) and roots (b) (g pot^{-1}) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. (n = 3). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level or by Student's *t*-test. YX and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively. a′b′ indicates comparison between root dry weights in the left and right compartments.

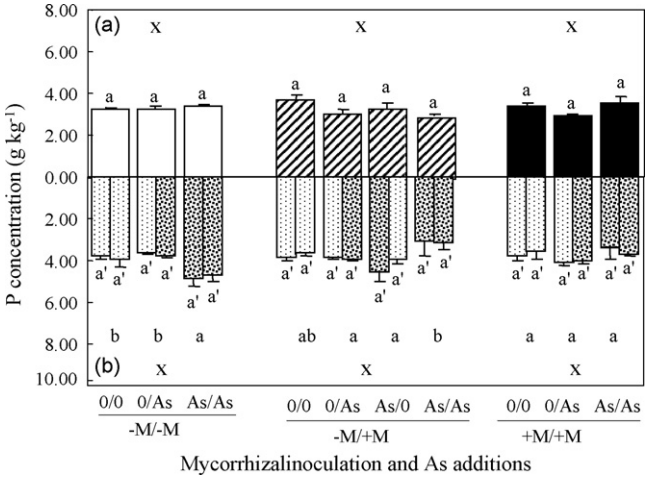


Fig. 3. Phosphorus concentrations of fronds (a) and roots (b) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. ($n = 3$). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level or by Student's *t*-test. YX and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively. a′b′ indicates comparison between root dry weights in the left and right compartments.

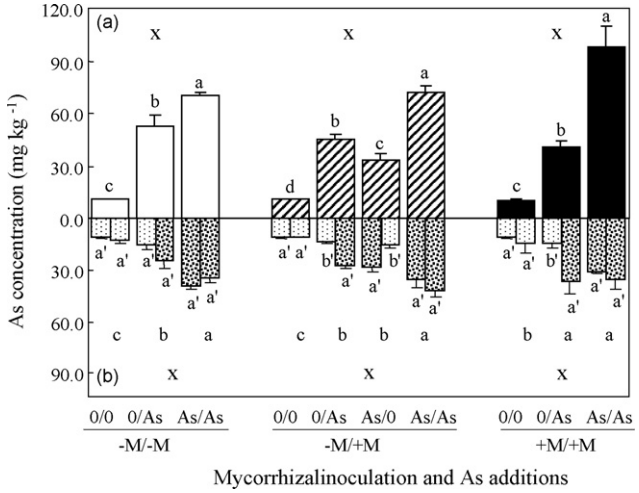


Fig. 5. Arsenic concentrations of fronds (a) and roots (b) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. ($n = 3$). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level or by Student's *t*-test. YX and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively. a′b′ indicates comparison between root dry weights in the left and right compartments.

influence on plant biomass (Fig. 4), indicating that total P uptake by plants reflected the changes in plant yield. Total P uptake of fronds and roots was almost doubled by mycorrhizal colonization. Partial inoculation (−M/+M) increased P content in roots grown in the inoculation compartment (except 0/As treatment). In general, As addition had no significant effect on total P uptake by fronds or roots of mycorrhizal plants. In contrast, P contents in fronds and roots of non-mycorrhizal plants decreased significantly (by 45% and 50%, respectively) when As was added to both compartments. As additions to one compartment tended to decrease root P uptake in that compartment when plants were un-inoculated or only partially inoculated.

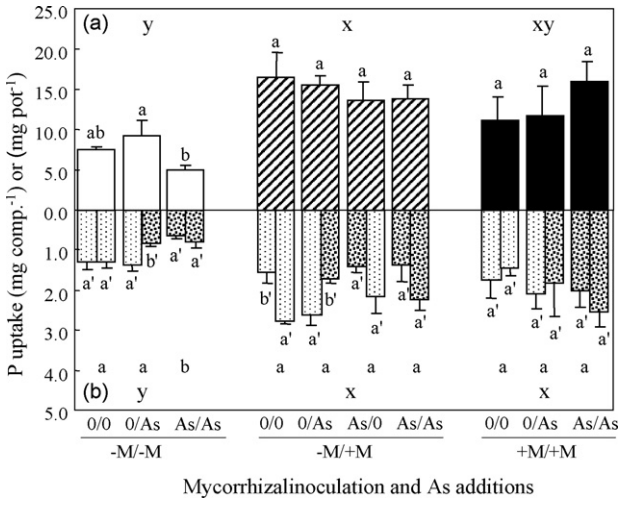


Fig. 4. Phosphorus uptake by fronds (a) and roots (b) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. ($n = 3$). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level or by Student's *t*-test. YX and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively. a′b′ indicates comparison between root dry weights in the left and right compartments.

Frond As concentrations were on average 2–3 times higher than root As concentrations in all treatments (Fig. 5). As concentrations in fronds and roots were significantly affected by As addition but not by mycorrhizal inoculation. As addition significantly increased As concentrations in both fronds and roots, and the effect was more pronounced in fronds than in roots. Irrespective of mycorrhizal inoculation, As concentrations in fronds and roots were highest when As was added to both root compartments (As/As), followed by As addition to one root compartment (0/As or As/0). The lower As concentration detected when no As was added indicates background As which may have originated from the substrate and/or the nutrient solution. In the partial inoculation treatment, As concentrations in fronds and roots were higher when As addition and mycorrhizal inoculation occurred in the same root compartment than when they were separately applied to the two compartments.

Frond As content was on average 6–25 times that of root As content (Fig. 6). Both partial and whole inoculation with *G. mosseae* significantly increased As content in fronds and roots, and the effect was more pronounced when As was added to both root compartments (As/As). In this treatment, compared with un-inoculated controls, frond As content of partially inoculated and whole inoculated plants increased by 2.2 times and 3.2 times, respectively, and the increase in root As content was within the range of 1.7–3.7 times. As content in fronds and roots was significantly increased by As addition. In the un-inoculated treatments, compared to the zero As addition treatment (0/0 As), frond As content increased by 4.6 times in the 0/As and by 3.0 times in the As/As treatment, and root As uptake increased by about 48% in both treatments. In partial or whole inoculation treatments, As uptake by fronds and roots of mycorrhizal plants was highest when As was added to both root compartments. In the partial inoculation treatment, frond As uptake was higher when As addition and mycorrhizal inoculation occurred in the same root compartment than when they were applied separately to the two root compartments.

Arsenic was distributed mainly in the fronds and the proportion of frond As to plant total As uptake was about 75–90% (data not shown). The translocation factor (TF) values (expressed as frond As concentrations/root As concentrations) were not significantly affected by mycorrhizal inoculation (Fig. 7). As addition

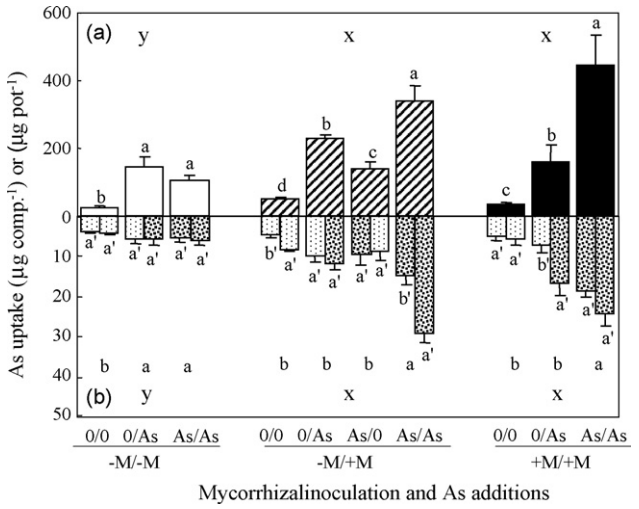


Fig. 6. Arsenic uptake by fronds (a) and roots (b) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. ($n = 3$). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level or by Student's *t*-test. YX and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively. a′b′ indicates comparison between root dry weights in the left and right compartments.

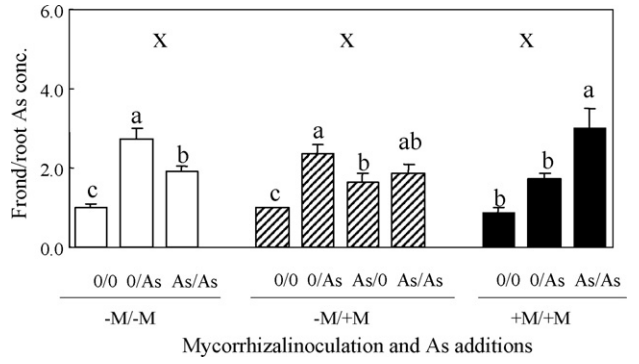


Fig. 7. Arsenic concentration ratios of fronds (a) and roots (b) of *Pteris vittata* as affected by mycorrhizal inoculation and As addition. Error bars: ±S.E. ($n = 3$). Open bars: un-inoculated in both compartments; italic bars: un-inoculated in left compartment and inoculated in right compartment; closed bars: inoculated in both compartments. Means above columns with the same letter are not significantly different by Duncan's multiple range test at the 5% level. X and ab indicate comparison among mycorrhizal inoculation and As addition treatments, respectively.

significantly increased the TF values. In un-inoculated and partially inoculated treatments, plants had the highest TF values in the 0/As treatment. In the whole inoculated treatment, TF values were the highest when As was added to both root compartments (As/As).

4. Discussion

P. vittata has been shown to be an effective arsenic hyperaccumulator, taking up As from the substrate and effectively translocating it from roots to fronds. In the present experiment, even though the plants were supplied with As solution for only 1 week, As was rapidly taken up by the plants. Frond As concentrations reached 40–100 mg kg^{-1} (Fig. 5) and As accumulated mainly in the fronds. The TF values were about 2–3 (Fig. 7), a range of values similar to the upper level (0.83–1.81) reported for *P. vittata* surveyed in the field in southeast China (Wu et al., 2007) and within the range reported for *P vittata* supplied with 133 or 276 μm Na_2HAsO_4 for 1 day or 5 days (Singh and Ma, 2006). In contrast, TF values of non-mycorrhizal *P. vittata* plants were about 50 when plants were grown in quartz sand for 45 days and fed with 15 mg kg^{-1} As. Inoculation with *G. mosseae* or *G. intraradices* increased the TF values greatly up to 730 and 292, respectively (Trotta et al., 2006). The TF values can be influenced by a wide range of plant and soil factors including soil properties (Wu et al., 2007), As supply level (Tu and Ma, 2002), plant growth period (Singh and Ma, 2006), and the associated mycorrhizas (Trotta et al., 2006). In the present experiment, inoculation with *G. mosseae* did not significantly affect the TF values and the pattern of inoculation and As addition showed a variable effect on TF values (Fig. 7). This could be due in part to the short period of exposure of *P. vittata* to the As solution and the relatively low percentage of root length colonized by the mycorrhizal fungus.

Studies have shown As(III) to be the predominant species present in the fronds of *P. vittata* and As(V) to be the main species in the roots (Tu et al., 2003; Kertulis et al., 2005). As in other plant species, arsenate was absorbed by roots of *P. vittata* via the phosphate uptake pathway (Wang et al., 2002). Since the predominant influence of AMF is to facilitate plant P uptake and mycorrhiza-induced phosphate transporters are exclusively expressed in mycorrhizal roots (Rausch et al., 2001; Harrison et al., 2002), the chemical similarity of As(V) and P(V) implies that AM fungi may make a large contribution to plant As uptake in a similar fashion to plant P uptake, providing that AMF do not display selective uptake of P over As. In this case, inoculation with AMF would be expected to result in greater As uptake by host plants and this might increase the efficiency of phytoremediation of As contaminated soils. On the other hand, if AMF preferentially take up P over As or As is retained in the fungal mycelium and/or in roots and fungal tissues, AMF may decrease As accumulation by the plant and thus confer increased plant tolerance to As. Some studies have indicated that increased As accumulation in *P. vittata* due to AMF is closely linked with AMF mediated enhanced plant P uptake and the consequent P/As ratios (Liu et al., 2005; Leung et al., 2006). Our results are partly in agreement with this. As contents in shoots and roots were significantly increased by inoculation with *G. mosseae* (Fig. 6), together with enhanced plant growth (Fig. 2) and increased P uptake (Fig. 4). However, unlike some published studies listed above, P concentration (Fig. 3) and/or P/As ratios (data not shown), and As concentrations in fronds and roots (Fig. 5) were not significantly affected by AMF in the present experiment. Similarly Trotta et al. (2006) reported that frond As concentration was not affected by inoculation with *G. mosseae* or *G. margarita*, and significantly lower As concentrations in roots of *P. vittata* resulted in substantially higher TF values in mycorrhizal plants. In another study, growth depression and reduced As concentrations and contents in mycorrhizal *P. vittata* plants (Chen et al., 2006) were presumably due to toxicity of another metal (U) in the substrate. The authors suggested that increased P over As might have increased plant tolerance to As toxicity. A recent study showed that arsenite was the main form translocated from roots to fronds, implying that P and As might follow different pathways within the plants (Su et al., 2008). Results from other studies and the present experiment indicate that interactions among AMF, As and P uptake by *P. vittata* plants is very interesting but can be very complicated and dependent on the experimental conditions. Clearly, more work is required to elucidate fully the underlying mechanisms.

In a recent study Chen et al. (2007) found that *G. mosseae* may protect alfalfa shoots from As toxicity by 'dilution effects' resulting from growth stimulation of AM plants and reduced portioning of As to shoots. In the present experiment partial inoculation with AMF and partial short-term supply of As allowed us to compare plant As uptake under conditions where plant growth and plant P nutritional level were comparable. Neither plant growth nor plant P concentration differed significantly among treatments −M/+M (0/As), −M/+M (As/0), and +M/+M (0/As), or between treatments −M/+M

(As/As) and +M/+M (As/As) (Figs. 2 and 3). Shoot As concentrations and contents were significantly higher in −M/+M (0/As) compared to treatment −M/+M (As/0), and in +M/+M (As/As) tended to be higher than in −M/+M (As/As) although the difference was not significant (Figs. 5 and 6). In this case As uptake and accumulation in *P. vittata* appeared to be enhanced by inoculation with *G. mosseae*. However, in contrast there was no increase in shoot As content when both parts of the root systems were inoculated with *G. mosseae* (e.g. +M/+M (0/As)) compared to partial inoculation (−M/+M (0/As, As/0), Fig. 6). These results indicate that the regulation of As uptake and translocation of As within *P. vittata* by AMF can be complicated. Results from current limited information about *P. vittata* associations (including the present experiment) and those from studies carried out using other plant species show that the interactions between AMF and As uptake may follow multiple mechanisms. The AMF-mediated effect on As uptake and accumulation in plants may vary with plant species, soil conditions, As supply level or fungal isolates inoculated. For example, recent work by Jankong and Visoottiviseth (2008) showed that inoculation with a mixture of *G. mosseae*, *G. intraradices* and *Glomus etunicatum* lowered As accumulation but had no significant effect on growth of the As hyperaccumulator *Pityrogramma calomelanos* or the non-hyperaccumulator *Tagetes erecta*, but growth and As accumulation in the non-hyperaccumulator *Melastoma malabathricu* was greatly enhanced by AMF. Mycorrhizal plants and/or hyphal mycelium can modify plant uptake of As by means of changes in the biotransformation of As at the interface between roots and rhizosphere soil (Ultra et al., 2007a,b), retention of As in external mycelium and/or possibly increased efflux of As (as arsenite) from mycorrhizal roots (Wang et al., 2008; Ultra et al., 2007b), downregulation of arsenate/phosphate transporters in the epidermis and root hairs (Meharg and Macnair, 1992; Gonzalez-Chavez et al., 2002) to reduce As uptake and/or upregulation of low affinity P transporters located in the membrane of mycorrhizal roots to increase P uptake (Harrison et al., 2002), and alteration of the translocation of As from roots to shoots (Dong et al., 2008; Trotta et al., 2006). Whether or not these mechanisms also apply to *P. vittata* mycorrhizal associations and under what conditions they will function effectively will require further investigation.

Heavy metal(loid)s are commonly heterogeneously distributed in soils (Keller et al., 2003). Studies on the responses of hyperaccumulators to heterogeneous metal(loid) supply are extremely limited compared to the large number of reports on plant responses to patchy nutrient supply (mainly N and P), even though hyperaccumulators are very effective in taking up heavy metals from soils. The sole study on *P. vittata* showed that plants acquired twice as much As when growing in heterogenous rather than in homogeneous conditions (Caille et al., 2003). Hyperaccumulators such as *T. caerulescens* respond positively to localized Zn enrichment by changing root traits (Schwartz et al., 1999) and/or increasing plant Zn uptake (Whiting et al., 2000). In the present experiment As concentrations increased in roots when portions of the root system were exposed to As (partial As addition, 0/As or As/0) irrespective of inoculation with *G. mossesse* (Fig. 5). However, this did not lead to increased As uptake in fronds and roots compared to treatment in which both root compartments were supplied with As (As/As), and the former was significantly lower than the latter (Fig. 6). This indicates that the total amount of As supplied into the substrate influences As uptake by *P. vittata*. The size, strength and position of the nutrient supply have been shown to have significant effects on plant responses to nutrients supplied in patches (Hodge et al., 1999; Hutchings and Wijesinghe, 2008). In the present experiment plants were exposed to As for only 1 week. Therefore it remains to be tested whether morphological and physiological plasticity of roots and/or root associated AMF reported in other plant species in response to N and P patches (Fitter, 1994; Hodge, 2006) can also help *P. vittata* plants to profit from As enriched sites in terms of As capture and uptake. In the present experiment, in contrast to partial As addition, plant growth (Fig. 2) and concentrations and contents of P (Figs. 3 and 4) and As (Figs. 5 and 6) in shoots and roots generally did not differ between partial inoculation and whole inoculation treatments, implying that partial inoculation with AMF can be as effective as whole inoculation with respect to plant As uptake.

The indigenous AMF associated with the dominant plant species growing on mine sites is suggested to assist plant survival in soils that are substantially polluted with toxic metals (Leung et al., 2007). Although the fungal isolate used in the present study did not originate from As contaminated soils, a field survey showed that *G. mosseae* was one of the most common species associated with *P. vittata* (Wu et al., 2007). In conclusion our results show that AMF can enhance growth and increase As uptake by *P. vittata* plants and may therefore have great potential for clean-up of As contaminated soil by using hyperaccumulators such as *P. vittata*. Since partial root inoculation seems to be as effective as whole inoculation with AMF in terms of enhancing plant growth and plant As uptake, the amount of inoculum applied and possibly the inoculation technique should also be considered when applying mycorrhizal inoculation to *P. vittata* in enhancing phytoremediation of As contaminated sites. However, our results also indicate that more work needs are required to elucidate the underlying mechanisms by which AMF mediate As uptake by *P. vittata*.

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