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 platyepidum* 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 mycorrhizal 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.

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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 platyepidum* 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 platyepidum* 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% H₂O₂ 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 K₂CrO₄ [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 KH₂PO₄ 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

Fig. 1. Diagram of the three-compartment cultivation system in experiment II. Nylon net (or 0.45 μm filter membrane) was used to separate the cultivation system into 3 compartments: root compartment (RC) for plant growth, buffer compartment (BC), and hyphal compartment (HC) for hyphal development only. BC was set to avoid Cr diffusion from HC to RC. 37 μm Nylon net only permits ERM to penetrate but not for roots, while 0.45 μm filter membrane does not permit penetration of ERM and roots.

uniformity, and 250 g soil without Cr contamination was put into BC. Compared with treatment “+M + Cr”, treatment “–M + Cr” had no AMF inoculation in RC, treatment “+M – Cr” had no Cr(VI) addition in HC, treatment “+M + F + Cr” had a 0.45 μm acetate filter membrane that can block ERM and roots to develop in HC instead of 37 μm nylon net (Fig. 1). By such an experimental design, it is possible to control other factors except ERM that potentially contribute to Cr transport, and thus to confirm whether ERM can take up and translocate Cr to plant roots. Each treatment had 4 replicates, resulting in a total of 16 pots. Plant growth conditions were the same to experiment I. The plants grew for 80 days before experimental harvest.

2.5. Harvest and sample analysis

2.5.1. Plant harvest

Plant shoots and roots were harvested separately and washed carefully with deionized water. Sub-samples of fresh roots were collected for the determination of AM colonization. Parts of fresh principal roots were also collected for Cr mapping using synchrotron radiation micro-focused X-ray fluorescence (SR $\mu\text{-XRF}$) microspectroscopy. Dry weights of shoots and roots were determined after oven-drying at 70°C for 48 h.

2.5.2. SR $\mu\text{-XRF}$ analyses

Fresh principal root samples at similar developmental stages were selected from both inoculated and noninoculated plants in experiment I where plant roots were exposed to Cr(VI) contamination. Principal roots were embedded by optimum cutting temperature (O.C.T.) compound (Sakura, Japan) and cut to sections of 40 μm thick with a cryotome (CM1850, LEICA, Germany) at –25°C, and then adhered to 3 M tape (cat. 810, Minnesota Mining and Manufacturing Company, Minn., USA). Sections in good conditions were selected for SR $\mu\text{-XRF}$ analysis after being freeze-dried at –25°C for 72 h. The SR $\mu\text{-XRF}$ microspectroscopy experiment was conducted at beamline 4W1B of Beijing Synchrotron Radiation Facility (BSRF), which runs 2.5 GeV electron with current from 150 mA to 250 mA. The detection limit for analysis was in the order of mg kg^{-1} . The incident X-ray energy was monochromatized by W/B4C double-multilayer-monochromator (DMM) at 15 keV and was focused on an area of 50 μm in diameter by the polycapillary lens. The two-dimensional mapping data were acquired by using a step size of 50 μm . The Si (Li) solid state detector was used to detect X-ray fluorescence emission lines with

live time of 20 s. SR $\mu\text{-XRF}$ spectra were processed by the PyMCA software package (Solé et al., 2007).

2.5.3. Plant P and Cr concentrations

Oven dried plant samples were milled and digested by HNO_3 with a microwave accelerated reaction system (Mars 5, CEM Co., Ltd., USA) in a three-step digestion program: the temperature was raised to 120°C in 8 min, holding for 3 min; and then raised to 160°C in 11 min, holding for 7 min; and finally to 190°C in 8 min with holding time of 20 min. The dissolved samples were then heated for additional 2 h at 140°C to remove NO_x . The final digestion solutions were diluted to 50 mL with Milli-Q water. P concentrations were analyzed by inductively coupled plasma-optical emission spectrometer (ICP-OES, Prodigy, Leemans, USA), and Cr concentrations were determined by inductively coupled plasma-mass spectrometry (ICP-MS, 7500a Agilent Technologies, USA). Blanks and internal standards of bush leaves (GBW07603, China Standard Research Center) and tea (GBW10016, China Standard Research Center) were used to ensure the accuracy of chemical analysis.

2.5.4. AM colonization

Sub-samples of fresh roots were cut into approximately 1 cm fragments, cleared in 10% KOH at 90°C for 1 h, and then rinsed in 5% HCl for 5 min, and stained with 0.05% Trypan blue at 90°C in water bath for 30 min, which was in accordance with a modified procedure of Phillips and Hayman (1970) by omitting phenol from the rinse. Thirty pieces of randomly selected stained roots were observed with a light microscope and the intensity of AMF colonization was assessed by the method described by Trouvelot et al. (1986) by using MYCOCALC software (www2.dijon.inra.fr/mychintec/Mycocalc-prg/download). The parameters measured included the frequency of mycorrhizal colonization (F%, the ratio of the number of root fragments colonized by AMF to the total number of root fragments analyzed), the intensity of the mycorrhizal colonization (M%, an estimate of the percentage of AM fungal structures in the whole root system) and the arbuscule abundance in the root system (A%, an estimate of the percentage of arbuscule occurrence in the whole root system), etc.

2.5.5. Soil hyphal length density

ERM in RC and HC were extracted from soil samples using a modified membrane filter technique (Jakobsen et al., 1992). Briefly, the soil samples were mixed and 4 g of the samples were blended with 250 mL deionized water, hyphae in 5 mL aliquots were then collected on 25 mm membrane filters (0.22 μm) and stained with 0.05% Trypan blue. Hyphal length density was determined by measuring intersections between blue-stained hyphae and the grids in the eyepiece in 25 fields of view at $\times 200$ magnification and then calculated by the modified Newman formula (Tennant, 1975).

2.5.6. Soil Cr speciation

Soil Cr(VI), DTPA-extractable Cr, acid-extractable Cr and reducible Cr in RC, BC and HC of different treatments were determined. Briefly, soil Cr(VI) concentrations were determined according to EPA method 3060A (USEPA, 1996), DTPA-extractable Cr concentrations were determined by using ICP-OES after extracting Cr from soil by 0.005 M DTPA–0.01 M CaCl_2 –0.1 M triethanolamine (TEA) solution (pH 7.30) (Lindsay and Norvell, 1978), acid-extractable and reducible Cr were extracted separately by 0.11 mol/L acetic acid and 0.5 mol/L hydroxylammonium chloride according to European community bureau of reference (BCR) three-step sequential extraction procedure (Rauret et al., 2000), and then analyzed by ICP-OES.

2.6. Statistics analyses

The translocation factor (TF), which represents the elemental translocation efficiency of plants, is expressed as the ratio of shoot Cr (or P) concentration to root Cr (or P) concentration (Tappero et al., 2007). All data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's test ($p < 0.05$) to determine the significance of differences between treatments.

3. Results

3.1. Experiment I

Similar to our previous study (Wu et al., 2014), inoculated plants showed very high frequency (100%) and intensity (81.5%) of mycorrhizal colonization, together with high arbuscule abundance (71.8%) regardless of Cr(VI) contamination, while non-inoculated plants remained uncolonized (Table 1). Fig. S1 displays the morphological comparison between mycorrhizal and nonmycorrhizal roots. The mycorrhizal roots exhibited numerous arbuscules and intraradical mycelium, as well as vesicles (Fig. S1). By contrast, there was no typical fungal structures in nonmycorrhizal roots (Fig. S1).

In general, P addition had no significant influence on plant shoot and root dry weights (Fig. 2a and b), while mycorrhizal colonization dramatically increased plant shoot dry weights (Fig. 2a, $p < 0.05$). Both AM colonization and P addition level of 150 mg kg^{-1} increased plant shoot P concentration (Fig. 2c, $p < 0.05$), while P addition and AMF inoculation did not influence plant root P concentration (Fig. 2d).

Both P addition and AMF inoculation decreased plant root Cr concentration, but only AM symbiosis significantly decreased shoot Cr concentration (Fig. 2e and f, $p < 0.05$). When plant P or Cr translocation factor (TF) were considered, 150 mg kg^{-1} P addition significantly increased TF value for P (Fig. 3a, $p < 0.05$). However, P addition did not affect TF value for Cr, while AM colonization significantly decreased Cr TF value (Fig. 3b, $p < 0.05$).

By SR μ -XRF analysis, we found that mycorrhizal colonization changed Cr distribution in roots, as compared with non-mycorrhizal roots where Cr signal was detected both in the cortex and vascular bundle, Cr signal was detected only in the cortex of mycorrhizal principal roots (Fig. 4). While for other elements such as Ca, K, Fe, Cu and Zn, AMF inoculation had no influence on their distribution. For both mycorrhizal and non-mycorrhizal principal roots, Ca, K and Fe were mainly located in the cortex, while Cu and Zn were detected both in the cortex and the vascular bundles (Fig. 4).

Table 1

Mycorrhiza development parameters in roots of dandelion (*Taraxacum platycephalum*) grown in 10 mg kg^{-1} Cr(VI) contaminated soils with different phosphate (P) additions and mycorrhizal inoculation (experiment I). Values are mean $\pm$ SD ($n = 4$). Means followed by the same letter are not significantly different (Duncan test, $p < 0.05$).

Treatment	F (%) ^f	M (%) ^g	A (%) ^h
Control ^a	9.64 $\pm$ 3.79b	1.21 $\pm$ 0.91b	0.12 $\pm$ 0.09b
P30 ^b	6.91 $\pm$ 0.35b	1.35 $\pm$ 0.28b	0.14 $\pm$ 0.03b
P60 ^c	8.84 $\pm$ 3.14b	0.80 $\pm$ 0.81b	0.08 $\pm$ 0.08b
P150 ^d	3.71 $\pm$ 5.23b	1.27 $\pm$ 1.81b	0.13 $\pm$ 0.18b
+M ^e	100 $\pm$ 0a	81.5 $\pm$ 2.93a	71.8 $\pm$ 5.51a

^a Control: no P amendment, without mycorrhizal inoculation.

^b P30: P amendment of 30 mg kg^{-1} , without mycorrhizal inoculation.

^c P60: P amendment of 60 mg kg^{-1} , without mycorrhizal inoculation.

^d P150: P amendment of 150 mg kg^{-1} , without mycorrhizal inoculation.

^e +M: no P amendment, with mycorrhizal inoculation.

^f F (%) represents the frequency of mycorrhizal colonization.

^g M (%) represents the intensity of the mycorrhizal colonization.

^h A (%) represents the arbuscule abundance.

3.2. Experiment II

Similar with experiment I, AM symbiosis developed well in mycorrhizal roots as mycorrhizal colonization frequency (F%) was approximately 100%, the intensity of colonization (M%) exceeded 60%, and the arbuscule abundance (A%) were generally higher than 49% (Table 2). Besides, there were no difference in F%, M% and A% among all three inoculated treatments (Table 2, $p < 0.05$). By contrast, no mycorrhizal colonization was detected in non-inoculated plants (Table 2). AMF inoculation treatments also exhibited higher hyphal length densities in RC than that of non-inoculation treatment (Table 2, $p < 0.05$). Treatments "+M + Cr" and "+M – Cr" had higher hyphal length densities in HC than treatments "–M + Cr" and "+M + F + Cr" (Table 2, $p < 0.05$).

Generally, AM colonization increased plant shoot and root dry weights except for treatment "+M – Cr" (Fig. 5a and b, $p < 0.05$), and there were no significant differences in plant dry weights among the three inoculated treatments (Fig. 5a and b). AM colonization increased plant P concentrations especially for roots, while decreased shoot Cr concentration significantly (Fig. 5c–f, $p < 0.05$). The treatment "+M + Cr" had a higher root Cr concentration than other three control treatments (Fig. 5f, $p < 0.05$), and root Cr concentration of "+M – Cr" was lower than that of "–M + Cr" and "+M + F + Cr" (Fig. 5f, $p < 0.05$). Among all 4 treatments, "+M + Cr" held the highest root and total Cr uptake (Fig. S2, $p < 0.05$), while there was no difference in shoot Cr uptake. Besides, treatment "+M + Cr" had the lowest TF value for Cr of all 4 treatments (Fig. S3).

4. Discussions

Through comparing the influences of exogenous P amendment with AM symbiosis on plant Cr(VI) tolerance, the present study revealed that AM symbiosis was multifunctional, and its benefits for host plants could not be simply replaced by P amendment. Furthermore, many previous studies (Chen et al., 2001, 2003, 2007b; Wu et al., 2014) implied the significance of metal immobilization by AM fungi in plant metal tolerance. Therefore, SR μ -XRF study on principal roots was carried out to test this possibility, and a compartment cultivation system was used to test if the extensive ERM contributed to Cr stabilization.

As one of the most important nutrients in plants, P is crucial for plant growth, development and reproduction. P also plays an important role in plant resistance to environmental stresses. For example, our recent study found that P addition improved the growth of *Medicago truncatula* L. under As contaminations (Zhang et al., 2015). However, the present study showed no significant improvement of plant growth by P addition in Cr(VI) contaminated soil, although certain P amendment level (150 mg kg^{-1}) increased shoot P concentration while decreased root Cr concentration (Fig. 2). The decreased Cr concentration may result from competition for root surface site between Cr(VI) and P (Chatterjee and Chatterjee 2000), as we previously found that Cr(VI) addition could decrease plant P concentration (Wu et al., 2014), and Qian's study (Qian et al., 2013) also showed that high P addition decreased plant Cr absorption.

The fact that P addition failed to promote plant growth might be caused by several reasons. Firstly, soil available P might be sufficient for non-mycorrhizal dandelion under experimental conditions as can be seen from the control treatment that plant shoot and root P concentration was 1.92 mg g^{-1} and 1.88 mg g^{-1} , which were much higher than that in our previous study (Wu et al., 2014) under the same Cr(VI) contamination level (Fig. 2). Accordingly, P amendment (below 60 mg kg^{-1}) did not increase plant P acquisition (Fig. 2). Although 150 mg kg^{-1} P amendment did increase shoot P concentration, it did not affect plant growth probably because of excessive P uptake. Secondly, it is well known

Fig. 2. Shoot and root dry weights (a and b), P and Cr concentrations (c–f) of dandelion plants (*Taraxacum platyepidum*) as influenced by P addition treatment and mycorrhizal inoculation in experiment I. Different letters above the columns indicate significant differences (Duncan test, $p < 0.05$) between treatments. The error bars represent SD ($n = 4$). The “control” represents no P was applied to the soil, “P30”, “P60” and “P150” represent 30, 60 and 150 mg kg⁻¹ P were applied to the soil respectively, and “+M” represents mycorrhizal inoculation without P application to the soil.

that soil phosphatases play an important role in P uptake by plants and mycorrhizal fungi, as these enzymes can mineralize organic P to inorganic P (Nannipieri et al., 2011), and therefore take an active part in P uptake by plants and mycorrhizal fungi. Cr contamination could possibly reduce soil phosphatase activities and thus decrease plant P uptake (Tyler, 1974; Kuperman and Carreiro, 1997). The decreased activities of soil phosphatases caused by Cr contamination probably could not be compensated by P application as previous study showed an inverse relation between P supply and acid phosphatase activity in soil (Spiers and McGill, 1979). However, AM symbiosis could possibly increase soil phosphatase activities under heavy metal contaminations (Wang et al., 2006), which would be beneficial for plant P acquisition. Thirdly, there may be different strategies for P absorption and translocation between non-mycorrhizal and mycorrhizal plants. AM symbiosis can mobilize P in the soil, or directly take up and transport P to plants (Feng et al., 2003; Karandashov and Bucher, 2005), while non-mycorrhizal root can only absorb mobile P in the soil

(Schachtman et al., 1998). Moreover, P in the mycelium mainly exists in the form of polyphosphate (poly P) (Kuga et al., 2008) and its transfer across the symbiotic interface is regulated by mycorrhiza-specific phosphate transporters (Karandashov and Bucher, 2005), while in the non-mycorrhizal roots there is no such regulation. The specific strategies of P uptake, transport and conservation in mycorrhizal plants could maintain a better P homeostasis than that of non-mycorrhizal plants. Lastly, AM symbiosis could immobilize Cr in roots and reduce its translocation to shoots, which was indicated by lower Cr TF value of mycorrhizal plants compared with that of control treatment, while exogenous P did not show such effects (Fig. 3).

The depression of Cr transport from roots to shoots by AM symbiosis was also proved by SR μ -XRF study on principal roots exposed to Cr(VI). Compared with non-mycorrhizal roots, where Cr was detected both in the cortex and vascular bundles, Cr was detected only in the cortex of mycorrhizal principal roots (Fig. 4). As Cr was mainly transported in the xylem (Skeffington et al.,

Fig. 3. P (a) and Cr (b) translocation factor (TF) of dandelion plants (*Taraxacum platycephalum*) as influenced by P addition and mycorrhizal inoculation in experiment I. Different letters above the columns indicate significant differences (Duncan test, $p < 0.05$) between treatments. The error bars represent SD ($n = 4$). The “control” represents no P was applied to the soil, “P30”, “P60” and “P150” represent 30, 60 and 150 mg kg⁻¹ P were applied to the soil respectively, and “+M” represents mycorrhizal inoculation without P application to the soil.

Fig. 4. Distribution of chromium (Cr) and mineral nutrients (Ca, K, Fe, Cu and Zn) in the thin sections of the principal roots of dandelion plants (*Taraxacum platycephalum*) inoculated with/without AM fungi under 10 mg kg⁻¹ Cr(VI) contamination (with no P application) as revealed by SR μ -XRF mapping at the microscopic scale in experiment I. Pixel size is 50 μ m.

1976), so the undetectable Cr in vascular bundle (including xylem) of principal root for mycorrhizal plants might indicate that large

quantities of Cr were retained in highly colonized fibrous roots, and little Cr could be translocated to aboveground parts. As for other

Table 2

Mycorrhiza development parameters and hyphal length density of dandelion (*Taraxacum platycephalum*) inoculated with *Rhizophagus irregularis* as influenced by different compartmentation (experiment II). Values are mean $\pm$ SD ($n = 4$). Means followed by the same letter are not significantly different (Duncan test, $p < 0.05$).

Treatment	F (%) ^e	M (%) ^f	A (%) ^g	Hyphal length density (m g ⁻¹)	
				RC ^h	HC ⁱ
–M + Cr ^a	0	0	0	0.46 $\pm$ 0.16b	0.38 $\pm$ 0.15c
+M + Cr ^b	100 $\pm$ 0a	66.6 $\pm$ 7.57a	52.6 $\pm$ 11.8a	2.35 $\pm$ 0.69a	1.28 $\pm$ 0.18b
+M – Cr ^c	99.2 $\pm$ 1.66a	62.6 $\pm$ 14.2a	49.7 $\pm$ 12.6a	1.76 $\pm$ 0.52a	2.11 $\pm$ 0.32a
+M + F + Cr ^d	99.2 $\pm$ 1.66a	76.1 $\pm$ 7.14a	66.4 $\pm$ 12.3a	2.22 $\pm$ 0.71a	0.68 $\pm$ 0.21c

^a –M + Cr represents no mycorrhizal inoculation in RC, 60 mg kg⁻¹ Cr(VI) addition in HC.

^b +M + Cr represents mycorrhizal inoculation in RC, 60 mg kg⁻¹ Cr(VI) addition in HC.

^c +M – Cr represents mycorrhizal inoculation in RC, no Cr(VI) addition in HC.

^d +M + F + Cr represents mycorrhizal inoculation in RC, 60 mg kg⁻¹ Cr(VI) addition in HC, and a 0.45 μ m acetate filter membrane was used instead of 37 μ m nylon net.

^e F (%) represents the frequency of mycorrhizal colonization.

^f M (%) represents the intensity of the mycorrhizal colonization.

^g A (%) represents the arbuscule abundance.

^h RC represents root compartment.

ⁱ HC represents hyphal compartment.

Fig. 5. Shoot and root dry weights (a and b), P and Cr concentrations (c–f) of dandelion plants (*Taraxacum platycephalum*) as influenced by mycorrhizal inoculation and different compartmentation in experiment II. Different letters above the columns indicate significant differences (Duncan test, $p < 0.05$) between treatments. The error bars represent SD ($n = 4$). “-M+Cr” represents no mycorrhizal inoculation in root compartment (RC), 60 mg kg⁻¹ Cr(VI) addition in hyphal compartment (HC); “+M+Cr” represents mycorrhizal inoculation in RC, 60 mg kg⁻¹ Cr(VI) addition in HC; “+M-Cr” represents mycorrhizal inoculation in RC, no Cr(VI) addition in HC; “+M+F+Cr” represents mycorrhizal inoculation in RC, 60 mg kg⁻¹ Cr(VI) addition in HC, and a 0.45 μm acetate filter membrane was used instead of 37 μm nylon net.

elements, Cu and Zn showed different distribution patterns from Ca, K and Fe, which may be caused by their chemical properties. Cu and Zn are micronutrient elements that can be combined with organic acids for translocation through xylem (Terzano et al., 2008; Collin et al., 2014), while Ca and K are macronutrient elements that can be transported in xylem or phloem (White, 2001; Ahmad and Maathuis, 2014). Fe was mainly accumulated in the cortex, which can be explained by the previous study showing that Fe was likely chelated to the nonproteinogenic amino acid nicotianamine (NA) in the epidermal (Curie et al., 2009).

Once AM symbiosis forms, extensive ERM are developed. These ERM may largely contribute to metal immobilization (Chen et al., 2001; Jone et al., 2000). In order to strictly investigate direct interaction between ERM and Cr, two mycorrhizal treatments – one without Cr(VI) addition to HC (treatment “+M-Cr”) and the other with 0.45 μm acetate fiber filter (treatment “+M+F+Cr”) – were included as controls in experiment II. Similar to our previous study (Chen et al., 2007a; Ren et al., 2015), the mycelium developed

well in the inoculated treatments, and passed through the 37 μm nylon net to HC, while 0.45 μm acetate fiber filter excluded mycelium development in HC. Little hyphae detected in soils of the non-inoculated treatments were probably dead or saprophytic hyphae, and were also considered to be present in the inoculated treatments. Besides, there were no difference among all mycorrhizal treatments for plant shoot and root dry weights (Fig. 5a and b), and thus we successfully diminished the growth dilution effects on Cr in plants.

The treatment “+M+Cr” clearly had a higher root Cr concentration than other 3 control treatments (Fig. 5f), and this treatment also had the highest root Cr uptake and total Cr uptake (Fig. S2), showing that ERM could take up and transport Cr from distance to mycorrhizal roots. However, there still exists possibility that Cr(VI) in the HC may diffuse to BC and RC. To check this possibility, the soil Cr speciation in each compartment of all treatments was detected. Cr(VI), DTPA-extractable Cr, acid-extractable Cr and reducible Cr concentrations in BC and RC were not significantly different among

different treatments (Table S2, $p < 0.05$), which proved that there was no significant diffusion of Cr from HC to RC, and thus confirmed that the high Cr accumulation in mycorrhizal roots of treatment “+M+Cr” was due to direct uptake by ERM.

All three mycorrhizal treatments had lower shoot Cr concentrations than non-mycorrhizal plants, which may result from “growth dilution effects”, or Cr immobilization in mycorrhizal roots. Besides, it is interesting to find that there was no differences in shoot Cr concentrations among inoculated treatments (Fig. 5e), showing that Cr uptake by ERM was possibly not translocated from roots to shoots. The lowest TF value of treatments “+M+Cr” also confirmed that AM symbiosis retained large quantities of Cr in roots (or ERM) and little Cr was translocated to shoots (Fig. S3). AMF itself can retain large quantities of heavy metals (such as Cu, Zn, Cd etc) and restrain delivery of these metals to plants (Joner et al., 2000; Chen et al., 2001; Nayuki et al., 2014), which may explain the Cr immobilization in mycorrhizal roots. In a word, experiment II no doubt proved the direct involvement of AM symbiosis in enhancing plant Cr tolerance, more exactly ERM can take up and immobilize large quantities of Cr in mycorrhizal roots.

5. Conclusions

In summary, the present work confirms that P supply in the Cr (VI) contaminated soil can not improve plant Cr tolerance as well as AM symbiosis does, and the direct immobilization of Cr by ERM and mycorrhizal roots plays an important role in plant tolerance to Cr contamination. However, this does not mean that P aid by AM symbiosis does not contribute to plant Cr tolerance, as P uptake and metabolism in mycorrhizal plants are much different from that in non-mycorrhizal plants. Although the present work confirms the uptake and immobilization of Cr by ERM, whether the Cr uptake by ERM was actually delivered to plant cells are still unknown and the underlying mechanisms of AMF tolerance to Cr (VI) needs further investigations. It is necessary to point out that the mechanisms by which AM fungi enhance plant Cr tolerance may depend on plant and AM fungi species, soil characteristics, etc. Therefore, various plants and AM fungi should also be considered when elucidating how AMF enhance plant Cr tolerance in the future.

Author's contributions

S. Wu and B. Chen designed the work. S. Wu, X. Zhang, Z. Wu, T. Li, Y. Hu and Y. Sun conducted the experiment and acquired the experimental data. Y. Wang prepared the AM fungal inoculum. S. Wu performed the data analysis and prepared the manuscript. B. Chen made critical revision for final submission.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2015.08.006>.

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