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RESEARCH ARTICLE

Roles of rhizosphere and root-derived organic acids in Cd accumulation by two hot pepper cultivars

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Abstract Cultivars of hot pepper (*Capsicum annuum* L.) have different abilities to accumulate Cd in their fruits. Previously, we suggested that low-Cd cultivars take up more Cd, but can better prevent the Cd translocation from roots to aerial parts. However, the mechanisms involved in those processes are still unclear. In this study, we explored the roles of rhizosphere soil Cd fractions and root secretions of low molecular weight organic acids in the uptake, translocation, and accumulation of Cd in a low-Cd and high-Cd cultivar. The results showed that there was no significant difference in exchangeable Cd between rhizosphere soils of the two cultivars, which might be related to their similar root's Cd uptake ability. The total content of low molecular weight organic acids released from roots of the low-Cd cultivar was almost equal to that released from roots of the high-Cd cultivar at the same Cd level; however, the composition of low molecular weight organic acids were determined by cultivars and Cd exposure levels. In the higher Cd (10 μM) treatment, the roots of the low-Cd cultivar excreted significantly less tartaric acid and more oxalic and acetic acids than those of the high-Cd cultivar. Additionally, there was no difference in the concentration of citric or succinic acid between the two cultivars. These results indicate that some kinds of low molecular weight organic acids efflux from hot pepper roots played an important role in the difference of Cd accumulation between low- and high-Cd cultivars.

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Abbreviations

Cd	Cadmium
LMWOAs	Low molecular weight organic acids
YCT	Yeshengchaotianjiao
JFZ	Jinfuzaohuangjiao

Introduction

Cadmium (Cd), as a non-essential element, is highly toxic to plants and can threaten human health through the contaminated food chain (Grant et al. 2008). Currently, Cd accumulation in agricultural soils has increased continuously due to anthropogenic activities, which is becoming a matter of great global concern (Pan et al. 2010; Wong et al. 2002). Cadmium in vegetable foodstuffs accounts for about 70 % of daily human intake of Cd (Günther et al. 2000). To reduce our intake of Cd from hot pepper (*Capsicum annuum* L.), the Cd concentration in fruits of hot pepper must be reduced. Previously, we found that there was great difference in fruit Cd concentration among hot pepper cultivars (Xin et al. 2013). Therefore, production of low-Cd cultivars of hot pepper can be a useful tool to reduce the amount of Cd entering the human diet. To do this, we must understand the mechanisms involved in Cd uptake, translocation in plants, and accumulation in fruits.

The processes of Cd entering the roots involve Cd release from soil colloids to the soil solution and Cd uptake from the soil solution (Greger and Landberg 2008). Consequently, the chemical behavior of Cd in soil is one of the major factors that influence the uptake of Cd by plants. Plant roots excrete various substances into the rhizosphere, so the chemical

effects of roots on the soil mainly occur in the rhizosphere rather than in the bulk soil (Gobran et al. 1999). It was reported that the Cd concentration in the rhizosphere soil of various wheat cultivars was significantly lower than that in the bulk soil (Greger and Landberg 2008). Furthermore, Hu et al. (2011) found that there were significant differences in Cd chemical forms of the rhizosphere soils between low- and high-Cd rice cultivars. Therefore, Cd fractions in the rhizosphere soils of hot pepper cultivars may be related to the difference of Cd accumulation among the cultivars.

Root release of organic acids into the rhizosphere is always occurring, which is based on the fact that the concentration of organic acids in the cytosol is about 1,000-fold higher than that in the soil solution (Jones 1998). The composition of organic acids released from roots is highly variable and depends on plant species, cultivars, and physiochemical environment. The interaction of Cd with organic acids in the plant–soil system may influence Cd speciation in soil and Cd transport in plant. Moreover, different compositions of low molecular weight organic acids (LMWOAs) may play an important role in determining the availability and uptake of Cd by crop cultivars with different Cd accumulation properties (Cieśliński et al. 1998; Liu et al. 2007). It was also reported that there were no differences in the concentration of LMWOAs in root exudates between low- and high-Cd wheat cultivars (Greger and Landberg 2008). Such researches have not yet provided consistent results with respect to the effects of LMWOAs efflux from roots on the differences in Cd uptake and accumulation among cultivars.

In the previous studies, we identified some low- and high-Cd hot pepper cultivars (Xin et al. 2013, 2014); however, limited information is available for the mechanisms of Cd variation between cultivars. The Cd fractions in the rhizosphere soil and the root release of LMWOAs can differ between hot pepper cultivars and thus be one of the reasons for their different abilities to accumulate Cd in the fruits. Therefore, the aims of this study were to test whether (1) Cd fractions in the rhizosphere soil, and (2) LMWOAs secreted by roots were mechanisms that could account for the difference of Cd accumulation between cultivars of hot pepper.

Materials and methods

Plant and soil materials

Two cultivars of hot pepper, Yeshengchaotianjiao (YCT) and Jinfuzaohuangjiao (JFZ), were used as the test plant materials in this study. The cultivar selection of hot pepper was based on our previous study that investigated the variations of fruit Cd concentration among 30 cultivars at different soil Cd levels. The Cd concentration in fruits of JFZ was 2.1- to 2.7-fold higher than that in fruits of YCT when grown in Cd-contaminated soils (0.28–2.69 mg kg^{-1} dry weight, DW), and thus JFZ and YCT were identified as high-Cd and low-Cd cultivars, respectively (Xin et al. 2013).

The experimental soil was collected from the surface layer (0–15 cm) of the farmland in Hengyang (112°41′ E, 26°52′ N), Hunan Province, China. After air drying at room temperature, the soil was ground to pass through a sieve with a 5-mm mesh size. The soil was a sandy loam, and its properties were determined using the analytical methods described by Lu (2000). The soil pH, total N, available P, available K, organic matter content, cation exchange capacity, and total Cd were 6.44, 1.46 g kg^{-1}, 104 mg kg^{-1}, 133 mg kg^{-1}, 18.9 g kg^{-1}, 89.7 mmol kg^{-1}, and 1.21 mg kg^{-1}, respectively. The tested soil was divided equally into three 15-kg portions. The first portion was served as a treatment (T1) without additional Cd^{2+}. The other two portions were used to create Cd treatments (T2 and T3) with target Cd concentrations of 2.0 and 3.5 mg kg^{-1}, respectively, which were obtained by adding the solution of $Cd(NO_3)_2{\cdot}4H_2O$. The T2 and T3 soils were respectively placed in a large basin, watered, and left to equilibrate outdoors under a waterproof tarpaulin for 6 months. This long period allowed for the equilibration of the various sorption mechanisms in the soils. For T2 and T3 treatments, the final soil total Cd concentrations were 2.03 and 3.44 mg kg^{-1}, respectively.

Pot experiment

A pot experiment was conducted in a greenhouse with an air temperature of 28–35 °C. Plastic pots (bottom diameter, 13 cm; top diameter, 18 cm; height, 15 cm) were filled with 2.5 kg (DW) of prepared soil. For each treatment, three pots (n=3) were planted for each cultivar. The experiment was arranged in a completely randomized design. Five seeds were planted in each pot on March 25, 2013, and watered daily with tap water. Within 15 days after germination, the plants were thinned to one per pot. A solid compound fertilizer (N/P/K= 15:15:15) was applied to the soil at the rate of 3.0 g pot^{-1} every 15 days.

Plant and soil analysis

The plants were harvested after the 120-day growth. Rhizosphere soil was collected according to the method reported by Greger and Landberg (2008). The plants were removed carefully from the pots and the bulk soil. Soil still adhering to the roots was considered to be rhizosphere soil and was separated from the roots by gently scraping the soil by hand. All rhizosphere soil samples were air dried, ground to pass through a 2-mm sieve, and kept in sealed plastic bags at room temperature until analyzed. A sequential extraction procedure was used to fractionate rhizosphere soil Cd into different fractions (Tessier et al. 1979). The extraction procedure

and corresponding fraction are listed in Table 1. The Cd concentration in each fraction solution was determined with an atomic absorption spectrophotometer (AAS) (Shimadzu AA-6300C, Japan).

Roots, stems, leaves, and fruits were separately rinsed with tap water, and roots were desorbed for 15 min in ice-cold 5 mM $CaCl_2$ solution (5 mM MES-Tris, pH 6.0). All samples were thoroughly washed with deionized water, dried at 105 °C for 30 min and then at 70 °C to a constant weight, and the fresh and dry weights were recorded. The dried plant samples were crushed to pass through a 0.149-mm sieve for chemical analysis. Concentrations of Cd in the dry plant samples were also determined using AAS after digestion with HNO_3–H_2O_2 (10:3, *v*/*v*) in a microwave oven (Microwave digester XT-9900A, Shanghai Xintuo Analytical Instruments Co., Ltd., China). A certified reference material (CRM) of plant GBW07605 (provided by the National Research Center for CRM, China) was used for quality assurance and quality control of the Cd analysis.

Hydroponic experiment

Seeds of two hot pepper cultivars were sown in well-washed sand for germination. Ten days after sowing, uniform seedlings were transplanted into flasks (one plant per flask) containing 200 mL Hoagland's solution without substrate (5 mM $Ca(NO_3)_2{\cdot}2H_2O$, 5 mM KNO_3, 2 mM $MgSO_4{\cdot}7H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA-Fe, 47 μM H_3BO_3, 1 μM $MnCl_2{\cdot}4H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.01 μM H_2MoO_4, and 0.25 μM $CuSO_4{\cdot}5H_2O$, pH 6.0). The solutions were replaced every 3 days throughout the experiment and the roots were always kept in the dark. A total of 18 flasks with seedlings of the 2 cultivars (9 flasks per cultivar) were arranged randomly in a growth chamber with a photoperiod of 16 h at a light intensity of 150 μmol m^{-2} s^{-1}, day/night temperatures of 28/22 °C and 60–70 % relative humidity. On the 21st day after transplantation, the seedlings were exposed to increasing Cd concentrations, 0 (control), 2 (Cd_2), and 10 (Cd_{10}) μM, by adding $nNO_3)_2{\cdot}4H_2O$ to the basal nutrient solution. This resulted in three replicates per cultivar in each treatment. These solutions were also replaced every 3 days in order to prevent changes in Cd concentration. The plants grew in these solutions for 21 days, and then the root exudates were collected. After the collection of root exudates, the Cd concentration in roots and shoots were analyzed according to the above method (2.3).

Collection and analysis of root exudates

Root exudates were collected using the method described by Rosas et al. (2007) with minor modifications. Roots of intact plants were thoroughly rinsed with deionized water, and then immersed in deionized water for 2 h under constant aeration. The short collection time was chosen to minimize degradation of organic acids by microorganisms. The solution containing root exudates was filtered with 0.45-μm filters and lyophilized. Low molecular weight organic acids present in the root exudates were measured using an HPLC system (Agilent Technologies 1200 Series, Santa Clara, CA, USA) equipped with a ultraviolet (UV) detector set at 214 nm. To quantify the concentrations of LMWOAs, lyophilized powder of the root exudates was dissolved in 300 μl sterile deionized water for HPLC injection. Chromatographic separation was carried out on a reversed phase Diamonsil C18 column (250×4.6 mm, 5-μm particle size; Dikma, USA). The mobile phase consisted of methanol: 0.3 % phosphoric acid (8:92, *v*/*v*). The flow rate was 0.8 ml min^{-1} and the injection volume was 50 μl. Standard organic acids (tartaric acid, oxalic acid, acetic acid, citric acid, succinic acid, and propionic acid) were run under the same conditions as controls. Identification of LMWOAs was performed by comparing retention times and absorption spectra with those of known standards.

Statistical analysis

The cadmium uptake ability of plants can be estimated by the net uptake of Cd via roots, which was calculated as the total amount of Cd in the whole plant divided by the root weight. The Cd translocation rate was calculated as the amount of Cd in the aboveground parts in relation to the total amount of Cd in the whole plant (Greger and Löfstedt 2004). Data were statistically analyzed with the independent samples *t* test and least significant difference (LSD) test based on one-way

Table 1 Sequential extraction method of different Cd fractions

Steps	Fractions	Reagent per 1.0 g of soil	Experimental conditions
1	Exchangeable	8 ml of 1 M $MgCl_2$, pH 7.0	Shake 1 h, room temperature
2	Carbonate bound	8 ml of 1 M NaOAc, pH 5.0 (HOAc)	Shake 5 h, room temperature
3	Fe/Mn oxide bound	20 ml of 0.04 M $NH_2OH{\cdot}HCl$ in 25 % (*v*/*v*) HOAc	6 h, 96 °C in bathing, occasional shaking
4	Organic matter bound	3 ml of 0.02 M HNO_3, 5 ml of 30 % H_2O_2, pH 2.0; or 3 ml of 30 % H_2O_2 (pH 2.0 with HNO_3); or 5 ml of 3.2 M NH_4OAc in 20 % (*v*/*v*) HNO_3	2 h, 85 °C in bathing, occasional shaking; 3 h, 85 °C in bathing, occasional shaking; Shake 0.5 h, room temperature
5	Residual	5 ml aqua regia and 1.5 ml $HClO_4$	8 h, 180 °C

ANOVA using Excel 2003 and SPSS 13.0. The data were checked for heteroscedasticity using Levene's test before the ANOVA was performed and showed no heteroscedasticity.

Results

Pot experiment

For both cultivars, there were no significant differences ($p>0.05$) between the T1 and T2 treatments in terms of the biomasses of roots, stems, and leaves except that the leaf biomass of YCT in the T2 treatment was significantly higher ($p<0.05$) than that in the T1 treatment (Fig. 1). Compared with the T1 and T2 treatments, the biomasses of roots, stems, and leaves of each cultivar significantly ($p<0.05$) decreased in the T3 treatment (Fig. 1). However, no significant variation ($p>0.05$) in the fruit biomass for each cultivar was observed among the three treatments (Fig. 1).

YCT always showed a significantly higher ($p<0.01$ or 0.05) concentration of Cd in its roots and significantly lower ($p<0.01$ or 0.05) concentrations in its stems, leaves, and fruits than JFZ (Table 2). The total Cd accumulation in YCT was significantly higher ($p<0.01$) than that in JFZ, while the Cd translocation rate in YCT was always significantly lower ($p<0.01$) than that in JFZ (Table 2). Additionally, there was no significant difference ($p>0.05$) in the root Cd uptake ability between YCT and JFZ (Table 2).

Table 3 shows the Cd fractions in the rhizosphere soils of YCT and JFZ. Residual Cd was not detected in all the treatments. The Cd concentrations in four other soil fractions increased with increasing soil Cd concentrations except for organic matter bound Cd for YCT in the T2 treatment. After the growth of hot pepper for 120 days, total Cd concentration in the rhizosphere soil was only 58.7–71.1 % of the soil total Cd concentration before planting. Carbonate bound Cd (43.0–46.0 %) was the predominant fraction, and Fe/Mn oxide bound Cd (25.9–35.9 %) was the second largest fraction in rhizosphere soils of the two cultivars in all the treatments. The proportions of exchangeable Cd were lower than those of organic matter bound Cd in the T1 treatment, but the opposite was the case in the other two treatments. In most cases, there were no significant differences in the Cd concentration of each soil fraction between YCT and JFZ in the same treatment except for Fe/Mn oxide bound Cd in the T2 treatment and organic matter bound Cd in the T1 and T3 treatments. Additionally, the total Cd concentration in the rhizosphere soil of YCT was significantly higher than that in the rhizosphere soil of JFZ in the T1 treatment; however, no significant differences were observed in the T2 and T3 treatments.

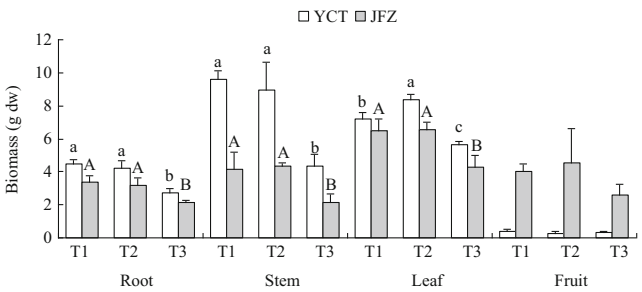

Fig. 1 Effect of Cd exposures on biomasses of the two cultivars of hot pepper in the pot experiment. Different *lowercase letters on bars* indicate a significant difference at $p<0.05$ among treatments in the same organ of YCT; different *capital letters* indicate a significant difference at $p<0.05$ among treatments in the same organ of JFZ. *Error bars* represent the standard deviation ($n=3$)

Hydroponic experiment

Compared with the control, the root biomass of YCT increased significantly ($p<0.05$) in the Cd_{10} treatment (Fig. 2). However, the shoot biomass of JFZ in the Cd_{10} treatment was significantly lower ($p<0.05$) than those in the control and Cd_2 treatment (Fig. 2).

YCT showed a higher concentration of Cd in its roots and a lower concentration in its shoots than JFZ (Table 4). The Cd translocation rates in YCT were always significantly lower ($p<0.05$) than those in JFZ. There was no significant difference in root's Cd uptake ability between the two cultivars in the Cd_2 treatment; however, YCT had higher root Cd uptake than JFZ in the Cd_{10} treatment (Table 4). In addition, the Cd concentrations of shoot and root and root Cd uptake increased greatly with increasing Cd supply, but the Cd translocation rate slightly decreased (Table 4).

The concentrations of five LMWOAs in the root exudates of YCT and JFZ are shown in Fig. 3 (propionic acid was not detected). The LMWOAs excreted by the roots of YCT and JFZ mainly consisted of succinic acid (43.7–73.6 %). Furthermore, there was no significant difference in the concentration of succinic acid for each cultivar with increasing Cd concentrations in the solutions. Additionally, compared with the control, the roots of YCT excreted more tartaric acid, acetic acid, and citric acid in the two Cd treatments. Nevertheless, the concentrations of tartaric acid, oxalic acid, and acetic acid in the root exudates of JFZ increased firstly and then decreased with increasing Cd supply. The total content of LMWOAs excreted by the roots of YCT and JFZ showed no significant difference ($p>0.05$) in each treatment. However, differences in concentrations of LMWOA except for citric acid and succinic acid between the two cultivars were observed in the control or Cd treatments. The roots of JFZ always excreted significantly more tartaric acid than those of YCT. In addition, there were also significant differences for oxalic acid, and acetic acid between the two cultivars in the Cd_{10} treatment, but no significant differences were observed in the control and Cd_2 treatment.

Table 2 Cd concentrations (in milligram per kilogram of dry weight) of different organs, total Cd accumulation (in microgram), Cd translocation rate (in percent), and Cd net uptake via roots (in milligram per kilogram of dry weight root) in low-Cd (YCT) and high-Cd (JFZ) cultivars of hot pepper

Treatment	Cultivar	Cd concentration				Total Cd accumulation	Cd translocation rate	Cd net uptake via roots
		Root	Stem	Leaf	Fruit			
T1	YCT	25.78±1.82**	3.18±0.26**	1.73±0.06**	0.38±0.06**	158.2±5.5**	27.3±2.3**	35.5±3.2 ns
	JFZ	12.50±0.86	6.04±0.46	3.63±0.38	0.76±0.12	93.6±5.2	55.3±4.8	28.3±5.0
T2	YCT	43.22±3.45**	5.21±0.25**	3.16±0.24**	0.83±0.17*	256.1±19.7**	28.9±6.2**	60.9±5.0 ns
	JFZ	25.80±0.97	10.20±1.47	7.22±0.48	1.34±0.13	179.5±6.5	54.5±4.1	57.1±5.8
T3	YCT	86.55±5.16*	11.08±1.15**	5.26±0.81**	1.19±0.03*	312.8±20.5**	24.4±1.8**	115.1±7.1 ns
	JFZ	55.90±10.67	24.31±1.03	11.11±1.00	2.11±0.49	224.8±6.0	46.5±7.0	104.1±6.3

Values shown are the mean±SD, $n=3$

ns not significant

*$p<0.05$; **$p<0.01$

Pearson's correlation analysis was carried out to investigate the relationships of Cd uptake, translocation, and accumulation in hot pepper plants and the concentrations of LWMOAs (Table 5). In the Cd_2 treatment, only the concentrations of tartaric acid and citric acid were negatively correlated with root Cd concentration and Cd translocation rate, respectively. In the Cd_{10} treatment, the concentration of tartaric acid was positively correlated with shoot Cd concentration and Cd translocation rate, but negatively correlated with root Cd concentration and root's Cd uptake ability, while the opposite was the case for oxalic acid and acetic acid. Furthermore, there were no significant correlations between the total contents of LMWOAs and Cd uptake, translocation, or accumulation.

Discussion

Hot pepper plants take up Cd from the soil, which is proved by the decrease of total Cd concentration in the rhizosphere soil (Table 3). YCT, low-Cd cultivar, removed 1.39- to 1.69-fold more Cd from soil than JFZ, high-Cd cultivar, but there was no such difference in root's Cd uptake ability between them (Table 2). JFZ had higher Cd concentrations in its above-ground parts than YCT, which is probably due to the higher Cd translocation rates in JFZ (Table 2). It demonstrated that roots play an important role in Cd uptake from soil and translocation to aerial organs.

The rhizosphere is a unique environment where numerous interactive progresses occurred, including root growth, respiration, water and nutrient uptake, and rhizodeposition (Hinsinger et al. 2005). Root–soil interactions in the rhizosphere can have an important influence on the bioavailability of Cd. In this study, there was no significant difference between the two hot pepper cultivars in the rhizosphere exchangeable Cd concentration. This might be the reason why there was no difference in root Cd uptake between the two cultivars. Compared with JFZ, the higher total Cd accumulation in YCT results from its more biomass. Additionally, the higher root Cd concentration in YCT than in JFZ suggests that the roots of YCT have a greater ability to retain Cd. It was worth noting that the proportions of exchangeable Cd

Table 3 Different fractions of Cd and total Cd concentrations in the rhizosphere soil of two hot pepper cultivars

Treatment	Cultivar	Fractionation of Cd (mg kg^{-1} dw)					
		Exchangeable	Carbonate	Fe/Mn oxide	Organic matter	Residual	Total
T1	YCT	0.03±0.00 (3.5 %)	0.37±0.01 (43.0 %)	0.29±0.01 (33.7 %)	0.17±0.01* (19.8 %)	ND	0.86±0.03*
	JFZ	0.03±0.00 (3.8 %)	0.35±0.01 (44.9 %)	0.28±0.01 (35.9 %)	0.11±0.02 (14.1 %)	ND	0.78±0.01
T2	YCT	0.18±0.01 (13.2 %)	0.61±0.02 (44.9 %)	0.40±0.02* (29.4 %)	0.16±0.01 (11.8 %)	ND	1.36±0.02
	JFZ	0.19±0.01 (14.7 %)	0.58±0.01 (45.0 %)	0.34±0.02 (26.4 %)	0.17±0.01 (13.2 %)	ND	1.29±0.05
T3	YCT	0.37±0.02 (18.0 %)	0.89±0.01 (43.4 %)	0.53±0.04 (25.9 %)	0.26±0.02* (12.7 %)	ND	2.05±0.03
	JFZ	0.32±0.03 (15.8 %)	0.93±0.03 (46.0 %)	0.56±0.02 (27.7 %)	0.21±0.00 (10.4 %)	ND	2.02±0.01

Values shown are the mean±SD ($n=3$). Percentage of total Cd for each fraction are shown in parentheses

ND not detected

*$p<0.05$

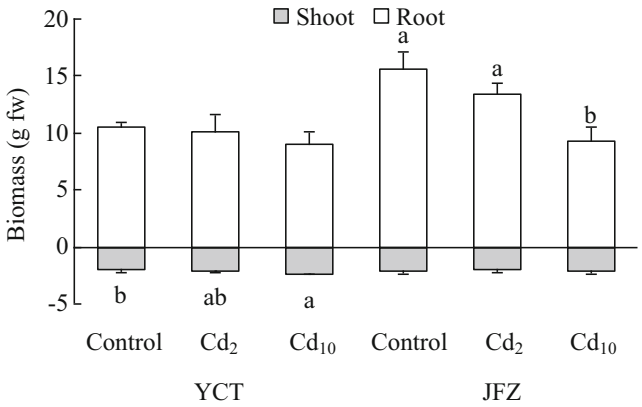

Fig. 2 Shoot and root biomass of two cultivars of hot pepper in the control and two Cd treatments in the hydroponic experiment. Different *lowercase letters on bars* indicate a significant difference at $p<0.05$ among treatments in the same cultivar. *Error bars* represent the standard deviation ($n=3$)

obviously increased in the T2 and T3 treatments compared with those in the T1 treatment (Table 3), possibly due to the low soil pH and the addition of cadmium nitrate. Similarly, Chen et al. (2011) also reported that the exchangeable Cd fraction greatly increased with increasing Cd supply in the acid Pinchen soil. As for the difference of other Cd fractions such as Fe/Mn oxide and organic matter forms between YCT and JFZ, a possible explanation could be given by considering the difference of root exudates between them. For example, rice roots can induce exchangeable Cd synthesis in their vicinities from Fe/Mn oxide bound Cd and other Cd species (Hu et al. 2011). Furthermore, it was also reported that the transformation of Cd from exchangeable Cd to organic matter bound Cd occurred in the rhizosphere soil of rice, which might be due to the root exudates, including arabinose, fucose, galactose, glucose, uronic acids, xylose, and so on, having the binding abilities to Cd (Lin et al. 2003). The transformation processes of Cd species in the rhizosphere soils of different cultivars are probably quite complex, and need to be further investigated.

Zabowski (1989)reported that the extraction of soil solution via centrifugation released limited soluble organics, and the extraction techniques inevitably led to contamination from damaged roots and microbial cells. Therefore, in order to facilitate the collection and analysis of LMWOAs, this study was performed in solution culture. Although the results of Cd uptake and translocation in the hydroponic experiment were not completely identical to those in the pot experiment, the characteristics of Cd accumulation in YCT and JFZ always remain stable in either growing environment. The LMWOAs excreted by roots were determined by the hot pepper cultivars and the Cd doses added to the nutrient solution (Fig. 3). The intensity of Cd exposure influenced the total LMWOAs efflux from the roots of YCT and JFZ, but the total content of LMWOAs did not seem to be related to the uptake and accumulation in the two cultivars. However, it was reported that low-Cd cultivars of durum wheat exuded more total LMWOAs than high-Cd cultivars in sterile solution culture (Cieslinski et al. 1997), but the contrary was the case in pot experiments (Cieśliński et al. 1998; Liu et al. 2007). Therefore, the LMWOAs efflux from plant roots in hydroponic solutions may differ from those in rhizosphere soils due to the difference of root morphology, microbial and nutrient status between hydroponic and soil environment. Despite the difference in plant's growing environment between the two culture systems, comparative studies previously reported strongly suggest that LMWOAs play an important functional role in the uptake and accumulation of Cd by plants.

In all of the LMWOAs examined, succinic acid was the most abundant, and its concentration was independent of cultivar and Cd exposure. Similarly, it was reported that different wheat cultivars exuded high amounts of succinic acid in untreated or Cd-treated soil, but the concentration of succinic acid greatly increased in the Cd-treated soil when compared with that in the untreated soil (Greger and Landberg 2008). However, Xie et al. (2013) found that oxalic acid accounted for the largest proportion of LMWOAs excreted from *Kandelia obovata* roots under Cd stress. Furthermore,

Table 4 Cd concentration (in milligram per kilogram of freshweight), Cd translocation rate (in percent), and Cd net uptake via roots (in milligram per kilogram of freshweight root) of the two cultivars of hot pepper exposed to Cd for 21 days in the hydroponic experiment

Treatment	Cultivar	Cd concentration		Cd translocation rate	Cd net uptake via roots
		Root	Shoot		
Cd_2	YCT	38.2±2.5*	0.99±0.04**	10.9±1.8**	42.8±2.3 ns
	JFZ	32.1±1.6	1.26±0.06	20.7±2.5	40.5±3.1
Cd_{10}	YCT	102.3±6.1**	2.94±0.08**	9.7±1.1**	113.2±5.5*
	JFZ	79.1±3.3	3.82±0.23	17.5±2.0	95.9±3.7

Values represent means±SD ($n=3$)

Cd_2 2 μM Cd, *Cd_{10}* 10 μM Cd, *ns* not significant

*$p<0.05$; **$p<0.01$

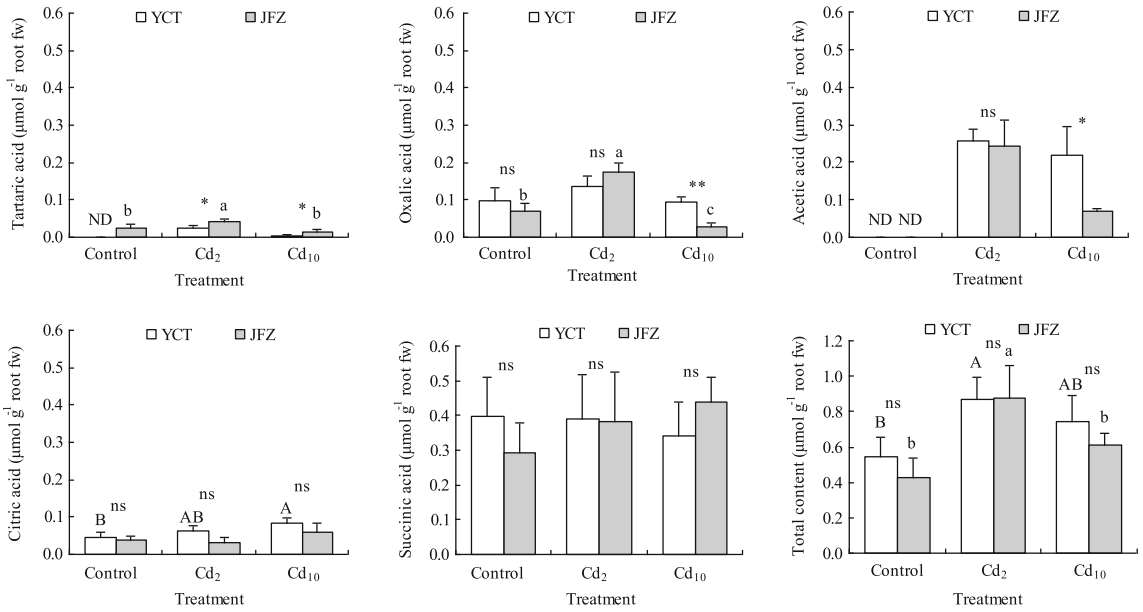

Fig. 3 LMWOAs excreted from the roots of two hot pepper cultivars in the control and Cd exposures. Different *lowercase letters* (for JFZ) and *capital letters* (for YCT) on bars indicate significant difference at the $p<0.05$ level among treatments. *Error bars* represent the standard deviation ($n=3$). *ns* indicates that the difference between the two cultivars in the same treatment is not significant; $*p<0.05$ and $**p<0.01$, significant difference between the two cultivars in the same treatment

Cieśliński et al. (1998) reported that soil type had a pronounced effect on the quantity of LMWOAs in the rhizosphere soil of durum wheat. These different results presumably reflect differences in experimental conditions and differences in plant species that have specific LMWOAs exudation mechanisms in response to Cd exposure.

In this study, large amounts of acetic acid were found in the root exudates in Cd treatments, and its concentration was higher in YCT than in JFZ in the Cd_{10} treatment. The secretion of acetic acid could be also a detoxification mechanism (Guo et al. 2007), namely through organic complexes of Cd to alleviate free Cd ion toxicity to hot pepper. These Cd complexes are not easily to be translocated to aerial parts, which might partly explain the reason that the concentration of acetic acid was negatively correlated with Cd concentration in the shoots of hot pepper. Di- and tricarboxylic acids are considered as potent metal complexes, and can promote metal, such as Cd and ferrum (Fe), accumulation in plant tissues (Jones et al. 1996; Nigam et al. 2001). However, it should be emphasized that these organic acids cannot improve the uptake of all metals by plants. For example, the addition of citric acid or oxalic acid to hydroponic or soil solutions had little effect on zinc (Zn) accumulation in wheat (Chairidchai and Ritchie 1993; Evans 1991). In this study, the concentrations of tartaric, oxalic, and citric acid in root exudates were quite low, which could be due to the formation of water soluble Cd-organic acid complexes (Cieśliński et al. 1998). The content of citric acid was not correlated with Cd concentration in hot pepper palnt tissues. Greger and Landberg also found that there was no difference in the concentration of citric acid in root exudate between low- and high-Cd wheat cultivars (Greger and Landberg 2008). However, Liu et al. (2007)

Table 5 Results of the Pearson correlation analysis ($n=6$) between Cd accumulation and concentration of LWMOAs for two cultivars of hot pepper

Treatment	Parameter	LMWOAs					
		Tartaric	Oxalic	Acetic	Citric	Succinic	Total
Cd_2	Shoot Cd concentration	0.802	0.665	−0.320	−0.760	−0.247	−0.216
	Root Cd concentration	−0.937**	−0.349	−0.275	0.769	0.240	0.049
	Root Cd uptake	−0.794	0.165	−0.777	0.382	0.030	−0.224
	Cd translocation rate	0.711	0.727	−0.315	−0.852*	−0.301	−0.265
Cd_{10}	Shoot Cd concentration	0.832*	−0.812*	−0.841*	−0.565	0.727	−0.430
	Root Cd concentration	−0.899*	0.847*	0.883*	0.473	−0.350	0.720
	Root Cd uptake	−0.918**	0.913*	0.888*	0.417	−0.352	0.707
	Cd translocation rate	0.813*	−0.891*	−0.813*	−0.535	0.341	−0.680

$*p<0.05$; $**p<0.01$

reported that citric acid constituted approximately 0.1 % of the total concentration of LMWOAs in the rhizosphere soil of rice, but high-Cd cultivar secreted significantly more citric acid than low-Cd cultivar. Tartaric and oxalic acids had no obvious effect on the uptake and translocation of Cd in hot pepper plant in the Cd_2 treatment. However, the concentrations of tartaric and oxalic acids in root exudates of YCT and JFZ decreased greatly by the 10 μM Cd treatment. Moreover, the two organic acids had adverse effect on the accumulation of Cd in hot pepper plants in the Cd_{10} treatment. These results suggest that differences in the Cd uptake and accumulation between YCT and JFZ may be modified by the changes in LMWOAs. Although mechanisms of Cd uptake and translocation in the plants are not quite clear at present, the processes of Cd accumulation in plant tissues generally include formation of Cd-organic acid complexes (Krotz et al. 1989). In general, the effect of LMWOAs on the uptake, translocation, and accumulation of Cd in hot pepper may depend upon cultivars and Cd supply concentrations.

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References

Chairidchai P, Ritchie G (1993) The effect of citrate and pH on zinc uptake by wheat. Agron J 85:322–328

Chen B-C, Lai H-Y, Lee D-Y, Juang K-W (2011) Using chemical fractionation to evaluate the phytoextraction of cadmium by switchgrass from Cd-contaminated soils. Ecotoxicology 20:409–418

Cieslinski G, Van Rees K, Szmigielska A, Huang P (1997) Low molecular weight organic acids released from roots of durum wheat and flax into sterile nutrient solutions. J Plant Nutr 20:753–764

Cieśliński G, Van Rees K, Szmigielska A, Krishnamurti G, Huang P (1998) Low-molecular-weight organic acids in rhizosphere soils of durum wheat and their effect on cadmium bioaccumulation. Plant Soil 203:109–117

Evans A Jr (1991) Influence of low molecular weight organic acids on zinc distribution within micronutrient pools and zinc uptake by wheat. J Plant Nutr 14:1307–1318

Gobran GR, Clegg S, Courchesne F (1999) The rhizosphere and trace element acquisition in soils (vol. 225). CRC Press, Boca Raton

Grant C, Clarke J, Duguid S, Chaney R (2008) Selection and breeding of plant cultivars to minimize cadmium accumulation. Sci Total Environ 390:301–310

Greger M, Landberg T (2008) Role of rhizosphere mechanisms in Cd uptake by various wheat cultivars. Plant Soil 312:195–205

Greger M, Löfstedt M (2004) Comparison of uptake and distribution of cadmium in different cultivars of bread and durum wheat. Crop Sci 44:501–507

Günther K, Ji G, Kastenholz B (2000) Characterization of high molecular weight cadmium species in contaminated vegetable food. Fresenius J Anal Chem 368:281–287

Guo T-R, Zhang G-P, Zhou M-X, Wu F-B, Chen J-X (2007) Influence of aluminum and cadmium stresses on mineral nutrition and root exudates in two barley cultivars. Pedosphere 17: 505–512

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168:293–303

Hu L, McBride MB, Cheng H, Wu J, Shi J, Xu J, Wu L (2011) Root-induced changes to cadmium speciation in the rhizosphere of two rice (*Oryza sativa* L.) genotypes. Environ Res 111:356–361

Jones DL (1998) Organic acids in the rhizosphere—a critical review. Plant Soil 205:25–44

Jones DL, Darah PR, Kochian LV (1996) Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. Plant Soil 180:57–66

Krotz RM, Evangelou BP, Wagner GJ (1989) Relationships between cadmium, zinc, Cd-peptide, and organic acid in tobacco suspension cells. Plant Physiol 91:780–787

Lin Q, Chen Y, Chen H, Yu Y, Luo Y, Wong M (2003) Chemical behavior of Cd in rice rhizosphere. Chemosphere 50:755–761

Liu J, Qian M, Cai G, Zhu Q, Wong MH (2007) Variations between rice cultivars in root secretion of organic acids and the relationship with plant cadmium uptake. Environ Geochem Health 29: 189–195

Lu R (2000) Methods of soil and agro-chemical analysis. Agricultural Science and Technology Press, Beijing

Nigam R, Srivastava S, Prakash S, Srivastava M (2001) Cadmium mobilisation and plant availability—the impact of organic acids commonly exuded from roots. Plant Soil 230:107–113

Pan J, Plant JA, Voulvoulis N, Oates CJ, Ihlenfeld C (2010) Cadmium levels in Europe: implications for human health. Environ Geochem Health 32:1–12

Rosas A, Rengel Z, de la Luz MM (2007) Manganese supply and pH influence growth, carboxylate exudation and peroxidase activity of ryegrass and white clover. J Plant Nutr 30:253–270

Tessier A, Campbell PG, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51: 844–851

Wong S, Li X, Zhang G, Qi S, Min Y (2002) Heavy metals in agricultural soils of the Pearl River Delta, South China. Environ Pollut 119:33–44

Xie X, Weiss DJ, Weng B, Liu J, Lu H, Yan C (2013) The short-term effect of cadmium on low molecular weight organic acid and amino acid exudation from mangrove (*Kandelia obovata* (S., L.) Yong) roots. Environ Sci Pollut Res 20:997–1008

Xin J, Huang B, Liu A, Zhou W, Liao K (2013) Identification of hot pepper cultivars containing low Cd levels after growing on contaminated soil: uptake and redistribution to the edible plant parts. Plant Soil 373:415–425

Xin J, Huang B, Dai H, Liu A, Zhou W, Liao K (2014) Characterization of cadmium uptake, translocation, and distribution in young seedlings of two hot pepper cultivars that differ in fruit cadmium concentration. Environ Sci Pollut Res 21:7449–7456

Zabowski D (1989) Limited release of soluble organics from roots during the centrifugal extraction of soil solutions. Soil Sci Soc Am J 53: 977–979