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RESEARCH ARTICLE

Characterization of cadmium uptake, translocation, and distribution in young seedlings of two hot pepper cultivars that differ in fruit cadmium concentration

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Abstract The reasons why some cultivars of hot pepper (*Capsicum annuum* L.) accumulate low levels of Cd are poorly understood. We aimed to compare the characteristics of Cd uptake and translocation in low-Cd and high-Cd hot pepper cultivars by determining the subcellular locations and chemical forms of Cd, and its distribution among different plant organs. We conducted a hydroponic experiment to investigate the subcellular distribution and chemical forms of Cd in roots, stems, and leaves of a low-Cd (Yeshengchaotianjiao, YCT) and a high-Cd cultivar (Jinfuzaohuangjiao, JFZ). The results showed that the concentrations of Cd in almost all subcellular fractions of roots, and in all chemical forms in roots, were higher in YCT than in JFZ. Compared with YCT, JFZ had higher Cd concentrations in almost all subcellular fractions of stems and leaves, and higher Cd concentrations in almost all chemical forms in stems and leaves. Additionally, YCT had significantly higher total Cd accumulation but a lower Cd translocation rate compared with JFZ. In general, the results presented in this study revealed that root-to-shoot Cd translocation via the xylem is the key physiological processes determining the Cd accumulation level in stems and leaves of hot pepper plants. Immobilization of Cd by the cell walls of different organs is important in Cd detoxification and limiting the symplastic movement of Cd.

Keywords *Capsicum annuum* L · Cadmium · Uptake · Subcellular distribution · Translocation · Genotypic difference

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Introduction

Cadmium (Cd), as a nonessential element, is highly toxic to plants and can pose a risk to human health through bioaccumulation and biomagnification along the food chain (Grant et al. 2008). Plant-derived foods are the main source of Cd entering the human body. In the past few decades, Cd contamination of agricultural soils has become increasingly serious because of mining and smelting, electroplating, sewage irrigation, and the use of phosphate fertilizers (Wong et al. 2002). Therefore, minimizing Cd accumulation in the edible parts of crops is a promising option for reducing the risk to human health (Uraguchi and Fujiwara 2013).

In recent years, researchers have found that the uptake and distribution of Cd varies greatly not only among plant species but also among cultivars (Alexander et al. 2006; Wang et al. 2007; Grant et al. 2008). On this basis, the concepts of pollution-safe cultivars (Yu et al. 2006), pollution-free cultivars (Liu et al. 2012), Cd-excluding cultivars (Li et al. 2012), and Cd-exclusive cultivars (Zhan et al. 2013) have been proposed. These are essentially low-Cd cultivars (Clarke et al. 2002), referring to crop cultivars whose edible parts accumulate Cd at a low enough level for safe consumption when grown in Cd-contaminated soils. Consequently, the breeding and production of low-Cd cultivars is considered to be an effective method to reduce soil Cd entering the human diet. For instance, the low-Cd durum wheat cultivar Strongfield is now sown on more than 25 % of the durum area in Canada (Grant et al. 2008). There have been several studies on screening for low-Cd crop cultivars (Xin et al. 2013a). However, the mechanisms underlying the lower Cd accumulation in low-Cd cultivars in comparison with high-Cd cultivars are not still fully understood, which has greatly slowed the breeding and selection of low-Cd crop cultivars.

Hot pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in the world. The cultivated area is

about 1.3 million hm^2 per year in China, producing 27 million tons of pepper (Xu et al. 2008). Angelova et al. (2009) reported that pepper accumulates Cd more easily than tomato (*Lycopersicon esculentum*) and aubergine (*Solanum melongena*). Moreover, large amounts of Cd taken up by the roots of chili peppers are translocated into shoots (Jidesh and Kurumthottical 2000), which facilitates the accumulation of Cd in the fruits. Thus, it is necessary to screen for low-Cd cultivars of pepper to ensure food safety. In our previous study, some low- and high-Cd cultivars of hot pepper were identified (Xin et al. 2013a); however, the mechanisms of the genotypic difference in Cd accumulation were still unknown. The levels of Cd in the fruits of different hot pepper cultivars may be influenced by several physiological differences among cultivars, including Cd uptake from the soil, sequestration of Cd (in subcellular compartments or as organic complexes), root-to-shoot Cd translocation via the xylem, and Cd movement into fruit via the phloem (Xin et al. 2013a). Previously, it was found that the difference in fruit Cd concentration among hot pepper cultivars was not correlated with root Cd uptake (Xin et al. 2013a). Therefore, the subcellular distribution and chemical forms of Cd, which greatly affect Cd transport in plants (Wu et al. 2005; Yu et al. 2012), may be responsible for the genotypic difference in Cd accumulation. Vacuolar sequestration and cell wall binding are generally considered to be the main storage methods for Cd in plant cells. In addition, the biological activity of Cd in plants is associated with its chemical forms, which may affect its migration and accumulation throughout the whole plant (Qiu et al. 2011). To our knowledge, there are only a few reports on the association of the subcellular distribution and/or chemical forms of Cd with differences in Cd accumulation among cultivars (Qiu et al. 2011; Yu et al. 2012; Xin et al. 2013b). However, such studies have not yet provided consistent results. For example, Qiu et al. (2011) found that a low-Cd Chinese flowering cabbage (*Brassica parachinensis* L.) cultivar always had a higher proportion of Cd bound to the cell wall in comparison with a high-Cd cultivar, but the proportions in the stem and leaves of low-Cd cultivars of water spinach (*Ipomoea aquatica* Forsk.) were similar or lower than those of a high-Cd cultivar (Xin et al. 2013b).

The aim of this study was to examine the subcellular distribution and chemical forms of Cd in the young seedlings of two hot pepper cultivars with distinctive fruit Cd concentrations to provide a better understanding of the uptake and translocation of Cd in a low-Cd cultivar.

Materials and methods

Plant materials and culture

The experiment was carried out at the Hunan Institute of Technology, Hengyang, China. Two hot pepper cultivars, Yeshengchaotianjiao (YCT) and Jinfuzaohuangjiao (JFZ), were used in this study. The fruit Cd concentration in JFZ was 2.1–2.7-fold higher than in YCT when grown in Cd-contaminated soils (0.28–2.69 mg kg^{-1}, dry weight) (Xin et al. 2013a). Therefore, YCT and JFZ were identified as low- and high-Cd cultivars, respectively.

Seeds of the two cultivars were surface sterilized in 0.5 % NaOCl for 20 min, rinsed with deionized water, and germinated in sterilized moist quartz sand at 25±1 °C. At the two-leaf stage (10 days old), three uniform seedlings were selected and transplanted into each beaker containing 400 mL Hoagland solution (5 mM $Ca(NO_3)_2 2H_2O$, 5 mM KNO_3, 2 mM $MgSO_4 7H_2O$, 1 mM KH_2PO_4, 0.1 mM EDTA-Fe, 47 μM H_3BO_3, 1 μM $MnCl_2 4H_2O$, 1 μM $ZnSO_4 7H_2O$, 0.01 μM H_2MoO_4, and 0.25 μM $CuSO_4 5H_2O$). All chemicals used in this work were analytical reagents, and the solutions were replaced every 3 days throughout the experiment. A total of 36 beakers with seedlings of the 2 cultivars (18 beakers per cultivar) were arranged randomly in a growth chamber with a 16-h light period at a light intensity of 300 μmol s^{-1} m^{-2}, 30 °C/25 °C day/night temperatures and 60–70 % relative humidity, and the roots were always kept in the dark. On the 10th day after transplanting, the 18 beakers with plants of the same cultivar were subjected to the following Cd additions: 0 (control), 2 (T1), and 10(T2) μM Cd as $Cd(NO_3)_2$ (pH 6.0) resulting in 6 replicates per treatment per cultivar. On the 15th day after treatment, the plants were harvested, with plants from three of the replicates of each treatment being used for the analysis of the subcellular distribution of Cd and the plants in the three remaining replicates being used for the analysis of the chemical forms of Cd. The plants in each beaker were separated into roots, stems, and leaves and used to give one composite aliquot of the each of the three tissues per beaker. Extracellular and apoplastic Cd were desorbed from the roots for 15 min in ice-cold 5 mM $CaCl_2$ solution (5 mM Mes-Tris, pH 6.0) (Han et al. 2006), and then all samples were thoroughly washed with deionized water. After weighing, the fresh plant samples were immediately frozen in liquid N_2 and kept frozen until Cd analysis.

Tissue fractionation

Frozen samples (about 2 g) were homogenized in pre-cooled (4 °C) extraction buffer (50 mM Tris–HCl, 250 mM sucrose, 1.0 mM $C_4H_{10}O_2S_2$, pH 7.5) (Weigel and Jäger 1980) with a chilled mortar and a pestle. The homogenate was sieved through a nylon cloth (80 μm), and the liquid was squeezed from the residue. The residue on the cloth was washed twice with homogenization buffer and was designated fraction I (FI); it mainly contained cell walls and cell wall debris. The filtrate was centrifuged at 20,000×*g* for 45 min. The supernatant solution was referred to as the soluble fraction (including the vacuole) and was designated fraction II (FII). The deposit

was treated as the organelles (excluding the vacuole) and was designated fraction III (FIII). All steps were performed at 4 °C. The subcellular fractions were dried at 70 °C to a constant weight, and then digested at 145 °C for 24 h with an oxidative acid mixture of HNO_3:$HClO_4$ (3:1, *v*/*v*).

Extraction of Cd in different chemical forms

Cadmium associated with different chemical forms was successively extracted by designated solutions in the following order (Wu et al. 2005): (1) 80 % ethanol, to extract inorganic Cd giving priority to nitrate/nitrite, chloride, and aminophenol cadmium; (2) deionized water (d-H_2O), to extract water-soluble-Cd organic acid complexes and $Cd(H_2PO_4)_2$; (3) 1 M NaCl, to extract pectate- and protein-integrated Cd; (4) 2 % acetic acid (HAc), to extract undissolved cadmium phosphate including $CdHPO_4$, $Cd_3(PO_4)_2$, and other Cd-phosphate complexes; (5) 0.6 M HCl, to extract cadmium oxalate.

Frozen materials (about 2 g), including roots, stems, and leaves, were homogenized in extraction solution with a mortar and a pestle, diluted at a ratio of 1:10 (*w*/*v*), and shaken for 22 h at 25 °C. After that, the homogenate was centrifuged at 5,000×*g* for 10 min, and the first supernatant solution was removed to a conical beaker. The sediment was resuspended twice in the same extraction solution, shaken for 2 h at 25 °C, centrifuged at 5,000×*g* for 10 min, and the supernatant was collected. The three suspension and centrifugation steps were the same for each of the five extraction solutions. Each of the pooled supernatant solution was then evaporated on an electric plate at 70 °C to a constant weight, and digested at 145 °C with an oxidative acid mixture of HNO_3:$HClO_4$ (3:1, *v*/*v*).

Chemical analysis

Cadmium concentrations in the digests were determined with an atomic absorption spectrophotometer (Shimadzu AA-6300C, Japan). 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 (QA/QC) of the Cd analytical procedure.

Statistics and calculations

All Cd concentrations were calculated on the basis of the fresh weight (FW) of samples before separation or extraction. Data were statistically analyzed with the independent samples *t* test and least significant difference (LSD) test based on one-way ANOVA using Excel 2003 and SPSS 13.0. The data were checked for heteroscedasticity with Levene's test before the ANOVA was performed and showed no heteroscedasticity.

To estimate Cd translocation to the aerial parts, the translocation rate (TR) (Wang et al. 2007) was calculated as follows: TR (%)=100×(Cd amount in the aerial parts)/(Cd amount in the whole plant).

Results

Biomass response to Cd stress

There were no significant differences ($p>0.05$) in the root biomass of each cultivar among the treatments (Table 1). The biomass of the stems and leaves of both cultivars decreased with increased Cd concentration in the nutrient solution; however, the reduction was only statistically significant in the T2 treatment (Table 1).

Cd subcellular distribution

The Cd concentrations in different organs of the two cultivars were found to increase with the level of Cd in the medium, and the highest Cd concentration occurred in roots, followed by leaves and stems (Fig. 1a). The total Cd concentration in the roots of YCT (low-Cd cultivar) was always significantly higher ($p<0.01$) than in JFZ (high-Cd cultivar), but Cd concentrations were lower ($p<0.05$ or $p<0.01$) in the stems and leaves of YCT than in those of JFZ.

The subcellular distribution of Cd in the two cultivars of hot pepper is shown in Fig. 1. Increasing Cd supply in the solution significantly increased Cd concentrations in all subcellular fractions of the two cultivars (Fig. 1b–d), with the majority of Cd associated with the soluble fraction (FII) and the cell wall (FI) and a minimal amount of Cd present in the organelle fraction (FIII) (Table 2). The Cd concentrations of all subcellular fractions in the roots of YCT were higher ($p<0.01$ or $p<0.05$) than those in JFZ except that no significant difference ($p>0.05$) in the concentration of FIII-Cd was observed in the T2 treatment (Fig. 1b). By contrast, the stems and leaves of YCT always had significantly lower ($p<0.01$ or $p<0.05$) concentrations of FI-, FII-, and FIII-Cd than those of

Table 1 Biomasses (g, FW) of the two cultivars of hot pepper (mean±SD, $n=3$)

Organ	Cultivar	Treatment		
		Control	T1	T2
Root	YCT	3.09±0.39	3.39±0.33	3.47±0.38
	JFZ	3.34±0.24	3.01±0.22	3.13±0.39
Stem	YCT	6.54±1.42 a	4.75±0.89 ab	3.60±0.80 b
	JFZ	8.58±1.25 a	6.96±0.77 a	4.03±0.64 b
Leaf	YCT	11.86±2.31 a	10.71±1.54 ab	8.43±1.00 b
	JFZ	15.65±1.66 a	13.61±0.62 a	10.16±0.81 b

Different letters in a row indicate significant differences between the treatments at the $p<0.05$ level

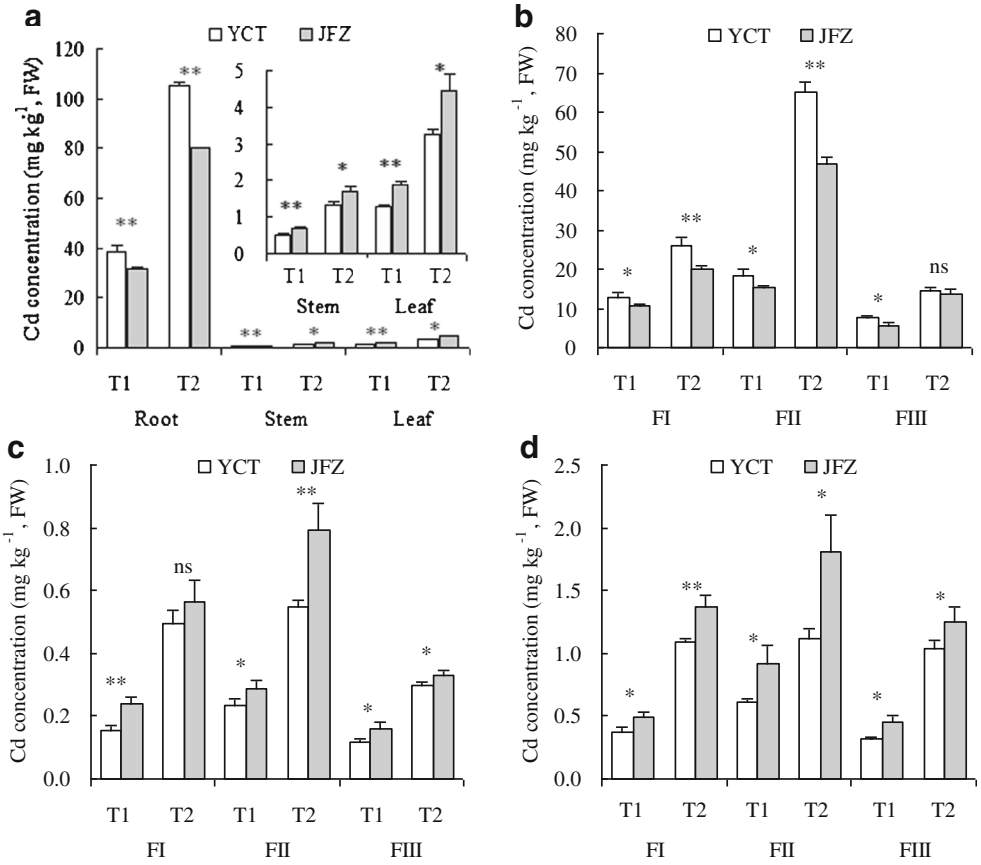

Fig. 1 Concentrations of Cd in roots, stems, and leaves (**a**), and different subcellular fractions of roots (**b**), stems (**c**), and leaves (**d**) of YCT and JFZ exposed to Cd levels of 2 (T1) and 10(T2) μM. *FI* cell walls and cell wall debris, *FII* soluble fraction (including the vacuole), and *FIII* organelle fraction (excluding the vacuole). *Error bars* represent the standard deviation (n=3). Significance levels were determined by the independent samples t test; *ns* not significant; * significant at the $p<0.05$ level; ** significant at the $p<0.01$ level

JFZ (Fig. 1c, d) except for the concentration of FI-Cd in stems in the T2 treatment (Fig. 1c).

Table 2 Percentages of Cd in subcellular fractions of roots, stems, and leaves of YCT and JFZ

Organ	Treatment	Cultivar	Cd percentage (%)		
			FI	FII	FIII
Root	T1	YCT	32.8±1.5	47.6±2.1	19.6±2.5
		JFZ	33.2±1.7	48.9±0.2	17.9±1.9
	T2	YCT	24.6±2.4	61.6±2.1	13.8±0.7
		JFZ	24.9±1.2	58.2±2.2	16.9±1.5
Stem	T1	YCT	30.2±1.8	46.3±1.5	23.5±3.2
		JFZ	34.6±2.4	41.9±4.3	23.5±2.1
	T2	YCT	37.0±1.4	40.9±0.7	22.1±0.7
		JFZ	33.3±2.4	47.0±3.1	19.7±1.6
Leaf	T1	YCT	28.7±1.8	47.3±0.8	24.0±1.8
		JFZ	26.4±2.6	49.6±4.4	24.0±4.2
	T2	YCT	33.8±1.3	34.3±1.0	31.9±0.9
		JFZ	30.9±1.3	40.8±2.2	28.3±1.2

Cd percentage (%) the fraction Cd concentration/(the sum of all fractions' Cd concentrations). Data presented are means±SD (n=3). *FI* cell walls and cell wall debris, *FII* soluble fraction (including the vacuole), *FIII* organelle fraction (excluding the vacuole)

In the roots of both cultivars, the percentages of FII-Cd increased and those of the other two subcellular fractions decreased with the increase of Cd level in the medium (Table 2). Generally, the Cd proportion of each subcellular fraction in the roots of YCT was similar to that in JFZ. In YCT stems, the percentage of FII-Cd decreased and FI-Cd increased with increasing Cd exposure level. However, it was the opposite in JFZ. The percentages of FIII-Cd were slightly reduced in both cultivars in the T2 treatment compared with the T1 treatment. Additionally, the percentages of FI- and FII-Cd in YCT were, respectively, lower and higher than those in JFZ in the T1 treatment, but the opposite was the case in the T2 treatment. In the leaves of both cultivars, the percentages of FII-Cd decreased but those of FI- and FIII-Cd increased with the increase of Cd exposure level. Notably, YCT always had lower percentages of FII-Cd and higher percentages of FI-Cd than JFZ. In addition, the percentage of FIII-Cd in YCT was similar to that in JFZ in the T1 treatment, but was higher in the T2 treatment.

Chemical forms of Cd

The Cd concentrations and the percentages of the various chemical forms of Cd in the roots, stems, and leaves are shown in Fig. 2 and Table 3, respectively. The concentrations of Cd in

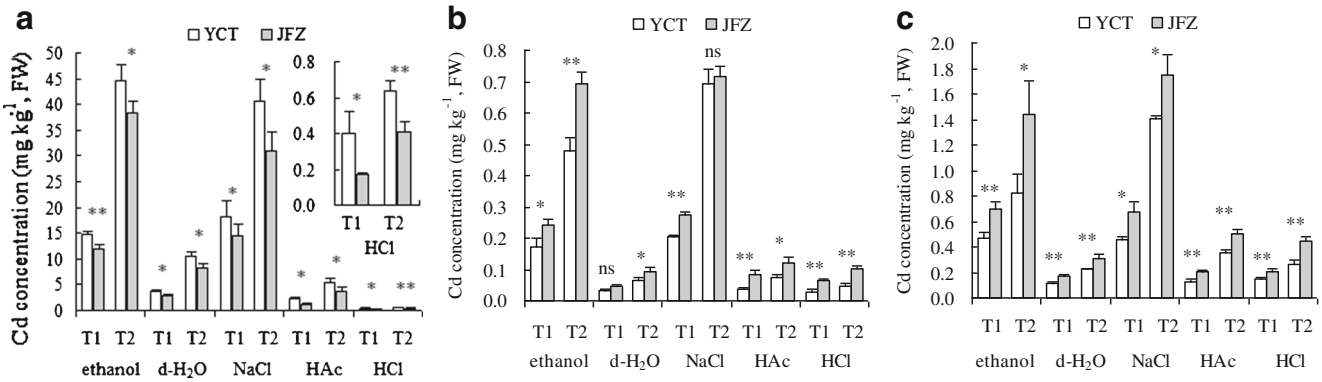

Fig. 2 Concentrations of different chemical forms of Cd in roots (**a**), stems (**b**), and leaves (**c**) of YCT and JFZ exposed to Cd levels of 2 (T1) and 10(T2) μM. *Error bars* represent the standard deviation (n=3). Significance levels were determined by the independent samples t test; *ns* not significant; * significant at the $p<0.05$ level; ** significant at the $p<0.01$ level

different chemical forms in all of the organs of the two cultivars increased with the Cd concentration in the medium. The amount of Cd extracted by 1 M NaCl and 80 % ethanol was predominant in the two treatments, representing more than 70 % of the total Cd in different organs. The proportion of Cd extracted by any one of other three extracting agents was about 10 % or even lower. In roots, the Cd concentration of each chemical form was always significantly higher ($p<0.05$ or $p<0.01$) in YCT than in JFZ (Fig. 2a). However, in stems and leaves, YCT had a lower ($p<0.05$ or $p<0.01$) concentration of Cd extracted by each extracting agent than JFZ in most cases (Fig. 2b, c).

With increased Cd application, the percentages of 80 % ethanol and d-H_2O-extractable Cd both increased in roots of the two cultivars but decreased in stems and leaves, except that the stems of JFZ showed an increase in the proportion of 80 % ethanol-extractable Cd. The percentages of Cd extracted by 1 M NaCl decreased in roots but increased in stems and leaves with the increase of Cd exposure. Additionally, the percentages of Cd extracted by 2 % HAc and 0.6 M HCl in roots, stems, and leaves of the two cultivars decreased with increasing Cd concentration in the medium, except that roots of JFZ and leaves of both cultivars showed a slight increase in the percentages of 2 % HAc-extractable Cd.

When comparing the two cultivars, the proportion of 80 % ethanol-extractable Cd in YCT roots was always lower than in JFZ roots; however, the percentages of d-H_2O-, 2 % HAc-, and 0.6 M HCl-extractable Cd in the roots of YCT were higher than those of JFZ. In addition, the percentage of 1 M NaCl-extractable Cd in YCT roots was slightly lower than in JFZ roots in the T1 treatment, but it was the opposite in the T2 treatment. In stems, the percentages of Cd extracted by 80 % ethanol and d-H_2O were higher in YCT in the T1 treatment, but were higher in JFZ in the T2 treatment. Furthermore, the percentages of Cd extracted by 1 M NaCl were always higher in YCT in comparison with JFZ, but those extracted by 2 %

Table 3 Percentages of different chemical forms of Cd in YCT and JFZ

Organ	Treatment	Cultivar	Cd percentage (%)				
			80 % ethanol	d-H_2O	1 M NaCl	2 % HAc	0.6 M HCl
Root	T1	YCT	37.7±2.6	9.3±1.5	46.4±3.5	5.6±0.5	1.0±0.3
		JFZ	39.1±3.8	9.0±0.3	47.4±4.0	3.9±0.5	0.6±0.0
	T2	YCT	43.8±2.2	10.3±0.8	39.8±1.9	5.4±0.7	0.6±0.1
		JFZ	46.9±0.8	10.2±1.4	37.8±1.9	4.6±0.7	0.5±0.0
Stem	T1	YCT	36.0±3.5	6.9±1.5	43.4±2.4	7.7±1.2	6.1±1.0
		JFZ	34.3±0.4	6.3±0.7	38.7±1.5	11.7±1.3	8.9±0.9
	T2	YCT	35.2±1.3	4.8±0.2	51.0±1.3	5.5±1.0	3.5±0.8
		JFZ	40.1±1.2	5.5±0.7	41.4±1.9	7.1±0.7	6.0±0.3
Leaf	T1	YCT	35.7±1.6	8.5±0.6	34.6±0.5	10.0±0.7	11.1±1.1
		JFZ	35.5±4.0	8.9±0.5	34.5±3.2	10.3±0.3	10.8±0.6
	T2	YCT	26.8±3.3	7.3±0.5	45.8±3.1	11.6±0.3	8.5±0.9
		JFZ	32.4±3.9	7.1±0.5	39.4±4.1	11.2±1.1	9.9±0.5

Cd percentage (%) the fraction Cd concentration/(the sum of all fractions' Cd concentrations). Data presented are means±SD (n=3)

Table 4 Cd accumulation (μg) in different organs of two cultivars of hot pepper (mean±SD, n=3)

Treatment	Cultivar	Root	Stem	Leaf	Total
T1	YCT	130.79±7.47 **	2.38±0.32 **	14.03±2.51 **	147.20±10.11 *
	JFZ	94.93±3.92	4.78±0.46	25.49±2.69	125.21±4.80
T2	YCT	365.76±38.29 *	4.79±0.87 *	27.41±4.62 *	397.97±43.73 *
	JFZ	250.98±30.46	6.75±0.68	45.23±8.52	302.96±38.96

The * and ** indicate that the difference between the two cultivars in the same treatment is significant at the $p<0.05$ level, and significant at the $p<0.01$ level, respectively

HAc and 0.6 M HCl were lower. In leaves, the percentage of 80 % ethanol-extractable Cd in YCT was obviously lower but the proportion of Cd extracted by 1 M NaCl in YCT was higher than JFZ in the T2 treatment. Additionally, the percentages of other chemical forms of Cd were similar between the two cultivars.

Cd accumulation and translocation

Cd accumulation in different organs (roots, stems, and leaves) and the total Cd accumulation in the whole plant for the two cultivars are shown in Table 4. YCT accumulated significantly more Cd in roots and less Cd in stems and leaves than JFZ. The difference in total Cd accumulation between the two cultivars was always significant. Cd was mostly accumulated in the roots of the two cultivars, and YCT had a higher ability to retain Cd in roots than JFZ (Table 5). Furthermore, the Cd distribution percentages in the stems and leaves of YCT were significantly lower ($p<0.01$) than those of JFZ. As a result, the Cd translocation rates in YCT were always markedly lower ($p<0.01$) than those in JFZ (Table 5).

Discussion

Genotypic differences in Cd accumulation can be associated with the subcellular distribution and chemical forms of Cd (Qiu et al. 2011; Yu et al. 2012; Xin et al. 2013b). In our previous study, we found that the low-Cd hot pepper cultivar YCT had a higher root Cd concentration than the high-Cd cultivar JFZ (Xin et al. 2013a), which was consistent with the results of this study (Fig. 1a). The lower Cd accumulation in the stems and leaves of YCT (Table 4) reflects the differential distribution of Cd among roots, stems, and leaves (Table 5). Similar large variations in Cd distribution among different organs were reported in six rice cultivars (Liu et al. 2007). In contrast, no difference among three durum wheat varieties was observed in Cd distribution between roots and shoots (Jalil et al. 1994). In the present study, a lower translocation rate in YCT compared with JFZ indicated that Cd was retained in the roots, perhaps by certain mechanisms that avoid long-distance translocation of Cd from roots to shoots. Therefore, it may be assumed that Cd sequestration in the plant cells could be responsible for the differences in Cd uptake, translocation, and distribution between the two cultivars. To test this hypothesis, in this study, the Cd subcellular distribution was investigated in young seedlings of YCT and JFZ differing in fruit Cd concentration. It was observed that the concentration of FI-Cd was higher in YCT roots than in JFZ roots, indicating that YCT can retain more Cd in the cell walls of its roots. This is also one of the reasons why the translocation rate of Cd was lower in YCT than in JFZ in our previous study (Xin et al. 2013a). Additionally, the Cd concentration in all subcellular fractions increased consistently with the Cd level in the growing medium, with the highest Cd accumulation in FII, followed by FI and FIII. Similarly, in barley (Wu et al. 2005), Cd was mostly accumulated in the soluble fractions of shoots and roots. However, in lettuce (Ramos et al. 2002), large amounts of Cd were found in the cell wall. Also, Qiu et al. (2011) found that the highest Cd level in a low-Cd cultivar of Chinese flowering cabbage was in the cell wall fraction, but in a high-Cd cultivar, the Cd concentration in cell walls was

Table 5 Cd distribution percentages (%) in different organs and translocation rates (TR, %) of two cultivars of hot pepper (mean±SD, n=3)

Treatment	Cultivar	Root	Stem	Leaf	TR
T1	YCT	88.90±1.12 *	1.61±0.18 *	9.49±1.03 *	11.10±1.12 *
	JFZ	75.83±2.07	3.82±0.31	20.35±1.76	24.17±2.07
T2	YCT	91.95±0.52 *	1.20±0.11 *	6.86±0.44 *	8.05±0.52 *
	JFZ	82.90±1.07	2.23±0.08	14.87±1.14	17.10±1.07

The * indicates that the difference between the two cultivars in the same treatment is significant at the $p<0.01$ level

similar to that in soluble fraction. These differences may be attributed to different experimental conditions, including Cd exposure levels and growing medium, and the variable levels of Cd uptake and translocation in different plant species and cultivars.

Cell walls, which are mainly composed of polyoses and proteins and provide negative charged sites on their surfaces that bind Cd ions and restrict their transportation across the cytomembrane, are the first barrier protecting the protoplast from Cd toxicity (Fu et al. 2011). The vacuole comprises 90 % of the cell's volume (Pittman 2005) and contains sulfur-rich peptides and organic acids (Weigel and Jäger 1980). In hot pepper, most Cd was stored in the soluble fraction. Although the concentrations of FI- and FII-Cd in the roots of YCT were always higher than those of JFZ, no significant difference in the concentration of FIII-Cd was observed between the two cultivars in the T2 treatment. Therefore, YCT may have greater ability than JFZ to compartmentalize Cd in subcellular compartments, such as the cell wall and vacuole, to avoid Cd accumulation in the cytosol. More importantly, the Cd concentrations in the soluble fractions of the stems and leaves of YCT were lower than those of JFZ, suggesting that YCT had lower Cd translocation to fruits than JFZ (Xin et al. 2013a). Similarly, Yu et al. (2012) reported that a high Cd concentration in the soluble fraction of rice leaves promotes Cd translocation from leaves to grains. Therefore, the higher fruit Cd concentration in JFZ might be ascribed to its higher Cd concentrations in the soluble fractions of the stems and leaves. However, an increase of free Cd ion concentration in the cytosol of plant cells is harmful to plant growth. In the present study, only the biomasses of stems and leaves decreased obviously in the T2 treatment in comparison with the control (Table 1), indicating that the shoots of the two hot pepper cultivars were more sensitive to Cd than roots. The reason for this may be that large amounts of Cd in the cytosol cause toxic damage to the organelles (Wu et al. 2003), especially chloroplasts, mitochondria, and the nucleus, and thus interrupt many physiological and biochemical processes in cells. Also, the two cultivars might have similar Cd tolerance in terms of their biomass response to Cd stress. However, more physiological and biochemical parameters, including net photosynthesis, water use efficiency, antioxidative enzyme activities, and the synthesis of phytochelatins (PCs) and desglycyl PCs (Jemal et al. 1998; León et al. 2002), need to be examined to clarify this view.

The biological activities of Cd in plants, which mean the reactivity or ability of Cd to interact with biological structures and tissues, are related to its chemical forms, which can be determined with different extracting agents (Wu et al. 2005). The cadmium extracted by 80 % ethanol and d-H_2O represents inorganic and organic water-soluble Cd, which have a higher ability to migrate and are more deleterious to plant cells than other chemical forms of Cd. The 1 M NaCl-extractable Cd, mainly bound to proteins and pectic acids, is more harmful to cells than undissolved Cd-phosphate (extracted by 2 % HAc) and Cd-oxalate (extracted by 0.6 M HCl) (Zhang et al. 2013). In this study, water-soluble Cd, including inorganic and organic forms, comprised 47.0–57.1 % of the Cd in the roots and was assumed to be sequestered into root vacuoles, thereby limiting its translocation from roots to shoots (Ueno et al. 2010). Although the concentration of water-soluble Cd in the roots of YCT was higher than in JFZ, YCT had lower Cd concentrations in stems and leaves. This suggests that JFZ can produce more xylem transport proteins than YCT to increase xylem loading of Cd. Similarly, Uraguchi et al. (2009) also reported that differences in the expression level of a xylem loading transporter(s) for Cd are responsible for the differential Cd accumulation between low- and high-Cd rice cultivars. In addition, the proportions of Cd extracted by 1 M NaCl, 2 % HAc, and 0.6 M HCl represented about half of the total Cd in roots, indicating that the Cd was transformed into non- or low-toxic complexes to protect the cells. It is worth noting that the concentrations of water-soluble Cd were lower in the stems and leaves of YCT than in those of JFZ. This may be the reason why the movement of Cd to fruits from stems and leaves in YCT was reduced compared with JFZ (Xin et al. 2013a).

In conclusion, significant differences in the subcellular distribution and chemical forms of Cd exist between the low- and high-Cd hot pepper cultivars. The soluble fraction is the largest subcellular fraction to store Cd in all organs of the two cultivars. In comparison with the high-Cd cultivar (JFZ), the low-Cd cultivar (YCT) has a lower water-soluble Cd concentration in both stems and leaves, which decreases the movement of Cd to fruits from stems and leaves via the xylem and phloem. The difference in translocation rate between the two cultivars may be the main cause of the genotypic variation in fruit Cd accumulation.

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