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Effects of cadmium stress on growth and amino acid metabolism in two Compositae plants

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ABSTRACT

Cadmium, a high toxic heavy metal, is one of the most serious contaminants in soil and a potential threat to plant growth and human health. Amino acid metabolism has the central role in heavy metal stress resistance of plants. In this paper, a pot experiment was carried out to study the effects of different concentrations of cadmium (0, 3, 6, 12, 30 mg kg^{-1}) on the growth, Cd accumulation and amino acid metabolism in two Compositae plants (*Ageratum conyzoides* L. and *Crassocephalum crepidioides*). The results showed that under cadmium stress, *C. crepidioides* accumulated more Cd in its shoot and was tolerant to Cd, whereas its low Cd-accumulating relative, *A. conyzoides*, suffered reduced growth. The Cd content in the aerial part of *C. crepidioides* exceeded the threshold of Cd-hyperaccumulator. Furthermore, the bioaccumulation factor (BCF) and biological transfer factor (BTF) values for Cd in *C. crepidioides* were > 1. Thus, *C. crepidioides* can be regarded as Cd-hyperaccumulator. The comparison between both studied plants indicated that Cd stress resulted in a differential but coordinated response of amino acid levels, which are playing a significant role in plant adaptation to Cd stress. Glu, Gln, Asp, Asn, Gaba, Val and Ala dominated the major amino acids. Higher Cd tolerance and Cd accumulation in *C. crepidioides* was associated with greater accumulation of free amino acids, especially for Gln and Asn, in *C. crepidioides* than in *A. conyzoides*.

1. Introduction

Increasing environmental pollution caused by heavy metals, originating mainly from industrial processes and urban activities, as well as the widespread application of pesticides, fertilizers, manure and sewage sludge, has posed a serious problem for safe food production and become a potential agricultural and global environmental problem (Bonet et al., 2014; Daş et al., 2016; Zhu et al., 2017a). Among the various heavy metals, Cadmium (Cd) occupies the top position in terms of hazardous effects posed to plants and human health, due to its high toxicity, mobility, and availability for all living organisms (Ali et al., 2015; Clemens et al., 1999; Wael et al., 2015). The deleterious effects following exposure to Cd in humans has been associated with cancers of the prostate, lungs, and testes, renal dysfunction, rhinitis, emphysema, and bone fractures (Jarup and Akesson, 2009). The accumulation of Cd also inhibits growth, development and productivity of plants via impaired amino acid biosynthesis, inhibition of enzyme activities, induction of oxidative stress, interference with mineral nutrition and metabolic imbalances (Liu et al., 2015; Nagajyoti et al., 2010). The visual symptoms of Cd toxicity in plants are chlorosis and necrosis of leaves, browning of roots and cell apoptosis (Zemanová et al., 2015b). However, plants have evolved a variety of adaptive mechanisms to protect against Cd stress. This is achieved by cellular exclusion, sequestration, chelation osmotic adjustment, metabolic utilization and production of antioxidant systems, etc. (Kushwaha et al., 2016; Li et al., 2016; Rahman et al., 2017; Sytar et al., 2013; Zhang et al., 2015).

Nitrogen metabolism is central in the plant response to heavy metals; it has been shown that Cd may interfere with nitrogen metabolism in plants (Chaffei et al., 2004). Upon exposure to metals, plants often synthesize a set of diverse low-molecular weight substances, particularly specific free amino acids (FAA), which are known as compatible solutes, and have been shown to serve as signaling molecules and play an important role in plants varied from acting as osmolyte, radical scavenger, regulation of ion transport, modulating stomatal opening to detoxification of heavy metals (Pavlíková et al., 2014; Sharma and Dietz, 2006; Xu et al., 2012). The changes appearing in free amino acids

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as a response to different stress factors are important for plant metabolism as they are not only known as precursors and functional components of proteins but also, for most of them, as precursors of other nitrogen containing compounds such as nucleic acids (Rai, 2002). The accumulation of Cd in plant organs cause damages to the photosynthetic apparatus (Gallego et al., 2012). The reduction in the photosynthetic rate results in a limited supply of metabolic energy and therefore to nitrogen assimilation restriction. Nitrogen flow through amino acids can thereby change in response to Cd stress (Zemanová et al., 2015a). Amino acids also affect synthesis and activity of some enzymes, gene expression and redox-homeostasis (Chaffei-Haouari et al., 2009; Islam et al., 2009). Furthermore, amino acids rich in carboxyl, amino, thiol and phenolic groups are involved in the synthesis of glutathione and phytochelatin, which are able to form complex metal cations diminishing its reactivity with other molecules at cellular level, and serving as long-distance metal-chelating compounds (Dave et al., 2013; Ghnaya et al., 2010; Irtelli et al., 2009; Richau et al., 2009).

Previous studies have focused attention on the role of the amino acids in Cd tolerance of plants. It has been recorded that Cd accumulation leads to the accumulation of proline in mung, wheat and barley (Leskó and Simon-Sarkadi, 2002; Vassilev and Lidon, 2011; Zhang et al., 2000). However, Costa and Morel (1994) found that Cd induced no accumulation of proline in lettuce but induced specific increases in the levels of asparagine, methionine and lysine. Zoghlami et al. (2011) showed that, in the roots of tomatoes after Cd exposure, asparagine, glutamine and branched chain amino acids (valine, isoleucine, phenylalanine and tryptophane) significantly accumulated; in contrast, few modifications occurred in the leaves in response to Cd, except for tyrosine. Xu et al. (2012) detected that a higher accumulation of proline in *S. nigrum* supports the observed higher Cd tolerance in S. *nigrum* than in *S. torvum*; a high accumulation of hydroxyproline in *S. torvum* roots may play a protective role in preventing Cd translocation from the roots to the aerial parts of the plant. Zemanová et al., (2013, 2014) reported that the major amino acid forms used for nitrogen transport are asparagine and histidine for the higher stress adaptation of *A. halleri* and glutamate for *N. caerulescens*. An increase of phenylalanine, threonine, tryptophan, ornithine and a decrease of alanine and glycine were observed in the responses of the two *Noccaea* metallophytes species to Cd stress (Zemanová et al., 2017). According to the data available in literature, amino acid metabolisms are differently affected by heavy metal treatments, plant species, genotypic difference, and even by different parts of the plant.

Phytoremediation technology is defined as the use of plants to remove contaminants from soils or to render them harmless, and is regarded as a cost-effective, environmental-friendly method for reclaiming soils contaminated by toxic metals (Lasat, 2002; Mahar et al., 2016; Marrugo-Negrete et al., 2016). Compositae plants have been shown to be excellent candidates for phytoremediation purposes due to their rapid growth, high biomass, strong breeding ability, adapted to growing in soils polluted with heavy metals, and low impact on the food chain and human health (Hernández and Pastor, 2008; Peng et al., 2006). We have already studied the content, subcellular distribution and chemical forms of heavy metals in three types of Compositae plants (*Artemisia lavandulaefolia*, *Ageratum conyzoides* L., *Crassocephalum crepidioides*) from one lead-zinc tailings area, and demonstrated that *C. crepidioides* demonstrated the basic characteristics of a Cd-hyperaccumulator, cell wall binding, vacuolar compartmentalization and distribution mainly in lower active chemical forms were supposed to be the main tolerance mechanisms to heavy metals (Zhu et al., 2017b, 2018). Free amino acids have been shown to have functional roles in metal tolerance of plants. However, there is little information on the free amino acid metabolism in Compositae plants under heavy metal stress. Therefore the present study was conducted to (1) confirm the identification of Cd hyperaccumulator for *C. crepidioides* by pot-culture upon exposed to various gradient of Cd stress, and (2) characterize the changes and differences in accumulation of free amino acids of two Compositae species with different Cd enrichment abilities -*Ageratum conyzoides* L. and *Crassocephalum crepidioides*, growing in pot experiment under Cd stress.

2. Materials and methods

2.1. Experimental design

The pot experiment was carried out in the greenhouse at the institute of geochemistry, Chinese Academy of Sciences, Guiyang, China. Topsoil (0–20 cm) was collected from a local abandoned vegetable garden, and its soil properties were as follows: a soil pH of 6.29; an organic matter content of 65.87 g kg^{-1}; a cation exchange capacity (CEC) of 19.86 cmol·kg^{-1}; total Cd concentrations of 0.62 mg kg^{-1} and available Cd concentrations of 0.25 mg kg^{-1}. The soil was air-dried, mixed thoroughly and sieved through 1 cm mesh. About 5.0 kg portions of the soil were transferred to plastic pots (30 cm in diameter and 25 cm in height). Cadmium was added at a rate of 0, 3, 6, 12, 30 mg kg^{-1} dry soil as an aqueous solution of $CdCl_2{\cdot}2.5H_2O$ for five different soil treatments and designated as CK, Cd3, Cd6, Cd12 and Cd30, respectively. After two months of the Cd addition, soil basal fertilizers were applied at 80 mg P kg^{-1} of dry soil and 100 mg K kg^{-1} of dry soil as KH_2PO_4. Additional N was added at 100 mg kg^{-1} of dry soil as CO $(NH_2)_2$.

The seeds of *Ageratum conyzoides* L. and *Crassocephalum crepidioides* were initially grown on artificial, non-polluted soil. After the first pair of healthy tender leaves appeared, the seedlings were thinned to four plants per pot and grown for ten weeks. Plants were kept in an ambient temperature of 22 ± 2 ℃, and a 16 h photoperiod of approximately 300 mE $m^{-2}{\cdot}s^{-1}$ intensity, and at 60% average relative humidity. Each pot was watered twice a week and the moisture level of the soil was maintained at 60–70% WHC. Each treatment was performed in three replicates.

2.2. Sample collection and chemical analysis

At the harvest, plants were gently removed from the pots and the fresh weights of the individual plant were subsequently determined. Roots, stems and leaves were further separated, then rinsed with tap water and carefully washed with deionized water later. Near half of the samples were dried by at 105 ℃ for 30 min, then oven dried at 65 ℃ to constant weight (around 3 days). The dried plant samples were milled to a fine powder and passed through a 2 mm sieve. The plant samples were digested with a 4:1 ratio of concentrated HNO_3-$HClO_4$. The residuals were re-dissolved by HNO_3 (2%) and diluted with distilled water. Water used for dilution and dissolution was purified using a Millipore deionizing system at 18.2 MΩ. HNO_3 and $HClO_4$ were superpure reagents. The solutions from the digested samples were stored at 4 ℃ until analysis. Cadmium concentration was determined by using inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, PerkinElmer company, US) in the Center for Environmental Remediation, Institute of Geographic Sciences and Natural Resources Research (IGSNRR), Chinese Academy of Sciences. The quality control included triplicate analyses, blanks and two standard reference plants.

The remaining half of the samples were frozen in liquid nitrogen and immediately lyophilized for free amino acid extraction. For total soluble amino acids extraction, the procedure was performed according to Xu and Xiao (2017). Briefly, about 150 mg plant sample (200 μL, 1 nmol·μL^{-1} α-aminobutyric acid and sarcosine added as internal standards) was homogenized with 1.8 mL of trifluoroacetic acid (TFA) 10% (v/v) under sonication for 5 min at 4 ℃. The homogenate was centrifuged at 12,000 rpm for 15 min (4 ℃), and the supernatant solution was collected. To recover the maximum amount of amino acids from the powder samples, the remaining sample was reextracted using 2 mL TFA (10% v/v) in the same manner. The supernatants were combined and filtered through a 0.22 mm membrane, and the collected solution