Chemosphere 119 (2015) 224–230

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Arbuscular mycorrhizal symbiosis influences arsenic accumulation and speciation in *Medicago truncatula* L. in arsenic-contaminated soil

Xin Zhang [1], Bai-Hui Ren [1], Song-Lin Wu, Yu-Qing Sun, Ge Lin, Bao-Dong Chen *

State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

HIGHLIGHTS

- DMA was detected only in shoots of mycorrhizal plants.
- Mycorrhizal inoculation increased the percentage of As(III) in total As in plants.
- AM fungi are potentially involved in As transformation in the plant-soil continuum.

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 12 June 2014
Accepted 14 June 2014
Available online 11 July 2014

Handling Editor: I. Cousins

Keywords:
Arbuscular mycorrhizal fungi
Arsenic speciation
Phosphorus
Medicago truncatula
Mutant

ABSTRACT

In two pot experiments, wild type and a non-mycorrhizal mutant (TR25:3-1) of *Medicago truncatula* were grown in arsenic (As)-contaminated soil to investigate the influences of arbuscular mycorrhizal fungi (AMF) on As accumulation and speciation in host plants. The results indicated that the plant biomass of *M. truncatula* was dramatically increased by AM symbiosis. Mycorrhizal colonization significantly increased phosphorus concentrations and decreased As concentrations in plants. Moreover, mycorrhizal colonization generally increased the percentage of arsenite in total As both in shoots and roots, while dimethylarsenic acid (DMA) was only detected in shoots of mycorrhizal plants. The results suggested that AMF are most likely to get involved in the methylating of inorganic As into less toxic organic DMA and also in the reduction of arsenate to arsenite. The study allowed a deeper insight into the As detoxification mechanisms in AM associations. By using the mutant *M. truncatula*, we demonstrated the importance of AMF in plant As tolerance under natural conditions.

1. Introduction

Arsenic (As) is ubiquitous in the lithosphere, hydrosphere and biosphere (Cullen and Reimer, 1989), and its wide use in many industries has caused soil contamination worldwide (Smith et al., 1998; Meharg and Hartley-Whitaker, 2002). Excessive As in the soil not only affects plant growth, but also poses a great threat to human health and ecological safety as a consequence of uptake by crops and migration into water (Fitz and Wenzel, 2002; Meharg, 2004).

The environmental chemistry of As is quite complex, and As may present in both inorganic forms such as arsenate [As(V)] and arsenite [As(III)], and organic forms such as monomethylarsonic acid (MAA), dimethylarsinic acid (DMA) and other forms in soils and plants (Quaghebeur and Rengel, 2005). Different As species exhibit different chemo-physical traits and therefore have different mobility, bioavailability and toxicity. Usually soluble inorganic As is more toxic than organic species, and As(III) is more toxic than As(V) (Fitz and Wenzel, 2002; Meharg and Hartley-Whitaker, 2002; Chakrabarty et al., 2009). As(V), as the main As species in aerobic soils, once absorbed, can be rapidly reduced to As(III) (Duan et al., 2005). Detoxification mechanisms of As(III) in plants include efflux from the roots, sequestration in an non-complex form in vacuoles, and transformation into less toxic organic forms (Lombi et al., 2002; Wang et al., 2002; Zhao et al., 2003).

Arbuscular mycorrhizal fungi (AMF), as an important group of soil fungi, can form symbiotic associations with more than 80% of the land plant families (Schwarzott et al., 2001). AMF can essentially improve plant mineral nutrition and plant water relations (Li et al., 2014), and enhance plant resistance to heavy metal contaminations (Hildebrandt et al., 2007). Recent studies show that the arbuscular mycorrhizas naturally occur in As-contaminated soils (Smith et al., 2010) and mycorrhizal inoculation can improve the As tolerance of tomato (Liu et al., 2005b), maize (Bai et al., 2008),

* Corresponding author. Address: No. 18, Shuangqing road, Haidian district, Beijing 100085, China. Tel.: +86 10 6284 9068; fax: +86 10 6292 3549.
E-mail address: bdchen@rcees.ac.cn (B.-D. Chen).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.chemosphere.2014.06.042
0045-6535/

white clover and ryegrass (Dong et al., 2008), plantain (Orłowska et al., 2012) and Chinese brake fern (Leung et al., 2013). As(V), as the main As species in aerobic environment, behaves similarly as phosphate and is a competitor for the phosphate transporters into plants (Asher and Reay, 1979). As AMF play key roles in plant acquisition of phosphorus (P) (Smith et al., 2003), it is therefore essential to take P–As interactions into account for understanding the involvement of AMF in plant uptake of As(V).

Recent studies have partially uncovered the underlying mechanisms of enhanced plant resistance to As by AM associations. Under As contaminations, AMF could effectively improve plant P nutrition and growth, resulting in a "dilution effect" on As in plant tissues (Chen et al., 2007; Xia et al., 2007; Xu et al., 2008). More importantly, AMF may also directly mediate As uptake by plants. Gonzalez-Chavez et al. (2002) found that AMF could reduce As(V) influx into excised roots of *H. lanatus*. Christophersen et al. (2009) reported that AM colonization reduced As(V) uptake in barley via down-regulation of the high-affinity Pi transports in plant roots that also absorbed As(V). As reported by González-Chávez et al. (2011), AMF may also contribute to As exclusion involving reducing As(V) to As(III) and pumping As(III) out of the fungal cells. Moreover, AM symbiosis could alleviate As(V) toxicity by mitigating oxidative stress (Yu et al., 2009; Garg and Singla, 2012), and influencing the distribution of different As species in plants (Yu et al., 2009; Chen et al., 2012). Ultra Jr et al. (2007) reported the occurrence of less toxic DMA only in the mycorrhizosphere. However, direct evidences are still lacking as for the role of AMF in detoxification of As particularly by influencing As speciation in host plants.

Nevertheless, most previous experiments investigating the interactions of AMF with host plants under As contaminations were performed under sterilized conditions. Such experiments might have neglected the impacts of other soil microorganisms and thus failed to reveal the significance of AM fungi under natural conditions. An experiment under unsterilized condition was conducted by Leung et al. (2010). However, as plants naturally can be colonized by indigenous AM fungi even for the uninoculated control, it was therefore insufficient to evaluate the mycorrhizal involvements in plant As metabolism. Due to the fact that any study on the role of AMF in As detoxification in host plants is ultimately directed to develop mycorrhizal technology for remediation of contaminated soils, it is essential to understand the role of AMF in plant tolerance to As under natural conditions. However such information so far is very limited.

In the present study, two pot experiments were carried out to investigate As speciation and detoxification in host plants as influenced by AMF. In the first experiment, wild type *Medicago truncatula* inoculated with/without AMF *Rhizophagus irregularis* was grown in sterilized As-contaminated soil amended with different levels of P, in order to investigate the involvements of AMF in plant As metabolism and the potential influences of P-As interactions on the mycorrhizal functions. In the second experiment, wild type and the non-mycorrhizal mutant TR25:3-1 (Sagan et al., 1995) of *M. truncatula* were grown in unsterilized As-contaminated soil to reveal the role of AMF in alleviation of As phytotoxicity under natural conditions.

2. Materials and methods

2.1. Host plants

Seeds of medic plants (*M. truncatula* L., wild type cv. Jemalong A17 and the mutant TR25:3-1) were obtained from the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The seeds were surface sterilized in 10% (v/v) H_2O_2 solution for 10 min, then immersed in deionized water for 10 h. They were then pre-germinated on moist filter paper for about 48 h at 27 °C till emergence of radicles. The seeds were selected for uniformity before sowing.

2.2. AMF inoculums

The AM fungus *R. irregularis* Schenck & Smith (BJ09) was provided by Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry. The fungus was propagated in pot culture with maize plants grown in a sandy soil for 10 weeks. Inoculum from pot culture was a mixture of spores, mycelium, sandy soil and root fragments containing approximately 1000 spores per 100 g soil.

2.3. Cultivation media

The experimental soil was collected from an As contaminated site (N25°35.22″, E113°00.21″) in Chenzhou City, Hunan Province, China. The soil had a pH value of 7.93 (1:2.5 soil to water), 5.25% organic matter, extractable P content of 13.00 mg kg^{-1} (extracted by 0.5 M $NaHCO_3$ following the methods described by Olsen et al., 1954) and extractable As of 4.54 mg kg^{-1} (extracted by 0.5 M $NaHCO_3$). The soil contained 93.53 mg As kg^{-1}, 14.03 mg Cu kg^{-1}, 566.18 mg Mg kg^{-1}, 358.22 mg Mn kg^{-1} and 103.18 mg Zn kg^{-1}. Total metal concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 2000 DV) following HNO_3-HF digestion. Before the experiment, the soil was passed through a 2 mm sieve and received basal nutrients without P as recommended by Pearson and Jakobsen (1993).

2.4. Experimental procedure

In the first experiment, experimental soil was sterilized by r-ray (20 kGy, 10 MeV electron beam) before use. Round plastic pots that can accommodate 1 kg soil were used for cultivation of test plants, the wild type of *M. truncatula*. Three P addition levels (0, 20 and 60 mg kg^{-1}) were arranged with appropriate amount of analytical grade KH_2PO_4 solution thoroughly mixed into the soil. Mycorrhizal treatments received 50 g of the fungal inoculum, which was also carefully mixed into the growth medium. Non-mycorrhizal treatments received an equivalent amount of autoclaved soil-sand mixture together with a filtrate (by using slow speed quantitative filter paper) of the fungal inoculum to provide a similar microflora except for the absence of the mycorrhizal fungus. There were two inoculation treatments in combination with three P addition levels, resulting in a total of 6 treatments. Each treatment has four replicates giving a total of 24 pots in a two-factor completely random design.

In the second experiment, wild type and the non-mycorrhizal mutant (which cannot be colonized by AM fungi) of *M. truncatula* were grown in unsterilized soil in same pots with the first experiment. There were two treatments and five replicates each, giving a total of 10 pots.

Ten pre-germinated seeds were sown in each pot and thinned to five one week after seedling emergence. The experiment was conducted in a controlled environment chamber with 16 h/25 °C day, 8 h/18 °C night, and a light intensity of 700 μmol m^{-2} s^{-1} provided by supplementary illumination. The plants grew for 16 weeks from 14 November 2012 to 5 March 2013. During the experimental period, de-ionized water was added as required to maintain soil moisture content of c.a. 55% water holding capacity by regular weighing.