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ARTICLE

Variation in Cadmium Accumulation among 30 Cultivars and Cadmium Subcellular Distribution in 2 Selected Cultivars of Water Spinach (*Ipomoea aquatica* Forsk.)

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To reduce the influx of cadmium (Cd), a toxic heavy metal, into the human food chain through vegetable intake, a pot experiment for the selection of a pollution-safe cultivar (PSC) of water spinach (*Ipomoea aquatica* Forsk.) was carried out. The experiment with 30 tested cultivars revealed that the maximum differences in Cd concentration between the cultivars containing the highest and the lowest Cd were 3.0−3.9-fold under low-Cd treatment (soil Cd = 0.593 mg kg^{-1}), 2.7−3.5-fold under middle-Cd treatment (soil Cd = 1.091 mg kg^{-1}), and 2.6−2.7-fold under high-Cd treatment (soil Cd = 1.824 mg kg^{-1}), large enough to define the Cd-PSCs. Concentrations of Cd in edible parts of six cultivars, cv. Daxingbaigu, Huifengqing, Qiangkunbaigu, Qiangkunqinggu, Shenniuliuye, and Xingtianqinggu, were lower than 0.2 mg kg^{-1}, the maximum level (ML) of Cd allowed by the Codex Alimentarius Commission (CAC) standard, even under middle-Cd treatment. Accordingly, these cultivars were treated as typical Cd-PSCs. Four cultivars, cv. Jieyangbaigeng, Xianggangdaye, Sannongbaigeng, and Taiwan 308, contained Cd in edible parts exceeding the ML even under low-Cd treatment, and they were defined as typical non-Cd-PSCs. The correlations of the Cd concentrations among the tested cultivars between the three treatments were significant at the $p < 0.05$ level. A conspicuous difference in Cd subcellular distribution in hydroponic plant tissues between cv. Qiangkunqinggu (a typical Cd-PSC) and cv. Taiwan 308 (a typical non-Cd-PSC) were observed. Cd absorbed by cv. Qiangkunqinggu seemed to be well-compartmentalized in root and in cell wall fragment, which may be one of the mechanisms leading to its low Cd accumulating property. The results indicated that water spinach, a leafy vegetable, could be easily polluted by soils contaminated with Cd, as 80% of the tested cultivars had exceeded the ML of Cd according to the CAC standard even under the middle-Cd treatment. Much of the evidence obtained from the present study proved that the high Cd-accumulating ability of water spinach is a stable biological property at cultivar level and, thus, is genotype dependent. Therefore, application of the PSC strategy to produce water spinach that is safer to consume is feasible and necessary.

KEYWORDS: Cadmium; soil contamination; subcellular distribution; pollution-safe cultivar; water spinach

INTRODUCTION

There is growing public concern over the potential accumulation of heavy metals in agricultural soils due to rapid urban and industrial development and increasing reliance on agrochemicals in the past several decades. Heavy metal contamination of agricultural soils has also become increasingly serious in China (*1*, *2*).

Of all metals, cadmium (Cd) needs to be especially taken care of in terms of food-chain contamination, because it is readily taken up by plants and easily translocated to different parts of the plant (*3*). Cd is harmful to people's health, and it is further known that food is the main source of nonoccupational exposure to Cd for human beings. For example, lifetime exposure to low-level soil contamination of Cd has been causing renal dysfunction in residents living near contamination sites in Japan (*4*) and China (*5*, *6*).

Potential methods to reduce Cd accumulation in crops include reduction of Cd influx into the soil system, site selection, and management practices, which decrease the concentration of Cd in the soil solution and its uptake and translocation by plants (*7*). However, in many developing countries, such measures are often difficult to put into practice in farmland because of the high cost and slow processing speed, as well as the high demand for foodstuff. An alternative strategy of screening, breeding, and using crop cultivars with the genetic tendency for low Cd

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uptake (*8*, *9*) or pollution-safe cultivars for Cd (Cd-PSCs), that is, the cultivars in which edible parts accumulate Cd at a low enough level for safe consumption when grown in Cd-contaminated soil (*10*, *11*), has been proposed to reduce the risk of soil contaminants entering the human food chain. Experiments have been conducted in rice (*Oryza sativa* L.) (*12*−*15*), wheat (*Triticum aestivum* L.) (*16*, *17*), maize (*Zea mays* L.) (*3*, *8*), soybean (*Glycine max* Merr.) (*14*, *18*), barley (*Hordeum vulgare* L.) (*19*), and potato (*Solanum tuberosum* L.) (*20*, *21*). However, there are many fewer studies conducted about PSCs of leafy vegetables (*22*, *23*).

Cd accumulation of plants is relative to the subcellular behavior of the Cd being absorbed (*24*). Cell vacuole is considered to be the organelle accumulating the greatest amount of Cd (*25*, *26*). Cd subcellular distribution was mainly reported at species level, whereas its intraspecies difference was rarely investigated, and little has been written on the association of Cd subcellular distribution with Cd accumulation at cultivar level.

Water spinach (*Ipomoea aquatica* Forsk.) is a very popular and commonly seen leafy vegetable in southern China; however, our previous research showed that the species can be easily contaminated by Cd in the soil (*27*). In this study, 30 cultivars of water spinach that are currently being used by farmers were investigated to screen the Cd-PSCs. Cd subcellular distributions of the selected typical Cd-PSC and non-Cd-PSC were compared to characterize the Cd subcellular behavior of Cd-PSC. It is hypothesized that the Cd subcellular distribution of Cd-PSC is similar to that of non-Cd-PSC, although their Cd concentrations in tissues are greatly different.

MATERIALS AND METHODS

Pot Experiment for Cd-PSC Screening. *Tested Cultivars.* There were 30 cultivars (**Table 1**) of water spinach used in this study. Seeds of the cultivars were mainly acquired from local seed companies in Guangzhou, Guangdong, and Nanning, Guangxi, China.

Soil Preparation. Garden soil was collected from a garden in Sun Yat-sen University, Guangzhou, and was air-dried and then mildly ground with a wooden roller to pass through a 5 mm sieve for the pot experiment. The soil pH was 7.05, and contents of organic matter, total N, available P, and available K were 1.46%, 0.23%, 55.8 mg kg^{-1}, and 93.9 mg kg^{-1}, respectively, and the total Cd concentration was 0.20 mg kg^{-1}.

Experimental Design. A pot experiment was carried out, and three treatments were conducted by adjusting soil Cd concentration. For each pot (18 cm in upper diameter and 16 cm in height), 1.5 kg of the prepared soil was filled followed by Cd adjustments. The soils spiked at 0.4, 0.8, and 1.6 mg kg^{-1} of Cd in the form of $Cd(NO_3)_2 \cdot 4H_2O$ were designated low-Cd, middle-Cd, and high-Cd, respectively, and their final Cd concentrations were 0.593, 1.091, and 1.824 mg kg^{-1}, respectively. Two weeks after the Cd treatments, 2.0 g pot^{-1} compound fertilizer (N:P:K = 26:6:13) was applied, and 10 seeds of each cultivar of water spinach were sown into a prepared pot on May 10, 2005. The experiment was laid out in a randomized complete block design with three replicates; that is, a total of 270 pots were randomly arranged. The plants were grown in a greenhouse at a temperature of 26−32 °C and watered daily with tap water to maintain moderate soil moisture. On the 10th day after germination, seedlings were thinned to six per pot. The first harvest (FH) for plant tissue was done (only for shoot) after a 40 day growth period, and the second harvest (SH) was carried out after another 40 day growth period for both shoot and root.

Soil and Plant Samplings and Chemical Analyses. Soil samples before and after Cd treatments were collected to analyze the soil properties and Cd concentrations. Soil pH was measured in a soil to water ratio of 1:2.5 (*28*). Organic contents were determined following the method of Nelson et al.(*29*). Total N was measured following the Kjeldahl method (*30*). Available P and available K were measured with molybdenum blue colorimetry (*31*) and atomic absorption spectrophotometry (AAS) (Perkin-Elmer AA 100, Norwalk, CT). Total soil Cd was determined by AAS, followed by mixed-acid digestion (HNO_3−$HClO_4$−HF) (*32*).

Table 1. Tested Cultivars of Water Spinach and Their Providers[a]

cultivar	provider	cultivar	provider	cultivar	provider
Changhebaigu	A	Honghailiuye	G	Sannongbaigeng	O
Daxingbaigu	B	Huifengqing	H	Sannongqingjing	O
Dalidayebaigan	C	Huihuangchunbai	I	Shenniudaye	P
Guangliantexuan	D	Jieyangbaigeng	D	Shenniuliuye	P
Guiliangbai	E	Jinhuachunbai	J	Taiwan 306	Q
Guiliangqing	E	Jinhuaxiye	J	Taiwan 308	Q
Hengxianjianye	F	Jinshachunbai	K	Taiwan 309	Q
Hengxianxiye	F	Kexingbaigeng	M	Xianggangdaye	A
Hengxianzhuye	F	Qiangkunbaigu	N	Xingtianqinggu	R
Honghaibaigeng	G	Qiangkunqinggu	N	Tianyoutaiwan	S

[a] Providers: A, Guangzhou Changhe Seeds Co., Ltd.; B, Liuzhou Huada Collecting Seeds Shop; C, Nanhai Dali Jiangzhiqing Seeds Shop; D, Guangzhou Guanglian Seeds and Seedlings Agency; E, Nanning Wulong Seeds Shop; F, Guangxi Hengxian Zilong Seeds Co., Ltd.; G, Guangzhou Honghai Seeds and Seedlings Co., Ltd.; H, Guilin Huifeng Seeds Co., Ltd.; I, Guangzhou Huihuang Nongyou Seeds Co., Ltd.; J, Shenzen Jinhuasheng Industry Co., Ltd.; K, Jinsha Vegetable Research Institute; M, Shandong Laizhou Jinxing Seeds Co., Ltd.; N, Guangxi Lingshan Qiangkun Seeds Co., Ltd.; O, Meizhou Sannong Seeds Co., Ltd.; P, Wuhan Shenniu Seeds and Seedlings Agency; Q, Guangzhou Aipunong Agriculture and Technology Co., Ltd.; R, Guangzhou Xingtian Seeds Co., Ltd.; S, Hongkong Tianyou Agriculture Unite Co.

The plant samples were washed thoroughly three times with tap water and infused three times with 2 L of deionized water for 30 s. The fresh weights (FW) of the root and shoot (including the leaf and stem) samples were measured, and proper blotting was done using filter papers. The roots and shoots were separately oven-dried at 70 °C to constant weight, crushed, and passed through a 100-mesh sieve after the dry weights (DW) of all the samples had been measured. Cd concentrations of the samples were determined with an atomic absorption spectrophotometer (Z-5300, Hitachi) followed by digestion using a microwave decomposition device (microwave digester 7295 manufactured by O. I. Analytical Co., Ltd.) with HNO_3 and $HClO_4$ (2:1, v/v). A plant CRM (GBW-07603, National Research Center for Certified Reference Materials, China; the certified Cd concentration is 0.057 mg kg^{-1}) was used to ensure the precision of the analytical procedure.

Safety Standard and Statistical Methods. The maximum Cd limitation (0.2 mg kg^{-1}) of the Codex Alimentarius Commission (CAC) standard (Codex Standard 248-2005, http://www.codexalimentarius.net/download/standards/374/CXG_039e.df/) was used to assess the safety of consuming the tested water spinach cultivars and to determine the Cd-PSCs.

Two-way ANOVA on shoot biomass, shoot Cd concentration, and correlation analyses (using Pearson product-moment correlation) were conducted using the software package SPSS 11.0. To compare the relative response of cultivars to the different levels of Cd exposures, we calculated the index of biomass response to stress (BRS) as (*10*)

$$\mathrm{BRS}\ (\%) = (B_H - B_L)/B_L \times 100$$

where B_H (g) and B_L (g) are the shoot biomasses (DW) under high and low Cd treatments, respectively.

To estimate Cd translocation to the edible part, we calculated the translocation rate (TR) (*33*) as follows:

$$\mathrm{TR}\ (\%) = \frac{\text{Cd accumulation in shoots, SH}}{\text{total Cd in whole plant}} \times 100$$

Hydroponic Experiment Comparing Cd Subcellular Distribution between Cd-PSC and Non-Cd-PSC of Water Spinach. *Plant Materials and Hydroponic Method.* Two water spinach genotypes, including a typical Cd-PSC and a non-Cd-PSC determined in the above-stated pot experiment, were used to compare their Cd subcellular distributions. The seeds were surface sterilized in 2% (v/v) H_2O_2 for 10 min, rinsed with deionized water, and germinated in sterilized moist quartz sand at 20 ± 1 °C. At the three-leaf stage, 10 uniform plants were selected and transplanted to a polystyrol-plate with five evenly spaced holes (two

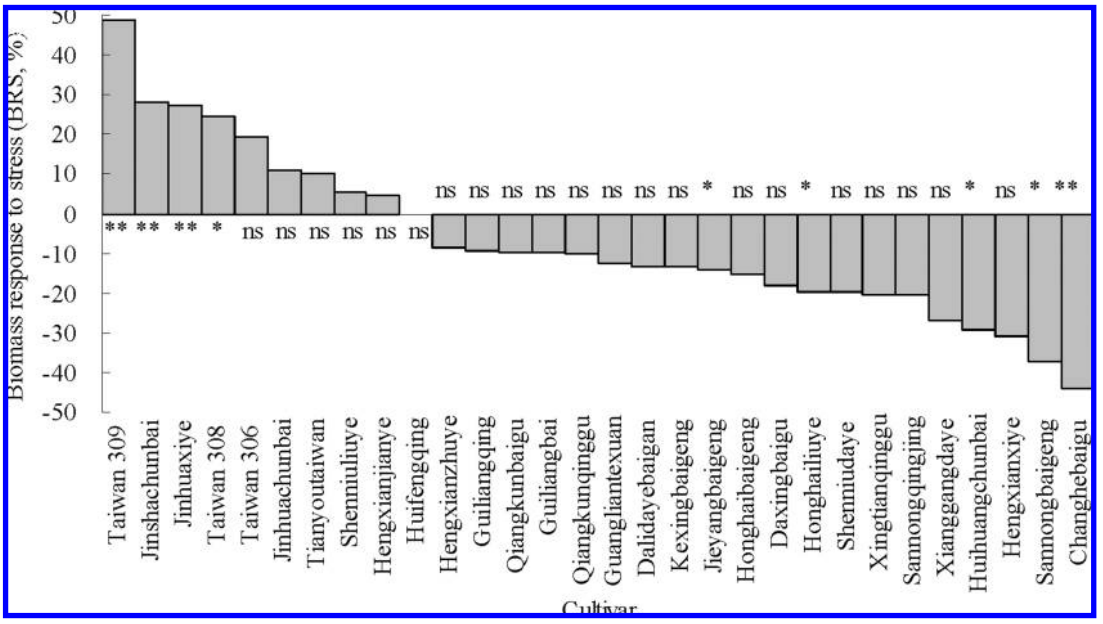

Figure 1. Shoot biomass response to stress (BRS) of the 30 tested cultivars. ns, *, and ** indicate that the differences of the shoot biomasses between the low-Cd and high-Cd treatments were not significant, significant at the $p < 0.05$ level, and significant at the $p < 0.01$ level, respectively.

plants per hole) suspended on the surface of the 1.5 L Hoagland nutrient solution in a 2 L container placed in a greenhouse. The solution pH was adjusted to 6.5 ± 0.1 with 1.0 mol L^{-1}NaOH or HCl. The nutrient solution in the growth containers was continuously aerated with pumps and renewed every day. On the seventh day after transplanting, three treatments with different Cd concentrations (0, 1.0, and 5.0 mg L^{-1}, respectively) were conducted by adding 0, 3.05, and 15.24 mg of $CdCl_2 \cdot 2.5H_2O$ into the containers. The level of the culture solution (1.5 L) was maintained by supplying deionized water every day. The experiment was laid out in randomized design, and three replicates (i.e., three containers) each with 10 plants were conducted for each treatment.

Sampling and Separation of Tissue Fractionations. In the two weeks after the Cd treatment was carried out, the shoot and root of the plants in the three containers for each treatment were harvested separately at the same time. The samples were washed thoroughly three times with tap water and three times with deionized water. The samples were pretreated according to method of ref *34* to get different subcellular fractions. Five grams of each frozen root, stem, and leaf tissue was, respectively, homogenized in a precooled extraction buffer [50 mM Tris-HCl, 250 mM sucrose, and 1.0 mM DTE ($C_4H_{10}O_2S_2$, Sigma D8255), pH 7.5] (*35*) 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 of the nylon cloth filtration was washed twice with the homogenization buffer; it contained mainly cell walls and cell wall debris and was designated fraction I (FI). The filtrate was centrifuged at 20000*g* for 45 min. The resultant supernatant solution was referred to as the soluble fraction (including the vacuole) and was designated fraction II (FII). The deposit was taken as the organelle (excluding the vacuole) and was designated fraction III (FIII). All of the steps were performed at 4 °C. Fractions I (cell-wall fraction) and III (organelle) were dried at 70 °C to constant weight and then digested at 145 °C for 24 h with an acid oxidative mixture of $HNO_3/HClO_4$ (2:1, v/v). Cd concentrations in the suspended FII (the soluble fractions) and in the digests were directly determined using the AAS methods above-mentioned.

RESULTS

Biomass Response to Cd Stress. The averages of shoot biomass harvested in FH and SH and root biomass under low-Cd treatment were 0.631 ± 0.088, 0.448 ± 0.149, and 0.272 ± 0.056 g $plant^{-1}$, respectively, which were higher than those under high-Cd treatment (0.578 ± 0.081, 0.400 ± 0.113, and 0.206 ± 0.056 g $plant^{-1}$, respectively). The variations of the biomasses from different Cd treatments were significant ($p < 0.01$) according to the two-way ANOVA. The biomass variations of the tissues from different cultivars were also significant ($p < 0.01$) under all Cd treatments.

According to the calculation of the BRS (**Figure 1**), there were 20 cultivars that had higher shoot biomasses under low-Cd than under high-Cd treatment, and the differences between the two treatments in five cultivars were significant ($p < 0.05$). There were nine cultivars that had lower shoot biomasses under low-Cd than under high-Cd treatment, and four of them were significantly different ($p < 0.05$). The results show that the species had moderate tolerance to Cd toxicity.

Cadmium Accumulation. Cd concentrations in shoots of FH and SH and root of the tested cultivars are shown in **Table 2**. The maximal differences of the Cd concentrations between the cultivars containing the highest and lowest Cd were 3.0-fold in shoot of FH, 3.9-fold in shoot of SH, and 2.5-fold in root under low-Cd treatment. They were 3.5-, 2.7-, and 2.9-fold, respectively, under the middle-Cd treatment and 2.7-, 2.6-, and 2.5-fold, respectively, under the high-Cd treatment. The variations in the shoots were generally larger than those in the roots. The variations among cultivars and Cd treatments and in the interaction between cultivar and Cd treatment were significant ($p < 0.0001$), indicating that the tested cultivars may respond to Cd stress to different degrees or in varying ways. Compared to the first harvest, the averages of Cd concentration in shoots of the second harvest were decreased by 10.6, 29.9, and 15.3% under low-, middle-, and high-Cd treatments, respectively, which could probably be attributed to the decrease of Cd bioavailability in the tested soil.

Selection of Cd-PSCs. Cd concentrations in shoots of FH and SH of the tested cultivars of water spinach under different Cd treatments and their correlation are shown in **Figure 2**. The results from FH and SH were well correlated, and the correlation coefficient was significant at the $p < 0.01$ level. The results implied that the Cd-accumulating pattern of the species is very possibly genotype dependent. According to the CAC Standard (Codex Standard 248-2005), the maximal level of Cd in leafy vegetable is 0.2 mg kg^{-1} (for safe consumption) (fresh weight basis). There were 32 pairs of Cd concentration data of the shoot that were lower than 0.2 mg kg^{-1} in both FH and SH (zone A). Among them, 26 were obtained under low-Cd treatment, and the

Table 2. Variations of Cd Concentrations in Different Tissues among the Tested Cultivars of Water Spinach and under Different Soil Cd Levels

	Range (Average) of Cd Concentration in Tissues (mg kg $^{-1}$, DW)		
tissue	low Cd	middle Cd	high Cd
shoot of FH[a]	0.972−2.890 (1.558)	1.301−5.802 (3.247)	2.713−7.423 (4.593)
shoot of SH[a]	0.778−3.018 (1.393)	1.371−3.698 (2.276)	2.253−5.825 (3.892)
root	1.717−4.360 (2.841)	3.500−10.12 (5.913)	5.765−14.54 (10.42)

Analysis of Two-Way ANOVA

	shoot of FH		shoot of SH		root	
source of error	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value
cultivar	0.0001	23.79	0.0001	19.22	0.0001	54.82
Cd treatment	0.0001	890.17	0.0001	754.92	0.0001	5789.06
cultivar × Cd treatment	0.0001	4.96	0.0001	3.62	0.0001	16.67

[a] FH, first harvest; SH, second harvest.

other 6 accumulated Cd of concentration lower than 0.2 mg kg^{-1} in shoot under both low- and middle-Cd treatments. We refer to the six cultivars, cv. Daxingbaigu, Huifengqing, Qiangkunbaigu, Qiangkunqinggu, Shenniuliuye, and Xingtianqinggu, as Cd-PSCs, meaning that they are safer to consume when cultivated in Cd-contaminated soils. The standard deviations of the data (n = 3) were relatively small for both harvests (the small figures among **Figure 2**) and were considered to be statistically reliable. The 15 data involved in zone B have Cd concentrations higher than the maximum level (ML) in only FH, and the values were lower in SH. The 12 cultivars that had Cd concentrations lower than the ML in SH under the middle-Cd treatment would be valuable for further investigation. There were three cultivars (cv. Jieyangbaigeng, Xianggangdaye, and Sannongbaigeng) and one cultivar (cv. Taiwan 308) that contained Cd higher than the ML in FH and both FH and SH under low-Cd treatment, respectively, and they would also be valuable as typical non-Cd-PSCs for further study.

Correlations of Cd concentrations of shoots in the two harvests between different Cd treatments are shown in **Figure 3**. The positive correlation coefficients were significant (p < 0.05) under the three treatments. The results well-demonstrated that the Cd-accumulating pattern in water spinach could be characterized as genotype dependent, which is less affected by the level of Cd contamination in soil. Typical Cd-PSCs such as cv. Qiangkunqinggu and cv. Huifengqing, as well as typical non-Cd-PSCs such as cv. Taiwan 308 always belonged to the groups with the lowest or highest Cd concentration and had no relationship with the Cd content in the soil or harvest timing. The high recurrence of the low or high Cd accumulation in the typical cultivars brought forth the genetic stability of the Cd accumulation as a cultivar character of water spinach.

Subcellular Distributions of Cd in the Typical Cultivars. Two water spinach cultivars, cv. Qiangkunqinggu (QK) and cv. Taiwan 308 (TW), were used to determine the subcellular distribution of Cd in different tissues. As a typical Cd-PSC, cv. QK had an insignificant negative BRS (−6.6%), implying low Cd sensitivity to stress from soil Cd. This cultivar accumulated the lowest Cd in shoot in almost all cases, and its edible parts were safe for consumption with regard to Cd concentration in both harvests, even under middle-Cd treatment, according to the CAC standard. Contrarily, cv. TW, as a typical non-Cd-PSC, seemed to have an induced growth increment under high Cd exposure because of its high and significantly positive BRS (34.6%). The concentrations of Cd in shoot were 1.8−3.4-fold

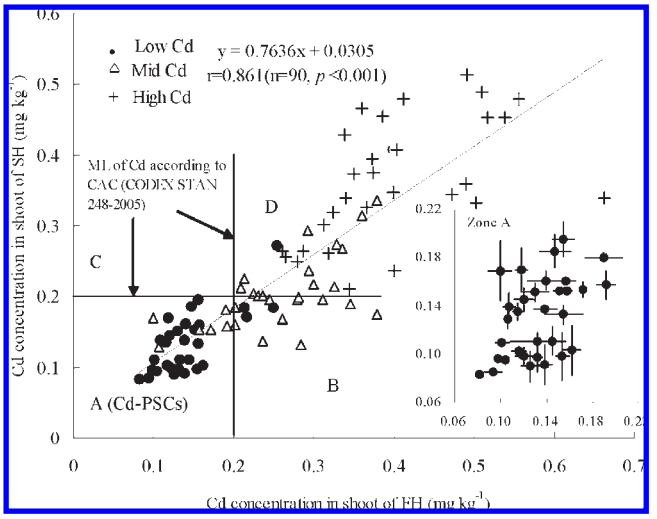

Figure 2. Cd concentrations (fresh weight basis) in shoots of FH and SH of the tested cultivars under low (●), medium (△), and high (+) Cd treatments and their correlation. Zone A involved all Cd-PSCs that were with Cd concentrations lower than the maximal limitation of Cd for leafy vegetables according to the CAC standard (Codex Standard 248-2005) in both harvests. Those in zone B were not true Cd-PSCs because they accumulated Cd higher than the ML in the first harvest, but may be valuable for further investigation. There were no data in zone C, indicating the Cd concentration was generally higher in the FH than in the SH. Zone D included the unsafe situations, and most of them were obtained from medium- and high-Cd treatments except cv. Taiwan 308. (Inset) Error bars (SD, n = 3) of the data in zone A (Cd-PSCs).

those of cv. QK. Even under the low-Cd treatment, its Cd concentrations in shoot exceeded the ML of the CAC standard in both harvests. The Cd concentrations in root of the two cultivars under middle- and high-Cd treatments appeared to be extreme in opposites compared with those in shoot, implying that more Cd absorbed by cv. QK (Cd-PSC) may be partitioned in roots and Cd translocation to the shoots was probably highly obstructed.

Similar results were obtained in the hydroponic experiment (**Table 3**), which was characterized by the lower Cd concentration in leaf and stem, but higher Cd concentration in root in cv. QK compared to cv. TW. Therefore, the Cd retention in root of cv. QK was more effective than cv. TW, which might be one of its main mechanisms to reduce Cd concentration in shoot.

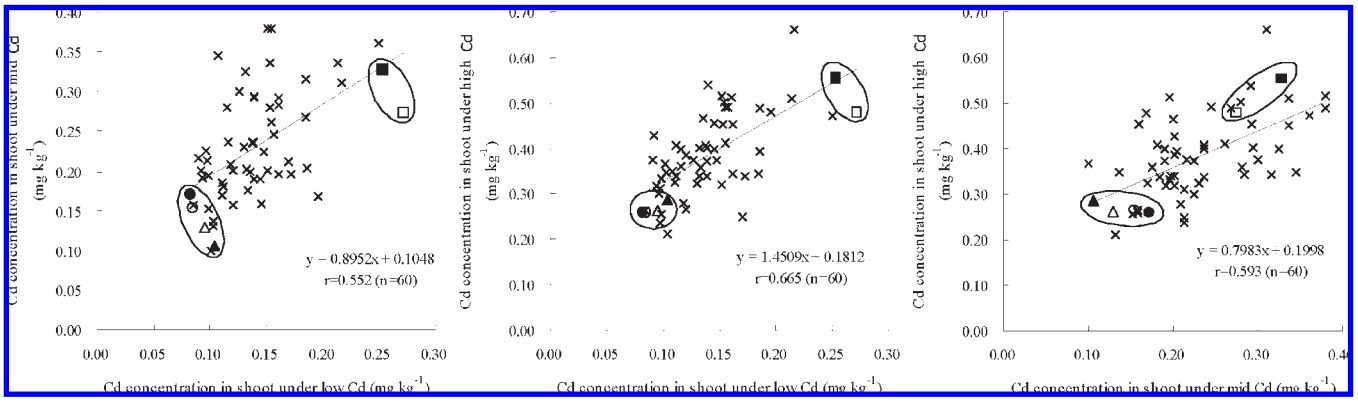

Figure 3. Correlations of Cd concentrations of shoots in the two harvests between different Cd treatments. Selected typical cultivars: (●, ○) cv. Qiangkunqinggu; (▲, △) cv. Huifengqing; (■, □) cv. Taiwan 308. Solid and open symbols indicate the first harvest and second harvests, respectively. Both cv. Qiangkunqinggu and cv. Huifengqing were typical Cd-PSCs, which accumulated lower Cd in the both harvests and under all Cd treatments. Cv. Taiwan 308 was a typical non-Cd-PSC, which accumulated higher Cd in the both harvests and under low- and middle-Cd treatments.

Table 3. Cd Concentrations (Milligrams per Kilogram, FW) in Root, Stem, and Leaf of Cv. QK and Cv. TW under Different Cd Treatments in the Hydroponic Experiment

Cd supplement	cultivar	leaf	stem	root
0 mg L^{-1}	QK	0.213	0.098	0.425
	TW	0.261	0.134	0.313
	difference[a]	1.2*	1.4*	0.7*
1.0 mg L^{-1}	QK	1.284	1.430	22.28
	TW	2.872	2.305	18.73
	difference[a]	2.2**	1.6*	0.8*
5.0 mg L^{-1}	QK	2.180	3.418	74.20
	TW	4.190	4.050	49.32
	difference[a]	1.9**	1.2ns	0.7**

[a] Difference = TW/QK; ns, *, and ** indicate not significant, significance at the $p < 0.05$ level, and significance at the $p < 0.01$ level, respectively ($n = 3$).

The subcellular distributions of Cd in leaf, stem, and root, expressed as Cd concentration in the different fractions, are shown in **Figure 4**. The Cd in FI is combined mainly in the cell wall, which is relatively insensitive to Cd stress. The Cd of FI in root and stem was higher in cv. QK than in cv. TW under high Cd exposure (5 mg L^{-1}), especially in root, where an almost 3-fold difference between the two cultivars was observed. The result revealed that the Cd retention or partition effects well functioned in root of cv. QK, whereas in FI of leaf, Cd concentrations of cv. QK were higher than those of cv. TW under Cd exposures, especially under high Cd stress. The increment of Cd was much greater in cv. TW than in cv. QK when the Cd exposure was increased from 1 to 5 mg L^{-1}. This indicated that there were some mechanisms restricting the transportation of Cd from root or stem to leaf in cv. QK, possibly relating to the protection of the photosynthetic system locating in leaf. The Cd in FII is contained mainly in the soluble fraction (including the vacuole) in the cell. Cv. QK accumulated much less Cd in FII of leaf and stem than did cv. TW, which should be the main reason why cv. QK is considered to be Cd-PSC, whereas cv.TW is not. For FII in root, the difference in Cd concentrations between the two cultivars was lessened, and a significantly higher Cd concentration in cv. QK than in cv. TW ($p < 0.05$) under the 1 mg L^{-1} Cd exposure was observed instead. For the Cd in FIII, combining mainly with organelle (excluding the vacuole) in the cell, a change should be noticeable when the Cd stress is increased from 1 to 5 mg L^{-1}. Cd in either shoot or root of cv. QK increased remarkably when the level of Cd exposure was increased from 1 to 5 mg L^{-1}. It may cause toxic damages to the plant because the organelles may lose their functions when combined with Cd. Such a phenomenon was, however, not observed in cv. TW and might have thus resulted in its higher Cd tolerance.

DISCUSSION

The Cd concentrations in the tested soils of the three treatments were 0.593 (low-Cd), 1.091 (middle-Cd), and 1.824 (high-Cd) mg kg^{-1}, respectively, and this range covered most cases of Cd contamination in agricultural soils in China (*36*, *37*). Under the high-Cd treatment, 30% of the tested water spinach cultivars had positive BRS, that is, yielding more than those under low Cd. It seemed, therefore, that the species is moderately tolerant to Cd toxicity compared to those that could grow well under rather high Cd stress (*38*). However, because only 16.7% of the cultivars yielded lower biomass under high-Cd than under low-Cd treatment, the hazard of Cd contamination in the soil might be not noticed through yield decrement of the plants. Thus, farmers could not be warned about Cd contamination in the soil simply by looking for toxic appearances, and problems in food safety would continuously occur as the contaminated products are consumed.

Water spinach could be easily polluted by Cd if the Cd concentration has reached a level >1 mg kg^{-1}, which is very common in sewage-irrigated areas and industrial areas in China (*37*). Even under the low-Cd treatment (containing 0.593 mg kg^{-1} Cd in soil), there were some cultivars that accumulated Cd at >0.2 mg kg^{-1}, which is above the ML of the CAC. Therefore, water spinach should not be cultivated in Cd-contaminated or potentially contaminated agricultural soils, such as those that have ever been irrigated by sewage or wastewater or those close to an industrial zone, especially mining areas. In the present study, 13.3% of the cultivars were found to be vulnerable to Cd contamination in soils (non-Cd-PSC), including cv. Taiwan 308, Xianggangdaye, Sannongbaigeng, and Jieyangbaigeng. In contrast, the typical Cd-PSCs (cv. Daxingbaigu, Huifengqing, Xingtianqinggu, Qiangkunbaigu, Shenniuliuye, and Qiangkunqinggu) accumulated Cd with concentration lower than the ML of the CAC standard even when grown under the middle-Cd (1.091 mg kg^{-1}) treatment, which was 3-fold the ML of Cd in soil (0.3 mg kg^{-1}) according to the Farmland Environmental Quality Evaluation Standard for Edible Agricultural Products (HJ332-2006). It was obvious that the Cd-accumulating property of most tested cultivars was coincident in the two harvests and under different Cd treatments. Together

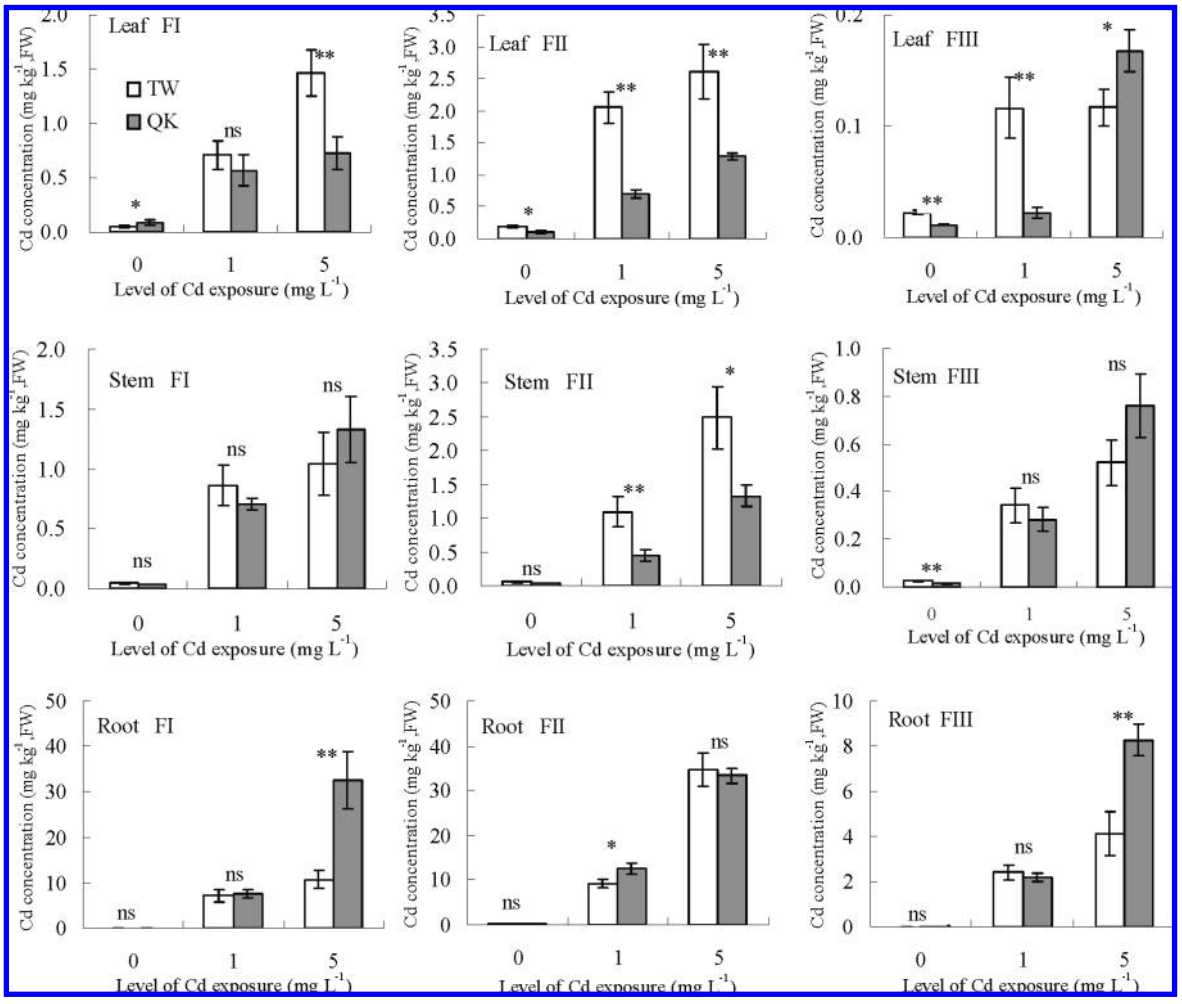

Figure 4. Cd concentrations in the three subcellular fractions of leaf, stem, and root grown under three Cd levels in the hydroponic experiment. ns, ∗, and ∗∗ indicate that the differences of data between cv. TW and cv. QK are not significant, significant at the $p < 0.05$ level, and significant at the $p < 0.01$ level, respectively. FI, cell walls and cell wall debris fractions; FII, soluble fraction (including the vacuole); FIII, organelle fraction (excluding the vacuole).

with the corresponding major difference in Cd accumulation between the typical non-Cd-PSC (cv. Taiwan 308) and the typical Cd-PSC (cv. Qiangkunqinggu) in the hydroponic experiment, Cd accumulation in water spinach is considered to be genotype-dependent or inherited. Similar Cd-accumulating properties were also found in the same cultivar series, such as the Qiangkun series (cv. Qiangkunbaigu and Qiangkunqinggu, both are typical Cd-PSC) and the Sannong series (cv. Sannongbaigeng and Sannongqingjing, both are typical non-Cd-PSC), further supporting the genetic stability of Cd accumulation in cultivars of water spinach.

There was no usable Cd-PSC whenever the Cd concentration went up to 1.824 mg kg^{-1}, and thus the feasibility of the Cd-PSC strategy is conditioned with the soil condition. Leafy vegetables tend to accumulate more heavy metals in edible parts when compared with fruit or even root vegetables (*20*, *39*). In the case of rice (*Oryza sativa* L.) and asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis* L.), both of which are fruit crops, Cd-PSC could be detected when they were grown in soil containing Cd as high as 1.85 mg kg^{-1} (*10*) and 11.0 mg kg^{-1} (*11*), respectively. Therefore, identifying Cd-PSC in leafy vegetables becomes more difficult than in fruits or other vegetables; the high vulnerability of leafy vegetables to Cd contamination makes the identification more important as well.

By using the typical Cd-PSCs and non-Cd-PSCs, differences of Cd subcellular distribution between the cultivars were compared as a preparatory investigation to explain the mechanism that led to the difference in Cd accumulation between the cultivars. The experiment proved that Cd accumulation in stem and leaf of cv. Qiangkunqinggu, a typical Cd-PSC, is more effectively obstructed from the Cd retention or compartment effects of root than in cv. Taiwan 308, a typical non-Cd-PSC.

The reduction of the cytosolic concentration of free Cd ion in plants is one of the defense strategies against Cd toxicity performed by various mechanisms including the compartmentalization of Cd in subcellular components (*19*). In maize (*34*) and lettuce (*24*), the highest level of Cd concentration was found in the cell wall, whereas in bean (*40*) and tobacco plants (*26*), most of the Cd was accumulated in vacuoles of roots and vacuolar sap, respectively.

It has been proven that the ability to synthesize PCs is one of the main reasons that Cd can be partitioned in root of plants (*41*−*43*). In addition, the cell wall of plant root contains protein and polyoses such as cellulose, hemicellulose, lignin, and mucilage glue. These compounds have a number of potential ligands such as hydroxyl, carboxyl, amino group, aldehyde group, phosphate, and thiol (*44*). These ligands can participate in a variety of reactions including ion exchange, adsorption, complexation, precipitation, and crystallization, leading to metal sequestration under metal toxicity (*45*). Thus, when Cd exposure was increased to a high level, the active Cd sequestration in the root cell wall of cv. Qiangkunqinggu, as a mechanism to perform Cd retention or partition in root, becomes noticeable, and similar

results were observed by Ramos et al. (*24*) and Ni and Wei (*46*). Although there is no further information about the mechanism that functioned in the difference in Cd accumulation between cv. Qiangkunqinggu and cv. Taiwan 308, the finding that the significant difference of Cd subcellular distribution can occur at cultivar level is of great interest.

The great increment of Cd in FI in leaf of cv. Taiwan 308 under 5 mg L^{-1} Cd treatment indicated that Cd sequestration in the cell wall may play another important role, that is, Cd detoxification (*47*), which may be one of the reasons that the Cd tolerance of cv. Taiwan 308 was so high. Furthermore, transporting Cd to the vacuole, which is included in FII, is another route for Cd detoxification (*48*, *49*), and cv. Taiwan 308 seemed to perform well in this aspect in leaf. The result is also consistent with those reported by Li and Zhu (*50*).

The differences of Cd subcellular distribution in the cultivars with opposite Cd-accumulating properties may have provided more evidence proving the genotype dependence of the Cd-accumulating pattern in water spinach. There are some studies about Cd accumulation of plants that involve the genetic aspect. Li et al. found that the expression of kernel Cd accumulation in sunflower hybrids was predominantly influenced by additive genetic effects (*51*). Clarker reported that the difference of Cd concentration in grain of durum wheat cultivars was controlled by a single, low-Cd dominant, gene, and genetic control had a stronger effect than environmental influence (*52*). Penner et al. linked successfully the random amplified polymorphic DNA (RAPD) markers to a gene governing Cd uptake (*53*). It would be valuable to further investigate the differences in PCs, MTs, and ligands between the two typical cultivars of water spinach, cv. Qiangkunqinggu and cv. Taiwan 308. Progress in the studies on the mechanisms for low Cd accumulation in crops certainly would enhance the possibility to breed crop cultivars that would accumulate a low level of Cd in edible parts, that is, breeding of PSC, which can effectively decrease the influx of toxic pollutants into the human food chain with almost no additional cost.

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