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Sodium chloride salinity reduces Cd uptake by edible amaranth (*Amaranthus mangostanus* L.) via competition for Ca channels

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ABSTRACT

Soil salinity is known to enhance cadmium (Cd) accumulation in crops. However, the mechanism by which this occurs independent of the surrounding soil remains unclear. In this study, root adsorption and uptake of salt cations and Cd by edible amaranth under NaCl salinity stress were investigated in hydroponic cultures with 0, 40, 80, 120, and 160 mM of NaCl and 27 nM Cd. The dominant Cd species in the nutrient solution changed from free Cd^{2+} to Cd chlorocomplexes as NaCl salinity increased. High salinity significantly reduced K, Ca, and Cd root adsorption and K, Ca, Mg, and Cd uptake. High salinity decreased root adsorption of Cd by 43 and 58 percent and Cd uptake by 32 and 36 percent in salt-tolerant and salt-sensitive cultivars, respectively. Transformation of Cd from free ion to chlorocomplexes is unlikely to have significantly affected Cd uptake by the plant because of the very low Cd concentrations involved. Application of Ca ion channel blocker significantly reduced Na, K, Ca, Mg, and Cd uptake by the roots, while blocking K ion channels significantly reduced Na and K uptake but not Ca, Mg, and Cd uptake. These results suggest that Na was absorbed by the roots through both Ca and K ion channels, while Cd was absorbed by the roots mainly through Ca ion channels and not K ion channels. Salinity caused a greater degree of reduction in Cd adsorption and uptake in the salt-sensitive cultivar than in the salt-tolerant cultivar. Thus, competition between Na and Cd for Ca ion channels can reduce Cd uptake at very low Cd concentrations in the nutrient solution.

1. Introduction

Cadmium (Cd) is one of the most common toxic pollutants found in soil. Cd in the soil poses health risks to humans because of its potential to enter the food chain (Li et al., 2012a). Cd accumulates in crops more readily than most other metals and can also be translocated into edible plant parts before any signs of phytotoxicity are observed (Tudoreanu and Phillips, 2004). Phytoavailability of Cd in the soil is controlled by its chemical speciation, soil properties, and genetic features of crops (Peris et al., 2007). Previous studies have demonstrated that chloride salinity in the soil, particularly NaCl, enhances the accumulation of Cd in crops (McLaughlin et al., 1994; Weggler-Beaton et al., 2004; Usman et al., 2005; Li et al., 2012b). McLaughlin et al. (1994) reported that the concentration of Cd in potato tubers grown in soil is increased by saline water irrigation. Soil salinity significantly enhances Cd accumulation in muskmelon leaves but does not affect Cd accumulation in muskmelon fruits (Gabrijel et al., 2009). This effect has been attributed to increase in Cd availability in the soil. Salinity increases the mobility of heavy metals in the soil (Ghallab and Usman, 2007; Acosta et al., 2011). High concentration of Cl^- in the soil can result in formation of the stable compounds $CdCl^+$ and $CdCl_2^0$, which can increase desorption and mobility of Cd in the soil (Norvell et al., 2000; Usman et al., 2005). High salinity also increases the concentrations of other major cations (i.e., Na, K, Ca, and Mg) that compete with Cd for sorption sites of the solid phase. Such competition could result in desorption of Cd and promotion of its phytoavailability (Du Laing et al., 2009).

It is well known that soil salinity affects several physiological and biochemical processes, such as plasma membrane permeability and transpiration rate, in plants (Mühling and Läuchli, 2003; Amer, 2010). This effect is closely related to the uptake and translocation of heavy metals in crops. Many previous studies focused on the effect of salt on Cd availability in the soil. However, only a few studies have considered the effects of salinity on the

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uptake and accumulation of Cd by crops independent of the properties of surrounding soil and the desorption processes from clay particles (Smolders and McLaughlin, 1996a, 1996b; Mühling and Läuchli, 2003; Huang et al., 2007; Lefèvre et al., 2009). In addition, the mechanism underlying this effect remains unclear. Understanding this effect is important for both cultivated crops and phytoextraction plants. Therefore, this study sought to investigate the adsorption and uptake of salt cations and Cd by the roots of edible amaranth under NaCl salinity stress using hydroponic culture. More specifically, the role of Ca and K ion channels in Cd and Na uptake was examined using channel blockers, and the effects of crop salt tolerance on Cd adsorption and uptake under NaCl salinity stress were investigated.

2. Materials and methods

2.1. Determination of cadmium in soil

Since very high doses of Cd are commonly used in physiological studies, undermining their direct relevance in the field, we used realistic Cd doses in plant hydroponic culture. The same Cd concentration as that found in soil solution separated from field soil was used in plant hydroponic culture. Based on our previous investigation on Cd contamination of farmland soil in the Pearl River Delta (Li et al., 2012a), a sample of typical Cd contaminated farmland soil was collected from this region after soil moisture was brought to field capacity using the method of Dewis and Freiras (1976). The collected fresh soil sample was divided into two parts. One part was centrifuged at 4000*g* for 5 min for separation of soil solutions. Another part was air dried at room temperature, and ground to pass through a 100-mesh nylon sieve. The total Cd content of the ground soil was determined using HCl–HNO_3–HF extraction. The concentration of Cd in each solution was determined by graphite furnace atomic absorption spectroscopy (GFAAS) (Shimadzu AA-7000, Japan) with Cd detection limit of 0.006 μM. The measured total Cd content of soil was 16 ± 2.7 μmol kg^{-1} dry weight soil, which is 6-fold higher than the farmland soil standard of China (National Standard of PR China, 2006). The measured Cd concentration in separated soil solution was 19 ± 2 nM. Therefore, 27 nM (19 nM divided by 70 percent) Cd was arbitrarily used in plant hydroponic culture to simulate the concentration of Cd in soil solution of a typical Cd-contaminated farmland soil in the Pearl River Delta.

2.2. Plant material and growth conditions

The salt-tolerant cultivar Taiwanbai (cultivar A) and the salt-sensitive cultivar Jianyeqing (cultivar B) were used in this study. Seeds of these commercially available cultivars were obtained from shops in Guangzhou city.

A hydroponic culture-based experiment was conducted. Four salinity treatment conditions with 40 (T1), 80 (T2), 120 (T3), and 160 (T4) mM of NaCl and one control (CK) condition with 0 mM of NaCl were designed for each cultivar. Each treatment and control experiment had five replicates. In all treatment and control conditions, the concentration of Cd, applied as $Cd(NO_3)_2$, was 27 nM. Since nutrient concentrations vary significantly in different soils, Hoagland nutrient solution was used to standardize the concentrations of other important compounds and thus make the results comparable with those of previous studies. The composition of full-strength Hoagland nutrient solution used in this study was 4.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 mM $MgSO_4 \cdot 7H_2O$, 5.0 mM KNO_3, 1.0 mM NH_4NO_3, 1.0 mM KH_2PO_4, 0.132 mM $MnSO_4 \cdot 4H_2O$, 0.1 mM H_3BO_3, 0.03 mM $ZnSO_4 \cdot 7H_2O$, 0.1 μM $CuSO_4 \cdot 5H_2O$, 0.1 μM $CoCl_2$, 1.0 μM $Na_2MoO_4 \cdot 2H_2O$, 5.0 μM KI, and 0.1 mM EDTA–FeNa.

Seeds were germinated at 25 °C in fine sand irrigated with 0.1 strength Hoagland nutrient solution. At 2 weeks after germination, five uniform seedlings with four leaves each were transferred to 1.5 L of 0.3 strength Hoagland nutrient solution (pH=5.5, buffered with Mes–Tris) for 4 d and then to 0.5 strength solution for 8 d. At 12 d after hydroponic culture, the seedlings were transferred to 0.8 strength solution to which 27 nM Cd and 40 mM NaCl were added. At 20 d after hydroponic culture, the seedlings were transferred to full-strength solution. In order to allow plants to adapt gradually to salt, the salt concentration was increased by 40 mM NaCl every 4 d until the targeted concentration was reached. The nutrient solution was aerated continuously and replaced every 4 d. The plants were grown in a glasshouse. The temperature range during the growth season was 20–34 °C, and the relative humidity was 60–85 percent. The plants were harvested after 50 d in hydroponic culture.

2.3. Desorption of elements from the roots

After washing with deionized water, fresh weights (FWs) of the roots, stems, and leaves of the vegetables in each vessel were recorded. The roots of each replicate were cut into 1.5 cm long pieces and put into a 50 mL centrifuge tube. The roots were then immersed in 10 mM EDTA–NH_4 and sonicated for 10 min. The supernatant was transferred to a 50 mL flask. The above process was repeated three times to completely remove adsorbed metals as previously described (Liu et al., 2011). The desorption solution was diluted to 50 mL with deionized water for determination of metal content. Samples of desorbed roots, stems, and leaves of vegetables were oven-dried at 60 °C until constant weight was achieved. After the dry weights were recorded, the samples were ground to fine powder and passed through a 60-mesh sieve in a pre-cleaned steel grinder. The fine powders were then stored in polythene zip bags.

2.4. Speciation and complexation modeling

Speciation and complexation of Cd, Na, Ca, and Mg with (in)organic ligands in the hydroponic culture solution under different NaCl salinity conditions were predicted using the chemical equilibrium model in Visual MINTEQ 3.0. EDTA was included by selecting the "show organic components" option when the program was run.

2.5. Treatment with ion channel blockers

Treatment and control experiments were conducted in Hoagland nutrient solution as follows: Ca ion channel blocker treatment, 2 μM $Cd(NO_3)_2$+50 mM NaCl+1 mM $LaCl_3$; K ion channel blocker treatment, 2 μM $Cd(NO_3)_2$+50 mM NaCl+5 mM tetraethyl ammonium chloride; and control treatment, 2 μM Cd $(NO_3)_2$+50 mM NaCl. All experiments were repeated three times.

Only cultivar A was used for this set of experiments. The hydroponic culture conditions were similar to those described in Section 2.2. Each replicate consisted of five uniform seedlings with four leaves each in Hoagland nutrient solution. Cd $(NO_3)_2$, NaCl, and ion channel blocker were added to the nutrient solution after culturing the plants for 50 d. The plants were harvested after 8 h of exposure to ion channel blockers. Plant roots were cleaned with deionized water and desorbed with EDTA–NH_4 for further uptake analysis.

2.6. Determination of metals

The ground soil sample was digested with 2 mL HCl, 6 mL HNO_3 and 2 mL HF, and the ground plant samples were digested with 10 mL HNO_3 in a microwave digestion system (MARS; CEM, USA) (Wang et al., 2011). The samples were heated for 5 min to 120 °C and maintained at that temperature for 3 min, then heated another 5 min to 150 °C and maintained for another 3 min, and were further heated for 6 min to 180 °C and maintained for 10 min for digestion. The concentrations of Na, K, Mg, and Ca in each digested solution and desorption solution were determined using flame atomic absorption spectrometry (FAAS) (Shimadzu AA-7000, Japan). The concentrations of Cd in the samples were determined using GFAAS (Shimadzu AA-7000, Japan).

Analytical reagent blanks were used in each batch of digestion and analyzed for the same elements as the samples. A plant standard reference material (GBW07602 (GSV-1)) was subjected to digestion and then analyzed to comply with the quality control protocol. Results of our analysis were accepted when the measured concentrations in the reference materials were within one standard deviation of the certified values. Average recoveries of Na, K, Mg, Ca, and Cd in certified reference materials were 103.1, 102.5, 96.5, 97.4, and 108.4 percent, respectively.

2.7. Data analysis and statistics

The amount of metals desorbed from the roots reflects metal adsorption by the roots. Likewise, the concentration of metals in the desorbed roots and shoots indicates metal uptake by the crop. For each metal, its uptake by the crop was calculated from weighted average of its concentrations in the desorbed roots, stems, and leaves relative to its biomass. Statistical analysis was performed using SPSS v17.0. The outcomes among different treatment conditions were compared by one-way ANOVA at the 0.05 significance level.

3. Results and discussion

3.1. Biomass

The biomasses of edible amaranth in the control and treatment groups are presented in Fig. 1. The biomasses of both cultivars decreased significantly with increasing salinity. Under control conditions, the biomasses of the two cultivars were similar. However, the biomass of cultivar A was significantly higher than that of cultivar B in the treatment groups. Zong et al. (2007) and