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RESEARCH ARTICLE

High cadmium pollution risk on vegetable amaranth and a selection for pollution-safe cultivars to lower the risk

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Abstract A pot experiment was carried out by growing 29 different genotypes (*Amaranthus* spp.) of vegetable amaranth under low- (0.12 mg·kg^{-1}) and middle- (0.40 mg·kg^{-1}) cadmium (Cd) exposure. The result showed that amaranth was vulnerable to cadmium (Cd) contamination in soil. Variations of Cd concentrations in both roots and edible parts among genotypes were significant ($P < 0.001$) in both treatments. Cd concentrations in edible parts of the tested genotypes grown under low- and middle-Cd levels were significantly correlated ($p < 0.01$), implying that Cd-accumulating property of amaranth is genotype-dependent. Differences in Cd chemical forms between cv. Nanxing-dayemashixian (cv. Nan), a selected typical pollution-safe cultivar (Cd-PSC), and cv. Pennongjianyexian (cv. Pen), a selected typical non-Cd-PSC, under different Cd exposure conditions were compared. It was found that the alternation of Cd in F_{NaCl} (Cd form extracted by 1 mol·L^{-1} NaCl) may be a key factor in regulating Cd accumulation of different amaranth genotypes and that the protein-binding Cd is considered to be associated with Cd translocation. The results indicated that amaranth is capable of enduring high level of Cd pollution when grown as vegetable crop, and accordingly, consuming vegetable amaranth would bring high health risk. Therefore, adopting Cd-PSC strategy would help reducing the risk of Cd pollution in amaranth. In this study, cv. Nan was identified as a Cd-PSC and recommended to be applied production practice.

Keywords Amaranth, Cadmium (Cd), Cd accumulation, pollution-safe cultivar (PSC), Cd chemical forms, health risk

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1 Introduction

Heavy metal contamination in soil has become a global issue since soils often receive heavy metals from natural occurrences such as heavy metal release from parent materials of soil, as well as through a variety of human activities, such as wastewater irrigation, sludge application, herbicides and pesticides usage, fertilizer application, atmosphere precipitation, and mining wastes. Among all the metal pollutants, cadmium is of major concern. Cadmium (Cd) is readily accumulated in soil, particularly in the surface soil and is easily taken up by plant roots and translocated to above-ground tissues [1], posing a potential threat to human health as it enters the food chain [2].

Phytoremediation is an innovative technology that utilizes plants to remove and/or degrade environmental contaminants such as heavy metals and organic compounds. In many developing countries with a large population, however, long-time fallow for phytoremediation cannot be afforded due to the high demand for foodstuff [3,4]. Thus, some researchers are currently trying to find a way to reduce the entrance of soil contaminants into the food chain without fallowing the land. The concept of PSCs (Pollution-safe cultivars), that is, the cultivars in which edible parts accumulate certain pollutant at a low enough level for safe consumption when grown in contaminated soil, has been proposed [3–8]. Actually, the PSCs strategy is based on the fact that the uptake and accumulation of heavy metals in plants not only vary greatly among species but also among genotypes. Differences in Cd absorption and accumulation among genotypes have been studied in rice (*Oryza sativa* L.) [9–11], barley (*Hordeum Vulgare* L.) [12], wheat (*Triticum sestivum* L.) [13–15], maize (*Zea mays* L.) [16,17], soybean (*Glycine max* Merr.) [18,19], potato (*Solanum tuberosum* L.) [20], lettuce (*Lactuca sativa* L.) [21], Chinese cabbage (*Brassica perkinensis* L.) [22] and so on.

However, most researches focused on cereal, and not much attention was paid to vegetables, even though vegetables (especially leafy vegetables) are the most vulnerable to soil Cd contamination [23].

Amaranth can be considered as cereal crop, vegetable crop, ornamental plant and weed plant. It is cultivated as a vegetable crop in vast areas of Asia, especially in China, for it contains abundant amounts of protein, Ca, Fe, Zn, carotenoids (pro-vitamin A), ascorbic acid (Vitamin C), dietary fiber and other common nutrients required for normal human growth [24].

However, amaranth is found to be a Cd accumulative species in previous studies [5,25], and this property of amaranth compromises food safety for human beings. Two genotypes of amaranth accumulated Cd in shoot at a level higher than 100 $mg \cdot kg^{-1}$ when grown in a soil containing 7.678 $mg \cdot kg^{-1}$of Cd. Therefore, selection for Cd-PSCs of amaranth is attempted in the present study as an innovative measure to reduce the risk of Cd pollution by investigating 29 genotypes of the species grown in soils contaminated with different levels of Cd. Considering that heavy metals in plants often exist in various complicated molecular forms with distinctive levels of mobility throughout plants [26], difference in the chemical forms of Cd between two genotypes were also compared with respect to low and high Cd accumulation levels. It is hypothesized that i) high risk of Cd pollution in vegetable amaranth occurs even when grown in soils with low Cd contamination and genotype difference in Cd accumulation of the species is unrelated to soil condition, and ii) the genotype differences in Cd accumulation patterns are associated with the different chemical forms of Cd among plant tissues. It is also expected that Cd-PSCs exist among the various genotypes in spite of the high Cd accumulating feature of amaranth.

2 Material and methods

Two pot experiments were conducted in the experimental garden of the Institution of Agricultural Science of Heshan, Guangdong, China (112°59'E, 22°42'N), during the summer of 2006 and 2007.

2.1 Genotypes of amaranth used for the Cd-PSC screening

There was a total of 29 amaranth genotypes (Table 1), including 27 commercial cultivars and 2 wild lines that were used for Cd-PSC screening.

2.2 Soils used for the Cd-PSC screening of amaranth

Two types of soil were used in the pot experiment to screen for Cd-PSCs of amaranth. One was uncontaminated native soil with low Cd concentration, collected from a vegetable field in Heshan, Guangdong Province. The other one was heavily Cd-contaminated soil which was spiked with $Cd(NO_3)_2$ in the summer of 2004 (28 months ago), and was used for cropping rice for twice-rotation [3]. Both soils were air-dried, mildly ground with a wooden roller to pass through a 1-cm sieve.

2.3 Experimental design for the Cd-PSC screening of amaranth

A pot experiment was conducted by using the two prepared soils. For low-Cd treatment, the uncontaminated native soil was applied directly, and the Cd concentration in the soil was 0.12 $mg \cdot kg^{-1}$ which measures up to the maximum level (ML) of Cd according to the Farmland Environmental Quality Evaluation Standards of China for Edible Agricultural Products (HJ332-2006, $Cd \leqslant 0.3\ mg \cdot kg^{-1}$). By mixing the native soil and the heavily Cd-contaminated soil (about 500∶3, w/w), soils containing up to 0.40 mg $\cdot kg^{-1}$ of Cd were obtained as another treatment (Middle-Cd). The Cd concentration in the middle-Cd soil slightly exceeded the ML of HJ332-2006 for Cd, but the basic properties of the soil were not altered by adding the contaminated soil (Table 2). Pots (22 cm in diameter and 18 cm in height) filled with 3 $kg \cdot pot^{-1}$ of the prepared soils were used. Basal fertilizer was supplied at the level of 0.9 g of nitrogen (as NH_4NO_3), 0.372 g of P and 0.468 g of K (as KH_2PO_4) per pot. After the experimental pots were balanced for a week, seeds of 29 amaranth genotypes were sown into the pot on July 18, 2006 with 16 seeds in each pot, and 3 replications were conducted. A total of 174 pots were randomly arranged in a greenhouse with air temperature at 28°C–32°C, and were watered daily to keep moisture in the soil. On the 10th and 20th day after sowing, the seedlings were thinned to 8 and 4 seedlings per pot, respectively. The whole plants were harvested after a 42-d growth period on August 29, 2006.

2.4 Chemical analysis of soils

The main parameters of the soils were measured using routine analytical methods for testing soil agricultural chemistry[28]. Soil pH and Eh (Oxidation-Reduction Potential) were determined by a pH-meter (PHS-3C, Shanghai, China) in a 2.5:1 water: soil suspension. Soil EC (electrical conductivity) was measured in a soil to water ratio of 1:5. Soil organic matter content and CEC (cation exchange capacity) were determined by methods referred to in Nelson and Sommers [27] and Lu [28], respectively. Total and available metal concentrations were determined by atomic absorption spectrophotometer (AAS, Hitachi Z-5300, Japan) following mixed acid digestion (HNO_3-HCl-H_2O_2) [29] and DTPA extraction, respectively. Total N was determined by Kjeldahl method [30] and available P was determined by molybdenum blue colorimetry [31].

Table 1 Cd concentrations in edible part of the tested genotypes of amaranth under low-Cd and middle-Cd

genotype	code	low-Cd	middle-Cd
Changhejianyeqing	Chang	0.086±0.000 a	0.176±0.006 abcd
Changhehuahongxian	Chang1	0.042±0.002 ghijk	0.150±0.008 defgh
Changheyuanyeqing	Chang2	0.067±0.012 bcde	0.160±0.012 cdefg
Fanjidahongxian307	Fan	0.055±0.008 efgh	0.150±0.006 defgh
Goldensunhongxian	Gold	0.050±0.002 efghij	0.129±0.004 ghij
Guangzhouyidianhong	Guang	0.059±0.003 defg	0.187±0.017 abc
Hanyuyidianhong	Han	0.059±0.011 defg	0.171±0.011 bcde
Heshan wild	He	0.052±0.003 efghi	0.123±0.010 hij
Jinhanhongyuanye	Jin	0.053±0.001 efghi	0.155±0.008 defg
Jinmudanyuanhuaxian	Jinm1	0.053±0.002 efghi	0.154±0.007 defg
Jinmudanyuanbaixian	Jinm2	0.076±0.010 abcd	0.206±0.006 a
Jinkedayehong	Jink1	0.083±0.013 ab	0.164±0.017 bcdef
Jinkebaiyuanye	Jink2	0.052±0.003 efghi	0.123±0.010 hij
Jiutouniaohongyuanye	Jiu	0.063±0.000 cdef	0.135±0.009 fghij
Liyuanhongxian (Netherlands)	Li1	0.038±0.002 hijk	0.113±0.007 ijk
Liyuanluxian (Brazil)	Li2	0.067±0.007 bcde	0.192±0.022 ab
Nanxingdayemashixian	Nan	0.037±0.004 ijk	0.108±0.005 jk
Nongfengyuanzhonghong	Nong1	0.058±0.006 defg	0.135±0.007 fghij
Nongyouxiangyouqing	Nong2	0.056±0.003 efg	0.140±0.001 efghi
Pennongjianyexian (Hongkong)	Pen	0.080±0.003 abc	0.174±0.003 bcd
Pennongtexuan330 (Thailand)	Pen330	0.027±0.001 k	0.091±0.007 kl
Pennongtexuan331 (Thailand)	Pen331	0.057±0.002 efg	0.140±0.003 efghi
Shenzhen wild	Shen	0.034±0.001 jk	0.073±0.003 l
Taixuanjingzhong356	Tai356	0.050±0.005 efghij	0.137±0.008 fghij
Youqingjianyexian	You	0.055±0.002 efghi	0.150±0.009 defgh
Yufengyouhuahong	Yu	0.055±0.004 efghi	0.166±0.020 bcdef
Yuhehongxian	Yu1	0.048±0.004 fghij	0.155±0.004 defg
Yuheluxian	Yu2	0.043±0.007 ghijk	0.107±0.006 jk
Zhengtaixiaoyuanye	Zheng	0.060±0.001 defg	0.188±0.001 abc

Notes: Same letters with each list indicate no significant difference at $p < 0.05$ level among cultivars

2.5 Pretreatment and digestion of the plant samples

The plant samples were washed with tap water and Milli-Q water for three times. Fresh weights of edible parts (stem and leaf) were measured, and all the samples were then oven-dried at 70°C to constant weight and were ground to pass through a 100–mesh sieve (149 micron). About 0.2 g dried material of each plant sample was digested in a microwave digestion device (Microwave digestion MDS-6 manufactured by Shanghai Sineo Microwave Chemistry Technology Co., Ltd. China) with 5 mL HNO_3 (65% v/v, Guaranteed reagent (G.R.)) and 2 mL H_2O_2 (30% v/v, G.R.)

2.6 Experimental design for research on chemical forms of Cd in two typical amaranth genotypes

The Cd chemical forms in the two typical amaranth cultivars (Cd-PSC and non-Cd-PSC) selected in the pot experiment were compared. Two soils with different Cd concentrations, low-Cd (0.13 $mg \cdot kg^{-1}$) and middle-Cd (0.61 $mg \cdot kg^{-1}$), were selected as treatments. Soil preparation and plant cultivation (done on June 22, 2007) were the same with the above-mentioned pot experiment. Three replications for the two treatments and the two genotypes were performed with a total of 12 samples. The whole plants were harvested after a 35-d growth period on July

Table 2 Properties of the tested soils (dry weight basis) for Cd-PSC screening and the study of Cd chemical forms (mean±SD, $n = 4$)

	Cd-PSC screen		Cd chemical forms study	
	low-Cd	middle-Cd	low-Cd	middle-Cd
pH	5.64	5.60	5.17	5.13
Eh/(mV)	60	62	87	89
EC/(us·cm^{-1})	431	453	245	266
Organic matter /%	2.18±0.10	2.15±0.02	2.11±0.04	2.23±0.04
CEC/(cmol·kg^{-1})	6.97±0.36	6.80±0.99	6.97±0.18	7.11±0.51
total K/(g·kg^{-1})	8.56±0.20	9.01±0.24	7.13±0.25	8.44±0.40
available K/(mg·kg^{-1})	171±8	177±5	20.1±0.1	24.2±0.7
total N/(g·kg^{-1})	1.49±0.03	1.48±0.027	1.66±0.01	1.34±0.05
available N/(mg·kg^{-1})	163±10	152±2	71.3±2.1	68.1±2.9
total P/(g·kg^{-1})	1.54±0.08	1.57±0.02	0.48±0.02	0.49±0.02
available P/(mg·kg^{-1})	138±11	170±7	143±3	136±3
total Cd/(mg·kg^{-1})	0.12±0.01	0.40±0.02	0.13±0.02	0.61±0.05
DTPA extractable Cd/(mg·kg^{-1})	0.04±0.00	0.13±0.00	0.05±0.00	0.29±0.01
Cd_{DTPA} / Cd_{total}	0.33	0.31	0.38	0.47

27, 2007. Leaf, stem and root were separately rinsed with tap water, and the root was immersed in 0.5 mmol·L^{-1} $CaCl_2$ for 30 min to remove the Cd adsorbed on the root surfaces. The tissues were then washed with tap water and Milli-Q water for three times. All the prepared plant samples were frozen at −20°C and were used within one month for the experiment concerning different chemical forms of Cd.

2.7 Extractions of different chemical forms of Cd

Six different solutions were used for Cd extraction in different chemical forms in the following order [32–34]: (1) 80% ethanol (F_E), extracting inorganic Cd giving priority to nitrate, chloride, and aminophenol cadmium; (2) Deionized water (F_D), extracting water-soluble Cd of organic acid complexes and dihydrogenphosphate; (3) 1 M NaCl (F_{NaCl}), extracting Cd integrated with pectates and protein; (4) 2% Acetic acid (F_{HAc}), extracting water-insoluble cadmium phosphate including $CdHPO_4$, $Cd_3(PO_4)_2$ and other Cd-phosphate complexes; (5) 0.6 M HCl (F_{HCl}), extracting cadmium oxalate, and so on; (6) Cd in residues (F_R).

Frozen leaf, stem and root tissues were put into a 80 mL centrifuge tube after being cut into small pieces of 1–2 mm^2, and then the first extractant was filled into the centrifuge tube at a ratio of 1∶10 (w/v), and shook for 22 h at 30°C. By centrifuging the homogenate at 5000 g for 10 min, the first supernatant solution was obtained and moved into a 100-mL conical flask bottle. The residues were extracted twice with the same extraction solution for another 2 h at 30°C and centrifuged at 5000 g for 10 min, and the resulted supernatant of the three suspending was pooled. All of the pooled supernatant solution was collected and evaporated on an electric-plate at 70°C to constant weight and digested with 10 mL HNO_3 and 2ml $HClO_4$. After collecting the former extraction solution, the retained plant materials were subjected to the next extraction solution with the same procedures. The residue was also collected and digested with 10 mL HNO_3 and 2 mL $HClO_4$ at the end of the sequential extraction.

2.8 Chemical analysis of Cd concentrations in plant samples

Cd concentrations in the prepared samples including the plant tissues and the extractions were determined using an Atomic Absorption Spectrophotometer (AAS, Hitachi Z-5300, Japan). A certified reference materials (CRM) of plant (GBW-07603, provided by the National Research Center for CRM, China) with Cd concentration of 0.057 mg·kg^{-1} was used to control for accuracy of the analytical procedures. The measurements of this material averaged to 0.059 mg·kg^{-1} Cd with a RSD of 1.69%.

2.9 Data analysis

An index described as biomass response to stress (*BRS*) [4,5], was calculated (as Eq. 1) to compare the relative growth response of the tested genotypes to different Cd exposure levels.

$$BRS = (B_{middle} - B_{low})/B_{low} \times 100\%, \quad (1)$$

where B_{middle} and B_{low} are the biomass of edible parts

under the middle-Cd and low-Cd exposures, respectively.

To evaluate the Cd translocation to edible parts, translocation rate (*TR*) [4] and *S*/*R* ratio [35] were calculated as follows: (as Eqs. 2 and 3)

$$TR = (C_{\text{edible part}} \times B_{\text{edible part}})/(C_{\text{edible part}} \times B_{\text{edible part}} + C_{\text{root}} \times B_{\text{root}}) \times 100\%, \quad (2)$$

$$S/R_{\text{ratio}} = C_{\text{edible part}}/C_{\text{root}}, \quad (3)$$

where $C_{\text{edible part}}$ and C_{root} are the Cd concentration in edible parts and roots (DW basis), respectively, and $B_{\text{edible part}}$ and B_{root} are the biomasses of edible parts and roots (DW basis), respectively.

The Cd transfer factor (*TF*) which reflects the transfer efficiency of Cd from soil to plant [36] is calculated as follow Equation 4:

$$TF = C_{\text{edible part}}/C_{\text{soil}}, \quad (4)$$

where $C_{\text{edible part}}$ is the average Cd concentrations in edible parts of the tested amaranth genotypes (FW basis), and C_{soil} is the Cd concentration in the corresponding soil (DW basis).

Data were analyzed by ANOVA and the calculated means were compared by a LSD test. Pearson's correlations between the Cd concentrations in edible part and root, the TRs under low- and middle-Cd treatments, the *S*/*R* ratios under low- and middle-Cd treatments, TRs and Cd concentration in edible part, and *S*/*R* ratios and Cd concentration in edible part were analyzed. Statistical package SPSS 13.0 and Excel 2003 for Windows were used for data analysis.

Two standards including General Standard for Contaminants and Toxins in Foods (Codex Standard 193–1995, Revision 4, 2008, WHO and FAO, Cd $\leqslant$ 0.2 mg $\cdot$kg^{-1}) and the Safety Qualification for Agricultural Product-Safety Requirements for Non-Environmental Pollution Vegetable (GB18406.1-2001, China, Cd $\leqslant$ 0.05 mg$\cdot$kg^{-1}) were applied to evaluate the safety of edible parts of the tested amaranth genotypes.

3 Results

3.1 Biomass response of vegetable amaranth to different Cd exposure

BRS for the tested amaranth genotypes are shown in Fig. 1. Biomasses of edible parts of the tested genotypes of amaranth were 9.46–19.80 g$\cdot$plant^{-1} (averaged 13.63 g $\cdot$plant^{-1}) and 8.89–21.54 g$\cdot$plant^{-1} (averaged 14.49 g $\cdot$plant^{-1}) under low- and middle-Cd exposures, respectively. Results of two-way ANOVA for the variations of biomasses of edible parts for genotypes ($p < 0.001$), soil Cd ($p < 0.01$), and interaction from genotype $\times$ soil Cd ($p < 0.05$) were all significant. Although soil Cd concentration in the middle-Cd treatment was 3.5 folds of that in low-Cd, the growth of most genotypes were not suppressed by the Cd in soil. Among the 21 genotypes (72%) that had a positive BRSs, 3 genotypes (cv. Tai356, Jink1, and Gold), had significantly higher biomass under middle-Cd than that under low-Cd ($p < 0.05$). None of the tested genotypes yielded significantly lower biomass under middle-Cd exposure, including the two wild types, Shen and He.

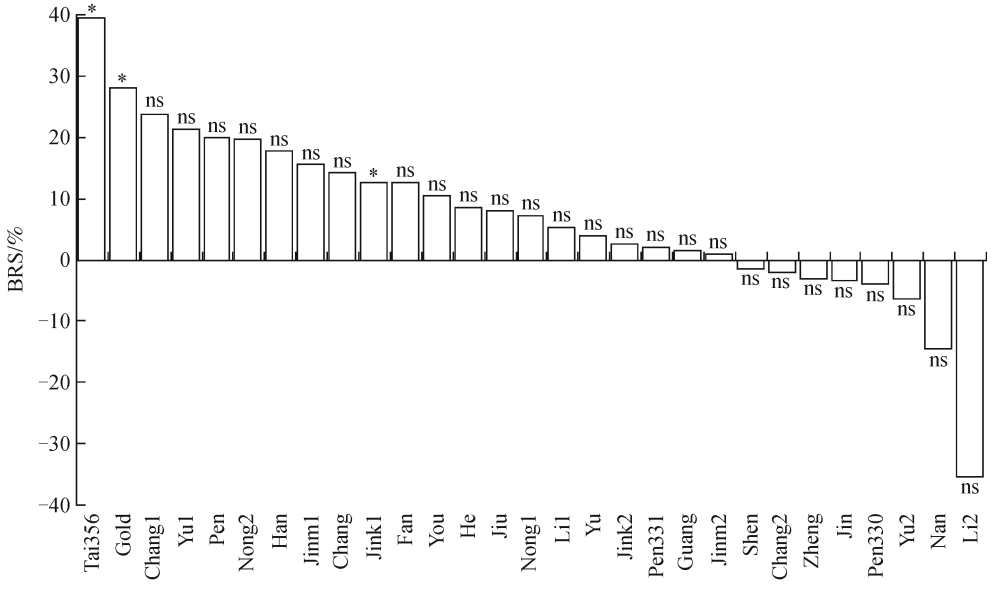

Fig. 1 *BRS* of 29 amaranth genotypes. ns and * represent that the differences of the biomass of edible between middle-Cd and low-Cd treatments were insignificance ($p > 0.05$) and significance ($p < 0.05$), respectively

3.2 Variations of Cd accumulation in different tissues of the amaranth genotypes

The average Cd concentrations (FW basis) in edible parts and root were 0.056 and 0.065 mg·kg^{-1}, respectively, under low-Cd exposure, and 0.147 and 0.172 mg·kg^{-1}, respectively, under middle-Cd exposure. The averages of both edible parts and root in middle-Cd were 1.6 folds higher than those in low-Cd.

Results of two-way ANOVA for the variations of Cd concentrations in different tissues showed that variations of genotypes, soil Cd, and interaction of genotype × soil Cd were all significant ($p < 0.001$). The maximal genotype differences of Cd concentration in edible parts were 3.1 and 3.8 folds for low- and middle-Cd treatments, respectively. The coefficients of inter-genotype variation for Cd concentration in edible parts were 22.2% and 22.1% under both low- and middle-Cd treatments, respectively. The averages of the coefficients of intra-genotype variation (the variation among different individuals of a genotype) for the respective treatments were only 11.1% and 12.3%, which was much lower than that of the inter-genotype variation.

3.3 Selection of typical Cd-PSCs of amaranth

The Cd concentrations in edible parts of the tested amaranth genotypes under low- and middle-Cd are shown in Table 1. Under low-Cd, Cd concentrations of all the tested genotypes were below ML of Cd according to the CODEX STAN 248-2005, and 8 out of the 29 tested genotypes (27.6%) had lower Cd concentration in edible parts than the ML of GB18406.1-2001 for Cd. Cd concentrations of Li1, Shen, He and Nan were significantly lower than those of Pen, Jink1, Nong2, Jinm2, Pen330, Chang, Jink2 and Jiu ($p < 0.05$). Under middle-Cd treatments, none of the tested genotypes measured up to the ML of GB18406.1-2001, and the four genotypes with the lowest Cd concentrations in shoot under low-Cd belonged also to the group with relative lowest Cd accumulation under middle-Cd. Thus, the four genotypes were categorized as typical Cd-PSCs.

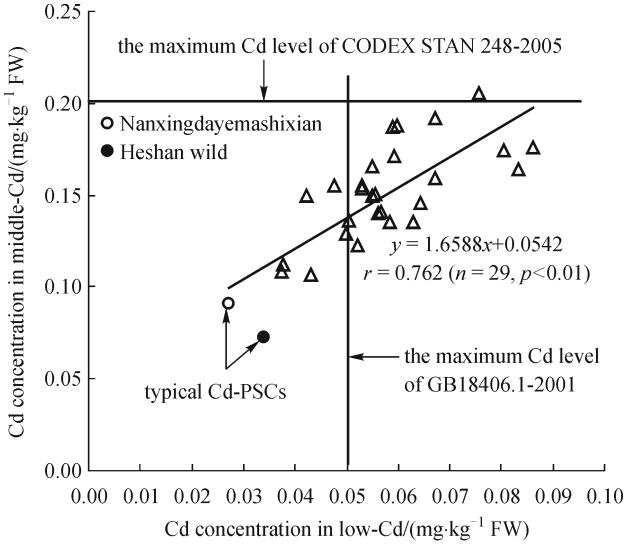

Fig. 2 Correlations of Cd concentrations in edible part of the tested amaranth genotypes between low- and middle-Cd

The Cd concentrations in edible part of the tested genotypes grown between under low- and middle-Cd were significantly positively correlated ($p < 0.01$) (Fig. 2). A similar correlation was observed in root ($r = 0.762$, $n = 29$, $p < 0.01$), indicating that the rank of the genotypes in Cd accumulation was not altered, even under different soil Cd treatments. The genotypes Nan and He, which accumulated exceptionally low concentration of Cd in edible parts under both the low-Cd and middle-Cd treatments, could be treated as typical Cd-PSCs for further studies.

3.4 Cd translocation of different amaranth genotypes

The average *TR*s under low- and middle-Cd were 90.5% (76.2%–95.0%) and 90.3% (75.0%–95.1%), respectively (Fig. 3), indicating that most Cd absorbed by the tested genotypes was transferred to edible part. There was a rather close correlation ($r = 0.860$, $n = 29$, $p < 0.01$) between the TRs under low- and middle-Cd.

Differing from the *TR*s, the S/R ratio of Cd widely varied among the tested genotypes, ranging from 0.50 to 2.22 with an average of 1.32 under low-Cd treatment, and from 0.51 to 2.18 with an average of 1.31 under middle-Cd treatment (Fig. 3). The correlation of the S/R ratios of Cd between under low- and middle-Cd was also significant ($r = 0.783$, $n = 29$, $p < 0.01$). cv. Nanxingdayemashixian (cv. Nan), one of the typical Cd-PSCs, had the lowest TRs and S/R ratios under both low- and middle-Cd treatment, and He, another typical Cd-PSC, had the second lowest TRs and the third lowest S/R ratios under the two Cd treatments. The features in Cd uptake and translocation might be a decisive factor of the low Cd accumulation in edible part of typical Cd-PSCs. The correlation coefficients between TRs and concentrations of Cd in edible parts were 0.703 ($n = 29$) and 0.764 ($n = 29$) under low- and middle-Cd, respectively, and those between S/R ratios and concentrations of Cd were 0.775 ($n = 29$) and 0.690 ($n = 29$), respectively. All of correlations were statistically significant at $p < 0.01$ level. Because cv. Pennongjianyexian (cv. Pen) and Nong2 had the highest values for both TRs and *S/R* ratios under the middle-Cd treatment and belonged to the group with the highest Cd concentration in the edible parts under both Cd exposures, these two genotypes are referred as typical non-Cd-PSCs for further studies.

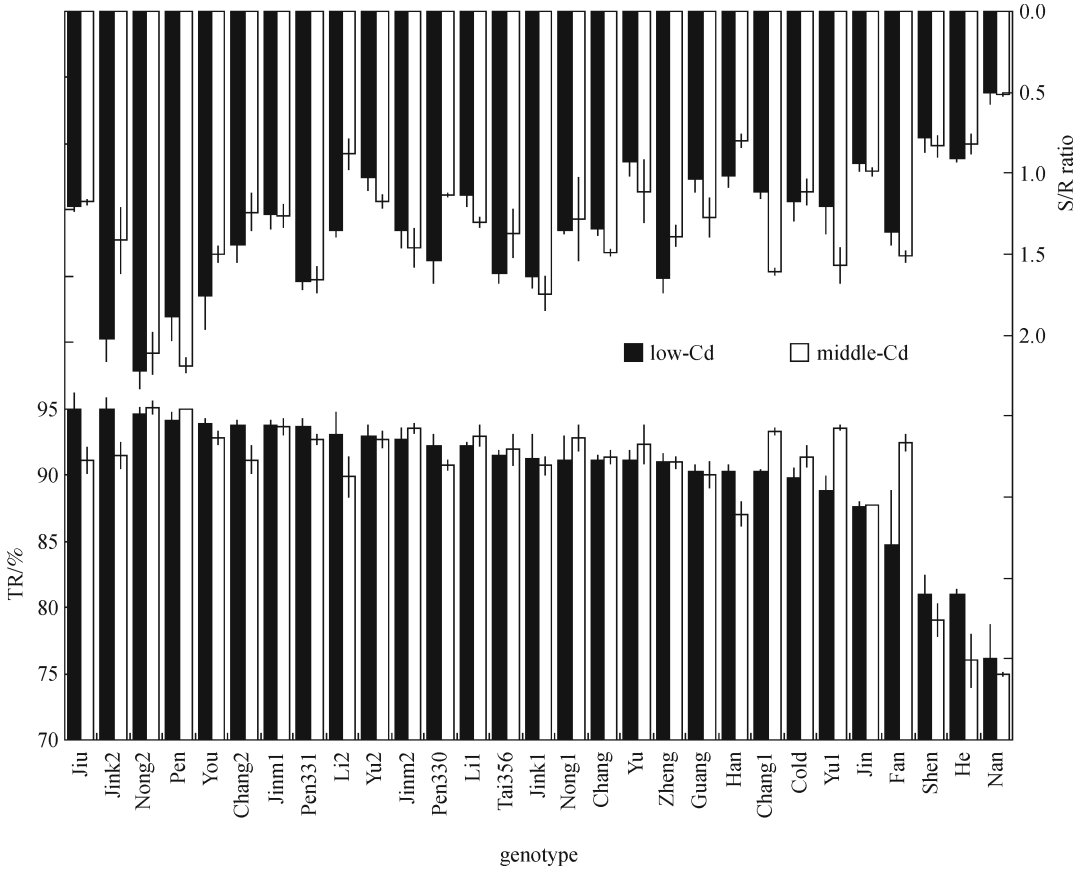

Fig. 3 *TR*s and S/R ratio of Cd for the tested amaranth genotypes under low- and middle-Cd. The error bars represent SD of means (n = 3)

3.5 Chemical forms of Cd in two typical amaranth genotypes

Two amaranth genotypes, i.e., cv. Nan, one of the typical Cd-PSCs, and cv. Pen, one of the typical non-Cd-PSCs, were adopted for this experiment according to the results of the above-mentioned. Under both low- and middle-Cd treatments, the total Cd concentrations in leaf and stem were higher in cv. Pen than in cv. Nan, and the differences between the two genotypes were amplified and became significant ($p < 0.05$) under the middle-Cd treatment. Concentrations of Cd in different chemical forms represented an overall pattern of $F_{HAC} > F_{HCl} > F_{NaCl} > F_{residue} > > F_E \approx F_D$ in different tissues under low-Cd treatment, and this pattern changed into $F_{HAc} \approx F_{NaCl} > F_{HCl} > F_{residue} > > F_E \approx F_D$ under middle-Cd treatment (Fig. 4). For the two tested genotypes, Cd in the F_{NaCl} of leaves, stems and roots increased significantly ($p < 0.01$) with increasing Cd exposure, but the increments were greater in cv. Pen (3.4–3.9 folds) than in cv. Nan (2.1–2.9 folds).

There were no significant differences in concentrations of Cd in different chemical forms between cv. Nan and cv. Pen under low-Cd exposure, except for F_{HCl} in leaf and F_{NaCl} in stem which was significantly higher ($p < 0.05$) in cv. Pen than in cv. Nan. Under middle-Cd exposure, F_{NaCl} was significantly higher in cv. Pen than in cv. Nan in leaf ($p < 0.01$), stem ($p < 0.01$) and root ($p < 0.05$), as well as F_{HCl} in leaf ($p < 0.05$) and stem ($p < 0.01$), implying that F_{NaCl} and F_{HCl} play an important role in contributing to the distinct Cd accumulation pattern between the two genotypes.

4 Discussion

4.1 Health risk via consuming amaranth

Amaranth was reported as a Cd hyperaccumulator because its Cd level was higher than 100 mg·kg^{-1} (DW basis) in aboveground parts when grown in soil containing 7.7 mg·kg^{-1} Cd [25]. Indeed, the Cd concentration in shoot of amaranth was found to be higher than that in root in the present study and also a study by Wang [25], which considered it to be one of the typical traits of hyperaccumulator [37]. The species seemed to be vulnerable to soil contamination because relatively high level of Cd would accumulate in the edible parts in comparison to other leafy vegetables [5]. Growths of all the tested genotypes under middle-Cd exposure (0.40 mg·kg^{-1}) were statistically similar to or better than those in absence of Cd exposure (0.12 mg·kg^{-1}). This may be attributed to the stress

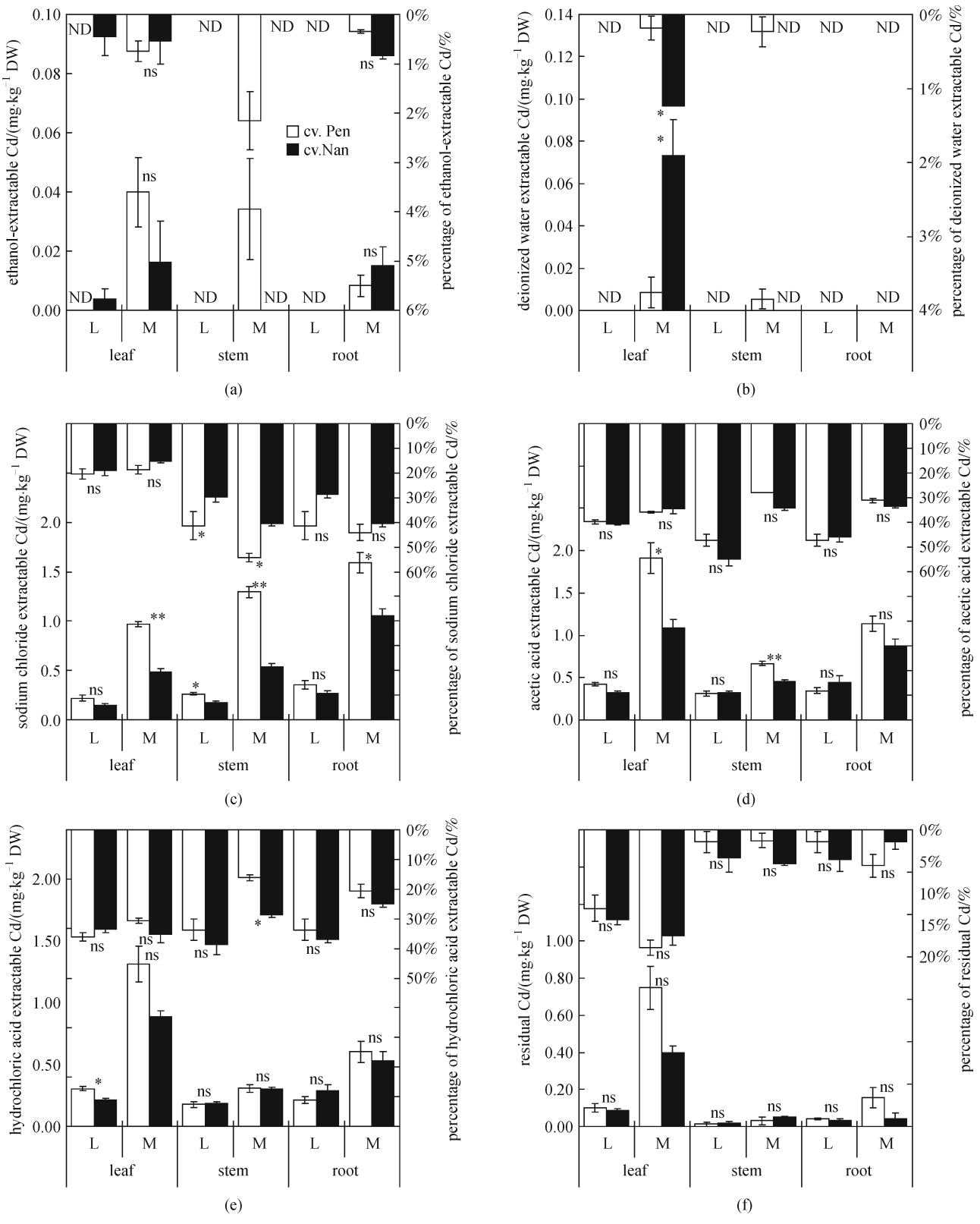

Fig. 4 Concentrations (DW basis) of Cd in different chemical forms (a–f) in tissues of two typical amaranth genotypes under low-Cd and middle-Cd exposures. ns, * and ** mean that the differences of the data between cv. Pen and cv. Nan were insignificant, significant at $p < 0.05$ level and significant at $p < 0.01$ level, respectively; ND, not dectected; cv. Pen, a non-Cd-PSC; cv. Nan, a typical Cd-PSC

response to the low Cd exposure that can also be observed in many other plant species [5]. Cd contamination was very likely to occur in the process of amaranth production as a food crop because producers could not get sufficiently warned when the plant was exposed to Cd contamination for the lack of toxic appearances.

Soil-to-plant transfer is one of the key pathway for human exposure to toxic metals through the food chain. The TF value is used to assess the health risk of crops associated with toxic metals in agricultural soils [36]. In the present experiments, the TF of Cd in amaranth reached up to 0.712, which was extremely high compared to the vegetables with low TF [36], such as *A. tuberosum* Rottler ex Prengel L. (0.035), *Nasturtium officinale* R.Br.L. (0.009), and *Vigna sinensis* L.(0.001). Cui et al. [36] also found that amaranth had a rather high TF (0.493) comparative to other vegetables with high TF.

PTWI (provisional tolerable weekly intake) is used by WHO to stress the importance of intake limitation over a period of time for contaminants that may accumulate in the body. The PTWI for Cd is ideally lower than 7 $\mu g \cdot kg \cdot b.w.^{-1} \cdot week^{-1}$ [38] and is sometimes divided by seven to derive a tolerable daily intake (1 $\mu g \cdot kg \cdot b.w.^{-1} \cdot day^{-1}$). Given that about 40% of Cd in our diet come from vegetables [39], the ML of daily Cd intake coming from vegetable would be approximately 0.4 $\mu g \cdot kg \cdot b.w.^{-1} \cdot day^{-1}$. According to the results of the present study, if the quantity of amaranth consumed by a person (60 kg in bodyweight) is 294 $g \cdot d^{-1}$, which is the average amount of daily vegetable consumption in China [40], the intake of Cd would be 0.722 $\mu g \cdot kg \cdot b.w.^{-1} \cdot day^{-1}$ for a person consuming the amaranth grown in soil containing 0.40 $mg \cdot kg^{-1}$ Cd, which is about two times of the ML. In other words, if an adult in China consumed 294 g of vegetable amaranth grown in slightly contaminated soil in a day, his/her Cd intake would exceed the highest daily Cd intake threshold from vegetables. This suggests that the consumption of amaranth grown in soil slightly contaminated by Cd will pose a rather high health risk. Thus, adopting the PSC strategy is particularly important to reduce the level of Cd exposure from amaranth products.

4.2 Cd-PSCs in amaranth

It is considered that screening of Cd-PSCs would be difficult for vegetable amaranth due to its high Cd accumulating potential. This also explained why only 0.4 $mg \cdot kg^{-1}$ of Cd was added to the soil treatment of the highest Cd concentration adopted in the experiment for Cd-PSCs screening . However, even under such low Cd exposure, there was a genotype that accumulated Cd in edible part with a concentration higher than 0.2 $mg \cdot kg^{-1}$, and all of the tested genotypes accumulated Cd with concentrations higher than 0.05 $mg \cdot kg^{-1}$. As Cd is a highly toxic heavy metal for human beings, the health status of the consumers would be considered protected when the Cd level of vegetables is kept below 0.05 $mg \cdot kg^{-1}$ (GB18406.1-2001). However, only 27.6% of the tested genotypes of amaranth measured up to the standard when grown in a relatively clean soil containing only 0.12 mg $\cdot kg^{-1}$ Cd. Among them, the two wild amaranth genotypes with the lowest Cd-accumulating ability are worth noting.

For some plants, Cd accumulation abilities are found to be heritable or genotype-dependent [15]. There were valuable findings supporting that the Cd accumulation of amaranth is genotype-dependent. Variations of Cd concentrations in tissues from different individuals within certain genotypes of amaranth were much smaller than those from other genotypes, which was a statistical proof for the genotype-dependent Cd accumulation pattern in amaranth. The relative ability to accumulate Cd among the tested genotypes of amaranth was generally consistent despite the soil Cd concentration, indicating that Cd accumulation patterns are genotype-specific. Similar characteristics were also observed in some other vegetables, such as water spinach [25], Chinese cabbage (*Brassica perkinensis* L.) [22], tomato (*Lycopersion esculentum*) [41], asparagus bean (*Vigna unguiculata* subsp. *Sesquipedalis* L., family Fabaceae) [4], pea (*Pisum sativum* L.) [42] and lettuce [43]. Furthermore, Cd accumulations of Cd-PSCs seemed to be relevant to the Cd distribution pattern in different tissues and the capability to transfer Cd from root to shoot.

Application of the Cd-PSCs identified in the present study is theoretically feasible in reducing Cd pollution of amaranth products. If Cd-PSCs (Nan and He) of amaranth grown in the soil containing 0.40 $mg \cdot kg^{-1}$ Cd are consumed, the intake of Cd will be 0.400 $\mu g \cdot kg \cdot b.w.^{-1} \cdot day^{-1}$ which is safe according to PTWI. Therefore, practice of the PSC strategy can certainly protect consumers of amaranth and other crops to some extent against soil Cd contamination which exist widely in China.

4.3 Mechanisms of the genotype difference in Cd accumulation of amaranth

A typical Cd-PSC (cv. Nan) with particularly low Cd mobility from root to shoot was used to further investigate the mechanism of Cd translocation in amaranth. Differences in Cd chemical forms between cv. Nan and cv. Pen, the selected typical Cd-PSC and non-Cd-PSC genotypes, under different Cd exposures were compared in the present study. It was found that the alternation of Cd in F_{NaCl} which bound usually to proteins and pectic acids may be a key factor in regulating Cd accumulation in different amaranth genotypes. Cd could easily combine with proteins [44,45] for its strong affinity to proteins or sulfhydryl compounds (-SH) and other side chains, and the protein-binding Cd is considered to be associated with Cd translocation [33]. In the non-Cd-PSC, the concentration or proportion of Cd in F_{NaCl} as well as the response to Cd exposure was always superior to those in Cd-PSC. This

implies that Cd translocation is performed more actively in the non-Cd-PSC than in the Cd-PSC, and thus results in the high Cd accumulation in shoot of the non-Cd-PSC. This is considered to be one explanation for the mechanism of the genotype difference in Cd accumulation of amaranth.

Another explanation is that the different responses in water-insoluble Cd, including the Cd in F_{HAc}, F_{HCl} and $F_{residue}$, to the increased Cd exposure may also be relevant to the genotype difference in Cd accumulations. The total proportions of the water-insoluble Cd in shoot and root of Cd-PSC were lower than those of non-Cd-PSC in the absence of Cd exposure, and the result was reversed when Cd exposure was added. The Cd exposure may have led to a lower mobility of Cd in the Cd-PSC than in the non-Cd-PSC. These findings demonstrate that the low Cd accumulation in the Cd-PSC obey certain rules and thus shall be stable.

4.4 Breeding of Cd-PSC to minimize Cd accumulation in amaranth

Because the distinctions in Cd accumulation between genotypes are significant enough for identifying Cd-PSC and non-Cd-PSC of many of the investigated crops, breeding Cd-PSCs is considered feasible. It was shown that tomato hybrids accumulate less cadmium in fruits than that their parents, which verified heterosis breeding as a viable method to accumulate less Cd in tomatoes [46]. Clarke [47] had demonstrated that a single gene (with low-Cd dominant) controlled Cd accumulation in the grain of durum wheat cultivars. Penner et al. [48] had successfully linked the random amplified polymorphic DNA (RAPD) markers for a single gene governing low Cd uptake in durum wheat and suggested that the markers could be used in breeding for low Cd uptake genotypes. Li et al. [49] used RNAi-mediated silencing of the phytochelatin synthase (PCS) gene *OsPCS1* to reduce cadmium accumulation in rice seeds. The results showed that Cd accumulation could be reduced by about half in RNAi rice seeds without any obvious influence over the plant growth. In fact, the idea of genetic selection to reduce Cd concentration in crops had already been put into practice. Grant et al. [15] had spent 10 years breeding for low-Cd durum wheat, and a cultivar named AC Napoleon was finally obtained. In Australia, three potato varieties (Wilwash, Russet Burbank and Russet) [50] (NCMS, 1999) and three peanut varieties (Florunner, Southern Runner and Streeton) [51] (NCMS, 2001) were recommended as low-Cd accumulators. In addition to these, more breeding programs were underway to produce low-Cd accumulating cultivars of rice and soybean [15].

For vegetable amaranth, due to shoot Cd accumulation of the wild lines are much lower than those of the cultivated varieties, it is suggested that the wild lines can be used as a parent for breeding new Cd-PSCs by crossing with one of the commonly cultivating varieties. However, because of the small floral organs and self-fertilisation (selfing) of amaranth, breeding of Cd-PSC for the species would be difficult. Chemical or physical mutagenicity may be one way to breed Cd-PSC of amaranth. In addition, some agricultural methods, such as fertilizer application, crop rotation or intercropping [52], and amendment of heavy metal deactivating materials [25] or microorganism [53] etc., were tested to be capable of reducing Cd accumulation in crops. Combining the agricultural methods with PSC strategy can certainly improve the quality of amaranth product.

5 Conclusions

In conclusion, the production of amaranth as a vegetable crop poses a risk to human health for its high Cd accumulating property even when grown in soil with rather low Cd contamination. The first hypothesis of this study is thus acceptable and amaranth shall not be planted in Cd heavily contaminated or potentially contaminated sites, such as areas close to industrial or mining zones, areas that had been irrigated with sewage or wastewater, and agricultural lands that had been using inferior fertilizer for a long period of time. The Cd accumulating properties of amaranth are very possibly genotype-dependent and related to the chemical forms of Cd, especially the NaCl extracted and the insoluble forms, which answered the second hypothesis of this study. Application of the Cd-PSCs identified by the present study will reduce the health risk and protect amaranth consumer from Cd exposure. Further experiments are of interest to clarify the function of Cd binding proteins and the effects of water-insoluble Cd salts on Cd translocation and detoxification.

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