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

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(Received June 10, 2014; revised January 12, 2015)

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 platyepidum* 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

Citation: Ren, B. H., Wu, S. L., Chen, B. D., Wu, Z. X. and Zhang, X. 2015. Cr stable isotope fractionation in arbuscular mycorrhizal dandelion and Cr uptake by extraradical mycelium. *Pedosphere*. 25(2): 186–191.

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 platyepidum* 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°36' N, 116°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 NaHCO₃; ^{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) H₂O₂ 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.

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 CrCl₃ containing 92.7% ⁵³Cr, with ⁵³Cr/⁵²Cr ratio (namely δ⁵³Cr value) of 2 × 10⁵. To avoid Cr diffusion from HC to RC, a piece of polytetrafluoroethylene (PTFE, penetrable by ERM)

(Mäder *et al.*, 1993) was inserted between the round holes and the 37- μm nylon net. For each treatment, 10 pre-germinated dandelion seeds were sown and then covered by 50 g soil. Seedlings were thinned to 2 per pot 6 d after emergence.

Each pot was watered daily with distilled water to keep moisture content at 150 g kg⁻¹ on a dry soil basis (around 55% water holding capacity). The experiment was conducted in a controlled plant growth chamber at a light intensity of 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$, a photoperiod of 16 h/8 h (light/dark), a temperature of 25 °C/20 °C (light/dark), and 70% relative humidity. The plants grew for 65 d before harvest. Considering the variations in environmental conditions, all experimental pots were randomly rearranged after every watering.

Harvest and chemical analyses

Plant shoots were carefully cut from the stem base, and plant roots were separated from soil, and then the whole plant samples were washed carefully with deionized water. Sub-samples of fresh roots were collected for the determination of AM colonization. Dry weights (DW) of shoots and roots were determined after oven drying at 70 °C for 48 h. Dried samples were milled and digested in HNO₃ using a microwave-accelerated reaction system (CEM Microwave Technology Ltd., Matthews, North Carolina, USA) in a three-step digestion program: the temperature was gently raised to 120 °C over 8 min with a holding time of 3 min, then to 160 °C over 11 min with a holding time of 7 min, and finally to 190 °C over 8 min with a holding time of 20 min. The dissolved samples were then diluted to 50 mL with Milli-Q water. Plant P concentrations were determined with an inductively coupled plasma-optical emission spectrometer (Prodigy, Leemans, Hudson, New Hampshire, USA) and plant Cr concentrations were determined with an inductively coupled plasma-mass spectrometer (7500a; Agilent Technologies, Palo Alto, California, USA). Blanks and internal standards of bush leaves (GBW07603; China Standard Research Center, Beijing, China) and tea (GBW10016; China Standard Research Center) were both used to ensure the accuracy of chemical analyses of Cr and P concentrations. To investigate the composition of Cr stable isotopes in plant shoots and roots, ⁵³Cr/⁵²Cr ratios were determined with a multicollector-inductively coupled plasma-mass spectrometer (Nu plasma HR; Nu Instruments Ltd., Wrexham, UK) with optimized settings. To calibrate mass discrimination for Cr isotopes, the Cr isotope composition in samples was measured with reference to an external Cr isotope

standard (NIST SRM 979 Cr(NO₃)₃·9H₂O) using the “standard-sample-standard” bracketing technique. The ⁵³Cr/⁵²Cr ratio ($\delta^{53}\text{Cr}$, ‰) of each sample is expressed as a per mil deviation from the NIST SRM 979 standard:

$$\delta^{53}\text{Cr} = \frac{(^{53}\text{Cr}/^{52}\text{Cr})_{\text{sam}} - (^{53}\text{Cr}/^{52}\text{Cr})_{\text{std}}}{(^{53}\text{Cr}/^{52}\text{Cr})_{\text{std}}} \times 1000 \quad (1)$$

where (⁵³Cr/⁵²Cr)_{sam} and (⁵³Cr/⁵²Cr)_{std} are the ratio of ⁵³Cr to ⁵²Cr for the sample and for the NIST SRM 979 standard, respectively.

Sub-samples of fresh roots were cleaned in 10% (v/v) KOH, rinsed by 1% (v/v) HCl and then stained with Trypan blue according to the method as described in Phillips and Hayman (1970). Percentages of root colonization were determined as described by Trouvelot *et al.* (1986) using the MycoCalc software. Extraradical mycelium in RC and HC was extracted from soil samples using a modified membrane filter technique (Jakobsen *et al.*, 1992). The soil samples were carefully mixed and duplicate 4-g samples were blended with 250 mL Milli-Q water, and hyphae in 5-mL aliquots were collected on 25-mm membrane filters (0.22 μm) and stained with Trypan blue. Hyphal length densities were determined by measuring intersections between blue-stained hyphae and the grids in the eyepiece in 25 fields of view at 200 × magnification, and then calculated by the modified Newman formula (Tennant, 1975).

Calculations and statistical analyses

Mycorrhizal dependency (MD), the ratio of mean DW of inoculated plant (DW_{AM}) to that of the uninoculated plant (DW_{non-AM}) (Menge *et al.*, 1978), was quantified as follows: MD = DW_{AM}/DW_{non-AM}. The difference in $\delta^{53}\text{Cr}$ values between shoots and roots ($\Delta^{53}\text{Cr}_{\text{shoot-root}}$, ‰) was calculated as follows: $\Delta^{53}\text{Cr}_{\text{shoot-root}} = \delta^{53}\text{Cr}$ in shoot – $\delta^{53}\text{Cr}$ in root (Jouvin *et al.*, 2012). Data were analyzed by an independent-samples *t*-test to compare mycorrhizal status using the SPSS statistical package (IBM SPSS Statistics 18).

RESULTS

The mean percentage of root colonization by AM fungi in the inoculated treatment was 76.8% while that in the uninoculated treatment was 0. The hyphal length density in the inoculated treatment (3.19±0.92 and 2.76±0.81 m g⁻¹ in RC and HC, respectively) was significantly (*P* < 0.01) higher than that in the uninoculated treatment (0.90±0.53 and 1.25±0.33 m

g^{-1} in RC and HC, respectively) in both RC and HC.

Dry weights of both shoots and roots were significantly ($P < 0.01$) increased by AM fungi colonization (Fig. 2) and the MD value was 3.79. The AM fungi inoculation significantly ($P < 0.01$) increased P concentrations in shoots and roots, decreased Cr concentrations in shoots ($P < 0.05$), but had no significant effect on Cr concentrations in roots (Fig. 2). The AM symbiosis dramatically increased root Cr contents ($P < 0.01$) but showed no effects on shoot Cr contents, resulting in decreased shoot-to-root ratios of Cr contents (Table II).

TABLE II

Cr contents in shoots and roots and shoot-to-root ratio of Cr content ($\text{Cr}_{\text{shoot}}/\text{Cr}_{\text{root}}$) of dandelion when inoculated with and without arbuscular mycorrhizal fungi

Treatment	Cr content		$\text{Cr}_{\text{shoot}}/\text{Cr}_{\text{root}}$
	Shoot	Root	
	$\mu\text{g pot}^{-1}$		
Uninoculated	$8.00 \pm 1.16^{\text{a}}$	13.80 ± 2.44	0.59 ± 0.13
Inoculated	10.30 ± 2.01	27.53 ± 2.95	0.38 ± 0.08

^a) Means $\pm$ standard deviation.

The $\delta^{53}\text{Cr}$ values of shoots in the inoculated treatment were lower than those in the uninoculated treatment (Fig. 3). In contrast, the $\delta^{53}\text{Cr}$ values of roots in the inoculated treatment were significantly ($P <$

0.05) higher than those in the uninoculated treatment (Fig. 3). The $\delta^{53}\text{Cr}$ values of shoots were significantly lower than those of the roots, and this gap became larger when inoculated with AM fungi, as the $\Delta^{53}\text{Cr}_{\text{shoot-root}}$ of inoculated plants (-431.36%) was much lower than those of the uninoculated plants (-40.14%).

DISCUSSION

Compared with the root organ culture method, the compartmented cultivation system was closer to natural conditions as it used soil as the growth medium and also contained a smaller HC that allowed plant roots to develop in a larger volume. The high percentage of root colonization in the inoculated treatment indicated that dandelion formed a strong symbiosis with *R. irregularis*. Similar to previous work (Mäder *et al.*, 1993; Chen *et al.*, 2005; Chen *et al.*, 2007a), mycelia developed well in the inoculated treatment and passed through the 37- μm nylon net and PTFE to the HC. Some hyphae, probably dead or saprophytic, were detected in soils of the uninoculated treatments, and were also considered to be present in the inoculated treatments.

AM symbiosis increased dry weights of plants, which may result from improvement of P nutrition and reduction of Cr uptake. Dandelion associated with AM

Fig. 2 Dry weights, P concentrations and Cr concentrations of shoots and roots of dandelion inoculated with and without arbuscular mycorrhizal fungi. The vertical bars indicate standard deviations of means ($n = 4$).

Fig. 3 $\delta^{53}\text{Cr}$ values of shoots and roots of dandelion inoculated with and without arbuscular mycorrhizal fungi. The vertical bars indicate standard deviations of means ($n = 4$).

fungi may have a selective absorption of P prior to Cr. The reduction of Cr concentrations in mycorrhizal plants compared with non-mycorrhizal plants most likely resulted from a “growth dilution effect”, as the improved plant growth by AM symbiosis can “dilute” metal uptake by plants (Chen *et al.*, 2007a, b).

The other possible mechanism responsible for improvement of mycorrhizal plant tolerance to Cr is that ERM may take up and retain Cr in AM structures (mycelia, vesicles, arbuscules), therefore inhibiting Cr translocation into plant tissues, similar to Ni and Zn (Kaldorf *et al.*, 1999). As in the present study, inoculated roots showed higher $\delta^{53}\text{Cr}$ values than the corresponding uninoculated roots (Fig. 3). This supported our hypothesis that ERM could take up Cr from a distance and transport it to the mycorrhizal roots. Furthermore, it seems that Cr taken up by ERM may be mainly immobilized in the roots and little Cr is transported to the shoots, as the shoot-to-root ratio of Cr contents was significantly decreased by AM symbiosis (Table II).

Along with lower Cr concentrations in shoots, mycorrhizal shoots also had lower $\delta^{53}\text{Cr}$ values than the corresponding non-mycorrhizal shoots (Fig. 3). It was interesting to find that shoots had lower $\delta^{53}\text{Cr}$ values than roots when inoculated with AM fungi while the same trend was not observed for the uninoculated treatments (Fig. 3). This supported our hypothe-

sis that Cr isotope fractionation did occur during Cr translocation from roots to shoots while the fractionation may be different when associated with AM fungi. Similarly to Cu and Zn (Guelke and Von Blanckenburg, 2007; Jouvin *et al.*, 2012), shoot Cr isotope signatures largely depend on both isotope pools in the roots and the mechanisms of Cr translocation from roots to shoots. Cr stable isotopes with high $\delta^{53}\text{Cr}$ values taken up by ERM from the HC may lead to high $\delta^{53}\text{Cr}$ values in the mycorrhizal roots. In the mycorrhizal roots, an enrichment of lighter Cr isotopes in the plant part may occur during Cr delivery across the symbiotic interface from the fungus to the nearby plant cell. Different mechanisms of Cr translocation from roots to shoots of plants inoculated with and without AM fungi may finally lead to different isotope composition of Cr in plant shoots. Cr in roots may diffuse to xylem and further be transported from roots to shoots in xylem (Skeffington *et al.*, 1976). The radical diffusion may further result in enrichment of lighter Cr isotopes in shoots (Rodushkin *et al.*, 2004). These processes can be enhanced by AM symbiosis for its significant improvement on plant growth.

CONCLUSIONS

In summary, this study confirmed the direct uptake and transport of Cr by ERM using a stable isotope tracer. Besides, there was an enrichment of lighter Cr isotopes in shoots during Cr translocation from roots to shoots for mycorrhizal plants. However, to fully explore the mechanisms underlying the enhancement of AM symbiosis on plant Cr resistance, further studies are required to determine how AM fungi absorb, transport, and transform Cr. Specifically, further research is urgently needed to elucidate the process of Cr stable isotope fractionation during Cr uptake and translocation by mycorrhizal plants.

ACKNOWLEDGEMENTS

This study was supported by the Knowledge Innovation Program of Chinese Academy of Sciences (No. KZCX2-YW-BR-17), the National Natural Science Foundation of China (No. 41101246), and the State Key Laboratory of Urban and Regional Ecology, Research Center of Eco-Environmental Sciences, Chinese Academy of Sciences (No. SKLURE2008-1-03). We thank Dr. Catherine DANDIE from Edanz Group Ltd. for English polishing.

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