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Variations in cadmium and nitrate co-accumulation among water spinach genotypes and implications for screening safe genotypes for human consumption[*]

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Abstract: Vegetables are important constituents of the human diet. Heavy metals and nitrate are among the major contaminants of vegetables. Consumption of vegetables and fruits with accumulated heavy metals and nitrate has the potential to damage different body organs leading to unwanted effects. Breeding vegetables with low heavy metal and nitrate contaminants is a cost-effective approach. We investigated 38 water spinach genotypes for low Cd and nitrate co-accumulation. Four genotypes, i.e. *JXDY*, *GZQL*, *XGDB*, and *B888*, were found to have low co-accumulation of Cd (<0.71 mg/kg dry weight) and nitrate (<3100 mg/kg fresh weight) in the edible parts when grown in soils with moderate contamination of both Cd (1.10 mg/kg) and nitrate (235.2 mg/kg). These genotypes should be appropriate with minimized risk to humans who consume them. The Cd levels in the edible parts of water spinach were positively correlated with the concentration of Pb or Zn, but Cd, Pb, or Zn was negatively correlated with P concentration. These results indicate that these three heavy metals may be absorbed into the plant in similar proportions or in combination, minimizing the influx to aerial parts. Increasing P fertilizer application rates appears to prevent heavy metal and nitrate translocation to shoot tissues and the edible parts of water spinach on co-contaminated soils.

Key words: Genotypic difference; Heavy metal; Nitrate; Soil pollution; Water spinach
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1 Introduction

Cadmium (Cd) is one of the most toxic and mobile heavy metals that affect human health through the food chain. Agricultural practices, intensive industrial activity, and urban expansion have accelerated the release of Cd into soil, water, and air (Lane et al., 2015). About 27 860 000 m^2 of agricultural soils in China are polluted with Cd (Liu et al., 2015) and Cd contamination has become one of the most important barriers to agricultural sustainability in China (Zhang et al., 2002). Cd in agricultural soils is taken up by plants and then enters humans and animals through the food chain (Kirkham, 2006). Phytoremediation is the best strategy to reduce the risk of Cd entry into food chain.

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Fertilizer application is one of the key factors in improving crop productivity. However, excessive use of chemical fertilizers, especially nitrogen (N) fertilizers, results in severe environmental problems (Hakeem et al., 2013). China is one of the largest consumers of N fertilizers in the world, but the average N use efficiency is low (only about 35%) (Wang et al., 2014), which results in waste of resources and environmental contamination, and also poses a serious hazard to human health (Xu et al., 2012; Chen et al., 2014). Nitrate is potentially carcinogenic and may increase the incidence of gastric, bladder, and oesophageal cancers (Gulis et al., 2002). However, nitrate can decrease blood pressure, thus reducing the risk of cardiovascular disease, myocardial infarction, and stroke. Daily consumption of nitrate-rich vegetables is associated with beneficial effects for patients with gastric ulcer, renal failure, and metabolic syndrome (Habermeyer et al., 2015).

Vegetables are nutritious and are assumed to be safe to consume; people are unaware that some parts of the vegetable may be contaminated with heavy metals and are a major source of human exposure to Cd and nitrate (Tang et al., 2016; Fan et al., 2017). Water spinach (*Ipomoea aquatica* Forsk.), an important leafy vegetable in eastern and southern Asia, can absorb Cd and nitrate naturally into their vacuoles (Wang et al., 2009; Xin et al., 2010). Therefore, evaluation of water spinach genotypes for low Cd and nitrate accumulation when grown on contaminated soils has become a research priority in minimizing human exposure to these co-contaminants.

Accumulation of Cd by plants varies with species and genotype. New strategies have been applied to breeding and screening heavy metal low accumulator vegetable genotypes, such as Chinese cabbage (*Brassica chinensis* L.) (Liu et al., 2010; Wang X et al., 2015), pakchoi (*Brassica rapa* L. ssp. *chinensis*) (Chen et al., 2012), soybean (*Glycine max* Merr.) (Arao et al., 2003; Sugiyama et al., 2011), welsh onion (*Allium fistulosum* L.) (Li et al., 2012), and sweet potato (*Ipomoea batatas* (L.) Lam.) (Huang et al., 2015). Nitrate concentration in high-accumulation genotypes was several times greater than that in low-accumulation genotypes, such as lettuce (*Lactuca sativa* L.) (Escobar-Gutiérrez et al., 2002; Burns et al., 2011a), leaf mustard (*Brassica juncea* (L.) Czern.) (Sharma et al., 2010), and taro (*Colocasia esculenta* (L.) Schott) (Kristl et al., 2016). Vegetable genotypes with a lower nitrate concentration need to be identified for agricultural production and improved human health.

Soils in southern China are often polluted by multiple contaminants. Among them Cd and nitrate contaminations are attributed to irrigation practices, use of low-grade organic fertilizers, and heavy application of N fertilizers, all of which can affect growth, metal tolerance, and metal accumulation in water spinach. Screening different vegetable genotypes with low absorption of Cd and nitrate provides an opportunity to safeguard human consumption.

Previous studies were limited to pot and hydroponics where correlations between different contaminants and nutrients were not observed. This study aimed to investigate co-accumulative remediation capacity of Cd and nitrate in field conditions among 38 genotypes of water spinach.

2 Materials and methods

2.1 Soil characterization

The experiment was performed in greenhouses (Chunyi farm) located at 30°23′37″ N and 120°2′13″ E, Hangzhou, Zhejiang Province, China with an average temperature of 30 °C throughout the day. The soil had been moderately contaminated by Cd and nitrate during several decades of intensive vegetable production (Tang et al., 2016). The depth of soil sampling for analysis was 10–15 cm, and four soil samples were collected to represent the area. The preliminary soil assessment and initial concentrations of metals in the soil (Table 1) were determined according to the previous methods (Li, 2000; Bao, 2008; Tang et al., 2016).

2.2 Sample collection and cultivation

Thirty-eight genotypes of water spinach (*I. aquatica* F.) were obtained from a local seed market in Hangzhou, China. Their genus, origin, and characteristics are listed in Table 2. Split plot design was used with three replicates for major plots and genotypes for the minor plots. Each minor plot was 10 m^2. Approximately 900 seeds of each genotype were soaked overnight in aerated deionized water at (23±0.3) °C, disinfected with 0.7 g/L NaClO for 30 min, drained and sown in the field in June 2014. Planting density and field management were the same as in conventional farming practice.

Table 1 Physicochemical properties of soil in the field experiment

pH	Organic matter (g/kg)	Total N (g/kg)	Total P (g/kg)	Total K (g/kg)	Available N (g/kg)	Available P (g/kg)
5.39±0.05	23.07±0.69	1.22±0.03	0.60±0.07	1.69±0.14	97.35±6.74	77.59±10.11

Available K (g/kg)	Total Cd (g/kg)	Total Pb (g/kg)	DTPA Cd (g/kg)	DTPA Pb (g/kg)	Nitrate-N (g/kg)
296.87±5.06	1.10±0.18	32.66±0.33	0.23±0.01	0.96±0.12	235.21±6.64

DTPA: diethylene triamine pentaacetic acid. Reprinted from Tang et al. (2016), Copyright 2016, with permission from Elsevier

Table 2 Tested genotypes of water spinach

Serial No.	Accession name	Origin*	Horticultural characteristics	
			Petioles	Blades
1	*JXDY*	Jiangxi	Green	Extra-big
2	*JADY*	Jiangxi	Green	Big
3	*GDLY*	Jiangxi	White	Small
4	*GZTB*	Guangdong	White	Extra-big
5	*G501*	Guangdong	Light yellow	Small
6	*GZQL*	Hong Kong	Green	Small
7	*XGDB*	Fujian	White	Big
8	*G268*	Fujian	Green	Small
9	*TWBD*	Jiangxi	White	Big
10	*TWLY*	Thailand	Green	Small
11	*TWBL*	Guangdong	White	Small
12	*TWYX*	Taiwan	Green	Small
13	*TWZY*	Indonesia	Green	Small
14	*T311*	Taiwan	White	Small
15	*TAIG*	Thailand	Green	Small
16	*TGLY*	Thailand	Green	Small
17	*T221*	Guangxi	Green	Small
18	*TGJY*	Thailand	Green	Small
19	*TGLL*	Jiangsu	Green	Small
20	*YXBL*	Guangdong	White	Small
21	*X606*	Guangdong	Light green	Small
22	*Y601*	Beijing	Green	Small
23	*CHFE*	Hebei	Green	Extra-big
24	*QCUI*	Tianjin	Green	Extra-big
25	*XIAO*	Jiangsu	Green	Small
26	*LZHU*	Thailand	Green	Small
27	*GDZY*	Thailand	Green	Small
28	*GLCQ*	Jiangxi	Green	Small
29	*DAYE*	Jiangxi	Green	Extra-big
30	*BQBA*	Fujian	Green	Small
31	*CBDY*	Jiangxi	White	Extra-big
32	*LIUY*	Jiangxi	Green	Small
33	*BGDY*	Hubei	White	Big
34	*LYQG*	Fujian	Green	Small
35	*HSZY*	Shanghai	Green	Small
36	*B888*	Jiangxi	White	Small
37	*DQGU*	Jiangxi	Light green	Small
38	*QGZY*	Hebei	Green	Small

* Region (province or city of China) or country (Thailand, Indonesia)

2.3 Fresh weight and biomass determination

The experiment was performed according to the previous method (Tang et al., 2017). All genotypes were grown in green house for four weeks without application of fertilizer. Plants excised at 0.5 cm above the base were used for fresh weight (FW) and biomass determination. Mature and immature leaves (leaf blades and petioles) were separated and FWs were estimated. After rinsing with tap water, ten representative plants selected from each genotype were combined together to make a composite sample. Subsamples were dried in an oven at 105 °C for 30 min, and then at 65 °C until a constant weight was attained and the biomass of shoots was then recorded.

2.4 Heavy metal analysis and nitrate determination of plant samples

The concentrations of Cd and other metals (K, Ca, Mg, Fe, Zn, Cu, Pb, Mn, and Se) of plant samples, which were digested with HNO_3 and $HClO_4$ (5:1, v/v), were determined using inductively coupled plasma mass spectrometer (ICP-MS, 7500a, Aligent, USA), according to the previous method (Bao, 2008). Fresh samples were placed in 10 ml deionized water and heated in a boiling water bath for 30 min. Then the nitrate concentration in the water extract was determined using an ultraviolet spectrophotometer (Lambda 350V-vis, Perkin Elmer, Singapore), according to salicylic acid colorimetric method described by Li (2000). Analyses of other mineral elements (N and P) and nutritional indices (chlorophyll, protein, vitamin C, and cellulose) were performed according to the pervious methods (Tang et al., 2016).

2.5 Statistical analysis

The statistical evaluation was represented as the mean±standard error (SE) of four replicates. The differences between 38 genotypes of water spinach were estimated by the least significant difference (LSD) method and the correlation was analyzed by Bivariate analysis (SPSS 20.0) and graphical representation was generated by Origin Pro 8.0.

3 Results

3.1 Fresh weight and biomass yields

FWs and biomass of 38 water spinach genotypes were determined (Figs. 1 and 2) after growth for four weeks. FW ranged from 16.62 g/plant (*LIUY*) to 10.22 g/plant (*BQBA*), approximately a 1.6-fold difference between the highest and the lowest levels with a mean value of 13.30 g/plant (Fig. 1). Biomass values ranged from 1.24 g/plant (*LIUY*) to 0.74 g/plant (*BQBA*), approximately a 1.7-fold difference with a mean value of 0.98 g/plant (Fig. 2).

3.2 Cd and nitrate uptake

Cd uptake ranged from 2.75 mg/kg dry weight (DW) (*GDZY*) to 0.07 mg/kg DW (*TWBL*), a difference of more than 39-fold with a mean value of 1.15 mg/kg DW (Fig. 3). Nitrate concentrations

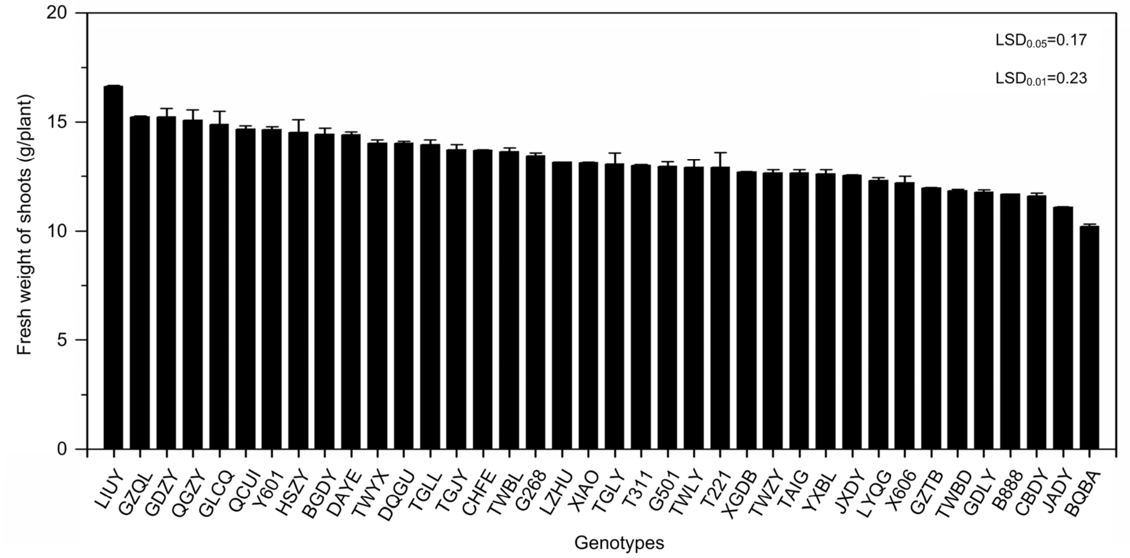

Fig. 1 Shoot fresh weights of 38 water spinach genotypes grown in co-contaminated soils

Bars represent standard error of the mean with four replicates. Data analysis was performed using LSD method

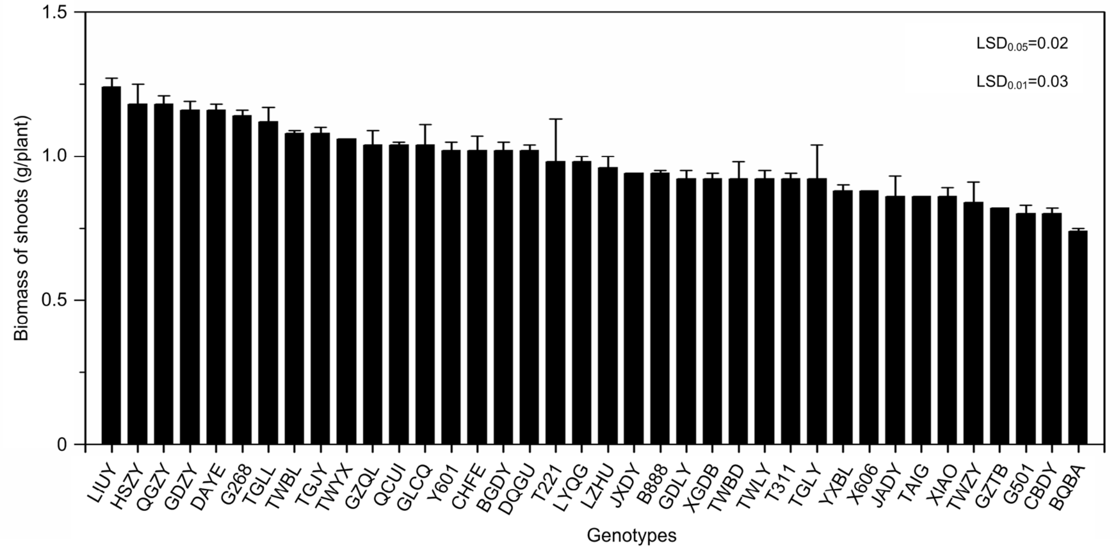

Fig. 2 Shoot biomass of 38 water spinach genotypes grown in co-contaminated soils

Bars represent standard error of the mean with four replicates. Data analysis was performed using LSD method among all genotypes ranged from 10983 mg/kg FW (*LIUY*) to 1809 mg/kg FW (*JXDY*), a 6-fold difference with a mean value of 5177 mg/kg FW (Fig. 4).

3.3 Correlations between Cd, nitrate, and other elements

No correlation was observed between Cd and nitrate concentrations (Fig. 5a). However, there was a significant positive correlation between Cd, Pb, and Zn concentrations (Fig. 6) and a significant negative correlation between Cd or Pb and P (Figs. 7a and 7b), and between Zn and P (Fig. 7c). There was a positive correlation between nitrate and chlorophyll concentrations (Fig. 8b), and a significant positive correlation between nitrate and vitamin C (Fig. 8a). However, no significant correlation between Cd, nitrate and biomass yield in water spinach (Figs. 5b and 5c) was observed.

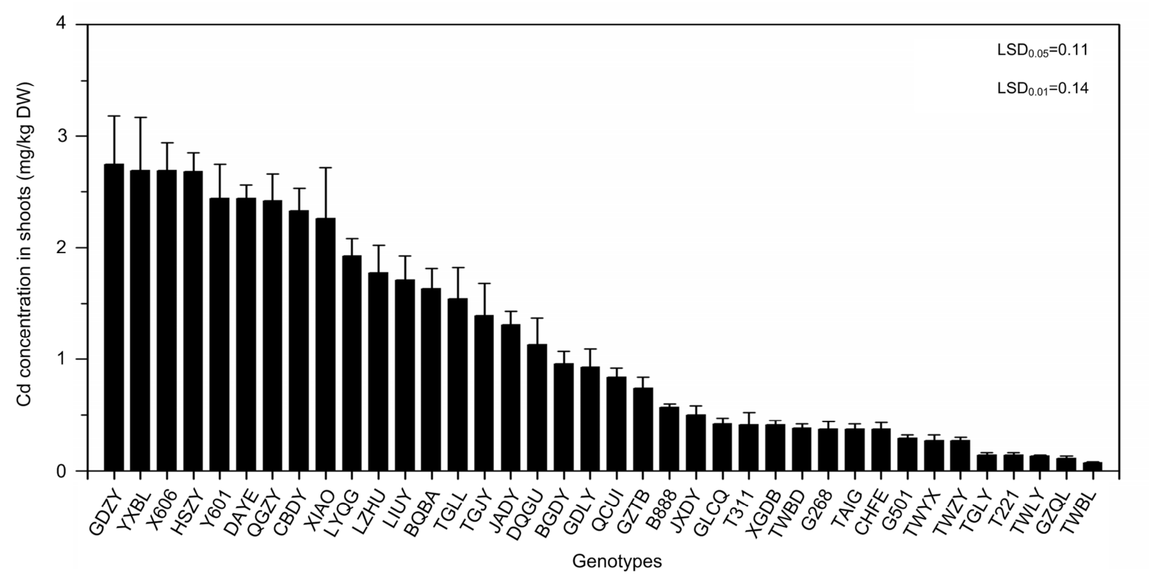

Fig. 3 Cd concentration of 38 water spinach genotypes grown in co-contaminated soils
Bars represent standard error of the mean with four replicates. Data analysis was performed using LSD method

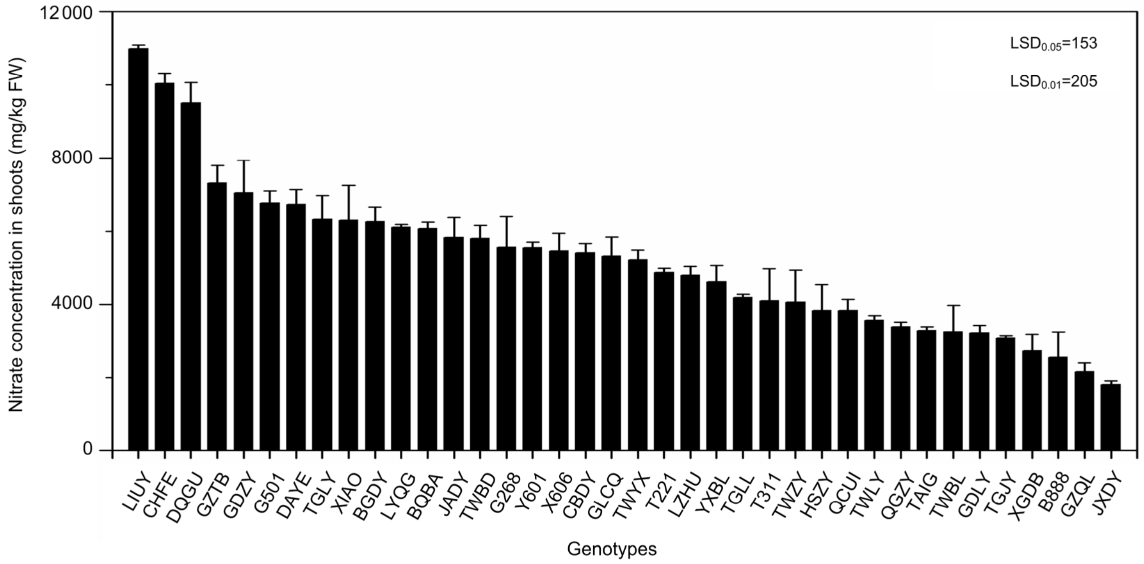

Fig. 4 Nitrate concentration in 38 water spinach genotypes grown in co-contaminated soils
Bars represent standard error of the mean with four replicates. Data analysis was performed using LSD method

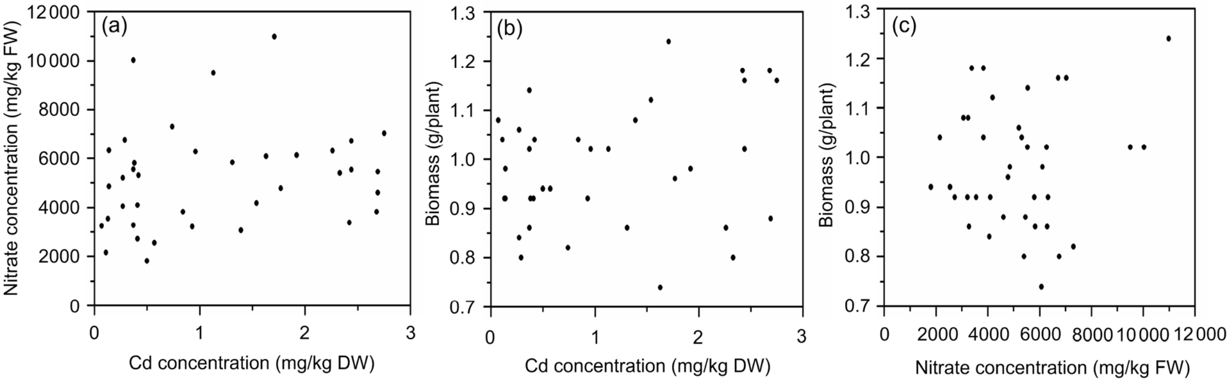

Fig. 5 Correlation coefficients between Cd, nitrate concentrations, and plant biomass of water spinach genotypes in Cd and nitrate contaminated soils

(a) Nitrate vs. Cd; (b) Plant biomass vs. Cd; (c) Plant biomass vs. nitrate

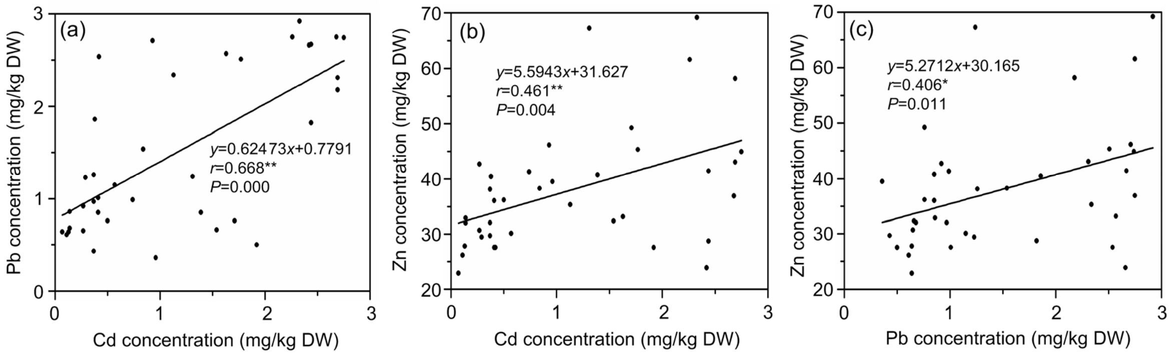

Fig. 6 Correlation coefficients between Cd, Pb, and Zn concentrations in water spinach genotypes in Cd and nitrate contaminated soils

(a) Pb vs. Cd; (b) Zn vs. Cd; (c) Zn vs. Pb. * Significance at $P<0.05$; ** Significance at $P<0.01$

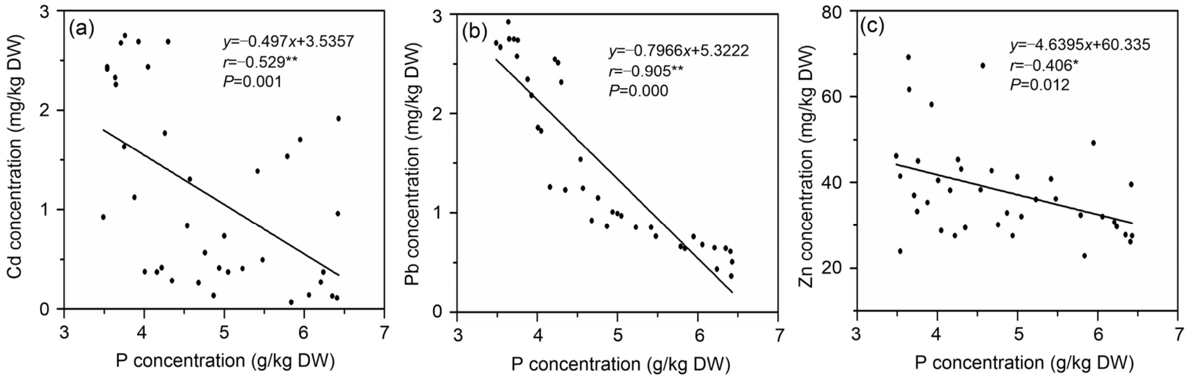

Fig. 7 Correlation coefficients between P concentrations and Cd, Pb, and Zn accumulation levels in water spinach genotypes in Cd and nitrate contaminated soil

(a) Cd vs. P; (b) Pb vs. P; (c) Zn vs. P. * Significance at $P<0.05$; ** Significance at $P<0.01$

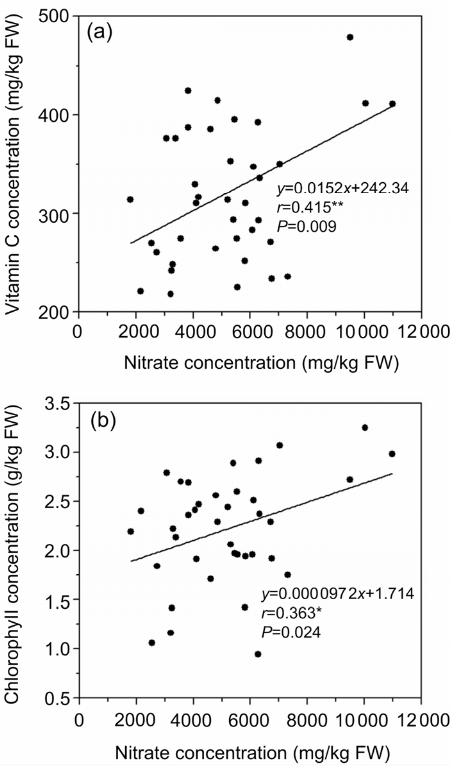

Fig. 8 Correlation coefficients between nitrate concentrations and vitamin C, chlorophyll accumulation levels in water spinach genotypes in Cd and nitrate contaminated soil

(a) Vitamin C vs. nitrate; (b) Chlorophyll vs. nitrate. * Significance at P<0.05; ** Significance at P<0.01

4 Discussion

Water spinach is a common leafy vegetable which provides rich nutrients to the diet. All the selected water spinach showed successive growth in Cd and nitrate co-contaminated soil. The FW or biomass yield is close to the mean value. Water spinach tolerates high Cd and nitrate stress relatively well. The results were consistent with previous studies (Wang et al., 2007). Since there is no visible symptom of toxicity when water spinach is grown in Cd and nitrate co-contaminated soils, the potential health risk may be high from inadvertently consuming contaminated water spinach. The National Food Safety Standard of China GB 2762-2012 (Ministry of Health of the People's Republic of China, 2012) sets the safe limit for Cd contamination of fresh leafy vegetables at 0.05 mg/kg FW. Since the water content of water spinach is 93%, it can be calculated that the safe consumption level of Cd in water spinach shoot is 0.71 mg/kg DW. Given this standard, 17 water spinach genotypes tested in our study can be safe for consumption. The maximum permissible concentration (MPC) of nitrate is 3100 mg/kg FW (Zhou et al., 2000), which means that only five water spinach genotypes can be safe for consumption. Using the combined standards for Cd and nitrate, four genotypes were recognized as safe, i.e. *JXDY*, *GZQL*, *XGDB*, and *B888* (Table 3), which are low Cd and nitrate co-accumulators and suitable to grow in slightly or moderately contaminated soils without any risk to human health.

Table 3 Cd and nitrate concentrations in the shoots of four safe water spinach genotypes

Genotypes	Cd (mg/kg DW)	Nitrate (mg/kg FW)
JXDY	0.50±0.08	1809.6±96.4
GZQL	0.11±0.02	2154.9±236.2
XGDB	0.41±0.04	2723.7±443.6
B888	0.57±0.03	2549.3±668.0

Data are expressed as mean±standard error with four replicates

Previous studies showed that low doses of heavy metals may promote plant hormone secretion and regulate plant growth and development (Liu et al., 2010). However, the distinct tolerance mechanisms of water spinach genotypes to Cd are not fully understood. Variations in Cd accumulation among genotypes may be related to uptake, transfer, and bioaccumulation of Cd in shoots (Uraguchi et al., 2009) and roots (Lux et al., 2011) and are influenced by several environmental factors such as light, cold, and humidity (Cheng et al., 2006; Li et al., 2015), including properties of the rhizosphere (Arao et al., 2009; Zheng and Zhang, 2011).

The accumulation of Cd in plants varies greatly not only among plant species but also among genotypes or cultivars within the same species (Yu et al., 2006; Liu et al., 2007; Wang et al., 2007; Martin et al., 2012). The results of the present study showed that different genotypes of water spinach had different Cd retention abilities, resulting in different Cd accumulations. Genotype-dependent Cd accumulation of water spinach largely depends on bioprocesses occurring in shoots and roots (Xin et al., 2013a). Some evidence indicates that Cd subcellular distribution may be associated with Cd tolerance and detoxification in plants. Distribution of Cd in plants is influenced by cross-membrane transport systems, the existence of intracellular binding sites, vacuole sequestration, xylem and phloem transport, and root retention (Grant et al., 2008). In most cases heavy metals are retained in plant root cell wall, restricting their translocation to shoot and minimizing plant damage (Wang Y et al., 2015). Similar results were reported by Xin et al. (2013a, 2013b) and Huang et al. (2016). Retention of Cd in the cell wall is the effective and most important mechanism for Cd detoxification in all water spinach tissues, especially in young leaves (Xin et al., 2013b), though this may vary between genotypes (Huang et al., 2016). The presence of thicker phloem and outer cortex cell walls in the low Cd cultivars may explain why low Cd cultivar roots were able to retain more Cd, thus reducing Cd translocation to shoots (Xin et al., 2013b).

Nitrate is taken up by plants from fertilizer, but this differs depending on the genotype and environment (Burns et al., 2011b). Nitrate concentration in plants depends mainly on environmental, genetic, and nutritional factors (Anjana et al., 2009). When plants are provided with excess nitrate, only a small portion taken up by roots may be immediately translocated to and assimilated by shoots, while the majority of the absorbed nitrate is stored in vacuoles in both roots and shoots (Luo et al., 2006). Nitrate accumulation in plant tissues acts not only as a temporary store, but also as a replacement osmoticum for other plant solutes for maintaining turgor and driving leaf expansion (Burns et al., 2010). Excessive N accumulation in plant tissues is attributed to the imbalance between uptake and assimilation (Cárdenas-Navarro et al., 1999). As a result, the uptake of nitrate may reduce osmatic pressure that limits the storage of organic solutes in vacuole (Wojciechowska and Kołton, 2014).

Although there is strong evidence for the genotypical effects on Cd or nitrate accumulation in vegetable plants (Arao et al., 2003; Burns et al., 2011a; Sugiyama et al., 2011; Wang X et al., 2015; Kristl et al., 2016), less is known about the higher specificity. The present study indicated that no correlation occurred between plant Cd, nitrate, and biomass, suggesting that yield, Cd and nitrate levels may be independent of each other. This suggests that these three traits could be improved separately or in combination for high yield with low concentrations of Cd and nitrate.

Heavy metals, such as Cd, Pb, and Zn, are phytotoxic when present in excessive amounts and can interfere with photosynthetic and respiratory activities, mineral nutrition, enzymatic activity, membrane functions, and hormone balance (Clijsters and van Assche, 1985). The significant positive correlation between Cd or Pb and Zn in water spinach genotypes may indicate that they have similar uptake mechanisms. Similar results were reported in previous studies (Wu and Zhang, 2002; Liu et al., 2003; Dong et al., 2006; Li et al., 2015). These heavy metals are probably transported by similar transporters in the form of compounds or chelate complexes, and the mobilizing function of root exudates is effective not only for Cd but also for Pb and Zn (Kabata-Pendias, 2011). Cohen et al. (1998) reported that *IRT1* may facilitate the transport of heavy metals in the form of divalent cations such as Cd^{2+}, Pb^{2+}, and Zn^{2+}. Almost all Cd hyper-accumulation plants could accumulate high concentrations of Pb and Zn (He et al., 2002). All of these suggest that these three metals may have similar transport mechanisms, although the interaction of Cd with some nutrients in soil is not fully understood.

P is a macronutrient and has an important role in plant physiology including metabolism. When P is deficient, plant growth and crop yield are reduced, as P is an essential element for the synthesis of nucleic acids, phospholipids, and adenosine triphosphate (ATP) (Yin et al., 2016). Our results indicated that there was a significant negative correlation between Cd, Pb, or Zn and P concentration in water spinach plants (Fig. 7). Similar results have also been reported (Keller and Römer, 2001; Zhang et al., 2002; Dheri et al., 2007). Application of P-containing materials influences the bioavailability of heavy metals such as Cd, Pb, and Zn in soil (Qiu et al., 2011). Jiang et al. (2007) reported that increased P in soil resulted in substantial precipitation of heavy metal-P complexes in the cell wall and vacuoles in maize (*Zea mays* L.), and a similar effect was reported in strawberry (*Fragaia ananassa* D.) (Nuzahath et al., 2013). P-heavy metal interactions reduce the availability of heavy metals in soil and limit their mobility in plants (Clemens, 2006). The supply of adequate and balanced mineral nutrients to crops has the potential to improve plant tolerance, growth, development, and productivity under stress environments (Mitchell et al., 2000; Astolfi et al., 2004). However, the proportion of macronutrient fertilizers in agriculture is often out of balance in China. Our present study points out that increasing the P supply to heavy metal (Cd, Pb, or Zn)-contaminated soils reduces Cd, Pb, or Zn concentration in water spinach.

Chlorophyll concentration is influenced by environmental factors and N fertilization (Barickman and Kopsell, 2016) and the form and ratio of N in plants (Borowski and Michalek, 2008). Previous research demonstrated that higher ratios of NO_3^--N to NH_4^+-N positively influenced chlorophyll concentrations in the leaf tissue of kale (*Brassica oleracea* L. var. *acephala*) (Kopsell et al., 2007) and leaf lettuce (*L. sativa* L.) (Stagnari et al., 2015). Chlorophyll concentration in low nitrate accumulation genotypes oilseed rape (*Brassica napus* L.) was reported to be significantly lower than that in high nitrate accumulation genotypes (Han et al., 2016). In the present study, there was a positive correlation between nitrate and chlorophyll concentration (Fig. 8b), which was consistent with previous studies. There was a significant positive correlation between nitrate and vitamin C in water spinach genotypes (Fig. 8a). Similar results in spinach (*Spinacia oleracea* L.) were previously reported by Conesa et al. (2009) and Koh et al. (2012). The levels of vitamin C and nitrate in leafy vegetables are two key indices for evaluating their nutritional quality (Konstantopoulou et al., 2010), and vitamin C content in leafy vegetables is mainly controlled by the form and ratio of N (Sørensen et al., 1994). The significant positive correlation between nitrate and vitamin C concentration in water spinach genotypes suggests that it would be difficult to reduce nitrate and increase vitamin C concentration simultaneously, so it is necessary to improve these two traits separately.

5 Conclusions

We have identified four genotypes of water spinach, *JXDY*, *GZQL*, *XGDB*, and *B888*, as low co-accumulators for Cd and nitrate, indicating that they should be the preferred genotypes for growing in slightly or moderately contaminated soils, minimizing risk to human health. It should be possible to combine high yields with low concentrations of Cd and nitrate as these variables are independent of each other. Increasing P fertilizer rates appears to increase tolerance in water spinach to Cd, Pb, and Zn toxicity and nitrate concentrations. Our experiment has shown that screening vegetable genotypes for those which do not exceed allowable levels of a contaminant is a cost-effective strategy for minimizing the risk of contaminants to human health via the food chain.

Compliance with ethics guidelines

Lin TANG, Wei-jun LUO, Zhen-li HE, Hanumanth Kumar GURAJALA, Yasir HAMID, Kiran Yasmin KHAN, and Xiao-e YANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：基于筛选安全的共低积累基因型评价空心菜对镉和硝酸盐积累的变异

目　的：筛选镉-硝酸盐共低积累空心菜基因型，并研究降低空心菜重金属含量，提高营养品质的农艺措施。

创新点：首次筛选得到镉-硝酸盐共低积累空心菜基因型，并研究空心菜可食部污染物、矿质元素和营养指标之间的相关性，提出进一步降低空心菜可食部镉和硝酸盐含量的农艺措施。

方　法：共 38 个空心菜基因型收集于世界各地，种植在连作了 7 年的中度镉-硝酸盐复合污染土壤上（Cd 1.10 mg/kg，NO_3^- 235.2 mg/kg），4 周后收获。用 HNO_3-$HClO_4$（体积比 5:1）消煮，电感耦合等离子体质谱仪（ICP-MS）测定各种金属元素，水杨酸-硫酸比色法测定硝酸盐含量，钒钼黄比色法测定磷含量，2,6-二氯靛酚滴定法测定维生素 C 含量，乙醇-丙酮（体积比 2:1）比色法测定叶绿素含量。

结　论：本试验筛选得到镉-硝酸盐共低积累空心菜基因型 4 个（Cd <0.71 mg/kg DW，NO_3^- <3100 mg/kg FW），分别是 *JXDY*、*GZQL*、*XGDB* 和 *B888*，可以在中轻度镉-硝酸盐复合污染土壤上安全生产。空心菜地上部镉与铅、锌含量呈正相关，而这 3 种元素均与磷量呈负相关。这些结果表明镉、铅和锌通过相同的途径被空心菜吸收，可以同时被治理。增加磷肥供应率可以抑制复合污染土壤中的镉和硝酸盐向空心菜可食部的转移。

关键词：基因型变异；重金属；硝酸盐；土壤污染；空心菜