Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) ISSN 1673-1581 (Print); ISSN 1862-1783 (Online) www.zju.edu.cn/jzus; www.springerlink.com E-mail: jzus@zju.edu.cn



Cadmium accumulation in different pakchoi cultivars and screening for pollution-safe cultivars^{*}

Ying CHEN^{1,2}, Ting-qiang LI^{†‡1}, Xuan HAN¹, Zhe-li DING¹, Xiao-e YANG¹, Ye-fei JIN²

(¹Ministry of Education Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China) (²College of Life Sciences, Shaoxing College of Arts and Sciences, Shaoxing 312000, China)

[†]E-mail: litq@zju.edu.cn

Received Nov. 28, 2011; Revision accepted Feb. 28, 2012; Crosschecked May 9, 2012

Abstract: The selection and breeding of pollution-safe cultivars (PSCs) is a practicable and cost-effective approach to minimize the influx of heavy metal to the human food chain. In this study, both pot-culture and field experiments were conducted to identify and screen out cadmium pollution-safe cultivars (Cd-PSCs) from 50 pakchoi (*Brassica rapa* L. ssp. *chinensis*) cultivars for food safety. When treated with 1.0 or 2.5 mg/kg Cd, most of the pakchoi cultivars (>70%) showed greater or similar shoot biomass when compared with the control. This result indicates that pakchoi has a considerable tolerance to soil Cd stress. Cd concentrations in the shoot varied significantly (P<0.05) between cultivars: in two Cd treatments (1.0 and 2.5 mg/kg), the average values were 0.074 and 0.175 mg/kg fresh weight (FW), respectively. Cd concentrations in the shoots of 14 pakchoi cultivars were lower than 0.05 mg/kg FW. In pot-culture experiments, both enrichment factors (EFs) and translocation factors (TFs) of six pakchoi cultivars were lower than 1.0. The field studies further confirmed that the Hangzhouyoudonger, Aijiaoheiye 333, and Zaoshenghuajing cultivars are Cd-PSCs, and are therefore suitable for growth in low Cd-contaminated soils (\leq 1.2 mg/kg) without any risk to food safety.

Key words:Cadmium pollution, Food safety, Pakchoi, Pollution-safe cultivarsdoi:10.1631/jzus.B1100356Document code: ACLC number: X173

1 Introduction

Soil contamination by heavy metals has gained considerable public attention because of their potential damage to human health and ecosystems even at low concentrations (Alloway, 1995). Cadmium (Cd) is one of the most toxic heavy metals in the environment due to its high mobility and severe toxicity to organisms (Li *et al.*, 2009). There are both natural and anthropogenic sources of Cd in soil, including shortor long-range mining, atmospheric deposition, irrigation water, utilization of fertilizers, and application of municipal sewage-wastes, compost sewage sludges, and manures (Das *et al.*, 1997; Arduini *et al.*, 2006; Singh *et al.*, 2006). In China, with the development of modern industry and agriculture, Cd has posed a serious problem for safe food production, and at least 13330 ha of farmland has been contaminated by varying levels of Cd (Liu *et al.*, 2009). It is therefore urgent that environmental and soil scientists to identify suitable approaches for reducing Cd accumulation in food crops.

There are several options available to remediate heavy metal contaminated soils. Conventional methods include soil excavation and landfill of the top contaminated soils ex situ; these methods are highly

[‡] Corresponding author

^{*} Project supported by the National High-Tech R & D Program (863) of China (No. 2009AA06Z316), the Science and Technology Commission of Zhejiang Province (No. 2008C33047), and the Fundamental Research Funds for the Central Universities, China

[©] Zhejiang University and Springer-Verlag Berlin Heidelberg 2012

effective, but too expensive due to the high costs involved in the disposal of the contaminated soil, transportation, and backfill of the original site with clean soil (Zhou and Song, 2004; Zhu et al., 2004). Phytoremediation has been widely considered as a cost-effective approach to remediate metal/metalloid contaminated soils, and considerable progress has been made in this area in recent years (Zhu et al., 2004). Phytoextraction, the use of green plants to clean up contaminated soil, has attracted attention as an environmentally friendly and low-input remediation technique (Chaney et al., 1997; McGrath and Zhao, 2003). This technology makes the use of hyperaccumulator plants that extract pollutants from the soil and accumulate them in their harvestable above-ground biomass. However, the practical utility of this technology is limited due to the low biomass of hyperaccumulators. The demand for food in China is constantly increasing due to the expansion of the population, thus farmers cannot afford to fallow agricultural soils for remediation, especially where soils are only slightly or moderately contaminated with pollutants. It is therefore urgent to find a new way to minimize the influx of heavy metal pollutants into the human food chain to reduce potential health risks (Yu et al., 2006).

Earlier results showed that the uptake and translocation of trace elements in plants vary greatly not only among plant species but also among cultivars of the same species (Zhang et al., 2002; Stolt et al., 2006; Guo et al., 2011). Crop species and cultivars differ in their genetic tendency to take up trace elements, and this fact provides new opportunities to minimize harmful elements in the food chain by selecting and breeding crops for low uptake potential (Grant et al., 2008). Based on this fact and earlier reports, Yu et al. (2006) proposed the concept of pollution-safe cultivars (PSCs), i.e., cultivars in which edible parts accumulate specific pollutants at a level low enough for safe consumption even when grown in contaminated soil. In recent years, as an alternative choice, selection and breeding of PSCs has attracted much more attention and interest (Grant et al., 2008). Many researchers have placed greater emphasis on producing Cd-PSCs of several grain crops (Clarke et al., 2006; Chen et al., 2007; Zeng et al., 2008). However, little work has been done on selection of Cd-PSC vegetables.

Most of the Cd (more than 70%) uptake by humans comes from vegetables (Ryan et al., 1982). It is therefore very important to minimize Cd accumulation in different parts of vegetables. Pakchoi (Brassica rapa L. ssp. chinensis), a cruciferous leafy vegetable, is widely planted in China, especially in southern China; identification of pakchoi genotypes with low Cd accumulation for food safety is therefore especially important. Variations in Cd concentration among cultivars have been reported in some vegetable species, including carrot (Harrison, 1986), cucumber (Harrison and Staub, 1986), lettuce (Thomas and Harrison, 1991), potato (Dunbar et al., 2003), and Chinese cabbage (Liu et al., 2009; 2010). However, to our knowledge, there is limited information on the screening of Cd-PSCs in pakchoi. Thus, pot-culture and field experiments were conducted with the objectives to (1) investigate genotypic differences in uptake and accumulation of Cd among 50 pakchoi cultivars, and to (2) identify and screen out Cd-PSCs among 50 pakchoi cultivars.

2 Materials and methods

2.1 Soil characterization

The soil used in the pot experiment was collected from the surface layer (0–20 cm) of the experimental station in the farm of Zhejiang University, Hangzhou, Zhejiang Province, China. The field experiment was conducted after the pot experiment on a farm in Cixi, Zhejiang Province, China. The site was slightly contaminated due to application of municipal sewagewastes. Basic physical and chemical properties of the soil were analyzed according to the routine analytical methods of agricultural chemistry in soil (Lu, 1999). Selected properties of the test soils are listed in Table 1.

2.2 Experimental design

2.2.1 Pot experiment

Surface layers (0–20 cm deep) of cultivated soils were air-dried, ground, and sieved to pass through a 4-mm mesh for pot experiment use. The dimensions of the pot were 180 mm×150 mm (diameter×height). Each pot contained 2.5 kg of soil samples. There were three treatments: CK (control), T1 (Cd 1.0 mg/kg) and T2 (Cd 2.5 mg/kg). To simulate field conditions, the

| Soil property | Pot-culture | Field-culture |
|---|-------------|---------------|
| Son property | soil | soil |
| pН | 6.64 | 6.15 |
| Organic matter (g/kg) | 17.5 | 13.8 |
| Total N (g/kg) | 1.02 | 1.25 |
| Available P (mg/kg) | 12.8 | 13.6 |
| Available K (mg/kg) | 78.6 | 91.6 |
| Total Cd (mg/kg) | 0.26 | 1.20 |
| Cation exchange capacity (CEC) (cmol/kg) | 12.0 | 14.4 |
| Particle composition (g/kg) | | |
| Sand (2.000-0.020 mm) | 450 | 390 |
| Silt (0.020-0.002 mm) | 340 | 347 |
| Clay (≤0.002 mm) | 210 | 263 |

 Table 1 Selected physiochemical characteristics of the two test soils

soils were equilibrated for one month after spiked with Cd. The seeds of 50 pakchoi cultivars were collected from seed companies from the Yangtze River and Pear River delta of China, and the names and sources of the selected 50 cultivars are listed in Table 2. Healthy seeds were surface sterilized with 1% (w/w) sodium hypochlorite (NaOCl) for 20 min, washed several times with distilled water, and then germinated for 24 h at 28 °C in the dark; uniformly germinated seeds (10 seeds per pot) were then sown in soil in April 2008. Two weeks after sowing, the seedlings were subsequently thinned to five plants. These pots were arranged in a randomized complete block design with four replicates for each treatment. The plants were allowed to grow for eight weeks under open field conditions and no fertilizers were applied. Soil moisture content was adjusted to 50% of the water-holding capacity by watering to weight weekly with deionized water. At the end of experiment, five plants were harvested and each plant was separated into root and shoot parts. These parts were washed thoroughly with tap water and rinsed with distilled water. The fresh weight (FW) was recorded. The samples were then dried at 70 °C for 72 h. Dry weights (DWs) of shoots and roots were recorded. Dry plant samples were ground using a stainless steel mill and passed through a 60 mesh sieve prior to Cd analysis.

2.2.2 Field experiment

According to the standards for the identification of Cd-PSCs put forward by Liu *et al.* (2009) with some modification, six pakchoi cultivars with low Cd accumulation were selected, and were further tested in a field experiment. Randomized block design with four replications was used in the field experiment. Each block was 24 m^2 (6 m long by 4 m wide). Seeds were sowed directly into the soil in September 2008. The management of water and fertilizer was the same as the pot experiment. The plants were allowed to grow for eight weeks. At the end of experiment, 50 plants were randomly sampled from each block. The pretreatment of the plants was the same as described in the pot experiment.

2.3 Chemical analysis

Plants were digested with a mixture of concentrated HNO₃ and HClO₄ (5:1, v/v) in polytetrafluoroethylene (PTFE) vessels. The digest was transferred to a 50-ml volumetric flask, filled to 50 ml, and filtered. Metal concentrations in the digest were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES; iCAP 6000, Thermo Scientific, Cambridge, UK). Soil samples were collected from both pots and field blocks after the plants were harvested, thoroughly mixed, air-dried in the laboratory, ground up using a mortar and pestle, and passed through a 100 mesh sieve. Total Cd in the soil was analyzed by digestion with concentrated HNO₃, HClO₄, and HF (5:1:1, v/v/v), and Cd concentration in the solution was determine by ICP-AES. Enrichment factor (EF) is calculated as the ratio of Cd concentration in the shoots to the Cd concentration in the soil, and it was used to evaluate the ability of pakchoi to accumulate Cd. The translocation factor (TF) was used to evaluate the capacity of pakchoi to translocate Cd from roots to shoots. It was calculated as the ratio of Cd concentration in the shoots of a plant to the Cd concentration in its roots (Baker and Whiting, 2002). The National Standard of the People's Republic of China (GB 2762-2005) (MHC, 2005) was used to evaluate the safety of pakchoi grown in Cd-contaminated soils. The maximum permissible concentration (MPC) of Cd for vegetables is 0.05 mg/kg FW (Yu et al., 2006; Liu et al., 2010).

2.4 Statistical methods

All data were statistically analyzed using the SPSS package (Version 11.0, SPSS Inc., Chicago, IL, USA). The data were analyzed with a two-way analysis

of variance (ANOVA). Cd accumulation values are expressed as means \pm standard deviation (SD) of the four replicates (*P*<0.05).

 Table 2 Names and sources of the selected 50 cultivars

| No. | Name | Source |
|-----------|--------------------------------|-----------------------------------|
| 1 | Jinguanqingjiang | Foshan, China |
| 2 | Siyueman | Hangzhou, China |
| 3 | Xizilv | Hangzhou, China |
| 4 | Gaogengbai | Hongkong, China |
| 5 | Zhouveheivoudonger | Shaoxing, China |
| 6 | Aihuangtou | Shaoxing, China |
| 7 | Yangzhouging | Yangzhou China |
| 8 | Oingvou 4 | Naniing China |
| 9 | Qingjou 1 Oingijang 456 | Shantou China |
| 10 | Viheviahao | Taiwan China |
| 11 | Sijigingijang | Shantou China |
| 12 | Zaoshenghuaiing | Janan |
| 12 | Aikangging | Hangzhou, China |
| 13 | Shanghajajag | Shanghai China |
| 14 | Uangahawaudangar | Jiangahay China |
| 15 | Hangzhouyoudonger | Rangzhou, China |
| 10 | Tuqing I | Shanghai, China |
| 1/ | Taiwanmingznu | Taiwan, China |
| 18 | Changgeng | Hangzhou, China |
| 19 | Teaiqing | Hangzhou, China |
| 20 | Lvlinghuo | Nanjing, China |
| 21 | Suzhouqing | Suzhou, China |
| 22 | Xiawangqing | Japan |
| 23 | Lifengqinggeng | Japan |
| 24 | Huaqing | Wuhan, China |
| 25 | Huahuangqinggeng | Japan |
| 26 | Qibao | Shanghai, China |
| 27 | Gaohuaqinggeng | Hongkong, China |
| 28 | Shanghaiaikang | Shanghai, China |
| 29 | Aijiaoheiye 333 | Guangzhou, China |
| 30 | Kexing | Mianyang, China |
| 31 | Datouqingjiang | Taiwan, China |
| 32 | Aijiaodatou | Guangzhou, China |
| 33 | Kangre 605 | Japan |
| 34 | Aijisuzhouqing | Suzhou, China |
| 35 | Jinxiawang | Shantou, China |
| 36 | Fubaokangre | Nantong, China |
| 37 | Sijiging | Shantou, China |
| 38 | Hangzhoubai | Hangzhou, China |
| 39 | Heidatou | Shanghai, China |
| 40 | Kangre 805 | Nantong China |
| 41 | Canbaicai | Hangzhou China |
| 42 | Huangiinhai | Chengdu China |
| 43 | Shanghaixiaging | Shanghai China |
| 44 | Ribenlyguan | Janan |
| <u> </u> | Wuvueman | Hangzhou China |
| т.) Д6 | Taiwanainaviu | Taiwan China |
| -10 17 | I wangingsiu | Vanazhou, China |
| 4/ /0 | Lyving | Naniing China |
| 4ð 40 | LVXIIIg Vongringler a sin a | Nanjing, Unina Vongehau, China |
| 49 | rangzirekangqing | Yangznou, China |
| 50 | Lvx1u 91-1 | Qingdao, China |

3 Results

3.1 Shoot biomass

After eight weeks of growth under different Cd levels, large differences in shoot biomass among cultivars were observed (Fig. 1). Shoot biomasses of 50 cultivars ranged from 19.8 to 70.1 g/pot for T1 and from 20.1 to 67.7 g/pot for T2. Compared with the control, the shoot biomasses of 32 cultivars under Cd treatments (T1 or T2) were not significantly different, indicating that these cultivars have high tolerance to Cd toxicity. For 13 other cultivars, however, the shoot biomass decreased significantly (P<0.05), especially for plants under the T2 treatment. In contrast, the shoot biomasses of the Aihuangtou, Yihexiabao, Changgeng, Shanghaiaikang and Kexing cultivars increased significantly during the eight weeks of growth in the T2 treatment, as compared with the control.

3.2 Cd accumulation in pakchoi in pot condition

The variation of shoot Cd concentrations among 50 pakchoi cultivars in pots was significant (P < 0.05) (Fig. 2). Under the control treatment, the Cd concentrations in shoots ranged from 0.022 to 0.055 mg/kg FW, and only four pakchoi cultivars exceeded 0.050 mg/kg FW, indicating that most of these pakchoi cultivars are PSCs when grown in noncontaminated soil. However, with the increase of Cd concentrations in the soil, the Cd accumulation in the shoots of pakchoi cultivars increased gradually. Under the T1 treatment, shoot Cd concentrations of 36 pakchoi cultivars were greater than 0.050 mg/kg FW, and exceeded MPC of Cd in vegetables. Using this standard, the 14 pakchoi cultivars that fell below the MPC of Cd were identified as Cd-PSCs; however, the shoot biomass of the Jinxiawang cultivar decreased significantly under Cd treatments, and thus 13 pakchoi cultivars were selected as the Cd-PSCs (Table 3). Under the T2 treatment, shoot Cd concentrations in all pakchoi samples except for Aijiaoheiye 333 exceeded 0.050 mg/kg FW (Table 3).

3.3 Enrichment and translocation factors of 13 selected pakchoi cultivars

EF and TF factors are two important indexes to evaluate a plant's ability to uptake and transport trace elements, respectively. Both EF and TF showed great

| Cultiver | Shoot Cd concentration (mg/kg FW)" | | |
|--------------------|------------------------------------|---------------------|-------------------|
| Cultival | Control | Cd 1.0 mg/kg | Cd 2.5 mg/kg |
| Aijiaoheiye 333 | 0.012 ± 0.001 | 0.036 ± 0.004 | 0.050 ± 0.012 |
| Aijisuzhouqing | 0.013±0.003 | 0.046 ± 0.005 | 0.119 ± 0.010 |
| Hangzhouyoudonger | 0.013±0.002 | 0.036 ± 0.006 | 0.095 ± 0.011 |
| Qingyou 4 | 0.019 ± 0.001 | $0.041 {\pm} 0.007$ | 0.111 ± 0.011 |
| Changgeng | 0.020 ± 0.003 | 0.049 ± 0.006 | 0.124 ± 0.018 |
| Zaoshenghuajing | 0.020 ± 0.004 | $0.041 {\pm} 0.007$ | 0.138±0.013 |
| Zhouyeheiyoudonger | 0.021±0.003 | $0.048 {\pm} 0.008$ | 0.199±0.011 |
| Lifengqinggeng | 0.022 ± 0.002 | 0.042 ± 0.006 | 0.137±0.012 |
| Jinguanqingjiang | 0.024 ± 0.002 | $0.040{\pm}0.007$ | 0.151±0.011 |
| Xiawangqing | 0.025 ± 0.003 | 0.042 ± 0.008 | 0.138±0.013 |
| Sijiqingjiang | 0.027±0.001 | 0.050 ± 0.005 | 0.178 ± 0.011 |
| Gaohuaqinggeng | 0.027 ± 0.003 | 0.040 ± 0.007 | 0.168±0.013 |
| Shanghaiqing | 0.030±0.005 | 0.047 ± 0.004 | 0.258±0.016 |

Table 3 Cd concentrations in the shoots of 13 selected pakchoi cultivars under three Cd treatments

^a Data are expressed as mean \pm SD (*n*=4)



Fig. 1 Shoot biomasses of 50 pakchoi cultivars under three Cd treatments



Fig. 2 Cd concentrations in the shoots of 50 pakchoi cultivars under three Cd treatments

variability among the 13 selected pakchoi cultivars, and the variability changed with Cd level in the soil (Fig. 3). The lowest EF under the three Cd treatments was found in Aijiaoheiye 333. In all treatments, the EFs of 10 cultivars of the selected 13 pakchoi cultivars were less than 1.0. These cultivars were Aijiaoheiye 333, Aijisuzhouqing, Hangzhouyoudonger, Qingyou 4, Changgeng, Zaoshenghuajing, Zhouyeheiyoudonger, Lifengqinggeng, Jinguanqingjiang, and Xiawangqing (Fig. 3a). The lowest TF was also found in Aijiaoheiye 333 (Fig. 3b); however, only seven pakchoi cultivars had TF less than 1.0 under the three Cd treatments. These cultivars were Aijiaoheiye 333, Aijisuzhouqing, Hangzhouyoudonger,



Fig. 3 Enrichment factor (EF) (a) and translocation factor (TF) (b) of the selected 13 pakchoi cultivars under three Cd treatments

Zaoshenghuajing, Zhouyeheiyoudonger, Jinguanqingjiang, and Sijiqingjiang (Fig. 3b). Thus six pakchoi cultivars, i.e., Aijiaoheiye 333, Aijisuzhouqing, Hangzhouyoudonger, Zaoshenghuajing, Zhouyeheiyoudonger and Jinguanqingjiang, with both EF and TF less than 1.0 were selected as PSCs and were then planted in field condition to confirm their low Cd accumulation potential.

3.4 Cd accumulation in pakchoi in field condition

Shoot Cd concentrations showed great variability among the six selected cultivars, and ranged from 0.039 to 1.080 mg/kg FW (Fig. 4). Cd concentrations in the shoots of Aijiaoheiye 333, Aijisuzhouqing, Hangzhouyoudonger, and Zaoshenghuajing were lower than 0.050 mg/kg, which is similar to the results from the pot-culture experiment. For Jinguanqingjiang and Zhouyeheiyoudonger, however, shoot Cd concentrations were 0.096 and 0.108 mg/kg, which exceeded the MPC of Cd in vegetables. The EFs in the six selected cultivars were all lower than 1.0; however, the TFs in Aijisuzhouqing, Jinguanqingjiang, and Zhouyeheiyoudonger were greater than 1.0. Therefore, only Aijiaoheiye 333, Hangzhouyoudonger, and Zaoshenghuajing were selected as Cd-PSCs based on the results of the field experiment.



Fig. 4 Cd concentrations, translocation factor (TF), and enrichment factor (EF) in six pakchoi cultivars in the field experiment

4 Discussion

Under various treatments of Cd, all cultivars grew normally without showing any toxic symptoms. For most of the tested cultivars (70%), no difference was recorded in the shoot biomass, as compared with the control; this result indicates that they had considerable tolerance to Cd. The stimulating effect of Cd on the growth of shoots occurred at Cd rates of 2.5 mg/kg for five pakchoi cultivars (Fig. 1). Various mechanisms have been suggested to explain the stimulatory effect, and one of the explanations is that metal ions may serve as activators of enzyme(s) in cytokinin metabolism, which accelerates the growth of plants (Kaminek, 1992; Nyitrai et al., 2004; Liu et al., 2010). Secondly, low dose stress may cause changes in plant hormones and cytokinins, which regulate plant growth and development. Similar findings were reported in previous studies for a broad range of species (Chapman, 2002; Yu et al., 2006). Accordingly, farmers may not receive sufficiently early warning of Cd pollution or toxic concentrations of Cd in vegetables based on biomass change alone (Yu et al., 2006). In many developing countries, many agricultural lands have been polluted with Cd, and most of these contaminated lands are being used for agricultural production. Therefore, the selection and breeding of Cd-PSCs is a feasible and effective approach to reduce the influx of Cd to the human food chain, especially in slightly and moderately contaminated agriculture soils (Liu et al., 2010).

The uptake and accumulation of trace elements in plants vary greatly, not only among plant species but also among cultivars within the same species (Zhang et al., 2002; Stolt et al., 2006; Grant et al., 2008). Under the same Cd level, shoot Cd concentrations showed great variability among 50 tested pakchoi cultivars, and similar findings were reported on rice (Liu et al., 2003a; 2003b; Yu et al., 2006), wheat (Stolt et al., 2006), maize (Kurz et al., 1999), asparagus beans (Zhu et al., 2007), and Chinese cabbage (Liu et al., 2009; 2010). These results demonstrate that genetic variability for Cd accumulation exists within a species and this fact makes it possible to select and breed Cd-PSCs. In recent years, as an alternative choice, selection and breeding of PSCs has attracted much more attention and interest (Kurz et al., 1999; Chen et al., 2007; Zeng et al., 2008), and great success has been achieved on sunflower (Li et al., 1997; Miller et al., 2006), rice (He et al., 2006; Liu et al., 2007), durum wheat (Clarke et al., 2005; 2006), barley (Chen et al., 2007), maize (Kurz et al., 1999), and soybean (Arao et al., 2003). However, only a few studies have been done on selecting Cd-PSCs vegetables. Liu et al. (2009; 2010) identified Lvxing 70, New Beijing 3, and Fengyuanxin 3 as Cd-excluder

genotypes from 40 Chinese cabbage genotypes. A screening standard is the basis for the screening of Cd-PSCs. However, there is not a unified screening standard up to now. Liu et al. (2009) recommended four standards for the identification of Cd-PSCs. Based on the literature and our research, we applied the following four criteria to select Cd-PSCs for food safety: (1) Cd concentration in the edible parts should be lower than the MPC; (2) EF<1.0; (3) TF<1.0; and (4) shoot biomass was not decreased significantly when growing in contaminated soils. In the pot experiment, when Cd concentration in shoots <0.050 mg/kg was used as a criterion, 14 pakchoi cultivars were selected as Cd-PSCs under the treatment of 1.0 mg/kg. When shoot biomass was taken into account, 13 pakchoi cultivars were selected as Cd-PSCs (Table 3). When EF<1.0 was used as a criterion, 10 cultivars could be judged as Cd-PSCs (Fig. 3a). When TF<1.0 was also used, only 6 pakchoi cultivars were regarded as Cd-PSCs (Fig. 3b). When tested in the field experiment, only Hangzhouyoudonger, Aijiaoheiye 333, and Zaoshenghuajing were regarded as Cd-PSCs. However, under the treatment of 2.5 mg/kg Cd, only Aijiaoheiye 333 could be considered as a Cd-PSC based on the shoot Cd concentration requirement of <0.050 mg/kg FW (Table 3). This result indicated that the screening of Cd-PSC is highly dependent on the level of soil Cd (Stolt et al., 2006). Overall, Hangzhouyoudonger, Aijiaoheiye 333, Zaoshenghuajing were selected as Cd-PSCs by both pot-culture experiment and field experiment, and these three pakchoi cultivars are therefore safe to consumers if they are planted in low Cd ($\leq 1.2 \text{ mg/kg}$) contaminated soils.

Although an increasing number of studies demonstrate that cultivar selection can be effective in decreasing Cd concentration in crops, there are still obstacles to utilize this approach to produce low-Cd cultivars (Grant *et al.*, 2008). Cd concentrations in plant tissues varied with Cd levels in soils, crop genotypes, and environmental factors. In this study, 98% (49/50) of the pakchoi cultivars had shoot Cd concentration >0.050 mg/kg when the soil Cd concentration was 2.5 mg/kg (Table 3); this result indicated that the selection of Cd-PSCs was not suitable in heavily Cd-contaminated soils. Physicochemical properties of the soils, such as cation exchange capacity (CEC), clay minerals and hydrous metal oxides, pH and buffering capacity, organic matter and moisture, can alter the phytoavailability of heavy metals in soil and thus affect Cd accumulation in crops. Further studies are therefore required to completely understand genotypic and soil properties, and their interaction mechanisms, to reduce Cd accumulation in pakchoi.

5 Conclusions

In this study, more than 70% of the tested cultivars produced greater or similar shoot biomass under Cd treatments; this result indicates that pakchoi has a considerable tolerance to soil Cd stress. Cd concentrations in the shoot varied significantly (P<0.05) between cultivars under two Cd treatments. Four parameters, i.e., Cd contents, EF, TF, and shoot biomass, were applied to select Cd-PSCs for food safety, and six pakchoi cultivars were selected as PSCs in the pot-culture experiment. Hangzhouyoudonger, Aijiaoheiye 333, and Zaoshenghuajing were finally identified as Cd-PSCs in the field culture experiment; these three pakchoi cultivars are safe to consumers if they are planted in low Cd (\leq 1.2 mg/kg) contaminated soils.

References

- Alloway, B.J., 1995. Heavy Metals in Soils. Blackie Academic & Professional, London, p.38-57.
- Arao, T., Ae, N., Sugiyama, M., Takahashi, M., 2003. Genotypic differences in cadmium uptake and distribution in soybeans. *Plant Soil*, **251**(2):247-253. [doi:10.1023/A: 1023079819086]
- Arduini, I., Ercoli, L., Mariotti, M., Masoni, A., 2006. Response of miscanthus to toxic cadmium applications during the period of maximum growth. *Environ. Exp. Bot.*, 55(1-2):29-40. [doi:10.1016/j.envexpbot.2004.09.009]
- Baker, A.J.M., Whiting, S.N., 2002. In search of the holy grail—a further step in understanding metal hyperaccumulation? *New Phytol.*, **155**(1):1-4. [doi:10.1046/j.1469-8137.2002.00449_1.x]
- Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Brewer, E.P., Angle, J.S., Baker, A.J.M., 1997. Phytoremediation of soil metals. *Curr. Opin. Biotech.*, 8(3):279-284. [doi:10.1016/S0958-1669(97)80004-3]
- Chapman, P.M., 2002. Ecological risk assessment (ERA) and hormesis. *Sci. Total Environ.*, **288**(1-2):131-140. [doi:10.1016/S0048-9697(01)01120-2]
- Chen, F., Dong, J., Wang, F., Wu, F.B., Zhang, G.P., Li, G.M., Chen, Z.F., Chen, J.X., Wei, K., 2007. Identification of barley genotypes with low grain Cd accumulation and its

interaction with four microelements. *Chemosphere*, **67**(10):2082-2088. [doi:10.1016/j.chemosphere.2006.10. 014]

- Clarke, J.M., McCaig, T.N., DePauw, R.M., Knox, R.E., Clarke, F.R., Fernandez, M.R., 2005. Strongfield durum wheat. *Can. J. Plant Sci.*, **85**(3):651-654. [doi:10.4141/ P04-119]
- Clarke, J.M., McCaig, T.N., DePauw, R.M., Knox, R.E., Clarke, F.R., Fernandez, M.R., 2006. Registration of 'Strongfield' durum wheat. *Crop Sci.*, 46(5):2306-2307. [doi:10.2135/cropsci2005.12.0454]
- Das, P., Samantaray, S., Rout, G.R., 1997. Studies on cadmium toxicity in plants: a review. *Environ. Pollut.*, **98**(1):29-36. [doi:10.1016/S0269-7491(97)00110-3]
- Dunbar, K.R., McLaughlin, M.J., Reid, R.J., 2003. The uptake and partitioning of cadmium in two cultivars of potato (*Solanum tuberosum* L.). J. Exp. Bot., 54(381):349-354.
- Grant, C.A., Clarke, J.M., Duguid, S., Chaney, R.L., 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. *Sci. Total Environ.*, **390**(2-3): 301-310. [doi:10.1016/j.scitotenv.2007.10.038]
- Guo, X.F., Wei, Z.B., Wu, Q.T., Qiu, J.R., Zhou, J.L., 2011. Cadmium and zinc accumulation in maizegrain as affected by cultivars and chemical fixation amendments. *Pedosphere*, **21**(5):650-656. [doi:10.1016/S1002-0160(11) 60167-7]
- Harrison, H.A., 1986. Carrot response to sludge application and bed type. J. Am. Soc. Hortic. Sci., 11(2):211-215.
- Harrison, H.A., Staub, J.E., 1986. Effects of sludge, bed, and genotype on cucumber growth and elemental concentration of fruit and peel. J. Am. Soc. Hortic. Sci., 11(2): 205-211.
- He, J.Y., Zhu, C., Ren, Y.F., Yan, Y.P., Jiang, D., 2006. Genotypic variation in grain cadmium concentration of lowland rice. J. Plant Nutr. Soil Sci., 169(5):711-716. [doi:10.1002/jpln.200525101]
- Kaminek, M., 1992. Progress in cytokinin research. *Trends Biotechnol.*, **10**:159-164. [doi:10.1016/0167-7799(92)902 04-9]
- Kurz, H., Schulz, R., Romheld, V., 1999. Selection of cultivars to reduce the concentration of cadmium and thallium in food and fodder plants. *J. Plant Nutr. Soil Sci.*, 162(3):323-328. [doi:10.1002/(SICI)1522-2624(199906) 162:3<323::AID-JPLN323>3.0.CO;2-M]
- Li, T.Q., Yang, X.E., Lu, L.L., Islam, E., He, Z.L., 2009. Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions. *J. Hazard. Mater.*, 169(1-3):734-741. [doi:10.1016/j.jhazmat.2009.04.004]
- Li, Y.M., Chaney, R.L., Schneiter, A.A., Miller, J.F., Elias, E.M., Hammond, J.J., 1997. Screening for low grain cadmium phenotypes in sunflower, durum wheat and flax. *Euphytica*, 94(1):23-30. [doi:10.1023/A:10029 96405463]
- Liu, J.G., Liang, J.S., Li, K.Q., Zhang, Z.J., Yu, B.Y., Lu, X.L., Yang, J.C., Zhu, Q.S., 2003a. Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium

stress. *Chemosphere*, **52**(9):1467-1473. [doi:10.1016/ S0045-6535(03)00484-3]

- Liu, J.G., Li, K.Q., Xu, J.K., Liang, J.S., Lu, X.L., Yang, J.C., Zhu, Q.S., 2003b. Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. *Field Crop Res.*, 83(3):271-281. [doi:10.1016/S0378-4290(03)00077-7]
- Liu, J.G., Qian, M., Cai, G.L., Yang, J.C., Zhu, Q.S., 2007. Uptake and translocation of Cd in different rice cultivars and the relation with Cd accumulation in rice grain. J. *Hazard. Mater.*, **143**(1-2):443-447. [doi:10.1016/j.jhazmat. 2006.09.057]
- Liu, W.T., Zhou, Q.X., Sun, Y.B., Liu, R., 2009. Identification of Chinese cabbage genotypes with low cadmium accumulation for food safety. *Environ. Pollut.*, **157**(6): 1961-1967. [doi:10.1016/j.envpol.2009.01.005]
- Liu, W.T., Zhou, Q.X., An, J., Sun, Y.B., Liu, R., 2010. Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars. J. *Hazard. Mater.*, **173**(1-3):737-743. [doi:10.1016/j.jhazmat. 2009.08.147]
- Lu, R.K., 1999. Analytical Methods for Soils and Agricultural Chemistry. China Agricultural Science and Technology Press, Beijing (in Chinese).
- McGrath, S.P., Zhao, F.J., 2003. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotech.*, 14(3):277-282. [doi:10.1016/S0958-1669(03)00060-0]
- Miller, J.F., Green, C.E., Li, Y.M., Chaney, R.L., 2006. Registration of three low cadmium (HA 448, HA 449, and RHA 450) confection sunflower genetic stocks. *Crop Sci.*, 46(1):489-490. [doi:10.2135/cropsci2005.04-0012]
- MHC (Ministry of Health of the People's Republic of China), 2005. GB 2762-2005: Maximum Levels of Contaminants in Foods. MHC, Beijing, China (in Chinese).
- Nyitrai, P., Bóka, K., Gáspár, L., Sárvári, É., Keresztes, Á., 2004. Rejuvenation of ageing bean leaves under the effect of low-dose stressors. *Plant Biol.*, 6(6):708-714. [doi:10.1055/s-2004-830385]
- Ryan, J.A., Pahren, H.R., Lucas, J.B., 1982. Controlling cadmium in the human chain: a review and rationale based on health effects. *Environ. Res.*, 28(2):251-302. [doi:10.1016/ 0013-9351(82)90128-1]

- Singh, S., Eapen, S., D'Souza, S.F., 2006. Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant, *Bacopa monnieri* L. *Chemosphere*, 62(2):233-246. [doi:10.1016/j.chemosphere. 2005.05.017]
- Stolt, P., Asp, H., Hultin, S., 2006. Genetic variation in wheat cadmium accumulation on soils with different cadmium concentrations. J. Agron. Crop Sci., 192(3):201-208. [doi:10.1111/j.1439-037X.2006.00202.x]
- Thomas, G.M., Harrison, H.C., 1991. Genetic line effects on parameters influencing cadmium concentration in lettuce (*Lactuca sativa* L.). J. Plant Nutr., 14(9):953-962. [doi:10.1080/01904169109364255]
- Yu, H., Wang, J.L., Fang, W., Yuan, J.G., Yang, Z.Y., 2006. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. *Sci. Total Environ.*, **370**(2-3):302-309. [doi:10.1016/j.scitotenv.2006. 06.013]
- Zeng, F.R., Mao, Y., Cheng, W.D., Wu, F.B., Zhang, G.P., 2008. Genotypic and environmental variation in chromium, cadmium and lead concentrations in rice. *Environ. Pollut.*, **153**(2):309-314. [doi:10.1016/j.envpol.2007.08.022]
- Zhang, G.P., Fukami, M., Sekimoto, H., 2002. Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Res.*, 77(2-3):93-98. [doi:10.1016/S0378-4290(02)00061-8]
- Zhou, Q.X., Song, Y.F., 2004. Principles, Methods of Contaminated Soil Remediation. Science Press, Beijing (in Chinese).
- Zhu, Y., Yu, H., Wang, J.L., Fang, W., Yuan, J.G., Yang, Z.Y., 2007. Heavy metal accumulation of 24 asparagus bean cultivars grown in soil contaminated with Cd alone and with multiple metals (Cd, Pb, and Zn). *J. Agric. Food Chem.*, **55**(3):1045-1052. [doi:10.1021/jf062971p]
- Zhu, Y.G., Chen, S.B., Yang, J.C., 2004. Effects of soil amendments on lead uptake by two vegetable crops from a lead-contaminated soil from Anhui, China. *Environ. Int.* **30**(3):351-356. [doi:10.1016/j.envint.2003.07.001]

502