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Short communication

The arbuscular mycorrhizal fungus *Glomus mosseae* can enhance arsenic tolerance in *Medicago truncatula* by increasing plant phosphorus status and restricting arsenate uptake

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G. mosseae was more tolerant than *M. truncatula* to As and may have conferred enhanced host tolerance by restricting root As uptake and enhancing P nutrition.

Abstract

A pot experiment examined the biomass and As uptake of *Medicago truncatula* colonized by the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* in low-P soil experimentally contaminated with different levels of arsenate. The biomass of *G. mosseae* external mycelium was unaffected by the highest addition level of As studied (200 mg kg⁻¹) but shoot and root biomass declined in both mycorrhizal and non-mycorrhizal plants, indicating that the AM fungus was more tolerant than *M. truncatula* to arsenate. Mycorrhizal inoculation increased shoot and root dry weights by enhancing host plant P nutrition and lowering shoot and root As concentrations compared with uninoculated plants. The AM fungus may have been highly tolerant to As and conferred enhanced tolerance to arsenate on the host plant by enhancing P nutrition and restricting root As uptake.

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Keywords: Arbuscular mycorrhiza; Arsenate; *Glomus mosseae*; *Medicago truncatula*; Phosphate uptake

1. Introduction

Plants growing on arsenic polluted soils tend to be mycorrhizal (Meharg and Hartley-Whitaker, 2002) and mycorrhizal fungi may therefore be important in re-vegetation or phytostabilization. *Hymenoscyphus ericae*, an ericoid mycorrhizal fungus, has co-evolved with its host plant, *Calluna vulgaris*, to resist As toxicity (Sharples et al., 2000a,b,2001). Al Agely et al. (2005) reported a pot experiment in which arbuscular mycorrhizal fungi (AMF) increased As uptake by Chinese

brake fern (*Pteris vittata*), suggesting that plant mycorrhizal status should be considered for effective phytoremediation.

It is well established that AMF can enhance host plant P uptake under limited P availability (Smith and Read, 1997). Arsenate and phosphate show similar behaviour, e.g. both compete for the same sorption sites on soil particles (Adriano, 2001). Uptake of As and P by plants and microorganisms is shown to be mediated by the same transporters (Asher and Reay, 1979; Willsky and Malamy, 1980a,b; Meharg and Macnair, 1990,1992; Bun-ya et al., 1996; Rosen, 2002), and thus enhanced plant acquisition of P may also lead to enhanced acquisition of As. Gonzalez-Chavez et al. (2002) reported enhanced arsenate resistance of both tolerant and non-tolerant plants colonized by AMF associated with suppression of high-affinity arsenate/phosphate transporters and decreasing arsenate uptake. Ahmed et al. (2006) showed that inoculation

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with *G. mosseae* lowered As concentrations in shoots and roots of lentil and the fungus may have promoted P uptake but excluded arsenic. The mechanisms by which AM fungi influence the relationship between arsenate resistance and arsenate/phosphate uptake are yet to be elucidated (Meharg and Hartley-Whitaker, 2002). Here, we attempted to acquire more information on the possible enhancement of plant arsenate tolerance by AM colonization.

2. Materials and methods

2.1. Plants, AM fungus and soil

Seeds of *Medicago truncatula* cv. Paraggio were surface sterilized by immersion in 30% v/v H₂O₂ for 10 min, rinsed with deionized water three times and germinated on moist filter paper at 20 °C. After two days, seedlings with radicles ~1–2 mm long were transplanted into 10-cm-diameter pots. *Glomus mosseae* BEG 167 was prepared by growing maize (*Zea mays* L.) and red clover (*Trifolium pratense* L.) in a low-P soil. The soil, a sandy clay loam with low Olsen P from the A horizon at Tangshan, Hebei province, north China, had the following properties: pH (in H₂O) 7.4, 8.24 g organic C kg⁻¹, 0.83 g Kjeldahl N kg⁻¹, 6.4 mg Olsen P kg⁻¹, and 9.87 mg total As kg⁻¹. Soil was sieved (<2 mm), autoclaved at 121 °C for 2 h and then air-dried before use. The dry soil was amended with 200 mg N kg⁻¹ (as NH₄NO₃) and 171 mg K kg⁻¹ (as K₂SO₄).

2.2. Experimental setup

The experimental treatments consisted of five addition levels of As (0, 10, 50, 100 and 200 mg kg⁻¹) as Na₃AsO₄ · 12H₂O and As was applied to the soil in aqueous solution. Soils were then maintained in plastic bags to equilibrate for three weeks prior to seedling transplantation. Plants were inoculated with *G. mosseae*-colonized roots and spores from pot culture and uninoculated controls received filtered inoculum washings. There were four replicate pots per treatment in a randomized block design, giving a total of 40 pots. Each pot received 800 g soil, 100 g inoculum (inoculated treatment) or autoclaved inoculum (uninoculated treatment) was placed on the soil surface, and a further 100 g soil was placed over the inoculum. Eight seedlings were transplanted in each pot and sand (120 g pot⁻¹) was placed on the soil surface. The experiment was conducted from 6 December 2003 to 11 February 2004 in a glasshouse in Beijing at 22–28 °C without supplementary illumination. Deionized water was added by weighing when water loss was approximately 10% of total pot weight.

2.3. Plant harvest and chemical analysis

Shoots and roots were harvested separately after two months' growth. Root systems were collected by hand and washed carefully with tap water and deionized water. Nodules were occasionally found on both mycorrhizal and non-mycorrhizal plant roots but their frequency of occurrence was very low (<5%). A randomly-selected portion of fresh roots from each pot was stained with Trypan blue (Phillips and Hayman, 1970) and both root length and the percentage of root length colonized were determined using the grid-line intersect method of Giovannetti and Mosse (1980). Shoots and roots were weighed after oven drying at 70 °C for 72 h and ground sub-samples (0.1–0.2 g) were digested with 5 ml 4:1 (v/v) HNO₃/HClO₄ for P and As analysis. Phosphorus was determined by the molybdenum blue method (Allen, 1989) and As using a Model AFS-920 Atomic Fluorescence Spectrometer (Beijing Titan Instruments Co. Ltd.). The extraradical mycelium was extracted from three replicate ~7-g sub-samples of soil from each pot using the aqueous membrane filtration technique (Boddington et al., 1999) and hyphal length was estimated using a modified membrane filter technique (Jakobsen et al., 1992).

2.4. Statistical analysis

Data were tested for normality using the Shapiro–Wilk test and the significance of treatment effects was analyzed by one- or two-way analysis

Table 1

Proportion of root length colonized, root length, AMF hyphal density in soil and AMF hyphal length per unit root length of *M. truncatula* colonized by *G. mosseae*

As addition level (mg kg ⁻¹)	Colonization (%)	Root length (m pot ⁻¹)	AMF hyphal density in soil (m g ⁻¹)	AMF hyphal length per unit root length (m m ⁻¹)
0	55.0 ± 6.7	85.2 ± 7.0a	0.397 ± 0.229	4.14 ± 2.45b
10	59.7 ± 8.4	98.8 ± 25.9a	0.753 ± 0.276	7.99 ± 3.01b
50	50.6 ± 11.3	92.8 ± 11.3a	0.745 ± 0.210	7.95 ± 1.74b
100	53.1 ± 5.8	77.2 ± 6.8ab	0.805 ± 0.389	10.40 ± 5.06b
200	46.4 ± 17.7	26.4 ± 5.7b	0.720 ± 0.192	27.89 ± 8.67a
Significance ^a	ns	P<0.001	ns	P<0.001

Data are presented as mean values ± SD (*n* = 4) and have been analyzed by one-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan's multiple range test at the 5% level.

^a By one-way analysis of variance; ns, not significant.

of variance using the SAS software package (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

Arsenate addition did not inhibit AM colonization of *M. truncatula* roots and the mean percentage of root length colonized was 53.0% whilst no colonization was observed in uninoculated plants (Table 1). Root length decreased markedly at the highest As application rate (200 mg kg⁻¹) but the hyphal density of *G. mosseae* showed no corresponding change. Consequently, AM hyphal length per unit root length increased substantially at this As level. In contrast, shoot and root dry matter biomass of *M. truncatula* decreased when the As addition level was >10 mg kg⁻¹ (Fig. 1) but root

Fig. 1. Mean shoot (a) and root (b) dry weight of non-mycorrhizal and mycorrhizal *M. truncatula* colonized by *G. mosseae*. Error bars: ±SD. Data have been analyzed by two-way analysis of variance.

colonization and hyphal density were unaffected even at an As application rate of 200 mg kg^{-1} (Table 1), indicating that the AM fungus was more tolerant to arsenate than was its host plant. Shoot and root dry weights of mycorrhizal plants were significantly higher than those of non-mycorrhizal controls at all As additional levels ($P < 0.001$). At 200 mg kg^{-1} added As, growth of non-mycorrhizal plants was poor but all plants survived. Growth of mycorrhizal plants was much greater than that of the non-mycorrhizal controls (Fig. 1). Our results are in agreement with other studies which have also reported higher tolerance of AMF to As as indicated by enhanced growth of mycorrhizal plants compared with non-mycorrhizal plants (Gonzalez-Chavez et al., 2002; Ahmed et al., 2006; Leung et al., 2006; Trotta et al., 2006; Chen et al., 2007).

Adding arsenate to the soil increased the As concentrations in shoots and roots of both mycorrhizal and non-mycorrhizal plants and the trend was less pronounced in plants inoculated with *G. mosseae* (Fig. 2). At and above

50 mg kg^{-1} added As, shoot As concentrations in both mycorrhizal and non-mycorrhizal plants remained constant but root As concentrations were elevated with increasing added As (Fig. 2). Root As concentrations were much higher than shoot concentrations regardless of plant mycorrhizal status. Our results support the conclusion that limiting the translocation of arsenate from roots to shoots is an effective mechanism involved in plant As tolerance (Meharg and Macnair, 1991). The substantial accumulation of As in roots may be phytotoxic because arsenate, an analogue of phosphate, disturbs the metabolism of phosphorus, for instance by interfering with ATP synthesis by forming unstable ADP-As. Increasing cytoplasmic P may also enable phosphate to compete more effectively with arsenate to decrease arsenate toxicity (Meharg, 1994). To relieve stress from root As accumulation, non-mycorrhizal plants maintain root P concentrations at the cost of decreasing shoot P concentrations (Fig. 3), whereas mycorrhizal plants readily

Significance due to	Shoots	Roots
Inoculation	$P < 0.001$	$P < 0.001$
As level	$P < 0.001$	$P < 0.001$
Interaction	$P < 0.001$	$P < 0.001$

Fig. 2. Mean shoot and root As concentrations of non-mycorrhizal and mycorrhizal *M. truncatula* colonized by *G. mosseae*. Error bars: $\pm$ SD. Data have been analyzed by two-way analysis of variance.

Significance due to	Shoots	Roots
Inoculation	$P < 0.001$	$P < 0.05$
As level	$P < 0.001$	$P < 0.01$
Interaction	$P < 0.001$	ns

Fig. 3. Mean shoot and root P concentrations of non-mycorrhizal and mycorrhizal *M. truncatula* colonized by *G. mosseae*. Error bars: $\pm$ SD. Data have been analyzed by two-way analysis of variance.

maintain root P concentrations without reducing shoot P concentrations.

Improving plant P nutrition is regarded as an important mechanism by which mycorrhizal plants withstand high As concentrations (Ahmed et al., 2006; Leung et al., 2006; Trotta et al., 2006; Ultra et al., 2007). Our results agree with this. The AMF enhanced shoot P concentrations, shoot and root P contents and plant total P contents at all As addition levels (Table 2). At the highest (200 mg kg⁻¹) As addition level the mean shoot and root P concentrations of mycorrhizal plants were 3.4 and 1.4 times higher than those of non-mycorrhizal controls and the corresponding values for shoot and root P contents were 25 and 8 times. In contrast, shoot and root As concentrations in mycorrhizal plants in soil amended with >10 mg As kg⁻¹ tended to be only half of the values for corresponding non-mycorrhizal controls (Fig. 2). In addition, specific shoot P uptake of mycorrhizal plants was significantly higher than that of non-mycorrhizal controls at all As addition levels but As showed the opposite trend (Table 3). Specific P uptake of non-mycorrhizal plants decreased with increasing As addition level but that of mycorrhizal plants remained relatively stable. Consequently, AM colonization increased the shoot and root P:As content ratio, especially at higher soil As levels (Table 2). It can be inferred that the AM fungus can enhance host plant P nutrition while

Table 3

Specific P and As uptake of shoots of non-mycorrhizal and mycorrhizal *M. truncatula*

Added As (mg kg ⁻¹)	Specific P uptake (mg g ⁻¹ root)		Specific As uptake (μg g ⁻¹ root)	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
0	6.54 ± 1.66a	9.86 ± 1.79a	4.11 ± 0.49d	2.27 ± 0.81c
10	6.18 ± 0.48a	9.01 ± 1.56ab	8.23 ± 3.34d	6.21 ± 2.36c
50	5.99 ± 0.75a	8.47 ± 0.71ab	54.54 ± 5.60c	28.14 ± 5.77b
100	4.08 ± 0.68b	7.45 ± 0.22b	72.69 ± 9.95b	30.16 ± 8.34b
200	3.54 ± 0.15b	10.45 ± 1.80a	97.33 ± 15.32a	70.37 ± 13.81a
Inoculation	<i>P</i> < 0.001		<i>P</i> < 0.001	
As level	<i>P</i> < 0.01		<i>P</i> < 0.001	
Interaction	<i>P</i> < 0.01		<i>P</i> < 0.001	

Data are presented as mean values ± SD (*n* = 4) and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan's multiple range test at the 5% level.

suppressing plant As uptake, and this agrees with a recent study by Chen et al. (2007). Plants take up arsenate via the phosphate uptake system (Asher and Reay, 1979; Meharg and Macnair, 1990, 1992) and the suppression of high affinity P uptake therefore assists plants to enhance arsenate tolerance (Meharg and Macnair, 1992). Interestingly, AMF can

Table 2

P and As contents and molar P/As content ratios of non-mycorrhizal and mycorrhizal *M. truncatula* colonized by *G. mosseae*

Added As (mg kg ⁻¹)	P content (mg pot ⁻¹)		As content (μg pot ⁻¹)		P/As content ratio (mol P/mmol As)	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
<i>Shoots</i>						
0	4.77 ± 0.13a	7.94 ± 0.16b	0.38 ± 0.13d	0.42 ± 0.12c	33.07 ± 12.17a	49.11 ± 15.26a
10	4.62 ± 0.20a	8.63 ± 0.40a	1.53 ± 0.11c	1.82 ± 0.51b	7.34 ± 0.81b	11.99 ± 2.52b
50	2.61 ± 0.23b	7.72 ± 0.34b	5.60 ± 0.82a	5.33 ± 0.85a	1.16 ± 0.27b	3.56 ± 0.55b
100	1.01 ± 0.30c	5.55 ± 0.06c	2.81 ± 0.67b	4.22 ± 1.39a	0.88 ± 0.22b	3.40 ± 0.91b
200	0.14 ± 0.03d	3.50 ± 0.36d	0.76 ± 0.12d	2.66 ± 0.74b	0.47 ± 0.16b	3.31 ± 0.64b
Inoculation	<i>P</i> < 0.001		<i>P</i> < 0.01		<i>P</i> < 0.01	
As level	<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.001	
Interaction	<i>P</i> < 0.001		<i>P</i> < 0.05		ns	
<i>Roots</i>						
0	1.12 ± 0.25ab	1.52 ± 0.22ab	3.06 ± 1.26b	2.03 ± 0.65b	0.98 ± 0.36a	1.96 ± 0.69a
10	1.36 ± 0.61a	1.64 ± 0.19ab	6.50 ± 3.54b	5.18 ± 1.73b	0.56 ± 0.20b	0.85 ± 0.36b
50	0.82 ± 0.13bc	1.74 ± 0.26a	25.98 ± 3.86a	26.01 ± 5.61a	0.08 ± 0.01b	0.17 ± 0.04c
100	0.48 ± 0.10cd	1.33 ± 0.36bc	23.08 ± 2.09a	23.75 ± 9.29a	0.05 ± 0.01b	0.14 ± 0.03c
200	0.12 ± 0.03d	1.01 ± 0.18c	6.47 ± 2.19b	28.18 ± 7.41a	0.04 ± 0.01b	0.09 ± 0.02c
Inoculation	<i>P</i> < 0.001		<i>P</i> < 0.05		<i>P</i> < 0.01	
As level	<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.001	
Interaction	ns		<i>P</i> < 0.001		<i>P</i> < 0.05	
<i>Total</i>						
0	5.95 ± 0.25a	9.46 ± 0.32b	3.44 ± 1.36d	2.45 ± 0.72b	4.55 ± 1.54a	10.14 ± 3.65a
10	5.98 ± 0.69a	10.27 ± 0.58a	8.04 ± 3.53c	7.00 ± 2.04b	2.06 ± 0.87b	3.81 ± 1.28b
50	3.43 ± 0.18b	9.46 ± 0.60b	31.58 ± 4.63a	31.34 ± 5.09a	0.27 ± 0.05b	0.74 ± 0.11c
100	1.49 ± 0.39c	6.88 ± 0.33c	25.88 ± 2.12b	27.97 ± 8.26a	0.14 ± 0.03b	0.64 ± 0.20c
200	0.26 ± 0.05d	4.51 ± 0.44d	7.23 ± 2.25cd	30.84 ± 7.17a	0.09 ± 0.02b	0.37 ± 0.11c
Inoculation	<i>P</i> < 0.001		<i>P</i> < 0.01		<i>P</i> < 0.001	
As level	<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.001	
Interaction	<i>P</i> < 0.001		<i>P</i> < 0.001		<i>P</i> < 0.01	

Data are presented as mean values ± SD (*n* = 4) and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan's multiple range test at the 5% level.

downregulate high-affinity phosphate transporters (MtPT1 and MtPT2) which may be responsible for absorption of phosphate from soil by *M. truncatula* (Liu et al., 1998; Chiou et al., 2001; Burleigh, 2001). Furthermore, studies on the kinetics of phosphate/arsenate uptake have suggested that AMF can downregulate phosphate/arsenate transporters in both arsenate resistant and non-resistant *Holcus lanatus* (Gonzalez-Chavez et al., 2002). Recent studies have established that the mycorrhizal pathway, in contrast with the direct root uptake pathway, can dominate plant P uptake, so that most (perhaps almost all) of the plant P comes from AMF (Smith et al., 2003). This implies that the probability of arsenate entering plants directly through the phosphate uptake system may decrease greatly. Taking together the studies by Burleigh (2001) and Smith et al. (2003) an interesting relationship between the capacities of AMF to provide P nutrition and downregulate the phosphate transporter can be suggested. Thus, for a particular AMF, the more P nutrition supplied, the more strongly the transcription of the phosphate transporter may be suppressed. More work is required to clarify the mechanisms by which AMF regulate P-As uptake by plants and enhance plant tolerance to As stress. In a recent study Ultra et al. (2007) reported that AMF seemed to be involved in the transformation of inorganic As into less toxic organic forms which consequently led to decreased As uptake by sunflower plants.

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