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Chromium detoxification in arbuscular mycorrhizal symbiosis mediated by sulfur uptake and metabolism

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ABSTRACT

It has been well demonstrated that arbuscular mycorrhizal (AM) symbiosis can improve plant chromium (Cr) tolerance, but the detoxification mechanisms are still unsolved. In the present study the role of AM symbiosis in plant sulfur (S) acquisition and metabolism related to Cr detoxification was investigated. *Medicago truncatula* plants were cultivated with/without AM fungus *Rhizophagus irregularis* in Cr(VI) contaminated soils at two soil S and phosphorus (P) concentrations. Sulfur uptake, transport and metabolism by plants were determined by biochemical and molecular methods. Meanwhile, Cr uptake and transformation were characterized by using spectroscopic methods, including synchrotron based X-ray absorption near edge structure (XANES) analysis. The results indicated that mycorrhizal inoculation improved plant S acquisition by up-regulating the expression of high affinity sulfate transporter genes (i.e. *MtSULTR1.1*, *MtSULTR1.2*) in plant roots. Mycorrhizal colonization also systematically regulated S transport and metabolism for Cr(VI) detoxification. XANES analysis revealed the Cr immobilization in AM roots by phosphate and histidine-like ligands rather than thiol groups. Correlation analysis further confirmed possible involvement of S metabolites in the relief of Cr induced oxidative stress. Mycorrhizal effects were more pronounced under low P condition. The study allowed a new insight into the mechanisms of enhanced plant Cr tolerance by AM symbiosis.

1. Introduction

Chromium (Cr) pollution has become a serious environmental problem worldwide due to excessive Cr containing effluents discharged by industrial activities (Zayed and Terry, 2003). Chromium usually exists in two oxidation status: Cr(III) and Cr(VI), among which Cr(III) is very stable with a low mobility, while Cr(VI) is highly mobile and much more toxic and carcinogenic in the environment (Losi et al., 1994). Chromium is not an essential element for plants, and its excess usually induces the production of reactive oxygen species (ROS) such as hydroxyl and superoxide radicals, leading to oxidative stress and cellular damages (Kováčik et al., 2015; Labra et al., 2006; Shanker and Pathmanabhan, 2004; Sharma et al., 2003). The investigation on the roles of biotic (e.g., mycorrhizal fungi) and abiotic (e.g., mineral nutrients such as sulfur) factors in regulating Cr uptake and detoxification in plants would be of great value for ecological restoration of Cr contaminated soils (Singh et al., 2013).

Arbuscular mycorrhizal (AM) fungi are ubiquitous soil fungi that can form symbiosis with most terrestrial plants (Smith and Read, 2008). In the AM symbiosis, AM fungi can take up phosphorus (P) from distance (Karandashov and Bucher, 2005; Pearson and Jakobsen, 1993) through phosphate transporters in the fungi, e.g. GiPT (Campos-Soriano

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et al., 2010) and efficiently deliver it to plant roots via specific phosphate transporters e.g. MtPT4 (Javot et al., 2007), and in return receive carbohydrates from plant partners (Jiang et al., 2017; Luginbuehl et al., 2017). More importantly, AM symbiosis can improve plant adaptation to various environment stresses that include drought, salinity, and metal(loid) contaminations (Chen et al., 2007a,b; Daei et al., 2009; Davies et al., 2001; Janouskova and Pavlikova, 2010; Wu et al., 2014; Xu et al., 2014). Our previous study well demonstrated that AM symbiosis markedly alleviated plant Cr toxicity through immobilizing Cr in roots (Wu et al., 2014). It was found that AM symbiosis could immobilize Cr in fungal structures (e.g. extra- and intraradical hyphae, spores) through complexation of Cr with phosphate or histidine analogues, and thus reduce Cr translocation from AM fungi to plant roots (Wu et al., 2016a,b, 2015). Besides direct Cr immobilization, AM symbiosis may also enhance plant Cr tolerance through improving plant mineral nutrition and facilitating the production of key metabolites, such as Sufur (*S*)-metabolites and compounds, that may contribute to detoxification of Cr(VI) in plants.

Previous studies demonstrated that AM symbiosis can improve plant S acquisition through up-regulating the expression of high affinity sulfate transporter genes (e.g. *MtSULTR 1.1* and *MtSULTR 1.2*) in plant roots (Casieri et al., 2012; Gigolashvili and Kopriva, 2014; Sieh et al., 2013), influence S transport within plants through regulating the expression of low affinity sulfate transporter genes (e.g. *MtSULTR2.1*, *MtSULTR2.2*, *MtSULTR3.1*, *MtSULTR4.1* etc) (Casieri et al., 2012; Sieh et al., 2013), and thus relieve S deficiency to plants (Wipf et al., 2014). In addition, AM symbiosis can also increase plant S acquisition through direct uptake and transport of S via extraradical mycelium (Allen and Shachar-Hill, 2009; Gigolashvili and Kopriva, 2014). The enhanced S uptake by plants can thus provide basis for synthesizing various S-bearing compounds that contribute to Cr detoxification (Holland and Avery, 2011). It is reported that some S metabolites such as cysteine (Cys), glutathione (GSH) and phytochelatins (PCs) can contribute to alleviation of Cr phytotoxicity (Gill et al., 2016; Qiu et al., 2013; Sobrino-Plata et al., 2014). Sulfur metabolites can either combine with Cr through their thiol groups and reduce Cr toxicity in plants or microorganisms (Howe et al., 2003; Levina et al., 2007; Wu et al., 2015), or serve as nonenzymatic antioxidants and take part in scavenging ROS induced by Cr stress (Sobrino-Plata et al., 2014). Considering the importance of S metabolism in Cr detoxification, and the involvement of AM symbiosis in plant S acquisition, it is hypothesized that AM symbiosis can potentially alleviate Cr phytotoxicity through influencing plant S uptake and S metabolism. This proposed mechanism may deepen our understanding of the physiological mechanisms underlying the Cr tolerance of AM symbioses, which is complementary to our previous studies (Wu et al., 2016a,b, 2015) that highlighted the translocation and transformation of Cr by AM symbioses.

The present study therefore aimed to investigate mycorrhizal effects on S uptake and metabolism in plant roots in relation to plant Cr tolerance, by using the model legume plant *Medicago truncatula* and AM fungus *Rhizophagus irregularis*. It was hypothesized that: (1) Mycorrhizal inoculation can enhance plant S uptake by up-regulation of the expression of key S transporter genes under Cr(VI) stress; (2) Mycorrhizal inoculation can facilitate the production of key S metabolites (i.e. Cys, GSH, PCs) in plants which consequently lead to Cr(VI) detoxification; (3) S metabolites take part in both Cr(III) chelation (through thiols) and the relief of oxidative stress induced by Cr(VI) in AM plants. In the experiment, the mycorrhizal effects on S uptake and metabolism were investigated at both low and high soil S concentrations. Given that P supply can largely influence AM symbiosis development and functions (Black and Tinker, 1977; Carbonnel and Gutjahr, 2014; Chu et al., 2013; Lee and George, 2005; Nouri et al., 2014), two soil P concentrations were also considered to examine how P status influence AM functions towards plant S nutrition and Cr tolerance.

2. Materials and methods

2.1. Growth substrate, host plants and AM fungal inoculum

Experimental soil was collected from Panggezhuang, Daxing district, Beijing (39.8360 N, 116.8180 E), passed through 2-mm sieve and sterilized by γ-radiation (20 kGy) before use. Details of the soil properties are displayed in Table S1. Before sowing, basal nutrients including N (120 mg kg^{-1}) and K (120 mg kg^{-1}) were carefully mixed into the soil.

Seeds of *Medicago truncatula* cv. Jemalong A17 were obtained from the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The seeds were scarified in sulfuric acid for approximately 10 min, carefully washed with Milli-Q water, and then pregerminated in darkness on moist filter paper until the emergence of radicles.

The AM fungus *Rhizophagus irregularis* BGC AH01 was provided by the Beijing Academy of Agriculture and Forestry Sciences. The fungal inoculum was a mixture of growth substrate, fungal spores (approximately 150 spores g^{-1}), mycelia and colonized root fragments.

2.2. Experimental design

The soil was amended with 20 mg kg^{-1} Cr(VI) (Our short-term Cr (VI) exposure experiment confirmed that 20 mg kg^{-1} Cr(VI) addition could pose toxicity to plants under experimental conditions, see Supplementary information (SI)), which was then incubated for 1 month to allow for equilibrium. Two soil S concentrations (0 and 60 mg kg^{-1} S in the form of K_2SO_4, realistic concentrations according to Casieri et al. (2012)) and two soil P concentrations (0 and 60 mg kg^{-1} P in the form of KH_2PO_4, realistic concentrations according to Wu et al. (2016a)) were designated to investigate their effects on mycorrhizal functions towards plant Cr tolerance. For each of the 4 combinations of P and S treatments, plants were inoculated with/without AM fungus. For AM inoculation treatment, 600 g amended soils were firstly put into the pot, and then 300 g soil containing 30 g fungal inoculum were added to the top. For non-inoculation controls, 30 g sterilized AM fungal inoculum together with 10 mL inoculum filtrate were added to reintroduce soil microbial communities except for AM fungi. Seedlings were thinned to 4 per pot one week after emergence.

In total, there were 8 treatments with 4 replicates, resulting in 32 pots. The experiments were conducted in a controlled environment greenhouse at 14/10 h (light/dark) and 25/20 °C. The light intensity was 500–1100 μmol $m^{-2}s^{-1}$, maintained by natural light and supplementary lights from high pressure sodium lamps. Each pot was daily watered by weighing to maintain water content of 15% on a weight basis.

2.3. Plant harvest and sample preparation

Plant roots, stems and leaves were separately harvested at 120 days after planting. Plant shoots and roots were carefully washed with distilled water and weighed to obtain the fresh weights. Subsamples of roots were prepared for AM colonization assessment. Part of the plant roots, stems and leaves were lyophilized with a freeze-dryer at −50 °C for 48 h. The dried samples were motor-homogenized in liquid nitrogen for chemical analysis (S, P and Cr concentration, and Cr speciation), the left fresh root and leaf samples were kept in liquid N_2 and then transferred to −80 °C for RNA extraction and biochemical analysis.

2.4. AM colonization assessment

Arbuscular mycorrhizal (AM) colonization was determined according to Phillips and Hayman (1970) by omitting phenol from the rinse. Briefly, sub-samples of fresh roots were cut into 1-cm fragments, cleared in 10% KOH, and rinsed in 2% HCl, and then stained with 0.05% Trypan blue. Thirty pieces of randomly selected stained root