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Use of low-calcium cultivars to reduce cadmium uptake and accumulation in edible amaranth (*Amaranthus mangostanus* L.)

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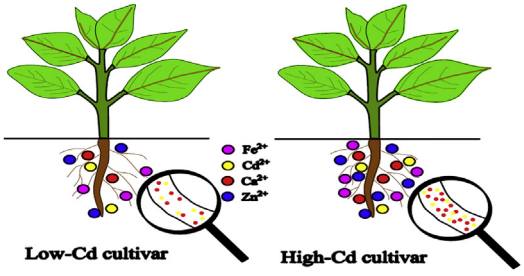
HIGHLIGHTS

- Low Ca cultivars resist the uptake of Cd.
- Ca-deficient culture led to increased Cd uptake in *Amaranthus mangostanus* L..
- Cd resistors secreted less dissolved sugars, amino acids, and organic acids.
- Cd resistors also possessed low root-shoot translocation efficiency for Cd.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed to investigate the mechanism of low Cd accumulation in crops using edible amaranth (*Amaranthus mangostanus* L.) as a model. Fifteen amaranth cultivars were grown in long-term contaminated soil, and the differences in soil Cd mobilization, root uptake, and root-shoot translocation between low- and high-Cd accumulating cultivars were examined. The transport pathways of Cd across the root were further identified in Hoagland nutrient solution using the Ca channel blocker La^{3+}, the ATP inhibitor 2, 4-dinitrophenol (DNP), and a nutrition-deficient culture. Cd concentrations in amaranth cultivars varied about six-fold and showed an elevated trend as the concentration of Ca and Zn increased ($p < 0.01$), but did not exhibit any correlation with Mg and Fe. The concentrations of essential metals (Ca, Mg, Zn, and Fe) in the rhizosphere of low-Cd cultivars were significantly lower than those of high-Cd cultivars, and decreased with decreasing levels of soluble rhizosphere exudates. These findings indicated that low co-mobilization of Cd with essential metals mediated by root-induced exudates of low-Cd cultivars contributed to its low accumulation in amaranth. Uptake of Cd was inhibited along with Ca by La^{3+} and DNP, but was promoted by Ca or Fe deficiency treatment. Therefore, the Ca pathway is likely the mode of Cd entry into amaranth roots, although Zn and Fe transporters may also be involved. Low-Ca cultivars exhibited lower Cd uptake capability than high-Ca cultivars. The low translocation efficiency of Cd from root to shoot also contributed to its low content accumulation in edible parts of amaranth.

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1. Introduction

Agricultural soil is generally subjected to persistent heavy metal contamination from sewage irrigation, fertilizer application, and atmospheric deposition (Shi et al., 2009; Wu et al., 2010). Heavy metals in the topsoil are easily absorbed by crops and enter the food chain. Among heavy metals, cadmium (Cd) is particularly notorious for its extensive distribution, high toxicity, and easy translocation from the soil to crops (Jamali et al., 2008a, 2009; Ding et al., 2013; Aziz et al., 2015; Marettová et al., 2015). Control of Cd uptake and accumulation by crops is urgently needed in order to reduce its risks to human health. A number of studies have revealed that the uptake and accumulation of Cd in some cultivars of the same crop species, such as rice (*Oryza sativa* L.) (Yu et al., 2006), wheat (*Triticum aestivum* L.) (Stolt et al., 2006), *Sorghum bicolor* L. (Jamali et al., 2008b), peanut (*Arachis hypogaea* L.) (Su et al., 2013), asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) (Zhu et al., 2007), potato (*Solanum tuberosum* L.) (Dunbar et al., 2003), water spinach (*Ipomoea aquatic Forsk*.) (Wang et al., 2009), and Chinese cabbage (*Brassica campestris* L. *ssp*. *Chinensis* var. *utilis* Tsen et Lee) (Liu et al., 2009), are significantly lower than those of other cultivars of the same species. Thus, the low-Cd-accumulating characteristics of crop cultivars can be employed to reduce their uptake and accumulation in crops.

In long-term contaminated agricultural soil, Cd is adsorbed on clays and organic matter (Janos et al., 2010; Rodrigues et al., 2012). The supply of available Cd is a key factor affecting Cd uptake and accumulation in crops (Luo et al., 2010; Lux et al., 2011). The Cd concentration in plants is substantially increased as its available content is enhanced in the soil (Liu et al., 2015). Cadmium availability is partly controlled by plant root exudates (such as organic acids, sugars, and amino acids), because of their direct chelating and dissolution effects on soil Cd, or influence on abundance and population of microorganisms of the rhizosphere (Rengel and Marschner, 2005; Dessureault-Rompre et al., 2008). Root exudates may vary in component and amount among different cultivars of the same crop, and such variations may affect Cd uptake. To date, the cultivar difference of root exudates in relation to Cd uptake in plants has not been studied thoroughly.

The transport of Cd in the root controls its entry into crops. As a nonessential element, Cd is assumed to be absorbed accidentally by plants via the transport systems of essential metals. For example, Ca channels permit passage of divalent ions such as Cd^{2+}, Mg^{2+}, and Zn^{2+} because of weak selectivity (Li and Cheng, 2007), the Zn-regulated transporters (ZRT) can transport it simultaneously with Cd (Eng et al., 1998), and iron-regulated transport (IRT) has strong affinity with Cd (Korshunova et al., 1999). Thus, Cd uptake in plants can be determined by calculating the transfer efficiency of essential metals such as calcium (Ca), magnesium (Mg), zinc (Zn), and iron (Fe). Based on this, we hypothesized that the cultivars with low uptake of these essential metals also absorb a small amount of Cd. Until now, only a few studies (Liu et al., 2003; Chen et al., 2007; He et al., 2015) have described correlations between essential metals (i.e., Ca, Mg, Zn, and Fe) and Cd among cultivars of a specific crop.

Edible amaranth (*Amaranthus mangostanus* L.), a common vegetable grown in South Asia, is often polluted with Cd (Zhou et al., 2013). Therefore, the current study aimed to: (i) investigate Cd mobilization in the rhizosphere of low-Cd cultivars of amaranth compared to high-Cd cultivars, and (ii) identify the main root uptake pathway of Cd and test the hypothesis that cultivars with low essential metal (similar to Cd) possess low Cd uptake capacity. Unlike previous studies (Yu et al., 2006; Zhu et al., 2007; Liu et al., 2009; Wang et al., 2009), a long-term contaminated farm soil sample was used in pot experiments instead of spiked soil to better recapitulate conditions in the field.

2. Materials and methods

2.1. Pot experiment

A pot experiment was conducted in a greenhouse at Jinan University in Guangzhou (temperature 21–32 °C, relative humidity 60–80%), Guangdong Province, China. Soil was collected from field farmland contaminated by wastewater irrigation in the 1990s. Immediately after collection, soil samples were air dried, crushed, and sieved through a 2-mm nylon sieve and mixed thoroughly. The soil had pH of 6.38, organic matter content of 35.4 g kg^{-1}, and cation exchange capacity of 20.9 cmol kg^{-1}. The total and exchangeable heavy metal contents of the prepared soil were determined by HCl-HNO_3-HF-$HClO_4$ and $MgCl_2$ extraction, respectively (Tessier et al., 1979). The results are listed in Table SM-1. This experiment was performed with 15 common amaranth cultivars including Baigengjianye (#1), Naichoudahong (#2), Baiguxiaoyuanyeyou (#3), Zhengtaiyuanye (#4), Hongyuanye (#5), Taiwanbai (#6), Quanhong (#7), Jianyeqing (#8), Yuanyehua (#9), Qingxian (#10), Liuye (#11), Youxuanliuye (#12), Huahong (#13), Liuyehuahong (#14), and Huahongyuanye (#15). The seeds of amaranth cultivars were purchased from shops in Guangzhou City. Each cultivar contained three replicates, and all the pots (diameter, 20 cm; depth, 15 cm) were randomly arranged. All soils were watered daily to 75% of field capacity using deionized water. The plants were harvested after 50 d.

2.2. Collection and analysis of rhizosphere soil

Soils firmly adhered to the roots of plants were collected for rhizosphere solution extraction. The collected rhizosphere soils were wrapped with fine-meshed aluminum foil, and loaded in syringes without a plunger and pinhead. The syringes were then placed in centrifuge tubes and centrifuged for 30 min at 8000 rpm to separate the soil solution. The soil solution was filtered through a 0.45 μm membrane for analysis (Degryse et al., 2008). The concentrations of metals (Ca, Mg, Zn, and Fe) in the solution were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermofisher iCAP 7000, UK). Cd concentration was determined by graphite furnace atomic adsorption spectrometry (GFAAS, Shimadzu AA-7000, Japan). Subsequently, the replicates of each cultivar were mixed to obtain sufficient amount of solution to measure low-molecular-weight organic acids (LMWOAs), sugars, amino acids, and dissolved organic carbon (DOC). The LMWOAs were analyzed in an ion chromatogram (ICS-900, Dionex, US) combined with an Ion IonpacAS11-HC column and an IonpacAG11-HC guard column (50 mm × 4 mm i.d.) (Johansson et al., 2008). A gradient elution was run to separate the LMWOAs, and KOH was used as eluent at 1 mL min^{-1} flow rate. The elution procedure of KOH was as follows: 1 mM for 10 min, 45 mM for 25 min, and 1 mM for the final 5 min. Sugars and amino acids were determined by anthranone and ninhydrin colorimetric analyses, respectively (Chutipongtanate et al., 2012). DOC was analyzed on a SHIMADZU TOC-VCSH (Smith and Cave, 2012).

2.3. Translocation of cadmium from rhizosphere solution to root

A low-Cd accumulating cultivar (#15) and a high-Cd accumulating cultivar (#12) were selected for hydroponics experiments in a greenhouse (Temperature 21–32 °C, Relative humidity 60–80%). Five seedlings with four leaves each were transferred to a 1.5 L modified 0.3 strength Hoagland nutrient solution (pH = 5.5, buffered with Mes-Tris) for 4 d in a plastic vessel, and then to 0.5 strength for 8 d, 0.8 strength for 8 d, and finally to full strength. The nutrient solution was continuously aerated and replaced every 4 d.

The full-strength Hoagland nutrient solution contained 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, 0.1 mM $FeSO_4 \cdot 7H_2O$, and 0.1 mM EDTA-Na_4 (Mei et al., 2014). After 50 d of culture, some plants were subjected to the following treatments for 8 h under light: Ca channel blocker treatment, 2 μM $Cd(NO_3)_2$ + 1 mM $LaCl_3 \cdot 7H_2O$; ATP blocker treatment, 2 μM $Cd(NO_3)_2$ + 50 μM DNP. Some were treated with 2 μM $Cd(NO_3)_2$ for 6 d under non-Ca, non-Zn, and non-Fe conditions. Plants grown in full-nutrient solution containing 2 μM $Cd(NO_3)_2$ served as control. There were three replicates for all treatment and control conditions. After treatment, the roots of the plants were cleaned with deionized water and desorbed with 10 mM EDTA-NH_4 (10 min) to remove adsorbed metals on the root surface. The concentrations of Ca, Mg, Zn, Fe, and Cd in the EDTA-washed roots were determined as the absorbed amount (Mei et al., 2014).

2.4. Measurement of metals in amaranth (Amaranthus mangostanus L.)

The roots and shoots of the plants were separated, weighed, and oven-dried, while their fresh weights (FW) and dry weights (DW) were recorded. After grinding to pass through a 250 μm sieve in a precleaned steel grinder, the samples were digested with 10 mL HNO_3 in a microwave digesting apparatus (CEM MARs XPRSS, USA). The digesting oven heated the samples for 5 min to 120 °C and maintained at the same temperature for 3 min, then heated for another 5 min to 150 °C and held for another 3 min, and finally heated for 6 min to 180 °C and held for 10 min. The essential metals (i.e., Ca, Mg, Zn, and Fe) and Cd in the solution were measured by ICP-AES and GFAAS, respectively. The plant standard reference material [GBW07602 (GSV-1)] and blanks were subjected to digestion, then analyzed to assess compliance with the quality control protocol. The analytical results were accepted only if the measured concentrations of the reference materials fell within one standard deviation of the certified values.

2.5. Data analysis

Statistical analysis was conducted using SPSS software (version 17.0). Significance of differences among amaranth cultivars was assessed by ANOVA. The ratios of decrease in metal concentration in the roots resulting from inhibition of La^{3+} (or DNP) were computed using Equation (1), where 'control' indicates the metal concentration in EDTA-washed roots of amaranth in hydroponics, and 'treatment' indicates metal concentration in EDTA-washed roots of amaranth exposed to $LaCl_3$ (or DNP) in hydroponics. The translocation factor (TF) was calculated using Equation (2).

$$\text{Ratio} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100\% \quad (1)$$

$$\text{TF} = \frac{[\text{Cd}]shoot}{[\text{Cd}]root} \quad (2)$$

3. Results

3.1. Cd concentration in edible parts of different cultivars of amaranth grown in contaminated soil

Cd concentrations in the edible parts (shoots) of 15 amaranth cultivars are presented in Figure SM-1. The values ranged from 0.047 mg kg^{-1} to 0.296 mg kg^{-1} and exhibited significant ($P < 0.05$) difference among cultivars. Most cultivars, except cultivar #11 and #12, were safe to eat because Cd concentrations in their shoots were less than the allowable limit of the National Standard of PR China, Maximum Level of Contaminants in Foods (GB2762-2012). Among them, cultivars #7 and #15 were selected as representatives of low-Cd accumulators because of their relatively low concentrations in shoots and high shoot biomass (Table 1). On the contrary, cultivars #11 and #12 were selected as representatives of high-Cd accumulators for subsequent research on the mechanism of Cd accumulation and translocation in edible amaranth.

3.2. Differences in Cd accumulation and root-shoot translocation in amaranth cultivars

Cd accumulation and translocation from root to shoot of amaranth cultivars are listed in Table 1. The root biomass of low-Cd accumulating cultivars had no obvious difference compared with high-Cd cultivars, while the shoot biomass of low-Cd accumulating cultivars was significantly ($P < 0.05$) higher than those of the high-Cd cultivars. The average Cd concentration in roots of low-Cd cultivars was significantly ($P < 0.01$) lower than those of high-Cd cultivars. The root-shoot translocation factors (TFs) of Cd in low-Cd cultivars were 2.81-fold ($P < 0.01$) lower than those in the high-Cd accumulating cultivars.

3.3. Variations in concentrations of soluble metals and low molecular-weight organics in the rhizosphere of amaranth cultivars

The low- and high-Cd accumulating cultivars were compared in terms of concentrations of Ca, Mg, Zn, Fe, and Cd in the rhizosphere solution (Table 2). The corresponding average values of the low-Cd cultivars were 4.62-, 4.91-, 6.21-, 3.80-, and 2.15-fold lower than those of the high-Cd cultivars ($P < 0.01$). Exudates in the rhizosphere soil are listed in Table 3, including amino acids, sugars, low-

Table 1
Biomass and Cd concentration in low-Cd (L) and high-Cd (H) cultivars of amaranth grown in long-term contaminated soil, data are means ± SD (n = 3).

	Cultivars	Biomass (g, fresh weight)		Cd concentration (mg kg^{-1}, fresh weight)		TF
		Root	Shoot	Root	Shoot	
L	#7	5.97 ± 1.05d	33.4 ± 7.2c	0.079 ± 0.009a	0.053 ± 0.021a	0.658 ± 0.010a
	#15	4.20 ± 1.06b	33.7 ± 1.5c	0.087 ± 0.007a	0.052 ± 0.010a	0.609 ± 0.003a
	Mean (N = 6)	5.09 ± 1.35	33.5 ± 4.6	0.083 ± 0.008	0.053 ± 0.015	0.633 ± 0.028
H	#11	4.45 ± 1.47c	31.8 ± 2.2b	0.196 ± 0.020c	0.266 ± 0.041b	1.35 ± 0.09b
	#12	3.00 ± 0.67a	17.0 ± 1.2a	0.134 ± 0.015b	0.296 ± 0.020b	2.21 ± 0.01c
	Mean (N = 6)	3.73 ± 1.30	24.4 ± 8.3	0.165 ± 0.037	0.281 ± 0.033	1.78 ± 0.47
H/L		0.733	0.727*	1.99**	5.36**	2.81**

Different lowercase letters denote significant differences by Duncan test at $p < 0.05$ within the column for each cultivar.
*Significance at 0.05 level by *t*-test; **Significance at 0.01 level by *t*-test.

Table 2
Metals concentrations in the rhizosphere solution of low-Cd (L) and high-Cd (H) cultivars of amaranth (mg kg^{-1} dry weight soil), data are means ± SD (n = 3).

	Cultivars	Ca	Mg	Zn	Fe	Cd
		(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(μg kg^{-1})
L	#7	76.2 ± 4.9a	3.92 ± 0.19a	0.93 ± 0.03a	1.62 ± 0.03a	2.53 ± 0.11a
	#15	98.7 ± 6.1b	5.74 ± 0.34b	4.33 ± 0.22b	1.40 ± 0.01a	2.61 ± 0.08a
	Mean (N = 6)	87.4 ± 13.3	4.83 ± 1.02	2.63 ± 1.87	1.51 ± 0.12	2.57 ± 0.09
H	#11	388 ± 15c	23.4 ± 0.5c	10.5 ± 0.6c	6.45 ± 1.00c	6.27 ± 0.13c
	#12	420 ± 13d	24.0 ± 0.4c	22.2 ± 1.0d	5.04 ± 0.21b	4.78 ± 0.07b
	Mean (N = 6)	404 ± 22	23.7 ± 0.5	16.4 ± 6.5	5.74 ± 1.00	5.52 ± 0.82
H/L		4.62**	4.91**	6.21**	3.80**	2.15**

Different lowercase letters denote significant differences by Duncan test at $p < 0.05$ within the column for each cultivar.
*Significance at 0.05 level by *t*-test; **Significance at 0.01 level by *t*-test.

Table 3
Concentrations of free amino acids, soluble sugar, low-molecular-weight organic acids (LMWOAs), and dissolved organic carbon (DOC) in the rhizosphere solution of low-Cd (L) and high-Cd (H) cultivars of amaranth (mg kg^{-1} dry weight soil), data are means ± SD (n = 3).

	Cultivars	Amino acids	Sugar	LMWOAs	DOC
L	#7	0.15 ± 0.01a	5.19 ± 0.07a	1.22 ± 0.05c	53.0 ± 0.1a
	#15	0.21 ± 0.02b	5.41 ± 0.05b	0.71 ± 0.02a	56.9 ± 0.1b
	Mean (N = 6)	0.18 ± 0.03	5.30 ± 0.13	0.96 ± 0.28	55.0 ± 2.1
H	#11	0.28 ± 0.02c	13.6 ± 0.0d	1.13 ± 0.02b	76.9 ± 01d
	#12	0.29 ± 0.01c	7.63 ± 0.01c	1.64 ± 0.03d	57.9 ± 0.0c
	Mean (N = 6)	0.29 ± 0.01	10.6 ± 3.3	1.38 ± 0.27	67.4 ± 10.4
H/L		1.61**	2.01**	1.44*	1.23*

Different lowercase letters denote significant differences by Duncan test at $p < 0.05$ within the column for each cultivar.
*Significance at 0.05 level by *t*-test; **Significance at 0.01 level by *t*-test.

molecular-weight organic acids (LMWOAs), and dissolved organic carbon (DOC). The exudates of the low-Cd cultivars were 1.61-, 2.01-, 1.44-, and 1.23-fold lower, respectively, than those of the high-Cd cultivars. However, the concentration of LMWOAs of the low-Cd cultivar #7 was slightly ($P < 0.05$) higher than that of high-Cd cultivar #11. The organic acid components are listed in Table 4. Detectable LMWOAs included succinic, acetic, malic, oxalic, and citric acids. Acetic, oxalic and citric acids comprised more than 80% of the LMWOAs. The percentage of each organic acid in the LMWOAs was similar across different cultivars. However, the concentrations varied among these cultivars. Thus, amaranth cultivars may differ not in the type but in the amount of organic acids in the rhizosphere solution. However, there was no obvious trend in variation of each kind of acid between low and high-Cd accumulators.

3.4. Cd concentration in the full plants of 15 amaranth cultivars grown in long-term contaminated soil

The relationships of Cd with Ca, Mg, Zn, and Fe in the full plants of 15 amaranth cultivars were analyzed as shown in Fig. 1. Cadmium concentration in the cultivars exhibited an elevated trend as the concentration of Ca and Zn increased ($p < 0.01$), but did not show any correlation with Mg and Fe. Table SM-2 shows a comparison of the metal concentrations between the low-Cd (#7, and #15) and high-Cd cultivars (#11, and #12). The average concentrations of Ca, Mg, Zn, Fe, and Cd in the full plants of the low-Cd cultivars were 2.22-, 1.70-, 4.67-, 1.50-, and 4.41-fold lower, respectively, compared to those of the high-Cd cultivars. In contrast to the results presented in Table 2, the variations of Ca, Zn, and Fe concentrations in the full plants of amaranth cultivars were lower than those in the rhizosphere solution. This indicated that amaranth cultivars displayed different transport capacity for Ca, Zn, and Fe in the roots. However, the variation of Cd levels in plants was greater than that in the rhizosphere solution. This suggested that apart from available Cd concentration in the soil solution, root uptake capacity also contributed to Cd content differences in the plants of amaranth cultivars. Taken together, our results show that low nutrition cultivars (#7, and #15) had taken up less Cd than high nutrition cultivars (#11, and #12).

3.5. Cd root absorption under the effect of $LaCl_3$, DNP, and nutrient deficiency

$LaCl_3$ is an efficient blocker of Ca channel in cell membranes (Moyen and Roblin, 1997). DNP, a highly effective uncoupler of oxidative phosphorylation, rapidly reduces ATP levels (Lu et al., 2009). Table 5 lists the concentrations of Ca, Mg, Zn, Fe, and Cd in the EDTA-washed roots of cultivars #12 and #15 exposed to $LaCl_3$ or DNP compared to the control. The decreased ratios of metal concentration indicate the magnitude of reduction in metal uptake. $LaCl_3$ significantly reduced the uptake of Ca, Mg, Zn, and Cd, but not Fe, in both cultivars, with the reduction ranging from 27.4% to 52.9%. DNP had strong influence on uptake of all metals in both cultivars. Under both La and DNP treatments, the decreases in Cd and Ca uptake were equivalent in the same cultivar. Fig. 2 presents the varied Cd concentrations in amaranths (cultivar #12 and #15) cultured in nutrient-deficient Hoagland solution. For both cultivars, the Cd concentration evidently increased under Ca and Fe deficiency, but no variation was observed in the absence of Zn.

Table 4
Concentration of low molecular weight organic acids (LMWOAs) in the rhizosphere solution of low-Cd (L) and high-Cd (H) cultivars of amaranth (mg kg^{-1} dry weight soil), data are means ± SD (n = 3).

	Cultivars	Succinic acid	Acetic acid	Malic acid	Oxalic acid	Citric acid
L	#7	0.00 ± 0.00a	0.83 ± 0.03c	0.06 ± 0.01a	0.20 ± 0.02c	0.13 ± 0.02b
	#15	0.02 ± 0.01b	0.49 ± 0.02a	0.07 ± 0.02ab	0.05 ± 0.01a	0.08 ± 0.01a
H	#11	0.03 ± 0.01b	0.74 ± 0.03b	0.09 ± 0.02b	0.14 ± 0.02b	0.13 ± 0.02b
	#12	0.02 ± 0.01b	1.22 ± 0.03d	0.07 ± 0.01ab	0.22 ± 0.02c	0.11 ± 0.02ab

Different lowercase letters denote significant differences by Duncan test at $p < 0.05$ within the column for each cultivar.

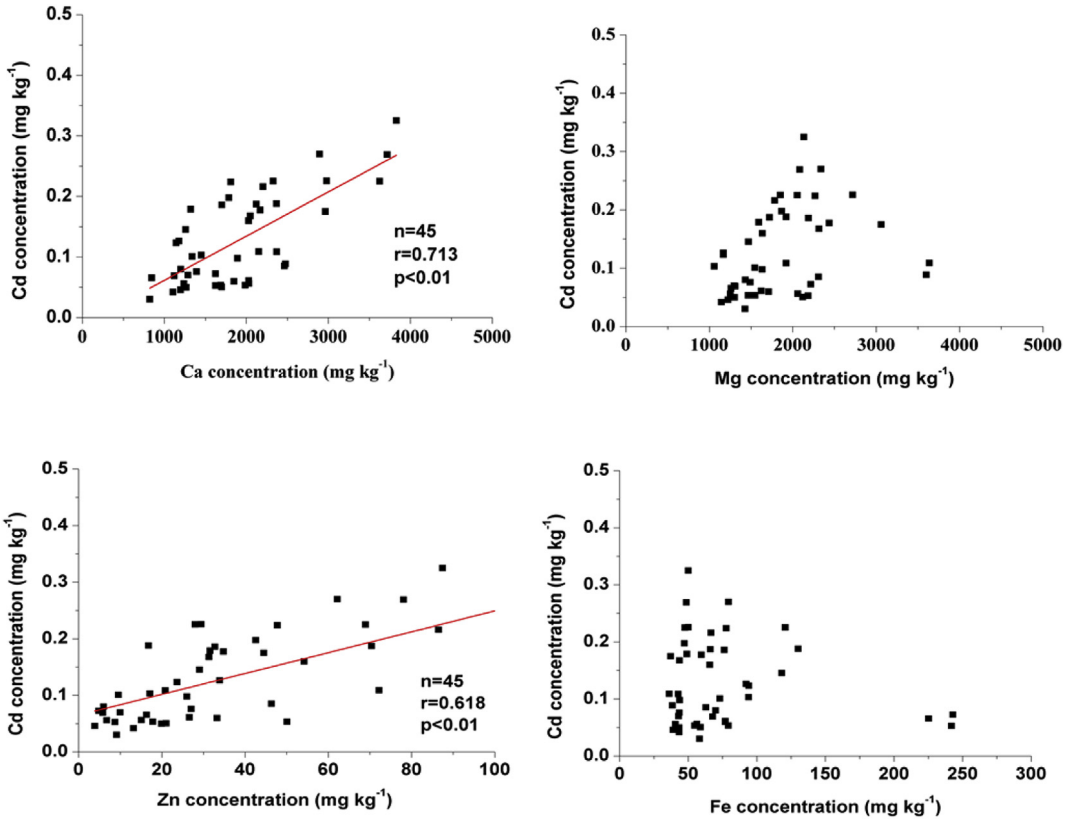

Fig. 1. Correlation among Cd and essential metals (i.e., Ca, Mg, Zn, and Fe) concentrations (mg kg^{-1}, fresh weight) in the full plants (root + shoot) of amaranth cultivars.

Table 5
Inhibition of metal uptake in amaranth roots by $LaCl_3$/DNP treatment (means ± SD, n = 3).

Cultivar	Treatment	Ca	Mg	Zn	Fe	Cd
#15	Control (mg kg^{-1}, FW)	667 ± 58a	303 ± 22a	7.88 ± 0.18a	36.5 ± 1.4a	1.39 ± 0.04a
	La treatment (mg kg^{-1}, FW)	420 ± 32b	198 ± 27b	5.72 ± 0.34b	35.5 ± 0.8a	0.90 ± 0.03b
	Decrease by La (%)	37.0	34.7	27.4	2.74	35.3
	DNP treatment (mg kg^{-1}, FW)	468 ± 33b	204 ± 27b	5.35 ± 0.25b	14.8 ± 0.4b	0.93 ± 0.07b
	Decrease by DNP (%)	29.8	32.7	32.1	59.5	33.1
#12	Control (mg kg^{-1}, FW)	1316 ± 72a	694 ± 65a	14.7 ± 0.7a	53.8 ± 0.9a	4.18 ± 0.31a
	La treatment (mg kg^{-1}, FW)	807 ± 13b	327 ± 31b	9.35 ± 0.34b	53.4 ± 0.2a	2.65 ± 0.14b
	Decrease by La (%)	38.7	52.9	36.2	0.725	36.6
	DNP treatment (mg kg^{-1}, FW)	826 ± 46b	234 ± 23b	8.38 ± 0.56b	37.0 ± 0.7b	2.14 ± 0.25b
	Decrease by DNP (%)	52.4	28.4	42.8	31.2	48.8

The roots were eluted with 10 mM EDTA-NH_4 to remove metals adsorbed on surface.
Lowercase letters a, and b indicate significant differences between control and treatment by *t*-test at $p < 0.05$ within the column.

4. Discussion

4.1. Cultivar difference in metal mobilization by root-induced exudates

The concentrations of Ca, Mg, Zn, Fe, and Cd in the rhizosphere solution of low-Cd cultivars were significantly ($P < 0.01$) lower than those of high-Cd cultivars of amaranth (Table 2). All metal concentrations measured in rhizosphere solutions of low-Cd cultivars were significantly lower than those of exchangeable metals in bulk soil (Table SM-1). However, the concentrations of Ca, Zn, and Fe in the rhizosphere solution of high-Cd cultivars were higher than those of exchangeable metals in bulk soil, while the concentrations of Mg and Cd in the rhizosphere solution were lower than their exchangeable concentrations. The results indicated a great difference in rhizosphere mobilization among amaranth cultivars. Under certain environmental conditions, the overall soluble metal concentrations in the soil are known to be influenced mainly by root exudates such as sugars, amino acids, phenolic acids, organic acids, and phytosiderophore (Cieslinski et al., 1998; Liu et al., 2008). Some exudates can directly dissolve metals in soil. In addition, these soluble organics may provide nutrition for microorganisms and alter rhizosphere microbial community structure, abundance, and activity. Rhizosphere microorganisms may also increase the availability of metals to plants by mineral bioweathering and secretion of organic acids, surfactants, and siderophores (Sheng et al., 2008; Li et al., 2010). Consistent with the trend in dissolved metal concentrations in the rhizosphere soil, low-Cd cultivars secreted less dissolved organics including sugars, amino acids, LMWOAs, and DOC (Table 3). Concentrations of exudates followed the order sugar > LMWOAs > amino acids. Sugars mainly offer carbon source for microorganism growth and may indirectly lead to Cd release from soil minerals. Some amino acids chelate Fe and result in Cd dissociation from iron oxide (Li, 2008). LMWOAs can

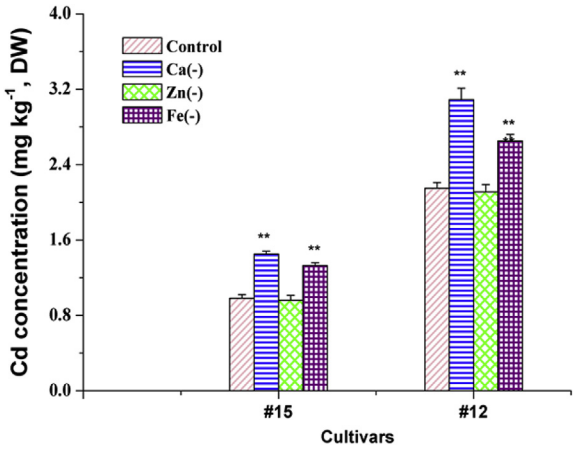

Fig. 2. Cd concentration variation in roots of amaranth cultivars exposed to 2 μM $Cd(NO_3)_2$ for 6 d, under nutrition-deficient conditions (DW). Control represents full-strength nutrition culture. Ca(-), Zn(-), and Fe(-) represent non-Ca, non-Zn, and non-Fe culture, respectively. Bars represent ± SD (n = 3). **indicates significant differences in metal accumulation from controls (*t*-test, $P < 0.01$).

not only directly chelate Cd, but also acidify rhizosphere soil, resulting in desorption of soil metals (Rengel and Marschner, 2005). For this reason, LMWOAs are believed to play a dominant role in mobilization of metals in the rhizosphere soil. In our study, the degree of metal mobilization depended strongly on the type and amount of exuded LMWOAs. However, there was no significantly difference in composition and content of LMWOAs between low- and high-Cd accumulating cultivars (Table 4). This suggested that LMWOAs were not the main mediators of differences in rhizosphere Cd activation between low-Cd and high-Cd accumulating cultivars of edible amaranth. Understanding the roles of sugars and amino acids in Cd activation in the rhizosphere of amaranth requires further investigation.

4.2. Cultivar difference in Cd uptake and translocation

A two-fold variation in Cd concentration in the rhizosphere solution isn't likely to account for about four-fold difference in plants when comparing the low-Cd and high-Cd cultivars (Table 2 and Table SM-2). Cd transport across root membrane also possibly contributes to this variation. Cd concentration in the plants was only positively correlated with Ca and Zn (Fig. 1), although Mg and Fe were also found to exhibit co-mobilization with Cd (Table 2). This suggested that sharing the same transport routes may be another factor giving rise to the correlation between Cd and Ca/Zn. Calcium can move across roots via two different pathways. One is the apoplastic bypass, in which Ca travels through spaces between cells without traversing a single plasma membrane (e.g., at the tips of roots where Casparian bands are undeveloped or at breaks in the endodermis). Thus, the apoplastic bypass is relatively nonselective and permits the flux of toxic metals (White, 2001). Another is the symplastic pathway, where Ca enters root cells and moves from cell to cell through plasmodesmata (White and Broadley, 2003). The symplastic pathway allows the plant to control the rate and selectivity of Ca transport into the plant. Calcium traverses the plasma membrane of root cells and enters the cytoplasm mainly through Ca^{2+}-permeable ion channels, which may be blocked by La^{3+}. However, some Ca channels exhibit weak selectivity and allow the influx of either bivalent cations (i.e., Cd^{2+}, Mg^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+}) or monovalent Na^{+} (White, 2001). Our hydroponic experiment (Table 5) demonstrated that $LaCl_3$ simultaneously inhibited the uptake of Ca, Mg, Zn, and Cd in amaranth. This suggested that these metals can pass through the cytoplasmic membrane of the amaranth root cells through Ca channels. The symplastic pathway involves an active uptake process and requires ATP metabolism, whereas the apoplastic bypass is driven by transpiration and root pressure (Saisho et al., 2001). The observed consistency in Cd and Ca variation under inhibition by La^{3+} or DNP (Table 5) suggested that most Cd and Ca ion followed the same transport routes regardless of the pathways involved. Cd uptake evidently increased under Ca and Fe deficiency, which can be ascribed to upregulation of activity and gene expression of Ca channels and Fe transporters (Fig. 2). Furthermore, decreasing Ca concentration increases electronegativity of the cell membrane and increases uptake of Cd (Wang et al., 2011; Kopittke et al., 2014). However, the present results showed that Cd uptake did not change under Zn deficiency. The strong correlation between Cd and Zn may be ascribed largely to their co-mobilization. Overall, the Ca pathway played a dominant role in Cd uptake, although Zn and Fe transporters also participated in the uptake of small amounts of Cd across the root cell membrane. Thus, the low-Ca cultivars exhibited lower risk of Cd accumulation than the high-Ca cultivars.

Apart from root uptake capacity, the translocation efficiency of Cd from root to shoot also determined Cd concentration in the shoots of plants. Our observation in TF indicated that low root-shoot transport capacity also partly contributed to low Cd accumulation in the edible parts of amaranth (Table 1).

In summary, our findings revealed that low rhizosphere mobilization, root uptake, and root-shoot translocation contributed to low Cd accumulation in the edible parts of amaranth. Amaranth cultivars with low concentrations of DOC (i.e., sugars, amino acids, and LMWOAs) dissolved less metal (i.e., Ca, Mg, Zn, Fe, and Cd) in the rhizosphere soils. However, the contributions of the various kinds of exudates remain unknown and need further research. Since mineral nutrient deficiency may stimulate secretion of root exudates, identifying a specific mineral and adding the appropriate mineral fertilizer may facilitate reduction of Cd uptake by plant. Moreover, planting low-Ca cultivars of crops would be an important strategy to reduce Cd accumulation in some of our staple agricultural products.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.12.085.

References

Aziz, R., Rafiq, M.T., Li, T.Q., Liu, D., He, Z.L., Stoffella, P.J., Sun, K.W., Yang, X., 2015. Uptake of cadmium by rice grown on contaminated soils and its bioavailability/Toxicity in human cell lines (Caco-2/HL-7701). J. Agric. Food Chem. 63, 3599–3608.

Chen, F., Dong, J., Wang, F., Wu, F.B., Zhang, G.P., Li, G.M., Chen, Z.F., Chen, J.X., Wei, K., 2007. Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. Chemosphere 67, 2082–2088.

Chutipongtanate, S., Watcharatanyatip, K., Teerada, H., Jaturongkakul, K., Thongboonkerd, V., 2012. Systematic comparisons of various spectrophotometric and colorimetric methods to measure concentrations of protein, peptide and amino acid: detectable limits, linear dynamic ranges, interferences, practicality and unit costs. Talanta 98, 123–129.

Cieslinski, G., Rees, K.C.J.V., Szmigielska, A.M., Krishnamurti, G.S.R., Huang, P.M., 1998. Low-molecular-weight organic acids in rhizosphere soils of durum wheat

and their effect on cadmium bioaccumulation. Plant Soil 203, 109–117.
Degryse, F., Verma, V.K., Smolders, E., 2008. Mobilization of Cu and Zn by root exudates of dicotyledonous plants in resin-buffered solutions and in soil. Plant Soil 306, 69–84.
Dessureault-Rompre, J., Nowack, B., Schulin, R., Tercier-Waeber, M.L., Luster, J., 2008. Metal solubility and speciation in the rhizosphere of *Lupinus albus* cluster roots. Environ. Sci. Technol. 42, 7146–7151.
Ding, Y.F., Qu, A.L., Gong, S.M., Huang, S.X., Lv, B., Zhu, C., 2013. Molecular identification and analysis of Cd-responsive microRNAs in rice. J. Agric. Food Chem. 61, 11668–11675.
Dunbar, K.R., McLaughlin, M.J., Reid, R.J., 2003. The uptake and partitioning of cadmium in two cultivars of potato (*Solanum tuberosum* L.). J. Exp. Bot. 54, 349–354.
Eng, B.H., Guerinot, M.L., Eide, D., Saier, M.H.J., 1998. Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. J. Membr. B 166, 1–7.
He, B.Y., Ling, L., Zhang, L.Y., Li, M.R., Li, Q.S., Mei, X.Q., Li, H., Tan, L., 2015. Cultivar-specific differences in heavy metal (Cd, Cr, Cu, Pb, and Zn) concentrations in water spinach (*Ipomoea aquatic* 'Forsk') grown on metal-contaminated soil. Plant Soil 386, 251–262.
Jamali, M.K., Kazi, T.G., Arain, M.B., Afridi, H.I., Jalbani, N., Memon, A.R., Shah, A., 2008a. Safely use of sewage sludge as fertilizer, after liming to grow *Zea mays* L. Pedosphere 18, 203–213.
Jamali, M.K., Kazi, T.G., Arain, M.B., Afridi, H.I., Jalbani, N., Sarfraz, R.A., Baig, J.A., 2008b. A multivariate study: variation in uptake of trace and toxic elements by various varieties of *Sorghum bicolor* L. J. Hazard. Mater. 158, 644–651.
Jamali, M.K., Kazi, T.G., Arain, M.B., Afridi, H.I., Jalbani, N., Kandhro, G.A., Shah, A.Q., Baig, J.A., 2009. Heavy metal accumulation in different varieties of wheat (*Triticum aestivum* L.) grown in soil amended with domestic sewage sludge. J. Hazard. Mater. 164, 1386–1391.
Janos, P., Vavrova, J., Herzogova, L., Pilarova, V., 2010. Effects of inorganic and organic amendments on the mobility (leachability) of heavy metals in contaminated soil: a sequential extraction study. Geoderma 159, 335–341.
Johansson, E.M., Fransson, P.M.A., Finlay, R.D., van Hees, P.A.W., 2008. Quantitative analysis of root and ectomycorrhizal exudates as a response to Pb, Cd and as stress. Plant Soil 313, 39–54.
Kopittke, P.M., Wang, P., Menzies, N.W., Naidu, R., Kinraide, T.B., 2014. A web-accessible computer program for calculating electrical potentials and ion activities at cell-membrane surfaces. Plant Soil 375, 35–46.
Korshunova, Y.O., Eide, D., Clark, W.G., Guerinot, M.L., Pakrasi, H.B., 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. Plant Mol. Biol. 40, 37–44.
Li, C.J., 2008. Mineral Nutrition of Higher Plants. China Agricultural University Press, Beijing, China, pp. 62–64.
Li, W.C., Ye, Z.H., Wong, M.H., 2010. Metal mobilization and production of short-chain organic acids by rhizosphere bacteria associated with a Cd/Zn hyperaccumulating plant, *Sedum alfredii*. Plant Soil 326, 453–467.
Li, Y., Cheng, R., 2007. Ca^{2+} channel. In: Li, Y. (Ed.), Ion Channelology. Hubei Science & Technology Press, Wuhan, China, pp. 33–34.
Liu, H.J., Zhang, J.L., Christie, P., Zhang, F.S., 2008. Influence of iron plaque on uptake and accumulation of Cd by rice (*Oryza sativa* L.) seedlings grown in soil. Sci. Total Environ. 394, 361–368.
Liu, J.G., Li, K.Q., Xu, J.K., Liang, J.S., Lu, X.L., Yang, J.C., Zhu, Q.S., 2003. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. Field Crop Res. 83, 271–281.
Liu, K., Lv, J.L., He, W.X., Zhang, H., Cao, Y.F., Dai, Y.C., 2015. Major factors influencing cadmium uptake from the soil into wheat plants. Ecotoxicol. Environ. Saf. 113, 207–213.
Liu, W.T., Zhou, Q.X., Sun, Y.B., Liu, R., 2009. Identification of Chinese cabbage genotypes with low cadmium. Environ. Pollut. 157, 1961–1967.
Lu, L.L., Tian, S.K., Yang, X.E., Li, T.Q., He, Z.L., 2009. Cadmium uptake and xylem loading are active processes in the hyperaccumulator *Sedum alfredii*. J. Plant Physiol. 166, 579–587.
Luo, J., Zhang, H., Zhao, F.J., Davison, W., 2010. Distinguishing diffusional and plant control of Cd and Ni uptake by hyperaccumulator and nonhyperaccumulator plants. Environ. Sci. Technol. 44, 6636–6641.
Lux, A., Martinka, M., Vaculik, M., White, P.J., 2011. Root responses to cadmium in the rhizosphere: a review. J. Exp. Bot. 62, 21–37.
Marettová, E., Maretta, M., Legáthb, J., 2015. Toxic effects of cadmium on testis of birds and mammals: a review. Anim. Reprod. Sci. 155, 1–10.
Mei, X.Q., Li, S.S., Li, Q.S., Yang, Y.F., Luo, X., He, B.Y., Li, H., Xu, Z.M., 2014. Sodium chloride salinity reduces Cd uptake by edible amaranth (*Amaranthus mangostanus* L.) via competition for Ca channels. Ecotoxicol. Environ. Saf. 105, 59–64.
Moyen, C., Roblin, G., 1997. Regulation of glycine uptake by calcium channel modulators in pulvinar cells of Mimosa pudica. J. Plant Physiol. 150, 97–102.
Rengel, Z., Marschner, P., 2005. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. New Phytol. 168, 305–312.
Rodrigues, S.M., Pereira, E., Duarte, A.C., Romkens, P.F.A.M., 2012. Derivation of soil to plant transfer functions for metals and metalloids. Plant Soil 361, 329–341.
Saisho, D., Nakazono, M., Tsutsumi, N., Hirai, A., 2001. ATP synthesis inhibitors as well as respiratory inhibitors increase steady-state level of alternative oxidase mRNA in Arabidopsis thaliana. J. Plant Physiol. 158, 241–245.
Sheng, X., He, L., Wang, Q., Ye, H., Jiang, C., 2008. Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J. Hazard. Mater. 155, 17–22.
Shi, W.Y., Shao, H.B., Li, H., Shao, M.A., Du, S., 2009. Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J. Hazard. Mater. 170, 1–6.
Smith, A.M., Cave, R.R., 2012. Influence of fresh water, nutrients and DOC in tow submarine-groundwater-fed estuaries on the west of Ireland. Sci. Total Environ. 438, 260–270.
Stolt, P., Asp, H., Hultin, S., 2006. Genetic variation in wheat cadmium accumulation on soils with different cadmium concentrations. J. Agron. Crop Sci. 192, 201–208.
Su, Y., Wang, X.M., Liu, C.F., Shi, G.R., 2013. Variation in cadmium accumulation and translocation among peanut cultivars as affected by iron deficiency. Plant Soil 363, 201–213.
Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.
Wang, J.L., Yuan, J.G., Yang, Z.Y., Huang, B., Zhou, Y.H., Xin, J.L., Gong, Y.L., Yu, H., 2009. Variation in cadmium accumulation among 30 cultivars and cadmium subcellular distribution in 2 selected cultivars of water spinach *(Ipomoea aquatic* Forsk.). J. Agric. Food Chem. 57, 8942–8949.
Wang, P., Kinraide, T.B., Zhou, D., Kopittke, P.M., Peijnenburg, W.J.G.M., 2011. Plasma membrane surface potential: dual effects upon ion uptake and toxicity. Plant Physiol. 155, 808–820.
White, P.J., 2001. The pathways of calcium movement to the xylem. J. Exp. Bot. 52, 891–899.
White, P.J., Broadley, M.R., 2003. Calcium in plants. Ann. Bot. 92, 487–511.
Wu, G., Kang, H.B., Zhang, X.Y., Shao, H.B., Chu, L.Y., Ruan, C.J., 2010. A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J. Hazard. Mater. 174, 1–8.
Yu, H., Wang, J.L., Fang, W., Yuan, J.G., Yang, Z.Y., 2006. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. Sci. Total Environ. 370, 302–309.
Zhou, Y.H., Xue, M., Yang, Z.Y., Gong, Y.L., Yuan, J.G., Zhou, C.Y., Huang, B.F., 2013. High cadmium pollution risk on vegetable amaranth and a selection for pollution-safe cultivars to lower the risk. Front. Env. Sci. Eng. 7, 219–230.
Zhu, Y., Yu, H., Wang, J.L., Fang, W., Yuan, J.G., Yang, Z.Y., 2007. Heavy metal accumulations of 24 asparagus bean cultivars grown in soil contaminated with Cd alone and with multiple metals (Cd, Pb, and Zn). J. Agric. Food Chem. 55, 1045–1052.