Environmental Pollution 241 (2018) 422–431

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Response of edible amaranth cultivar to salt stress led to Cd mobilization in rhizosphere soil: A metabolomic analysis☆

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ARTICLE INFO

Article history:
Received 11 December 2017
Received in revised form
23 April 2018
Accepted 4 May 2018

Keywords:
Metabolic profiles
Salt stress
Tricarboxylic acid cycle
Organic acid excretion
Soil Cd mobility

ABSTRACT

The present study aimed to investigate the metabolic response of edible amaranth cultivars to salt stress and the induced rhizosphere effects on Cd mobilization in soil. Two edible amaranth cultivars (*Amaranthus mangostanus* L.), Quanhong (low-Cd accumulator; LC) and Liuye (high-Cd accumulator; HC), were subject to salinity treatment in both soil and hydroponic cultures. The total amount of mobilized Cd in rhizosphere soil under salinity treatment increased by 2.78-fold in LC cultivar and 4.36-fold in HC cultivar compared with controls, with 51.2% in LC cultivar and 80.5% in HC cultivar being attributed to biological mobilization of salinity. Multivariate statistical analysis generated from metabolite profiles in both rhizosphere soil and root revealed clear discrimination between control and salt treated samples. Tricarboxylic acid cycle in root was up-regulated to cope with salinity treatment, which promoted release of organic acids from root. The increased accumulation of organic acids in rhizosphere under salt stress obviously promoted soil Cd mobility. These results suggested that salinity promoted release of organic acids from root and enhanced soil Cd mobilization and accumulation in edible amaranth cultivar in soil culture.

1. Introduction

Soil salinization can reduce crop growth and grain yield and is a long-standing environmental problem in the world (Wang and Li, 2013). Worse yet, a large proportion of salt-affected soil is simultaneously contaminated with heavy metals (Hu et al., 2013). Salinity could enhance the accumulation of heavy metals in crops (Acosta et al., 2011; Li et al., 2012; Weggler et al., 2004). Thus, co-occurrence of heavy metals and salinity in farmlands greatly threatens food safety. The impact of salinity on Cd mobilization can be reflected in changes of heavy metal species and distribution between the solid and solution phases of soil (Smolders et al., 1998), as well as changes in soluble heavy metal species in rhizosphere soil due to increased metal-Cl complexes. Salinity can increase soluble Cd concentration in soil by forming stable and soluble Cd-chloride complexes and/or desorbing Cd from solid soil and mineral surface via ion exchange (Acosta et al., 2011). Salt stress also induces osmotic stress and ion injury by disrupting ion homeostasis in plant cells (Shulaev et al., 2008). Stress resistance is primarily accomplished via metabolic adjustment through synthesis of enzymatic antioxidants and organic osmotic substances, such as proline, glycine betaine, soluble sugars, and free amino acids (Garg and Manchanda, 2009; Sleimi et al., 2015). Such synthesis would inevitably alter the release of exudates by metabolic adjustment in response to salt stress.

Exudates released from plants and microbes can alter the mobility of soil heavy metals by acidification (Li et al., 2013), changing soil redox potential (Wu et al., 2010), and complexation (Tao et al., 2016). A previous study indicated that cadmium (Cd)-dissolved organic matter complexes were the dominant Cd species in soil solutions after *Sedum alfredii* grew (Li et al., 2013). Differential pulse anodic stripping voltammetry measurement also indicated that Cd lability in the rhizosphere solution of durum wheat was mainly attributed to lability of organically-bound heavy metals (Bravin et al., 2012). However, the impact of salinity-induced exudates and the key metabolic pathway change on rhizospheric soil Cd mobilization has remained poorly understood. Metabolites

☆ This paper has been recommended for acceptance by Prof. W. Wen-Xiong.
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https://doi.org/10.1016/j.envpol.2018.05.018
0269-7491/

in the rhizosphere can provide realistic insights into what happens under field conditions. Thus, acquiring a wide range of metabolites from roots and the rhizosphere is necessary for demonstrating plant metabolic responses to salt stress and its impact on Cd mobilization in the rhizosphere (van Dam and Bouwmeester, 2016). In recent years, metabolomics analysis has been developed as a powerful tool to investigate metabolites diversity (Obata and Fernie, 2012) and has been widely applied for metabolite profiles of plants (Gavaghan et al., 2011; Shulaev et al., 2008) and root exudates (van Dam and Bouwmeester, 2016).

As a toxic heavy metal, Cd may pose health risks to humans via consumption of vegetables. Edible amaranth (*Amaranthus mangostanus* L.), a favorite vegetable among consumers, is widely grown in Southeast Asia. Edible amaranth exhibits good adaptability in saline soil compared to other common vegetables (Li et al., 2009). Increased Cd accumulation in edible amaranth under salt stress treatment was also observed in a pot soil culture experiment (He et al., 2017). On the other hand, salinity (sodium chloride) in hydroponic cultures could reduce Cd accumulation in edible amaranth (Mei et al., 2014). Thus, increased Cd accumulation in edible amaranth grown in saline soil may be a result of increased Cd mobility in soil. The present study aimed to (1) determinate Cd mobilization directly or indirectly induced by salinity in rhizosphere soil of cultivars; (2) analyze the metabolic profiles of edible amaranth in response to salt stress; and (3) evaluate the impact of altered metabolites triggered by salinity on Cd mobilization in rhizosphere soil.

2. Materials and methods

2.1. Soil pot trial

Soil pot experiments were conducted in a greenhouse on the campus of Jinan University, Guangzhou (Guangdong Province, China). Soil used in pot culture was collected from a suburb farmland in Guangzhou that has long been contaminated with Cd. Fresh soil samples were air dried and passed through a sieve of 2.0 mm after removing coarse objects. The physicochemical properties of the soil have been described by previous study (He et al., 2015). In brief, soil pH, salinity, organic matter content, and cation exchange capacity were 6.38, 0.47 g kg^{-1}, 35.4 g kg^{-1}, and 20.86 cmol kg^{-1}, respectively. The total concentration of Cd was 1.81 mg kg^{-1}, which exceeded the Farmland Environmental Quality Evaluation Standard for Edible Agricultural Products (HJ332-2006, China).

Two contrasting Cd-accumulator edible amaranth cultivars, namely, *Quanhong* (low-Cd accumulator) and *Liuye* (high-Cd accumulator), were screened for pot experiments (He et al., 2017). The same two cultivars of edible amaranth were chosen for the present study. Seeds were sowed directly into rhizosphere bag containing 500 g sieved soil. Each pot had a diameter of 22 cm; and a depth of 15 cm, and contained three rhizosphere bags with 2.5 kg of sieved soil. After a 10-d seed germination period, the seedlings were thinned to one plant per rhizosphere bag. Soil was watered daily to maintain 75% maximum water holding field capacity. The plants were grown at temperature ranging from 25 to 35 °C and relative humidity ranging from 60% to 85%. Plants were treated with two different NaCl-level: control (no NaCl added) and salt stress (NaCl level: 3.0 g kg^{-1} dry soil). A soil treated with salt but without plant was used as bulk soil. Soils were thoroughly mixed with salt solution and then incubated for 4 weeks before used. Each treatment condition had six replicates per cultivar.

Plants were harvested after a 45-d soil culture, and rhizosphere soil and plant were carefully separated. After washing thrice with deionized water, fresh roots, shoot and whole plants in each pot were weighted and recorded. The roots were then immersed in 0.5 mmol L^{-1} $CaCl_2$ and sonicated for 10 min to remove sorbed metals. Take some part of fresh root and shoot were frozen with liquid nitrogen and stored at −80 °C in an ultra-low temperature freezer (Haier DW-86L626, China) for metabolomic analysis. The other part of roots and shoot were oven dried at 105 °C for 0.5 h, and then dried to a constant weight at 80 °C in preparation for Cd determination.

After plants were harvested, bulk soils and rhizosphere soils were collected separately. Soil adhering to the roots after shaking was defined as rhizosphere soil. About 100 g rhizosphere soil was collected per pot. Every two replicates were grouped into one to collect enough soil solution by centrifugation. Soil was directly saturated with deionized water and then maintained at equilibrium for extraction. The centrifuged rhizosphere soil was air dried and then their dry weight per pot was recorded. Extracted solutions were then centrifuged twice at 6000 rpm for 20 min and filtered through a 0.45 μm membrane filter. The centrifuged soil solution was divided into two parts, one for immediate Cd determination, and the other part was stored at −80 °C in an ultra-low temperature freezer (Haier DW-86L626, China) for metabolomic analysis.

2.2. Hydroponic culture trial

Seeds of two contrasting Cd-accumulator edible amaranth cultivars were sown in well-washed sand for germination. Three weeks after sowing, uniform seedlings were transplanted into 1.5 L Hoagland nutrient solution in a plastic vessel in a greenhouse. The growth condition was the same with as that for the pot experiment. The full Hoagland nutrient solution used in present study was agreement with Mei et al. (2014). The nutrient solution was continuously aerated and replaced every 3 days. After 6 days of full-nutrient culture, plant were treated with two different NaCl-level: control (0.25 μmol L^{-1} $Cd(NO_3)_2{\cdot}4H_2O$ + 0 NaCl) and salt stress (0.25 μmol L^{-1} $Cd(NO_3)_2{\cdot}4H_2O$ + 51.3 mmol L^{-1} NaCl). Six replicates per cultivar were grown in each treatment group. Plants were harvested after 10-d salt stress treatment. Part of the plants was used for root exudates collection (Lu et al., 2007). The roots rinsed in distilled water containing an antimicrobial agent to stop exudates degradation by microbes during collection. The rest part of plants was weighted for fresh roots and shoots. Cd adsorbed on the roots was washed with 0.5 mmol L^{-1} $CaCl_2$ before they were oven dried.

2.3. Determination of Cd

Before Cd analysis, 0.3 g of dry plant sample (root or shoot) was digested using HNO_3, and stored in bottles. The plant standard reference material [GBW07602 (GSV-1)] and blank were digested and analyzed as part of a quality control protocol. Cd concentrations were quantified by graphite furnace atomic absorption spectrometry (Shimadzu AA-7000, Japan).

2.4. Metabolites determination by GC-MS (gas chromatography-mass spectrometry)

The protocols of root and leaf sample collection, derivatization and GC-MS analysis were based on a method described by Lisec et al. (2006). A scheme about sample preparation was presented in Fig. S1 of Supplementary Material. In brief, 100 mg fresh root or leaf was rapidly frozen with liquid nitrogen and homogenized in ball mill. Then, 1.4 mL of 100% methanol was added as extractant. The mixture was shaken and the supernatant was transferred to an Eppendorf tube. Another 0.75 mL of chloroform and 1.5 mL of deionized water were added to the mixture, which was then vortexed and centrifuged. A total of 0.3 mL of supernatant was