JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Q2 *Rhizophagus irregularis* influences As and P uptake by alfafa and the neighboring non-host pepperweed growing in an As-contaminated soil

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ARTICLE INFO

Article history:
Received 3 March 2017
Revised 15 June 2017
Accepted 6 July 2017
Available online xxxx

Keywords:
AM fungi
Arsenic contamination
Non-host plant
Interaction

ABSTRACT

It was documented that arbuscular mycorrhiza fungi (AMF) play an important role in protecting host plants against arsenic (As) contamination. However, most terrestrial ecosystems contain a considerable number of nonmycorrhizal plants. So far little information is available for the interaction of such non-host plants with AMF under As contaminations. By using a dual compartment cultivation system with a plastic board or a nylon mesh separating roots of non-host pepperweed from roots of the AM-host alfafa plants, avoiding direct root competition, the two plant species were grown separately or partially separated (with rhizosphere effects) in the presence or absence of the AMF *Rhizophagus irregularis* in As-contaminated soil. The results indicated that mycorrhiza caused phosphorus (P) concentration decrease in the non-host pepperweed, but promoted the P concentration of the AM host alfafa. Mycorrhiza is potentially helpful for non-host pepperweed to adapt to As contamination by decreasing root As concentration and showing no suppressing effect on biomass production. The study provides further evidence for the protective effects of AMF on non-host plants against As contamination, and improved our understanding of the potential role of AMF for non-host plant adaptation to As contaminated soils.

Introduction

Arsenic (As) is ubiquitous in the environment, which is highly toxic even at low concentrations. In recent decades, public concern regarding this element has been increasing, because As is regarded as a well-known class 1, nonthreshold carcinogen (Smith *et al.*, 2002). Arsenic mining industry (Zhu *et al.*, 2008), irrigation with As-contaminated groundwater (Williams *et al.*, 2006) and application of As-containing pesticides (Williams *et al.*, 2007) have been found to be the main reasons for the elevated As concentration in soil. Plant uptake of As from contaminated soils may contribute a significant pathway of human exposure *via* the food chain. Understanding As uptake in plant is thus critical for formulating countermeasures to minimize the ecological risk of As contaminations (Meharg and Hartley-Whitaker, 2002).

It was documented that higher plants that are adapted to As-contaminated soils are generally symbiotic with arbuscular mycorrhizal fungi (AMF) (Sharples *et al.*, 2000; Gonzalez-Chavez *et al.*, 2002), while the mycorrhizal associations could influence

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http://dx.doi.org/10.1016/j.jes.2017.07.005
1001-0742/

Please cite this article as: Xin, Z., et al., *Rhizophagus irregularis* influences As and P uptake by alfafa and the neighboring non-host pepperweed growing in an As-contaminated soil, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.07.005

As uptake and accumulation by plants. However, up to date most of the investigations focused on As uptake, accumulation and distribution of a single mycorrhizal plant (Chen *et al.*, 2007; Liu *et al.*, 2009; Zhang *et al.*, 2015), or the interactions between two mycorrhiza plants under As contamination (Dong *et al.*, 2008). It has been well known that there are some plant families, an estimated 18% of all vascular species, that do not associate with AMF (Brundrett, 2009). These plants have been named as "non-host" or "non-mycorrhizal" (NM) plants and are widely distributed (Francis and Read, 1994). Brassicaceae is one of these families that have long been known as non-host for AMF except a few of plant species such as *Thlaspi praecox* and *Lepidium bonariense* (Giovannetti *et al.*, 1994; Vogel-Mikus *et al.*, 2005; Massenssini *et al.*, 2014). Even though a functional mycorrhization does not occur in a non-host plant, the invasion of hyphae deriving from the host plant neighbors to the non-host plant roots is persistent (Tong *et al.*, 2015). On the other hand, Brassicaceae family has been proven to have genetic and physiological adaptations that allow plants to accumulate, translocate, and resist high amounts of arsenic (Wang *et al.*, 2009; Ramirez-Andreotta *et al.*, 2013). Therefore, we specifically addressed the role that AMF play in non-host plant adaptation Q5 to As contamination. To our knowledge, the interaction of non-host plants with AMF under As contamination is so far poorly understood.

In the present study, a model mycorrhizal plant alfalfa (*Medicago sativa* L.) was selected as arbuscular mycorrhizal Q6 (AM) host plant, and pepperweed (*Lepidium apetalum* L.), a weed of the Brassicaceae family widely distributed in rubbly slopes, meadows, steppes, fallow lands, and along the roads (Prokopiev *et al.*, 2013) was chosen as non-AM host plant, whose leaves can be eaten and the whole plant can be used as Q7 a medicine (Duke and Ayensu, 1984; Kunkel, 1984). By using a dual compartment cultivation system with a plastic board or a nylon mesh separating roots of pepperweed from roots of alfalfa plants and avoiding direct root competition, the two plant species were grown separately or partially separated (with rhizosphere effects) in the presence or absence of the AMF *Rhizophagus irregularis* in an As-contaminated soil. It was hypothesized that although non-host pepperweed could not form symbiosis with AMF, while the involvement of AMF might decrease As concentration of non-host pepperweed, thus potentially helpful for non-host pepperweed to adapt to As contamination. The aim of the present study is to shed light on the effects of AMF on growth, mineral nutrition, As uptake of non-AM host plant under As contaminations, which may contribute to our understanding of the potential role of AMF for non-host plant adaptation to As-contaminated soils.

1. Material and methods

1.1. Host plants

Seeds of alfalfa and pepperweed plants were respectively obtained from the Beijing Gold Garden Agriculture Technology Institution and Research Center of National Vege Engineering and Technology. The seeds were surface sterilized in 10% (V/V) H_2O_2 solution for 10 min, then immersed in deionized water for 10 hr. They were then pre-germinated on moist filter paper for about 48 hr at 27°C until emergence of radicles. The seeds were selected for uniformity before sowing.

1.2. AMF inoculums

The AMF *Rhizophagus irregularis* Schenck and 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 6000 spores per 100 g soil.

1.3. Cultivation system

Two alfalfa and three pepperweed seedlings were grown in each individual compartmented PVC boxes (12 cm × 8 cm × 10 cm). Roots of both plant species were separated by a PVC board (board compartment mode, BC), to avoid that both roots and hyphae get intermingled with the other plant species, or by a nylon net with 37 μm mesh size (mesh compartment mode, MC), which only allows the penetration by AM fungal hyphae (Fig. 1). The hyphae penetrating freely through the nylon mesh can directly interact with the root of pepperweed, which is convenient for investigating the interactions of AMF with non-hosts in the present study. In contrast, in the BC modes, neither the roots nor the hyphae of alfalfa could reach the rhizosphere of pepperweed, which was regarded as a control in the present study.

1.4. Cultivation media

The experimental soil was collected from the experimental field of Chinese Agriculture University, Beijing, China. The soil had a pH value of 7.47 (1:2.5 soil to water (*m/V*)), extractable phosphorus (P) content of 10.50 mg/kg (extracted by 0.5 mol/L $NaHCO_3$ following the methods described by Olsen *et al.*, 1954) Q8 and extractable As of 0.21 mg/kg (extracted by 0.5 mol/L $NaHCO_3$). The soil contained 5.6 mg/kg As, 27.39 mg/kg Cu, 125.51 mg/kg Mg, 527.92 mg/kg Mn and 84.31 mg/kg Zn. Total As concentration were analyzed by inductively coupled plasma-mass spectroscopy (ICP-MS, Agilent7500, Agilent Technology, USA) and other total metal concentrations were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 2000 DV, Perkin Elmer, USA) 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). A 2:1 (W/W) mixture of the soil and river sand, also passed through 2-mm sieve and sterilized by irradiation (20 kGy, 10 MeV electron beam), was used as growth medium, and this mixture is referred to as "soil" hereinafter.

1.5. Experimental procedure

The 5 mg/kg As in the form of $Na_3AsO_4{\cdot}12H_2O$ (As[V]) were added to the soil and then carefully mixed to ensure uniformity. The soil was incubated for one month to allow metal equilibrium. A mixture of 900 g soil and 30 g fungal inoculum was divided equally and put into the two compartments of each box for AM

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