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Chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant chromium tolerance

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi, as important plant mutualists, can protect host plants against environmental stresses, including heavy metal contaminations. It is generally accepted that improvement of plant P nutrition by AM symbiosis plays an important role in plant tolerance to heavy metals. In the present study, we tested if exogenous P amendment to the chromium (Cr) contaminated soil could match the positive effects of AM symbiosis on plant Cr tolerance for the highly mycorrhizal dependent plant—dandelion (*Taraxacum platypecidum* Diels.). Experimental results showed that P addition could not enhance plant growth as well as AM symbiosis did. AM fungi could immobilize Cr in mycorrhizal roots besides enhancing plant P acquisition. Cr distribution pattern in principal roots as revealed by synchrotron radiation micro-focused X-ray fluorescence (SR μ-XRF) analysis supported the stabilization of Cr in mycorrrihzal roots. Furthermore, by using a three-compartment cultivation system, we demonstrated that extraradical mycelium (ERM) could take up and transport Cr to mycorrhizal roots, but restrained Cr translocation from roots to shoots, and thus contributed to Cr immobilization in roots and relieved Cr phytotoxicity.

1. Introduction

As the seventh most abundant element on earth, chromium (Cr) is essential in glucose metabolism of human beings and animals (Katz and Salem, 1994; Shrivastava et al., 2002). By contrast, Cr is a non-essential element for plants, which can interfere with photosynthesis and respiration processes, lead to oxidative damage, inhibit important enzymatic activities, and even cause plant death (Shanker et al., 2005; Singh et al., 2013a). Naturally Cr has two stable forms, hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)], of which Cr(VI) is highly mobile, and more toxic than Cr(III) for its mutagenic and carcinogenic effects (Losi et al., 1994; Singh et al., 2013a). Chromium is widely used in the chemical industries such as electroplating, leather tanning, pigment production, etc. In the past decades, improper discharge of Cr into environment during Cr processing has resulted in severe Cr contaminations, and subsequently threatened the ecosystem stability (Mohanty and Patra, 2011).

In the natural ecosystem, plants usually establish intimate contact with rhizosphere microorganisms, among which arbuscular mycorrhizal fungi (AMF) are most common and can form symbiotic associations with more than 80% terrestrial plants (Smith and Read, 2008). AMF obtain carbohydrates from their host plants and in return they provide plants with mineral nutrients such as phosphorus (P), nitrogen (N), etc (Smith and Read, 2008). Additionally, AMF can relieve plant drought stress (Li et al., 2014), protect host plants from pathogens (Singh et al., 2013b), improve soil structure (Rillig and Steinberg, 2002), and even play an important role in maintaining plant biodiversity and ecosystem stability (van der Heijden et al., 1998). Various studies have demonstrated that AM symbiosis take an active part in plant resistance to heavy metal contamination including As, Cd, Cu and Cr etc (Chen et al., 2007a,b, 2005; Davies et al., 2001; Wu et al., 2014). For example, Davies et al. (2001) found that AMF could enhance Cr tolerance of sunflower (*Helianthus annuus*) under Cr

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stress. Our recent work has also indicated that AM symbiosis could greatly enhance Cr tolerance of both dandelion (*Taraxacum platypecidum* Diels.) and bermudagrass (*Cynodon dactylon* (linn.) Pers.) under Cr(VI) contamination conditions (Wu et al., 2014).

Although AM symbiosis can protect host plants against Cr stress, little information is available as for the underlying mechanisms. One possible mechanism is that AM symbiosis can improve plant growth through enhancing plant P uptake, which subsequently result in so-called "growth dilution effects" on metals in plants (Chen et al., 2007a). AMF is well known for its positive effects on plant P nutrition especially under stressful conditions. For example, AM symbiosis substantially increased P uptake efficiency of dandelion plants under Cr(VI) contaminations (Wu et al., 2014). However, we do not know if this is the main way AM fungi enhance plant Cr(VI) tolerance, and if AM function can be replaced by exogenous P addition.

Another explanation for the alleviation of plant Cr toxicity by AM symbiosis is that the extensive extraradical mycelium (ERM) may directly immobilize large quantities of Cr and restrict its translocation from roots to plant shoots, just like Cd (Nayuki et al., 2014) and U (Weiersbye et al., 1999; Rufyikiri et al., 2002). ERM has a high cation exchange capacity (CEC) and can adsorb metals on fungal surface (Joner et al., 2000; Chen et al., 2001). Even if ERM can transport metals to mycorrhizal roots, the metals may not be actually delivered to plants across the symbiotic surface between AMF and root cells (Joner and Leyval, 1997; Nayuki et al., 2014). Therefore, the second question is that whether ERM can take up and retain Cr in plant roots, and thus relieve Cr phytotoxicity.

To address the above two questions, we carried out two experiments in which dandelion plants, together with AM fungus—*Rhizophagus irregularis* were adopted to establish mycorrhizal associations. In the first experiment, different P addition treatments, along with mycorrhizal inoculation treatments were arranged in Cr(VI) amended soils to investigate if AM fungi enhance plant Cr(VI) tolerance mainly through improving plant P acquisition, and whether AM function can be replaced by exogenous P addition. We predicted that AMF enhance plant Cr tolerance mainly through improving plant P nutrition, and appropriate P application would increase plant growth under Cr (VI) contamination as well as AM symbiosis did. In the second experiment, we used a compartment cultivation system to investigate if ERM can directly take up and transport Cr to plants, and also play an important role in Cr immobilization in mycorrhizal roots, which could potentially relieve Cr phytotoxicity.

2. Material and methods

2.1. Growth substrate

A calcareous sandy soil with low nutrient level was collected from Panggezhuang, Daxing district, Beijing (39°36′N, 116°18′E). As analyzed by a laser diffraction technique using a Longbench Mastersizer 2000 (Malvern Instruments, Malvern, England), the soil consisted of 12.1% (v/v) clay (0–5 μm), 51.7% (v/v) silt (5–50 μm) and 36.2% (v/v) sand (50–2000 μm). Soil properties are described in details in Table S1. The soil was passed through a 2 mm sieve and then sterilized by radiation (γ rays, 20 kGy, 10 MeV electron beam). Before experiment, basal nutrients with 30 mg kg^{-1} P, 120 mg kg^{-1} N and 120 mg kg^{-1} K were carefully mixed into the soil.

2.2. Host plant

Based on our previous study (Wu et al., 2014), we used highly mycorrhizal dependent plant dandelion (*Taraxacum platypecidum* Diels.) as host plant. Seeds of dandelion were purchased from Beijing Greatgreen Ecological Technology Development Company, Beijing, China. The seeds were surface sterilized with 10% H_2O_2 for 15 min, washed carefully with Milli-Q water, and then pre-germinated on moist filter paper until the appearance of radicles.

2.3. AM fungus

The AM fungus *Rhizophagus irregularis* Schenck & Smith (BGC AH01) were provided by Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry. The fungi were propagated on *Sorghum bicolor* (L.) Moench in a sandy soil for 12 weeks. Inoculum from the pot culture is a mixture of plant root fragments, mycelium, spores (c.a. 150 spores g^{-1}) and sandy soil.

2.4. Experimental procedure

2.4.1. Experiment I

This experiment aimed to reveal the importance of plant P nutrition in plant Cr(VI) tolerance. The soils were amended with 10 mg kg^{-1} Cr in the form of K_2CrO_4 [Cr(VI)], and then carefully mixed to ensure uniformity. After that, the soil was placed for over a year to allow metal equilibrium. Phosphorus (0, 30, 60, 150 mg kg^{-1}) in the form of KH_2PO_4 were added to the soil and carefully mixed for homogeneity. Besides, an AM inoculated treatment without P addition was arranged. Considering the main purpose of this experiment was to investigate if P amendment could relieve plant Cr(VI) toxicity in comparison with mycorrhizal treatment, it is unnecessary to detect mycorrhizal effects under each P addition level. Thus, totally 5 treatments were arranged, namely "control", "P30", "P60", "P150" and "+M", where "P" represents phosphorus, "+M" represents mycorrhizal inoculation. Each treatments had 4 replicates, resulting in 20 pots in total.

For AM inoculated treatment ("+M"), 300 g Cr(VI) contaminated soil was firstly put into the pot, and then 300 g Cr(VI) contaminated soil that contained 30 g fungal inoculum was added. As for non-inoculated treatment at each P addition level, 30 g sterilized inoculum and 10 mL inoculum filtrate (passed through a 15 μm filter to remove AMF) was added instead to reintroduce soil microbial communities except AMF. Each pot was sown with 10 pre-germinated dandelion seeds. 10 days after emergence seedlings were thinned to 2 per pot, and each pot was daily watered with deionized water to maintain moisture content of 15% on a dry weight basis (around 55% of water holding capacity). The experiment was conducted in a controlled growth chamber at a light intensity of 700 μmol m^{-2} s^{-1}, 16 h: 8 h and 25°C: 20°C (light: dark), 70% relative humidity. The plants grew for 2 months before experimental harvest.

2.4.2. Experiment II

This experiment aimed to exploit whether ERM could take up and transport Cr to plants. A compartment cultivation system was used, which was a rectangular box (12 cm high, 14 cm wide and 10 cm deep) with three compartments: a root compartment (RC) at one side of 8 cm width, a hyphal compartment (HC) at the other side of 4 cm width, and a central buffer compartment (BC) of 2 cm width (Fig. 1). All three compartments were separated by a 37 μm nylon net that only allow penetration by hyphae but not by roots. RC was set for plant growth, HC was set for extraradical mycelium development, and BC for avoiding Cr diffusion from HC to RC. There were no Cr(VI) addition for RC and BC, while for HC, 60 mg kg^{-1} Cr (VI) or no Cr(VI) was added. Four treatments were arranged in the present work, namely "−M + Cr", "+M + Cr", "+M − Cr", "+M + F + Cr". For treatment "+M + Cr", dandelion in association with AM fungi was introduced to RC using the methods described in experiment I (but the total soil was 1 kg), and 500 g soil with 60 mg kg^{-1} Cr(VI) were added in HC and carefully mixed to ensure