

Cr Stable Isotope Fractionation in Arbuscular Mycorrhizal Dandelion and Cr Uptake by Extraradical Mycelium



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ABSTRACT

As common soil fungi that form symbioses with most terrestrial plants, arbuscular mycorrhizal (AM) fungi play an important role in plant adaptation to chromium (Cr) contamination. However, little information is available on the underlying mechanisms of AM symbiosis on plant Cr resistance. In this study, dandelion (*Taraxacum platycepium* Diels.) was grown with and without inoculation of the AM fungus *Rhizophagus irregularis* and Cr uptake by extraradical mycelium (ERM) was investigated by a compartmented cultivation system using a Cr stable isotope tracer. The results indicated that AM symbiosis increased plant dry weights and P concentrations but decreased shoot Cr concentrations. Using the Cr stable isotope tracer technology, the work provided possible evidences of Cr uptake and transport by ERM, and confirmed the enhancement of root Cr stabilization by AM symbiosis. This study also indicated an enrichment of lighter Cr isotopes in shoots during Cr translocation from roots to shoots in mycorrhizal plants.

Key Words: arbuscular mycorrhizal fungi, Cr contamination, Cr translocation, hyphae, mycorrhizal plant

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INTRODUCTION

Chromium (Cr) is the seventh most abundant element on earth (Katz and Salem, 1994) and is essential in glucose metabolism of human beings and animals (Shrivastava *et al.*, 2002). However, excess Cr is highly toxic to all living organisms (Mohanty and Patra, 2011). There exists widespread Cr contamination in soil because of extensive use of Cr in industry (such as leather production, electroplating, and textile dyeing) and agriculture during the past several decades (Mohanty and Patra, 2011). As a non-essential element for plants, Cr causes oxidative damage, depresses important enzymatic activities, interferes with photosynthetic and respiration processes, and even results in plant death (Shanker *et al.*, 2005; Singh *et al.*, 2013). So it is hard to grow plants in Cr-contaminated soils.

It is well known that plants can closely associate with soil microorganisms, which often benefits plant growth under stress conditions. Arbuscular mycorrhizal (AM) fungi can form symbioses with the majority of terrestrial plants, and they play an important role in plant adaptation to Cr stress (Davies *et al.*, 2002; Estaún *et al.*, 2010). Under high levels of

Cr contamination, AM fungi promoted the growth of *Plantago lanceolata* (Estaún *et al.*, 2010) and *Leucaena leucocephala* (Gardezi *et al.*, 2005). AM fungi also enhanced Cr tolerance and accumulation of sunflower (*Helianthus annuus*) under Cr stress (Davies *et al.*, 2001).

Although it has been demonstrated that AM associations can protect host plants against Cr contamination, little information is so far available on the underlying mechanisms. One potential mechanism is that extraradical mycelium (ERM) may take and retain metals in their own structure, and thus relieve metal toxicity to plants (Chen *et al.*, 2001). For example, ERM could take up and transport Cd-109 and Zn-65 to mycorrhizal roots (Joner and Leyval, 1997; Jansa *et al.*, 2003; Hutchinson *et al.*, 2004). However, there are few reports on Cr uptake and translocation by ERM. Dandelion (*Taraxacum platycepium* Diels.), a widespread herb, was chosen as it has proven to be highly dependent on mycorrhiza under Cr contamination conditions (Wu *et al.*, 2014). The metal isotope fractionation occurred when metals were absorbed and transported by plants (Guelke and von Blanckenburg, 2007; Jouvin *et al.*, 2012). We hypothesized that ERM could take up and transport Cr from a distance to my-

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corrhizal roots, and that the Cr stable isotope fractionation occurred during Cr transport from roots to shoots of mycorrhizal plants. The aims of this study were to investigate the Cr uptake and transport by ERM using a compartmented cultivation system together with Cr isotope tracers and to elucidate the Cr isotope fractionation during Cr transport in plants inoculated with and without AM fungi.

MATERIALS AND METHODS

Growth substrate

Soil was collected from Panggezhuang in Daxing District of Beijing, China ($39^{\circ}36' N$, $116^{\circ}18' E$). Detailed soil properties are described in Table I. The soil was passed through a 2-mm sieve and then sterilized (20 kGy, 10 MeV electron beam). Before sowing, basal nutrients (30 mg P kg^{-1} , 120 mg N kg^{-1} , and 120 mg K kg^{-1}) were carefully mixed into the soil.

TABLE I

Some physical and chemical properties of soil used in the study

Property	Value
pH (1:2.5 in water)	8.53
Soil organic matter (g kg^{-1})	10.2
Extractable P ^{a)} (mg kg^{-1})	5.90
Extractable Cr ^{b)} (mg kg^{-1})	1.37
P (mg kg^{-1})	819
Cr (mg kg^{-1})	67.3
N (mg kg^{-1})	836
S (mg kg^{-1})	324
Zn (mg kg^{-1})	83.3
Mn (mg kg^{-1})	553
Fe (mg kg^{-1})	22 806
Cu (mg kg^{-1})	55.5

^{a)}Extracted by 0.5 mol L^{-1} NaHCO_3 ; ^{b)}Extracted by 2 mol L^{-1} HCl .

Host plant

Dandelion seeds were purchased from the Beijing Greatgreen Ecological Technology Development Company, Beijing, China. The seeds were first surface sterilized with 10% (v/v) H_2O_2 for 20 min, washed carefully with Milli-Q water, and then pre-germinated on moist filter paper until the radicles appeared.

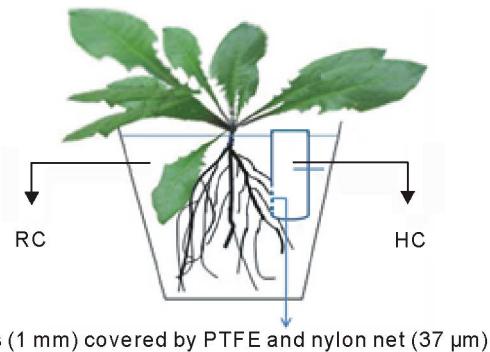
AM fungus

The AM fungus *Rhizophagus irregularis* Schenck & Smith (BGC AH01) was obtained from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry, China. The fungus was propagated in pot culture of *Sorghum bicolor* (L.) Moench in a sandy soil for 10 weeks. Inoculum from the pot

culture consisted of a mixture of mycelium, spores (*ca.* 150 spores g^{-1}), sandy soil and root fragments.

Experimental design

The aim of the experiment was to investigate Cr uptake and transport by *R. irregularis* in association with dandelion. A compartmented cultivation system was used, including a root compartment (RC) and a hyphal compartment (HC), with the Cr stable isotope tracers in the HC (Fig. 1). The experiment was designed with 2 treatments: inoculated and uninoculated with AM fungus. The inoculated treatment allowed ERM to develop in the HC, while in the uninoculated treatment, there was no ERM in the HC. Each treatment had 4 replicates, with a total of 8 pots.



Holes (1 mm) covered by PTFE and nylon net (37 μm)

Fig. 1 Diagram showing the compartmented cultivation system in this study. Nylon net (37 μm), polytetrafluoroethylene (PTFE), and plastic vial with holes (1 mm in diameter) were used together to separate the cultivation system into two compartments: root compartment (RC) for plant growth, and hyphal compartment (HC) (the columned vial) for hyphal development only.

Experiment procedure

A plastic pot constituted the main RC with a 50-mL cylinder with many round holes (diameter of 1 mm) near the blocked bottom. The holes were covered by a 37- μm nylon net, which allowed penetration by ERM but not by roots. Therefore, the plastic cylinder served as the HC (Fig. 1). For the RC, a mixture of 870 g soil and 30 g fungal inoculum or a mixture of 870 g soil and 30 g sterilized inoculum was filled into each pot to make the inoculated or uninoculated treatments. For the uninoculated treatment, 10 mL inoculum filtrate was also added to soil to reintroduce soil microbial communities except for AM fungi. For the HC, 30 g soil was amended with 60 mg kg^{-1} Cr stable isotopes, mainly in the form of CrCl_3 containing 92.7% ^{53}Cr , with $^{53}\text{Cr}/^{52}\text{Cr}$ ratio (namely $\delta^{53}\text{Cr}$ value) of 2×10^5 . To avoid Cr diffusion from HC to RC, a piece of polytetrafluoroethylene (PTFE, penetrable by ERM)

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