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The arbuscular mycorrhizal fungus *Glomus mosseae* gives contradictory effects on phosphorus and arsenic acquisition by *Medicago sativa* Linn

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Abstract

Mycorrhizal fungi may play an important role in protecting plants against arsenic (As) contamination. However, little is known about the direct and indirect involvement of arbuscular mycorrhizal fungi (AMF) in detoxification mechanisms. A compartmented pot cultivation system ('cross-pots') is used here to investigate the roles of AMF *Glomus mosseae* in plant phosphorus (P) and As acquisition by *Medicago sativa*, and P–As interactions. The results indicate that fungal colonization dramatically increased plant dry weight by a factor of around 6, and also substantially increased both plant P and As contents (i.e. total uptake). Irrespective of P and As addition levels, AM plants had shoot and root P concentrations 2 fold higher, but As concentrations significantly lower, than corresponding uninoculated controls. The decreased shoot As concentrations were largely due to "dilution effects" that resulted from stimulated growth of AM plants and reduced As partitioning to shoots. The study provides further evidence for the protective effects of AMF on host plants against As contamination, and have uncovered key aspects of underlying mechanisms. The possible application of AMF in remediation practices is discussed.

Keywords: Alfalfa; Arbuscular mycorrhiza; Phosphorus; Arsenic

1. Introduction

Arsenic (As) is ubiquitous in the environment, and is highly toxic to most biological systems. Elevated As concentrations in soils have been found in areas impacted by mining and smelting industries, and by coal burning throughout the world (Nriagu, 1994). The application of As-containing agrochemicals to domestic and agricultural land has also caused accumulation of As in soils (Woolson et al., 1971; Murphy and Aucott, 1998). Plant uptake of As from contaminated soils represents a significant pathway of human exposure. Understanding plant uptake metabolism of As is thus critical for estimating the risks associated with soil As contamination and for formulating countermeasures to minimize the accumulation of As in plants consumed directly by humans, farm animals or wildlife (Meharg and Hartley-Whitaker, 2002).

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Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with the vast majority of land plants, and are known to benefit the phosphorus (P) nutrition of host plants by increasing P acquisition (Smith and Read, 1997). The pathway includes 1) P uptake by extensive and highly branched extraradical hyphae, 2) translocation via hyphae towards plant roots, 3) release of Pi at the arbuscular interface and efficient transfer of Pi to root cells. Arsenate, the dominant As species under aerobic conditions, is known to be an analogue of phosphate (Pi), and Pi can effectively inhibit plant uptake of arsenate (Meharg and Macnair, 1991, 1992). Due to the similarity between Pi and arsenate, understanding the role of AMF in plant uptake of arsenate is essential in predicting the biogeochemistry of As in the terrestrial environment.

In recent studies of the role of mycorrhizal fungi in adaptation of host plants to As-contaminated soils, Sharples et al. (2000a,b) reported that the key mechanism by which the ericoid mycorrhizal fungus *Hymenoscyphus ericae* could improve resistance of *Calluna vulgaris* to As toxicity was through As exclusion. In a similar manner to bacteria and yeasts (Rosen, 1999), the fungus achieves arsenate resistance by reducing arsenate to arsenite, and pumping arsenite out of the fungal cells (Sharples et al., 2000a,b). In a more recent study on arsenate resistance of *Holcus lanatus* conferred by AMF, Gonzalez-Chavez et al. (2002) found that regardless of their arsenate resistance, the AMF strains tested could all reduce arsenate influx into *H. lanatus* plant roots. Decreased As concentration but increased As content in the As hyperaccumulating fern *Pteris vittata* due to colonization by AMF was observed by Liu et al. (2005). There are two possible reasons for AMF-mediated arsenate resistance: 1) AMF colonization may down-regulate the high-affinity Pi transport system in plant roots that also absorbs arsenate; 2) AMF may increase the efflux of As (as arsenite) from mycorrhizal roots. However, overall information on AMF-As interactions is very limited and more studies are needed, particularly to reveal the underlying mechanisms of protective effects of AMF on host plants under As contamination, and to look further into the mycorrhiza-mediated P–As interactions.

By using a compartmented ('cross-pot') cultivation system based on the design of Pearson and Jakobsen (1993), the present study aimed to 1) reveal direct and indirect involvement of AMF in As uptake by plants, 2) compare P and As uptake via the mycorrhizal pathway and their possible interactions, 3) elucidate the underlying mechanisms of protective effects of AMF on host plants, and 4) discuss the possible use of AMF in remediation of As-contaminated soil environments.

2. Materials and methods

2.1. Host plants and AM fungus

Seeds of *Medicago sativa* Linn. cv. Chuangxin were pre-germinated on moist filter paper for about 36 h and were selected for uniformity before sowing. Plants were grown without or with inoculum of the AM fungus *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. The original inoculum was procured with the courtesy of Prof. Y.S. Wang of the Institute of Plant Nutrition and Fertilizers (IPNF), Beijing Academy of Agronomy and Forestry with an inventory number Glm93 at the laboratory of the IPNF. Inoculum from pot cultures of sorghum plants was a mixture of spores, mycelium, sand and root fragments containing approximately 1000 spores per 100 g.

2.2. Cultivation system

The compartmented cultivation system ('cross-pot') consists of a standing PVC tube (5.7 cm diameter × 25 cm long) for plant growth (root and hyphae compartment, RHC) and two side-arms (4.2 cm diameter × 8 cm long) fixed tightly to the standing tube. The side-arms are separated from the RHC by 37 μm nylon meshes that allow only hyphal penetration. In one side-arm, two phosphorus (KH_2PO_4-P) addition levels (25 and 100 mg P kg^{-1}) (hyphal compartment with P added, HCP), and in the opposite one, three arsenic ($Na_3AsO_4 \cdot 12H_2O$ As) addition levels (0, 25 and 100 mg As kg^{-1}) were arranged (hyphal compartment with As added, HCA) (Fig. 1).

2.3. Growth medium

A calcareous loamy soil was collected from the experimental station of the Institute of Genetics and Developmental Biology, the Chinese Academy of Sciences, Changping County, Beijing, China. The soil had a pH of 8.53 (1: 2.5 soil to water), extracs P content of 11.35 mg kg^{-1} (using the methods described by Olsen et al., 1954) and extractable As of 0.25 mg kg^{-1} (extracted by 0.5 mol L^{-1} $NaHCO_3$). The soil was passed through a 2 mm sieve and partially sterilized (10 kGy, 10 MeV electron beam).

2.4. Experimental procedure

Each side-arm of the cross-pot was filled with 125 g sterilized loamy soil with P or As addition, then with 30 g soil without any amendments to establish a buffer layer and minimize possible movement of added P or As