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RESEARCH ARTICLE

Low root/shoot (R/S) biomass ratio can be an indicator of low cadmium accumulation in the shoot of Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis var. utilis* Tsen et Lee) cultivars

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Abstract

Chinese flowering cabbage is a commonly consumed vegetable that accumulates Cd easily from Cd-contaminated soils. Cultivations of low-Cd cultivars are promising strategies for food safety, but low-Cd-accumulating mechanisms are not fully elucidated. To address this issue, 37 cultivars were screened to identify high- and low-Cd cultivars upon exposure to sewage-irrigated garden soil pretreated with different Cd concentrations (1.81, 2.90, and 3.70 mg kg^{-1}dry soil). The results showed that shoot Cd concentrations differed among the cultivars by maximum degrees of 2.67-, 3.71-, and 3.00-fold under control and treatments, respectively. Soil-pot trial and hydroponic trial found no significant difference in Cd and Ca mobilization, uptake, and transport ability by root per weight between high- and low-Cd cultivars. Interestingly, a stable R/S ratio difference among cultivars ($p < 0.01$) was observed, and the cultivar variation of Cd accumulation in shoots was mainly dependent on their R/S ratios. R/S ratio was also statistically positively associated with Cd and Ca accumulation in high- and low-Cd cultivars ($p < 0.05$), both in soil and hydroponics culture. This was mainly due to the lower root biomass of low-Cd cultivars resulted in lower total release of root exudates, lower total Cd and Ca mobilization in rhizosphere soil, and lower total Cd and Ca uptake and transport. The higher shoot biomass of low-Cd cultivars also has dilution effects on Cd concentration in shoot. Overall, low R/S ratio may be regarded as a direct and efficient indicator of low Cd accumulation in the shoot of Chinese flowering cabbage. These findings provided the possibilities to screening low-Cd cultivars using their R/S ratio.

Keywords Low-Cd cultivars · R/S biomass ratio · Cd mobilization · Cd uptake and translocation · Ca channel

Introduction

Cadmium (Cd)-contaminated cropland is a serious concern because of the high health risk associated with soil-to-food chain transfer (Bian et al. 2014; Dahlin et al. 2016; Dar et al. 2017; Lin et al. 2016). Selection and cultivation of crop cultivars with genetic tendency to accumulate low Cd concentrations in their edible parts are currently proposed as a viable solution to reduce the risk of soil Cd to the human food chain (Wang et al. 2009). This approach has been successfully applied to rice (*Oryza sativa* L.) (Wang et al. 2011; Zeng et al. 2008), barley (*Hordeum vulgare* L.) (Chen et al. 2007), carrot (*Daucus carota* L.) (Harrison 1986), potato (*Solanum tuberosum* L.) (Dunbar et al. 2003), and some *Brassia* species (Liu et al. 2010; Qiu et al. 2011a). However, some of the mechanisms contributing to low accumulation of Cd in cultivars have yet to be fully understood.

The Cd content in aboveground plant tissues is influenced by the ability of roots to mobilize Cd in rhizosphere soil. Greger and Landberg (2008), He et al. (2015), and Xu et al. (2017) found low Cd concentrations in the rhizosphere solution

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considerably contributed to low Cd accumulation in shoot of wheat (*Triticum aestivum* L.) and amaranth (*Amaranthus mangostanus* L.) cultivars. Variable metal concentrations in rhizosphere solutions among cultivars for a specific soil are further attributed to metal mobilization by rhizosphere exudates. As reported on water spinach (*Ipomoea aquatica* Forsk) by He et al. (2015) and on rice by Liu et al. (2007), dissolved organic carbon (DOC) and organic acids in rhizosphere soil were significantly lower in low-Cd cultivar than in high-Cd cultivar. Chiang et al. (2006) found significant positive correlations between Cd contents in tobacco (*Nicotiana tabacum* L.) and sunflower (*Helianthus annuus* L.) and organic exudates contents in rhizosphere soils. Therefore, low secretion levels of root exudates may be one of the crucial factors contributing to low Cd accumulation in crop cultivars.

Apart from rhizosphere effects, different uptake and transfer mechanisms for soil Cd may also result in variable Cd concentrations in crop cultivars. Cellular compartmentation of Cd and formation of insoluble Cd (e.g., phosphate precipitation and pectate-bound forms) further hindered Cd translocation from roots to shoots, and thus resulted in low accumulation of shoot Cd (Xu et al. 2017; Ye et al. 2012). On the other hand, differential expressions of Cd transporter genes (e.g., ZIP and HMA families) may influence the ability of Cd across the plasmalemma (Ueno et al. 2011). Zhou et al. (2016) confirmed that the gene *YSL1* responsible for Cd uptake across the plasmalemma were less expressed in low-Cd cultivars of pakchoi compared with high-Cd cultivars under Cd stress. Sasaki et al. (2012) showed that knockout of gene *Nramp5* in the plasmalemma of paddy rice significantly delayed the transport of Cd and Mn from the external solution to root cells. The aforementioned mechanisms are all likely to contribute to the low Cd accumulation. However, whether another mechanism may contribute to the variability in Cd accumulation levels among cultivars has remained unclear.

The root/shoot (R/S) biomass ratio is one of the important parameters used to characterize carbohydrate allocation between shoots and roots (Lambers et al. 2006). The ratio can influence not only the water, nitrogen, and phosphorus uptake by roots from rhizosphere soil (Cheng et al. 2011; Dai et al. 2005; Walk et al. 2006) but also the metals uptake. Greater accumulation of metals (i.e., Ni, Zn, and Mn) in the shoots was found to correlate with higher R/S ratio in two contrasting genotypes of hyperaccumulator *Thlaspi goesingense* (*Thlaspi arvense* L.) (Kramer et al. 1997) and transgenic thale cress (*Arabidopsis thaliana* L.) and tobacco (*Nicotiana tabacum* L.) (Werner et al. 2010). Namely, higher R/S ratio may be one of the key drivers for heavy metal accumulation in plants. Additionally, biotic and abiotic factors can change R/S ratio, including growth habit, agronomic trait, soil properties, and topographical factors. Allard et al. (2013) observed that R/S ratios in wheat (*Triticum aestivum* L.) were influenced by crop tillering dynamics and N nutritional status. Similar results have been reported in quite a few researches (Sun et al. 2017; Veresoglou et al. 2012; Yang et al. 2018). Although previous studies did demonstrate that R/S ratio may be changed by internal and external factors, little attention has been paid to whether the difference in R/S ratio among the contrasting cultivars was relatively steady. For instance, R/S ratios in three sweet stevia cultivars (*Stevia rebaudiana* (Bertoni) Hemsl.) increased along with the increase of salinity stress; however, the difference of R/S values among these three cultivars had remained the same (Sheng et al. 2010). Our preliminary study also observed a relatively stable difference in R/S ratio between cultivars of Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis var. utilis* Tsen et Lee) grown in either the soil or hydroponic medium.

Chinese flowering cabbage is a vegetable commonly planted in Southeast Asia (Wu et al. 2008), with relatively strong ability to uptake Cd (Qiu et al. 2011b; Wang et al. 2007). We suppose that the relatively stable difference in R/S ratio among the cultivars of Chinese flowering cabbage will cause the difference of Cd uptake and accumulation in their shoots. Since Cd, a nonessential metal, is believed to be primarily absorbed through Ca^{2+}channels (Welch et al. 1999), we also speculate that Cd and Ca accumulation is likely to be influenced by a "common factor," which may be the R/S ratio critically involved in the regulation and control of soil Cd and Ca mobilization, uptake, and transport in Chinese flowering cabbage cultivars. If these hypotheses are correct, the patterns of soil Cd and Ca mobilization, uptake, and transport by roots should be consistent with each other. The present study was conducted to verify this hypothesis by (1) investigating the variation in the shoot Cd concentrations of different Chinese flowering cabbage cultivars and identifying contrasting cultivars; (2) determining the relationship between R/S ratio and the aforementioned Cd mobilization in rhizosphere soil, uptake, and translocation in contrasting cultivars; and (3) further confirming the role of R/S ratio in Cd accumulation by identifying the correlation between Cd and Ca. The expected results may be used as a specific marker identifying the Cd-accumulating levels in such vegetables.

Materials and methods

First soil-pot trial: measurement of biomass, Cd and Ca in 37 cultivars

Thirty-seven Chinese flowering cabbage cultivars (*Brassica campestris* L. ssp. *chinensis var. utilis* Tsen et Lee.), which are widely cultivated in China, were selected for comparing the variability of Cd concentrations in shoots of different cultivars. The names and providers of tested seeds are listed in Table S1. The potting soil was collected from a sewage-irrigated vegetable field in the suburb of Guangzhou, China (113° 23′ E/23° 07′ N). The

field was irrigated with wastewater 20 years ago and now contains abundant heavy metals. Prior to the experiment, the main soil parameters were measured with routine analytical methods widely used in agricultural soil testing (Lu 1999). The physicochemical properties of the soil are described in Table S2. The total Cd concentration (1.81 mg kg^{-1} dry soil) in soil exceeded the limit (0.30 mg kg^{-1}) set by the Farm Land Environmental Quality Evaluation Standard for Edible Agricultural Products (HJT 332–2006, China).

The soil-pot trial was set up in a greenhouse at Jinan University (120° 58′ E/23° 58′ N). The Cd-contaminated garden soil served as treatment (T_0) without addition of Cd. Two other Cd treatments (T_1 and T_2) were prepared by mixing T_0 soil with Cd $(NO_3)_2{\cdot}4H_2O$ solution. The two Cd treatments were established based on the Cd levels in polluted soil of Guangdong Province, China (Liu et al. 2007), an area renowned for Chinese flowering cabbage cultivation. The soil was watered and incubated outdoors under a waterproof tarpaulin for 4 months. For T_1 and T_2 treatments, the total Cd concentrations in soil were 2.9 and 3.7 mg kg^{-1}, respectively, at the end of incubation.

Soaked seeds were sown in 555 black PVC pots (37 cultivars × 3 treatments × 5 replicates) after soil addition (2.5 kg pot^{-1}). A plastic dish was placed at the bottom of each pot to collect potential leachate during the experiment. The culture conditions were as follows: 12 h photoperiod, 25 °C light/18 °C dark cycle, and 70–75% relative humidity. The plants were harvested for Cd and Ca analysis after 50 days of seed germination (Yoon et al. 2006). The biomass of dried samples was recorded and the samples were ground to powder for the digestion (Wei et al. 2005). Concentrations of Cd and Ca in the digested solutions were determined using a Shimadzu AA-7000 atomic absorption spectrometer. A certified bush leaf reference material (GBW07603, China) was used to monitor metal recovery from the plant samples. Cd and Ca recoveries were all around 90 ± 10%. Tolerance index (TI) of Cd, a modified version of that proposed by Sun et al. (2009) and Wei and Zhou (2008), were calculated by

$$\mathrm{TI}\% = (\text{biomass in Cd treatment}/\text{biomass in control}) \times 100 \quad (1)$$

Enrichment factor (EF) and translocation factor (TF) were calculated as follows (Chen et al. 2004):

$$\mathrm{EF} = \mathrm{shoot_M}/\mathrm{soil_M} \quad (2)$$

$$\mathrm{TF} = \mathrm{shoot_M}/\mathrm{root_M} \quad (3)$$

where M is the metal (Cd or Ca), $\mathrm{soil_M}$, $\mathrm{root_M}$, and $\mathrm{shoot_M}$ are the concentration of M in the soil (mg kg^{-1}DW), the root (mg kg^{-1}DW), and the shoot (mg kg^{-1}DW), respectively.

Second soil-pot trial: comparison of soil Cd and Ca mobilization, uptake, and transfer by roots between high- and low-Cd cultivars

The second soil-pot trial was conducted in the aforementioned greenhouse. The potting soil was collected from the same sewage-irrigated vegetable field mentioned above and not mixed with extra Cd during subsequent processing (T_0 treatment; 1.81 mg Cd kg^{-1} dry soil). Available Cd concentration is only 0.25 mg kg^{-1} in soil. The level of Cd treatment in previous study is no negative effect on their biomasses (Liao et al. 2010; Mei et al. 2014). A blank control group (unpolluted soil; 0.21 mg Cd kg^{-1} dry soil) was also designed in order to reconfirm that Cd treatment had no obvious toxicity to plant biomass. The unpolluted soil was collected from non-polluted areas near the field, and its physicochemical properties were listed in Table S2. Plant samples grown in unpolluted soil were used only for biomass determination. Based on their Cd-accumulating abilities found in the first soil-pot trial, three low-Cd cultivars ('*Jiudian*50', '*Meixinjianye*', and '*Youxin*3') and three high-Cd cultivars ('*Guangte*49', '*Ao*100', and '*Xin*701') were selected for the soil-pot trial. They were randomly selected from the lowest-Cd or highest-Cd cultivars, respectively. The seeds were sown into a 300-mesh nylon rhizobag (300 g soil $rhizobag^{-1}$). Then, 108 nylon rhizobags were used during the trial (6 cultivars × 3 replicates × 3 rhizobags × 2 soil types (sewage-irrigated soil and unpolluted soil). A total of 1600 g soil was then placed around the rhizobags, resulting in total soil amount of 2.5 kg in each pot. Following 50 days of seed germination, plant samples were harvested, dried, and weighed. Soil samples were only collected from the rhizosphere of the six cultivars grown in sewage-irrigated vegetable soil. Soil samples collected from rhizobags were considered as rhizosphere soil (He et al. 2015).

The harvested roots were removed the adsorbed metals in root surface using 10 mM EDTA-$(NH_4)_2$ following the method of Mei et al. (2014). Processed samples were oven-dried and digested for determination of Cd and Ca concentrations. The collected fresh soil samples were centrifuged at 4000×*g* for 5 min to separate rhizosphere soil solution. Total Cd and Ca concentrations in the collected soil solution were determined. The dissolved organic carbon (DOC) concentration was determined by high-temperature catalytic oxidation (Shimadzu TOC-V_{CSH}, Japan) (Cauwet 1994). Total soluble sugar concentration was calculated using the color reaction of anthranone (Irigoyen et al. 1992). Concentrations of organic acids were determined via anion chromatograph (Dionex ICS-900, USA). Concentrations of total free amino acids were measured by color reaction of ninhydrin (Rosen 1957). To compare the abilities of soil Cd and Ca mobilization, uptake,

and transfer among cultivars, the following indices were calculated:

Muptake by root per weight

$$= (\text{shoot}_M \times \text{shoot}_b + \text{root}_M \times \text{root}_b)/\text{root}_b \quad (4)$$

$$\text{Mtransfer by root per weight} = \text{shoot}_M \times \text{shoot}_b/\text{root}_b \quad (5)$$

Soil M mobilization by root per weight

$$= (\text{total}_M + \text{rhizo}_M \times \text{RMC})/\text{root}_b \quad (6)$$

$$\text{Root/shoot (R/S) biomass ratio} = \text{root}_b/\text{shoot}_b \quad (7)$$

where M is the metal (Cd or Ca), root_b is the biomass of dry root (g), shoot_b is the biomass of dry shoot (g), total_M is the M amount in whole plant (mg), rhizo_M is the M concentration in rhizosphere solution (mg L^{-1}), and RMC is the rhizosphere moisture content (L).

First hydroponic trial: comparison of Cd and Ca uptake and transfer by roots between high- and low-Cd cultivars

A hydroponic trial was implemented in the aforementioned greenhouse. Six cultivars in second soil-pot trial were also selected for the hydroponic trial. Seedlings with consistent growth of only four leaves were selected, washed with deionized water, and placed in 36 (6 cultivars × 3 replicates × 2 hydroponics (with and without Cd)) black PVC pots containing 1.5 L of Hoagland solution with 2 μmol L^{-1}Cd $(NO_3)_2$ for 50 days of culture. Similarity, another blank control group (hydroponics without Cd) was also designed in order to reconfirm that Cd treatment was no negative effect on their biomasses. Plant samples grown in hydroponics without Cd were used only for biomass determination. The environmental conditions and processing steps of the harvested root and shoot samples were in accordance with second soil-pot trial. The dried samples were weighed and digested for determination of Cd and Ca concentrations. To compare the abilities for Cd and Ca uptake and transfer among cultivars when grown in hydroponics with added Cd, the indices were similarly calculated using the aforementioned formulas (4, 5, and 7).

Second hydroponic trial with an ion channel blocker: determination of root Cd and Ca concentrations

The second hydroponic trial with an ion channel blocker, namely $LaCl_3$, was implemented in the aforementioned greenhouse. Two low-Cd cultivars (*Youlvcutai* and *Jianyezaolv*) and two high-Cd cultivars (*Dongbai*60 and *Xinkangre*) were chosen for the hydroponic trial. They were randomly selected from the lowest-Cd or highest-Cd cultivars, respectively. Seedlings were placed in 32 (4 cultivars × 2 treatments × 4 replicates) black PVC pots for 50 days of culture. The plants were harvested after 8-h exposure to the ion channel blocker and Cd treatment (2 μmol L^{-1}Cd $(NO_3)_2$ + 2 mmol L^{-1} $LaCl_3$). Blank experiments in the solution without La^{3+} were also performed in parallel and served as controls.

Plant roots were cleaned with deionized water, cut into 1.5 cm long pieces, and then immersed in 10 mM EDTA-NH_4 and sonicated for 10 min (Liu et al. 2011). The supernatant solution (desorptive solution) was diluted to 50 mL with deionized water to determine Cd and Ca concentrations. Desorbed root samples were oven-dried and digested for determination of Cd and Ca concentrations.

Data analysis

Data were analyzed using SPSS 11.5. The data of plant biomass was described using their dry weight (DW). Cd concentration in plant was expressed on a plant dry weight basis (mg kg^{-1} DW). Cd and exudates concentrations in rhizosphere soil solution were expressed on a soil dry weight basis (mg kg^{-1}/μg kg^{-1} DW). Differences among treatments and cultivars were analyzed using one-way ANOVA (independent sample *t* test) and two-way ANOVA.

Results and discussion

Tolerance indices and enrichment factors of Cd in 37 cultivars

The tolerance indexes (TIs) of 20 cultivars upon T_1 and/or T_2 treatments were greater than 100%, with 7 cultivars possessing TIs greater than 150% (Fig. 1). These findings indicated that these seven cultivars exhibited higher tolerance to Cd stress than other cultivars. The TIs of the other 17 cultivars were as low as 50%, suggesting poor tolerance to Cd stress. Similar positive and negative responses of biomass to Cd stress were also observed in 40 cultivars of Chinese cabbage (*Brassica campestris* L. ssp. *pekinensi*) and 24 cultivars of asparagus bean (*Vigna unguiculata subsp. Sesquipedalis* L.) (Liu et al. 2010; Zhu et al. 2007).

The shoot Cd concentrations and enrichment factors (EFs) of 37 cultivars are presented respectively in Table 1 and Fig. S1. Upon T_0 treatment, Cd concentrations in all tested cultivars did not exceed the national standards of China (Cd ≤ 0.2 mg kg^{-1} fresh weight for leaf vegetable; GB 2762–2012). Upon T_1 and T_2 treatments, 37 cultivars had shoot Cd concentrations higher than the maximum allowable level of the standard. The results indicated that all tested cultivars could be considered as Cd-safe cultivars under the treatment with 1.81 mg Cd kg^{-1} dry soil (sewage-irrigated garden soil), but might trigger serious health risks when Cd concentrations in the soil reached 2.90 and 3.70 mg kg^{-1} dry soil. Likewise, Liu et al. (2010) reported that

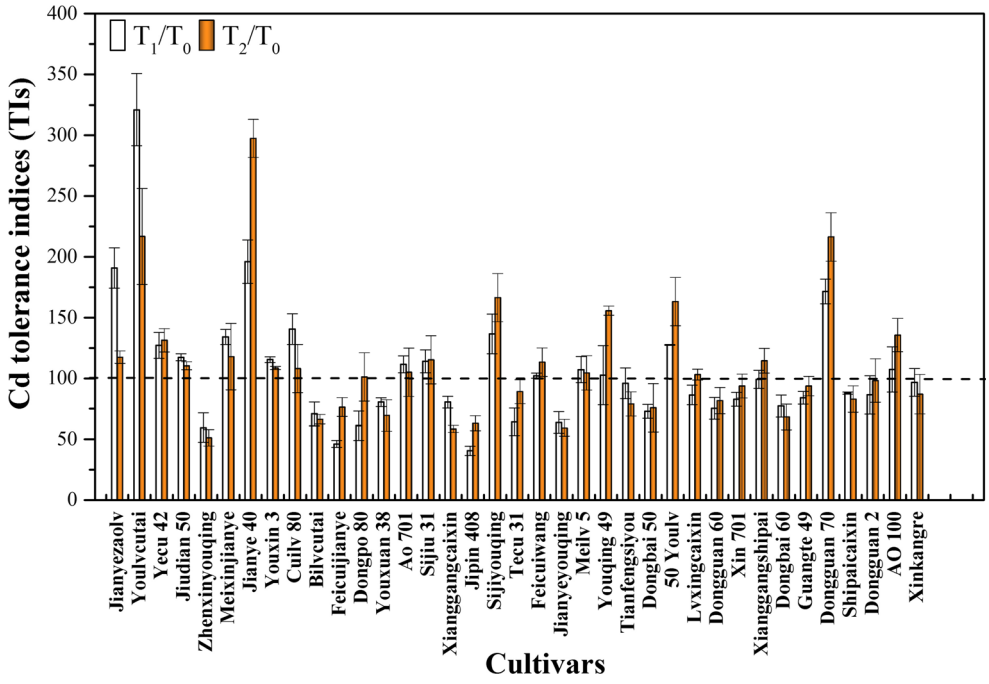

Fig. 1 Cd tolerance indices (TIs, shoot biomass% of control) of 37 Chinese flowering cabbage cultivars when grown in contaminated soil with different Cd treatments. Values are means (± SD; $n = 5$)

no Cd-safe Chinese cabbage cultivars were found under 2.5 and 5.0 mg Cd kg^{-1} dry soil scenarios. Significant variation ($p < 0.01$) was observed among the Cd concentrations in the 37 tested cultivars exposed to Cd stress, which was consistent with the result of a study on water spinach (He et al. 2015). The average EFs of 37 cultivars increased with the increasing soil Cd concentration (Fig. S1). Under T_0, T_1, and T_2 treatments, the EF values were in the ranges of 0.33–0.87, 1.15–4.25, and

Table 1 Cd concentrations (mg kg^{-1} DW) in aboveground tissues (shoot) of 37 Chinese flowering cabbage cultivars when grown in contaminated soil with different Cd treatments

Cultivar	Cd treatments			Cultivar	Cd treatments		
	T_0	T_1	T_2		T_0	T_1	T_2
Jianyezaolv	0.63 ± 0.11	4.02 ± 0.07	8.18 ± 0.38	*Feicuiwang*	1.16 ± 0.02	7.39 ± 0.13	12.8 ± 0.33
Youlvcutai	0.91 ± 0.01	4.09 ± 0.14	10.3 ± 0.29	*Jianyeyouqing*	0.96 ± 0.02	7.77 ± 0.21	13.0 ± 0.15
*Yecu*42	0.74 ± 0.03	5.08 ± 0.13	9.75 ± 0.29	*Meilv*5	1.18 ± 0.05	7.84 ± 0.14	11.3 ± 0.27
*Jiudian*50	0.92 ± 0.03	5.51 ± 0.11	10.4 ± 0.57	*Youqing*49	1.09 ± 0.03	8.32 ± 0.20	18.7 ± 0.30
Zhenxinyouqing	1.08 ± 0.02	5.55 ± 0.20	10.9 ± 0.26	*Tianfengsiyou*	1.41 ± 0.04	8.52 ± 0.22	15.4 ± 0.36
Meixinjianye	0.86 ± 0.02	5.87 ± 0.08	9.46 ± 0.31	*Dongbai*50	1.43 ± 0.06	8.68 ± 0.20	16.9 ± 0.43
*Jianye*40	0.96 ± 0.02	5.95 ± 0.18	9.52 ± 0.19	50*Youlv*	1.27 ± 0.03	8.71 ± 0.17	18.9 ± 0.45
*Youxin*3	0.94 ± 0.04	6.11 ± 0.19	10.6 ± 0.31	*Lvxingcaixin*	1.57 ± 0.06	8.82 ± 0.18	14.9 ± 0.22
*Cuilv*80	1.04 ± 0.03	6.14 ± 0.16	10.8 ± 0.40	*Dongguan*60	1.37 ± 0.03	9.18 ± 0.23	16.3 ± 0.19
Bilvcutai	0.88 ± 0.05	6.21 ± 0.13	9.82 ± 0.27	*Xin*701	1.29 ± 0.05	9.82 ± 0.24	17.1 ± 0.32
Feicuijianye	1.13 ± 0.03	6.23 ± 0.17	12.0 ± 0.40	*Xianggangshipai*	1.37 ± 0.02	9.90 ± 0.26	21.4 ± 0.36
Dongpo80	1.11 ± 0.07	6.34 ± 0.13	12.8 ± 0.41	*Dongbai*60	1.60 ± 0.07	9.97 ± 0.15	19.4 ± 0.28
*Youxuan*38	1.10 ± 0.04	6.43 ± 0.20	12.1 ± 0.50	*Guangte*49	1.32 ± 0.05	10.1 ± 0.28	21.1 ± 0.32
*Ao*701	1.12 ± 0.06	6.47 ± 0.22	10.4 ± 0.35	*Dongguan*70	1.56 ± 0.03	10.2 ± 0.23	22.8 ± 0.42
*Sijiu*31	1.05 ± 0.03	6.92 ± 0.17	11.9 ± 0.34	*Shipaicaixin*	1.28 ± 0.05	11.9 ± 0.34	17.4 ± 0.34
Xianggangcaixin	1.03 ± 0.04	6.99 ± 0.15	13.5 ± 0.42	*Dongguan*2	1.23 ± 0.05	11.3 ± 0.23	19.5 ± 0.39
*Jipin*408	1.25 ± 0.08	7.18 ± 0.24	12.6 ± 0.26	*AO*100	1.48 ± 0.06	12.4 ± 0.29	22.3 ± 0.36
Sijiyouqing	1.25 ± 0.02	7.24 ± 0.19	12.0 ± 0.32	*Xinkangre*	1.68 ± 0.08	14.9 ± 0.20	24.5 ± 0.41
*Tecu*31	1.20 ± 0.03	7.26 ± 0.13	14.5 ± 0.35				
Means of all tested cultivars	1.17 ± 0.24	7.92 ± 1.15	14.4 ± 0.35				
ANOVA *F* ratio							
Cd	**	**	**				
Cultivar	**	**	**				
Cultivar × Cd	**	**	**				

Asterisk ** means significant effect of each factor on the indicator at $p < 0.01$ by two-way ANOVA tests. Values are means (± SD; $n = 5$)

1.82–5.46, with average values of 0.67, 2.57, and 3.85, respectively. Cultivar '*Jianyezaolv*' and '*Xinkangre*' had the lowest (0.33–1.82) and highest (0.87–5.46) EFs, respectively, in the three Cd treatments.

Overall, cultivars with low ('*Jiudian*50', '*Meixinjianye*', '*Youxin*3', '*Jianyezaolv*', and '*Youlvcutai*') and high ('*Xin*701', '*Guangte*49', '*Ao*100', '*Dongbai*60', and '*Xinkangre*') Cd-accumulating abilities were identified. They were selected mainly on the basis of their Cd-accumulating abilities found in the first soil-pot trial. Furthermore, compared with T_0 treatment, shoot biomasses of most cultivars with high Cd concentrations decreased under T_1 and T_2 treatments, whereas most cultivars with low Cd concentrations increased their shoot biomasses. Among the 17 cultivars with high tolerance indices (TIs ≧ 100%), almost 11 cultivars had a low or medium Cd concentration in shoots (Fig. 1). In general, with increased soil Cd stress, most low-Cd cultivars exhibited stronger Cd tolerance than higher-Cd cultivars. Hence, selection and breeding of Cd-safe cultivars based on the TIs may be a direct and effective control strategy in reducing the potential risk of Cd entering into the human food chain.

Stability of cultivar difference in R/S biomass ratio

In our preliminary experiment, no significant differences ($p > 0.05$) were observed in R/S ratio of Chinese flowering cabbage cultivars between unpolluted soil (0.21 mg Cd kg^{-1} dry soil) and sewage-irrigated garden soil (1.81 mg Cd kg^{-1} dry soil) (Table S3). A relatively stable difference ($p < 0.05$) in R/S ratio was found between the identified low- and high-Cd Chinese flowering cabbage cultivars grown in either the unpolluted soil or sewage-irrigated garden soil, which showed a significantly lower R/S ratio in low-Cd cultivars in contrast with high-Cd cultivars ($p < 0.05$; Table S3). Similar results also were observed in hydroponics without and with Cd (2 μmol L^{-1}Cd $(NO_3)_2$) (Table S4). In the present study, the R/S ratio in low-Cd cultivars (average 0.33) was still significantly lower than high-Cd cultivars (average 0.54) when grown in Cd-contaminated soil ($p < 0.01$; Table 3). Analogously, a lower R/S ratio was also observed in low-Cd cultivar (average 0.37) than high-Cd cultivar (average 0.52) when grown in hydroponics with Cd. ($p < 0.01$; Table 4). These results indicated that cultivar difference in R/S ratio of Chinese flowering cabbage was relatively stable and might be even largely depending on its genetic factors. Therefore, the variation of the R/S ratio in Chinese flowering cabbage cultivars might potentially have a substantial link with Cd-accumulating ability and could be used for investigating the mechanism of cultivar difference in Cd-accumulating ability.

Roles of root biomass in release of root exudates and their effects on soil Cd mobilization

In the second soil-pot trial, the average contents of Cd, DOC, lactic acid, formic acid, amino acids, and soluble sugar in the rhizosphere soil solutions from low-Cd cultivars were 0.78-, 0.69-, 0.52-, 0.88-, 0.46-, and 0.82-fold of those from high-Cd cultivars, respectively ($p < 0.05$; Table 2). Furthermore, significant positive correlations ($p < 0.05$) were found between Cd and DOC, as well as amino acids and soluble sugar in the rhizosphere soil solution (Table 2). These results suggested that secretion levels of organic matters potentially influenced

Table 2 Cd, DOC, lactic acid, formic acid, amino acid, and soluble sugar concentrations in rhizosphere soil solution of high- and low-Cd cultivars when grown in Cd-contaminated soil

Cultivar	Cd (μg kg^{-1}DW)	DOC (mg kg^{-1}DW)	Lactic acid (mg kg^{-1}DW)	Formic acid (mg kg^{-1}DW)	Amino acids (μg kg^{-1}DW)	Soluble sugar (μg kg^{-1}DW)
L–*Jiudian*50	0.82 ± 0.01	48.98 ± 0.96	10.89 ± 0.18	0.64 ± 0.02	0.58 ± 0.03	8.87 ± 0.09
L–*Meixinjianye*	0.90 ± 0.03	45.39 ± 5.26	9.84 ± 0.49	3.47 ± 0.09	0.64 ± 0.03	8.96 ± 0.21
L–*Youxin*3	0.82 ± 0.01	52.80 ± 2.81	13.82 ± 0.17	2.97 ± 0.14	0.69 ± 0.02	8.49 ± 0.10
L–average	0.84 ± 0.04	49.06 ± 4.41	11.52 ± 1.80	2.36 ± 1.31	0.63 ± 0.05	8.77 ± 0.25
H–*Xin*701	0.97 ± 0.08	70.39 ± 3.77	22.80 ± 0.08	3.53 ± 0.02	1.40 ± 0.02	10.78 ± 0.09
H–*Guangte*49	0.98 ± 0.01	76.67 ± 1.00	24.76 ± 0.20	2.81 ± 0.09	1.24 ± 0.03	11.12 ± 0.03
H–*A0*100	1.30 ± 0.02	63.69 ± 2.74	18.54 ± 1.49	1.71 ± 0.08	1.47 ± 0.03	10.29 ± 0.02
H–average	1.08 ± 0.16 **	70.25 ± 6.10 **	22.03 ± 2.85 **	2.69 ± 0.06 ns	1.37 ± 0.10 **	10.73 ± 0.37 **
L/H(fold)	0.78	0.69	0.52	0.88	0.46	0.82
Correlation analysis						
R^2 value		0.237	0.211	− 0.012	0.638	0.333
P value		0.040 < 0.05	0.055 > 0.05	0.671 > 0.05	0.000 < 0.01	0.012 < 0.05

H and L represent high-Cd and low-Cd cultivars, respectively. Asterisk **, *denote significant difference between the average values of low- and high-Cd cultivars at the 0.01 and 0.05 level, respectively. The "ns" means no significant. The same below. *P* values denote correlative relationship between Cd and root exudates contents (DOC, lactic acid, formic acid, amino acids, and soluble sugar, respectively) in the rhizosphere solution. *P* values < 0.01 and 0.05 were significance at the 0.01and 0.05 level, respectively. Values are means (± SD; $n = 3$)

Cd mobility in rhizosphere soil. As a principal component in root exudates, DOC modifies Cd speciation through formation of Cd-organics complexes (Hu et al. 2011; Xin et al. 2015b), thereby influencing Cd bioavailability in rhizosphere soil of high- and low-Cd cultivars. High concentration of DOC (e.g., organic acids and amino acids) in high-Cd cultivars could result in low rhizosphere soil pH, possibly due to the occurrence of carboxyl and phenolic OH functional groups. Rhizosphere acidification further promotes the release of insoluble Cd from the solid phase to the rhizosphere solution (Chen and Zhu 2006; Dessureaultrompré et al. 2010; Yanai et al. 2006). This explained why high-Cd cultivars had significantly higher Cd concentration in the rhizosphere solutions (0.97–1.30; average 1.08 μg kg^{-1} dry soil) than low-Cd cultivars (0.82–0.90; average 0.85 μg kg^{-1}dry soil) ($p < 0.05$; Table 2). Similar results have also been observed for edible amaranth cultivars (*Amaranthus mangostanus* L.) (Xu et al. 2017) and *Sedum alfredii* (Xin et al. 2013). In the present study, Cd concentrations in rhizosphere soil solution were found to be positively correlated ($p < 0.05$) with root and shoot Cd concentrations in high- and low-Cd cultivars (Tables 2 and 3), which was consistent with observations in *Brassicanapus* (*Brassica napus* L.) by Selvam and Wong 2009 Taken together, these results implied that Cd accumulation in plant was considerably determined by the ability of the root system to mobilize insoluble soil Cd, and that root exudates played crucial roles in the mobilization process. Hence, lower soil Cd mobilization in the rhizosphere soil from low-Cd cultivars partially contributed to low Cd accumulation in shoots.

Interestingly, no significant difference was observed in the root Cd mobilization ability by root per weight between high- (average 12.35 mg kg^{-1} DW) and low-Cd (average 12.41 mg kg^{-1} DW) cultivars ($p > 0.05$; Table 3). However, many researches did demonstrate that root Cd mobilization ability can directly influence the Cd concentration in the rhizosphere soil solutions (Guo et al. 2018; Xu et al. 2017). That is, cultivar difference in Cd concentration of the rhizosphere soil solutions was dependent on the amount/weight (biomass) of roots actually involved in the release of root exudates. In the present study, a lower root biomass was also observed in low-Cd cultivar (average 0.48) than high-Cd cultivar (average 0.66) ($p < 0.05$; Table 3). The lower root biomass of low-Cd cultivars resulted in their lower total release of root exudates and their lower total Cd mobilization in rhizosphere soil.

Roles of R/S biomass ratio in Cd uptake and translocation by plant roots

In the second soil-pot trial, Cd concentrations in root and shoot, and translocation factors (root-to-shoot) of low-Cd

Table 3 Soil Cd and Ca mobilization, uptake, and transfer by root per weight, R/S ratio, and Cd and Ca translocation factor in high- and low-Cd cultivars when grown in Cd-contaminated soil

Cultivars	L–*Jiudian*50	L–*Meixinjianye*	L–*Youxin*3	L–average	H–*Xin*701	H–*Guangte*49	H–*A0*100	H–average	L/H (fold)
Root biomass (g $plant^{-1}$ DW)	0.56 ± 0.03	0.44 ± 0.01	0.43 ± 0.01	0.48 ± 0.06	0.89 ± 0.02	0.56 ± 0.01	0.52 ± 0.03	0.66 ± 0.17*	0.73
Shoot biomass (g $plant^{-1}$ DW)	1.58 ± 0.02	1.20 ± 0.03	1.62 ± 0.02	1.47 ± 0.14	1.43 ± 0.03	1.28 ± 0.02	0.93 ± 0.01	1.21 ± 0.10*	1.22
R / S biomass ratio	0.36 ± 0.02	0.37 ± 0.01	0.27 ± 0.01	0.33 ± 0.05	0.62 ± 0.00	0.44 ± 0.01	0.56 ± 0.02	0.54 ± 0.08**	0.61
Root Cd concentration (mg kg^{-1} DW)	1.13 ± 0.02	1.07 ± 0.02	1.13 ± 0.02	1.11 ± 0.04	1.26 ± 0.01	1.36 ± 0.02	1.36 ± 0.00	1.33 ± 0.05**	0.83
Shoot Cd concentration (mg kg^{-1} DW)	0.83 ± 0.02	0.76 ± 0.01	0.91 ± 0.02	0.83 ± 0.07	1.27 ± 0.01	1.44 ± 0.01	1.52 ± 0.01	1.41 ± 0.11**	0.59
Cd translocation factor (TF)	0.73 ± 0.02	0.71 ± 0.01	0.81 ± 0.01	0.75 ± 0.04	1.01 ± 0.01	1.06 ± 0.01	1.12 ± 0.01	1.06 ± 0.05**	0.71
Soil Cd mobilization by root per weight (mg kg^{-1} DW)	10.40 ± 0.03	12.92 ± 0.03	13.72 ± 0.04	12.35 ± 1.62	8.47 ± 0.03	12.97 ± 0.02	15.81 ± 0.04	12.41 ± 3.61 ns	1.00
Cd uptake by root per weight (mg kg^{-1} DW)	3.49 ± 0.19	3.14 ± 0.08	4.56 ± 0.16	3.74 ± 0.66	3.31 ± 0.03	4.64 ± 0.12	4.08 ± 0.14	4.01 ± 0.59 ns	0.93
Cd transfer by root per weight (mg kg^{-1} DW)	2.35 ± 0.17	2.07 ± 0.08	3.43 ± 0.14	2.62 ± 0.63	2.04 ± 0.02	3.28 ± 0.10	2.72 ± 0.14	2.68 ± 0.54 ns	0.98
Root Ca concentration (g kg^{-1} DW)	4.43 ± 0.03	4.53 ± 0.09	4.41 ± 0.08	4.46 ± 0.08	5.09 ± 0.07	5.17 ± 0.05	5.42 ± 0.12	5.22 ± 0.17**	0.85
Shoot Ca concentration (g kg^{-1} DW)	17.52 ± 0.96	20.22 ± 0.12	19.01 ± 0.10	18.91 ± 1.17	21.67 ± 0.09	31.79 ± 0.11	31.54 ± 0.24	28.33 ± 5.00**	0.67
Ca translocation factor (TF)	3.95 ± 0.01	4.46 ± 0.09	4.31 ± 0.09	4.24 ± 0.23	4.26 ± 0.06	6.15 ± 0.05	5.82 ± 0.01	5.41 ± 0.09**	0.78
Soil Ca mobilization by root per weight (g kg^{-1} DW)	188 ± 6.70	230 ± 4.80	264 ± 1.92	227 ± 36.05	173 ± 3.10	239 ± 2.80	364 ± 1.80	259 ± 94.56 ns	0.88
Ca uptake by root per weight (g kg^{-1} DW)	53.93 ± 2.56	59.54 ± 1.86	75.90 ± 2.16	63.23 ± 10.03	40.03 ± 0.29	77.67 ± 1.79	61.77 ± 2.33	59.82 ± 16.43 ns	1.06
Ca transfer by root per weight (g kg^{-1} DW)	49.50 ± 2.57	55.31 ± 1.95	71.49 ± 2.23	58.77 ± 10.06	34.95 ± 0.31	72.50 ± 1.83	56.35 ± 2.42	54.60 ± 16.39 ns	1.08

Values are means (± SD; $n = 3$)

cultivars were 0.83-, 0.59-, and 0.71-fold of those in high-Cd cultivars, respectively ($p < 0.05$; Table 3). In the first hydroponic trial, Cd concentrations in root and shoot, and translocation factors of low-Cd cultivars were 0.88-, 0.77-, and 0.88-fold of those in high-Cd cultivars, respectively ($p < 0.05$; Table 4). The two trials confirmed that the difference in Cd accumulation between previously identified high-Cd ('*Xin*701', '*Guangte*49', and '*A0*100') and low-Cd cultivars ('*Jiudian*50', '*Meixinjianye*', and '*Youxin*3') were repeatable and stable. These results indicated that the low-Cd cultivars absorbed less Cd through the roots than the high-Cd cultivars, and less Cd was transferred to aboveground parts (shoots) via the xylem or/and phloem. Many studies reported that xylem loading was an important transport process resulting in difference of shoot Cd accumulation (Clemens et al. 2002; Uraguchi et al. 2009). Xin et al. (2015a) speculated that cultivar difference in shoot Cd accumulation of hot pepper (*Capsicum annuum* L.) was most likely connected with the expression levels of Cd transporter(s). However, in the present study, no significant difference was observed in root Cd uptake and transport ability by root per weight between high- and low-Cd cultivars ($p > 0.05$; Tables 3 and 4). Thus, cultivar difference in Cd accumulation might not depend on the expression levels of Cd transporter(s).

In the second soil-pot trial, the average root biomasses of low-Cd cultivars (0.48 g) grown in Cd-contaminated soil were only 0.73-fold of those of high-Cd cultivars (0.66 g), but the average shoot biomasses of low-Cd cultivars (1.47 g) were 1.22-fold of those of high-Cd cultivars (1.21 g) (Table 3). In the first hydroponic trial, the average root biomasses of low-Cd cultivars (0.33 g) grown in hydroponics with Cd were 0.79-fold of those of high-Cd cultivars (0.42 g), and the average shoot biomasses of low-Cd cultivars (0.89 g) were 1.10-fold of those of high-Cd cultivars (0.81 g) (Table 4). Therefore, the amount/weight (biomass) of roots actually involved in the uptake and transfer of Cd may be the critical impact factor of shoot Cd accumulation. High shoot biomass diluted more Cd and further reduced Cd concentration in shoots. Vymazal (2016) also expounded that concentration was not enough to evaluate accumulation of heavy metals in plants, but must take biomass into account. It has been sufficiently reported that plant biomass will depend on Cd concentration in the roots and shoots of plants exposed to Cd (biomass is a consequence of Cd concentration). Usually, Cd

Table 4 Cd and Ca uptake and transfer by root per weight, R/S ratio, and Cd and Ca translocation factor in high- and low-Cd cultivars when grown in hydroponics with Cd $(NO_3)_2$ added

Cultivars	L–*Jiudian*50	L–*Meixinjianye*	L–*Youxin*3	L–average	H–*Xin*701	H–*Guangte*49	H–*A0*100	H–average	L/H (fold)
Root biomass (g plant^{-1} DW)	0.38 ± 0.04	0.35 ± 0.01	0.27 ± 0.03	0.33 ± 0.05	0.40 ± 0.01	0.45 ± 0.03	0.40 ± 0.01	0.42 ± 0.03**	0.79
Shoot biomass (g plant^{-1} DW)	0.96 ± 0.01	0.91 ± 0.02	0.81 ± 0.01	0.89 ± 0.07	0.80 ± 0.02	0.93 ± 0.01	0.71 ± 0.01	0.81 ± 0.01ns	1.10
R / S biomass ratio	0.40 ± 0.04	0.38 ± 0.00	0.33 ± 0.03	0.37 ± 0.04	0.49 ± 0.02	0.48 ± 0.03	0.57 ± 0.01	0.52 ± 0.04**	0.71
Root Cd concentration (mg kg^{-1} DW)	45.31 ± 1.00	46.57 ± 0.90	46.76 ± 1.30	46.21 ± 1.16	59.00 ± 0.03	47.33 ± 1.91	51.80 ± 1.30	52.71 ± 5.23**	0.88
Shoot Cd concentration (mg kg^{-1} DW)	15.71 ± 1.26	17.45 ± 0.25	15.15 ± 0.33	16.10 ± 0.61	22.39 ± 0.18	19.99 ± 0.51	20.67 ± 0.58	21.02 ± 1.14**	0.77
Cd translocation factor (TF)	0.35 ± 0.02	0.37 ± 0.00	0.32 ± 0.00	0.35 ± 0.02	0.38 ± 0.00	0.42 ± 0.01	0.40 ± 0.00	0.40 ± 0.02**	0.88
Cd uptake by root per weight (mg kg^{-1} DW)	85.21 ± 5.19	91.94 ± 1.25	92.55 ± 4.36	89.90 ± 4.93	104.37 ± 1.89	88.88 ± 2.57	88.74 ± 3.36	93.77 ± 8.29 ns	0.96
Cd transfer by root per weight (mg kg^{-1} DW)	39.90 ± 4.58	45.37 ± 0.35	45.79 ± 4.58	43.69 ± 4.32	45.36 ± 1.88	41.41 ± 2.46	36.41 ± 1.64	41.06 ± 4.26 ns	1.06
Root Ca concentration (g kg^{-1} DW)	3.61 ± 0.08	3.40 ± 0.02	3.23 ± 0.02	3.41 ± 0.17	4.91 ± 0.03	3.79 ± 0.04	4.95 ± 0.03	4.55 ± 0.57**	0.75
Shoot Ca concentration (g kg^{-1} DW)	22.78 ± 0.11	20.00 ± 0.10	18.68 ± 0.18	20.49 ± 1.82	27.24 ± 0.16	28.88 ± 0.12	26.99 ± 0.21	27.70 ± 0.90**	0.74
Ca translocation factor (TF)	6.31 ± 0.11	5.88 ± 0.03	5.78 ± 0.03	6.00 ± 0.25	5.54 ± 0.05	7.62 ± 0.05	5.46 ± 0.03	6.21 ± 1.06 ns	0.97
Ca uptake by root per weight (g kg^{-1} DW)	61.50 ± 5.53	55.41 ± 0.10	59.68 ± 5.76	58.86 ± 4.83	60.10 ± 2.16	63.63 ± 3.72	52.47 ± 1.31	58.73 ± 5.43 ns	1.00
Ca transfer by root per weight (g kg^{-1} DW)	57.89 ± 5.51	52.00 ± 0.12	56.45 ± 5.77	55.45 ± 4.79	55.19 ± 2.17	59.84 ± 3.69	47.53 ± 1.28	54.19 ± 5.83 ns	1.02

Values are means (± SD; $n = 3$)

concentration was higher in roots, so its biomass was lower and the relationship R/S was also lower. However, no significant differences ($p > 0.05$) in average biomass of roots and shoots were observed between unpolluted soil and sewage-irrigated garden soil (Table S3). Similar results ($p > 0.05$) also were observed in hydroponics with and without Cd (Table S4). Thus, the Cd level in this study had no significantly negative effect on their biomasses. Many literatures also reported that low level of Cd treatment had no obvious toxicity to their biomasses (Liao et al. 2010; Zong et al. 2007). Based on the results of the second soil-pot trials (Table 3 and S3) and the first hydroponic trials (Table 4 and S4), the R/S ratios of low-Cd cultivars were not significantly different between Cd-polluted soil and unpolluted soil, and between hydroponics cultures with and without Cd. Similar results were obtained in the R/S ratios of high-Cd cultivars. In all cultivating mediums, the R/S ratios of low-Cd cultivars were distinctly lower than those of high-Cd cultivars ($p < 0.01$). This indicated that difference of R/S ratios between high- and low-Cd cultivars was repeatable and depended on genetics, other than their cultivating medium. Therefore, R/S ratio could be considered as one of the key factors affecting cultivar variations in Cd accumulation in shoots of Chinese flowering cabbages. Xu et al. (2018) also reported that low R/S ratios may be one of the important factors influencing cultivar variations in terms of Cd, Zn, and Cu accumulation in tomato fruits (*Solanum lycopersicum* L.).

Previous researches have reported that several mechanisms might be involved in Cd cultivar variation, such as rhizosphere effects (Stritsis 2011), root morphology (Huang et al. 2015; Xia et al. 2016), transporter genes in cell membrane (Mendoza-Cózatl et al. 2011; Solti et al. 2011), and distribution characteristics within the organs (Wang et al. 2009). The control of cultivar-wide variation in Cd accumulation in shoots by R/S ratio could be a mechanism unique to Chinese flowering cabbage. Indeed, most of our previous study demonstrated that R/S ratio did not underlie the cultivar-wide variation in Cd accumulation in edible amaranth cultivars (*Amaranthus mangostanus* L.) (Mei et al. 2014) and water spinach cultivars (*Ipomoea aquatica* Forssk.) (He et al. 2015). For Chinese flowering cabbage, the lower Cd concentration in shoots of low-Cd cultivars can be attributed to its lower R/S ratio.

Roles of R/S ratio in Cd accumulation based on relationship between Cd and Ca in cultivars

In the first soil-pot trial, the average concentrations of shoot Ca in 37 cultivars was found to be significantly elevated in shoot tissues under soil Cd treatment compared to those with T_0 treatment ($p < 0.05$; Fig. S2), probably due to Cd exchangeable competition for soil Ca adsorption sites. In general, shoot Ca concentrations were lower in most low-Cd cultivars than in high-Cd cultivars. Shoot Cd and Ca concentrations were positively correlated with each other in 37 cultivars under T_0 ($R^2 = 0.52$; $p < 0.01$), T_1 ($R^2 = 0.59$; $p < 0.01$), and T_2 ($R^2 = 0.44$, $p < 0.01$) treatments (Fig. 2a). These results indicated that the uptake of Cd and Ca in different cultivars might be influenced by a "common factor."

In the second soil-pot trial, Ca concentrations in root and shoot, and translocation factors of low-Cd cultivars were 0.85-, 0.67-, and 0.78-fold of those in high-Cd cultivars, respectively ($p < 0.05$; Table 3). However, no significant difference was observed in the root soil Ca mobilization ability by root per weight, root Ca uptake, and transport ability by root per weight between high- and low-Cd cultivars ($p > 0.05$, Table 3). The results were in complete agreement with Cd accumulation in cultivars (Table 3). Besides, the R/S ratio

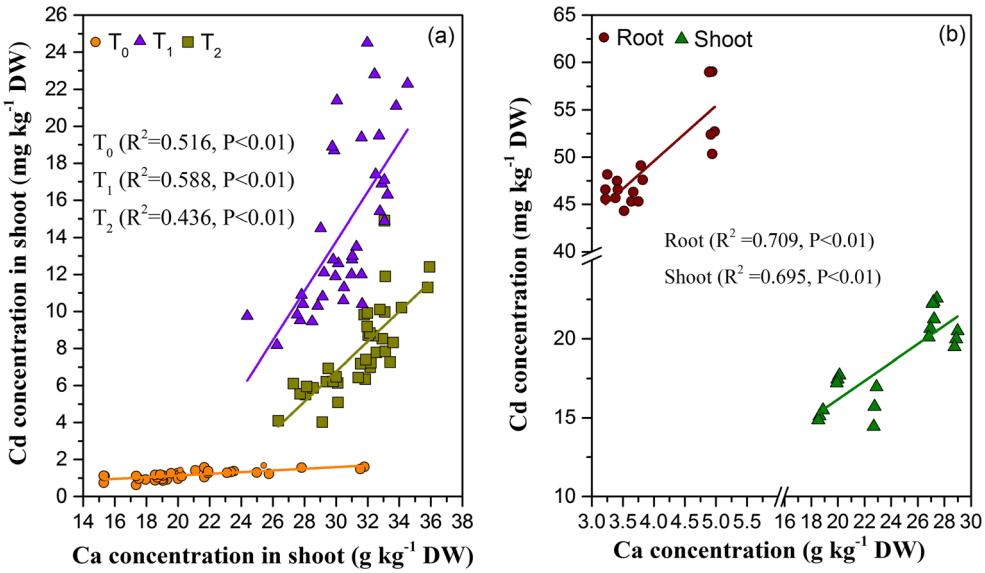

Fig. 2 Correlation relationship between total Cd and Ca concentrations in the shoots of 37 Chinese flowering cabbage cultivars (**a**), and roots and shoots of high- and low-Cd cultivars in hydroponic trial (**b**). $P < 0.01$ denotes significance at the 0.01 level

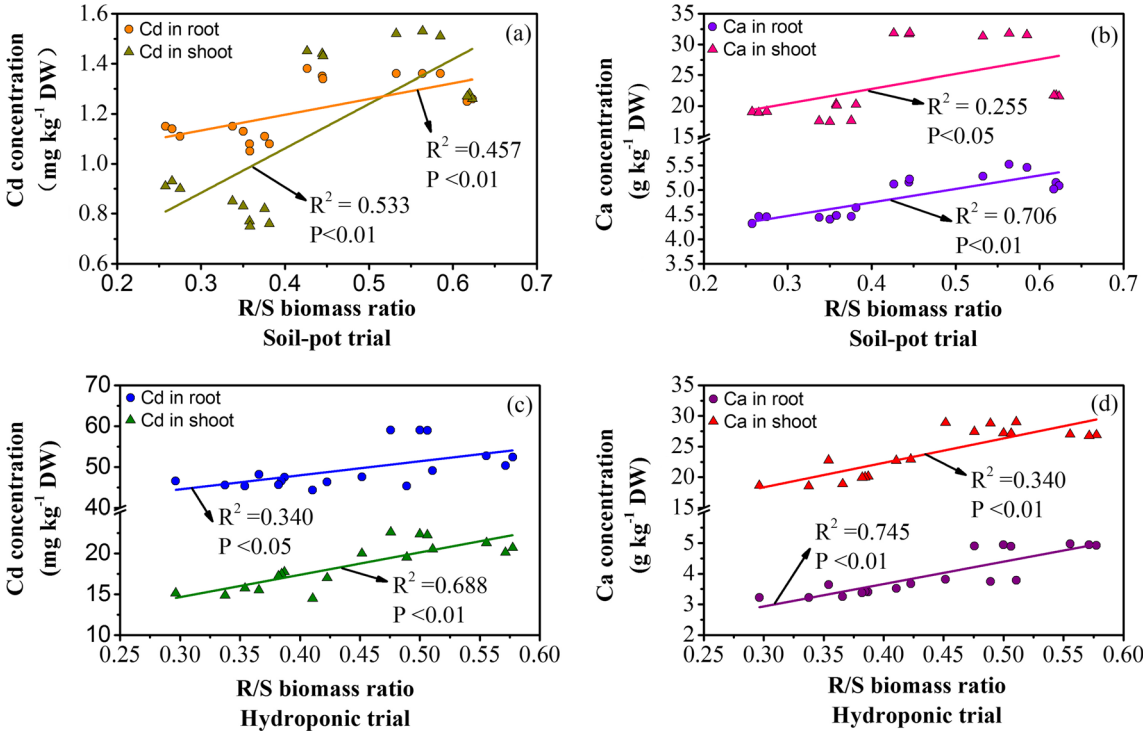

Fig. 3 Correlation relationship between R/S biomass ratio and Cd or Ca concentrations in the roots and shoots of high- and low-Cd cultivars in the second soil-pot trial (**a**, **b**) and the first hydroponic trial (**c**, **d**). *P* value denotes significance at the 0.01and 0.05 level, respectively

was positively correlated with root Cd ($R^2 = 0.46$; $p < 0.01$) or Ca ($R^2 = 0.71$; $p < 0.01$) and shoot Cd ($R^2 = 0.53$; $p < 0.01$) or Ca ($R^2 = 0.26$; $p < 0.05$) (Fig. 3a, b). This suggested that cultivar variations of R/S ratio might cause cultivar variations in

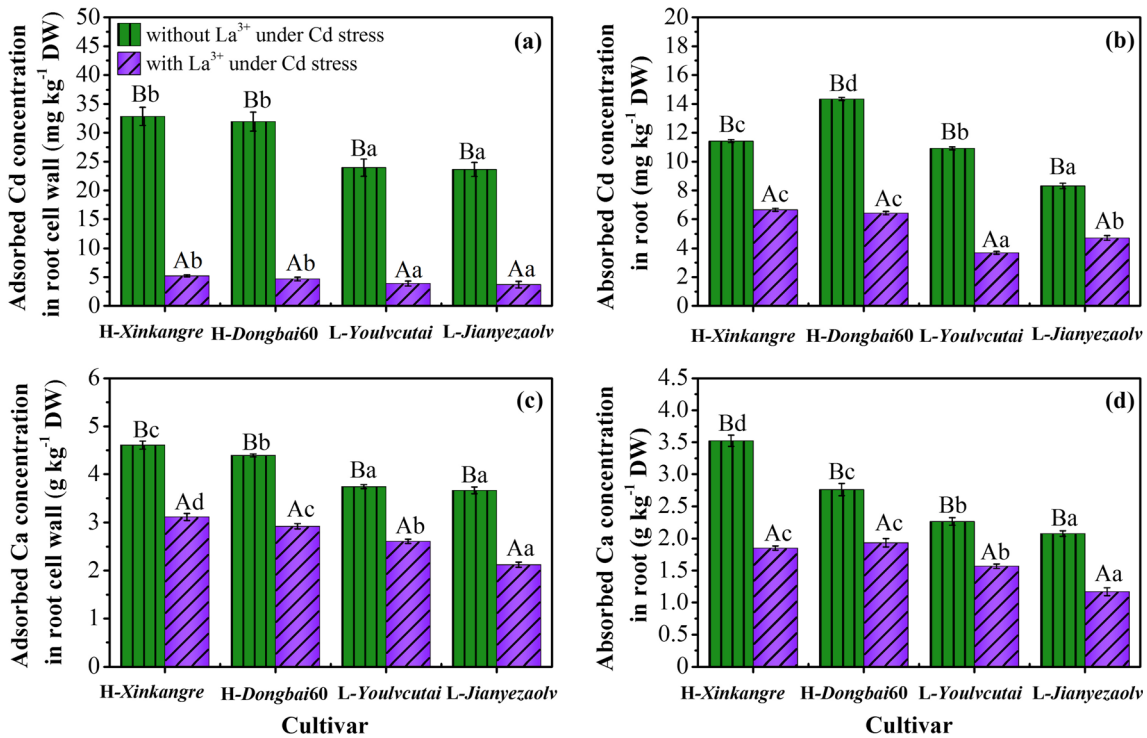

Fig. 4 Adsorbed Cd and Ca concentrations in root cell walls (**a**, **c**) and absorbed Cd and Ca concentrations in desorbed roots grown in solution (**b**, **d**) without and with $LaCl_3$ added for high- and low-cultivars. H and L represent high-Cd and low-Cd cultivars, respectively. The capital letters (A and B) on histograms denote significant differences at $p < 0.05$ between treatments of the same cultivars, and the lowercase letters (a, b, and c) denote significant differences at $p < 0.05$ between cultivars under the same treatment. Values are means (± SD; $n = 4$)

Ca mobilization in rhizosphere soil, Ca uptake and transfer by root, and further resulted in cultivar variations of Ca accumulation in shoots of cultivars and the positive correlations between Cd and Ca.

In the first hydroponic trial, Ca concentrations in root and shoot of low-Cd cultivars were 0.75- and 0.74-fold of those in high-Cd cultivars, respectively ($p < 0.05$; Table 4). No significant difference was observed in root Ca uptake and transport ability by root per weight between high- and low-Cd cultivars ($p > 0.05$; Table 4). Ca concentrations in root and shoot were significantly positively correlated with Cd in root and shoot of cultivars ($p < 0.01$; Fig. 2b). The results were attributed to 0.71-fold of R/S ratio of low-Cd cultivars compared to high-Cd cultivars. Lower root biomass of low-Cd cultivars may result in fewer channels in root plasma membrane for Cd and Ca transport (Kubo et al. 2011; Lu et al. 2013). This reconfirmed that cultivar variations of R/S ratio controlled both Cd and Ca accumulation in shoots of cultivars in hydroponic cultures. Similarly, statistically positive correlation was found between R/S ratio and Cd or Ca, which also supported the results (Fig. 3c, d).

In the second hydroponic trial, results showed that $LaCl_3$ significantly decreased Cd and Ca concentrations in the root cell walls and root protoplasts of the cultivars exposed to Cd stress ($p < 0.01$; Fig. 4). La^{3+} may displace Cd^{2+} and Ca^{2+} from the cell walls (He et al. 2015; Mei et al. 2014) and compete with Cd^{2+} for transport systems in the plasma membrane, such as Ca^{2+} channels (Mendoza-Cózatl et al. 2011; Solti et al. 2011). These results suggested that Cd uptake and transport are also largely dependent on Ca^{2+} channels due to the similarity in ion radius and charge between Cd^{2+} and Ca^{2+} in Chinese flowering cabbage. Consistent with this interpretation, (Li et al. (2017)) also found that Ca^{2+} channels in the plasmalemma were the main pathway allowing Cd ions to migrate into the wheat cells. The results further explained why the R/S ratio might influence both Cd and Ca accumulation in shoots of Chinese flowering cabbage cultivars in hydroponic cultures. Overall, the close relationship between Cd and Ca accumulation provided further evidence that R/S ratio was an indicator closely associated with Cd accumulation in the Chinese flowering cabbage cultivars.

Conclusions

Shoot Cd concentrations and Cd tolerance index varied obviously ($p < 0.01$) upon exposure to Cd stress among 37 Chinese flowering cabbage cultivars. No significant differences were observed in soil Cd mobilization, uptake, and transport ability by root per weight between high- and low-Cd cultivars grown in Cd-polluted soil. However, a stable R/S ratio difference among cultivars was observed. The cultivar variation of Cd accumulation in shoots was mainly dependent on their R/S ratios. The low-Cd cultivars with lower R/S ratio had lower root biomass and higher shoot biomass. Namely, their roots released less exudates, mobilized less Cd in rhizosphere soil, absorbed and transferred less Cd to the shoots, and their shoots diluted more Cd, resulting in lower Cd concentration in shoots. In all cultivating mediums, including Cd-polluted soil, unpolluted soil, and hydroponics cultures with and without Cd, the R/S ratios of low-Cd cultivars were significantly lower than those of high-Cd cultivars ($p < 0.01$). Cultivar variation of Ca accumulation in crops was in complete agreement with that of Cd accumulation, suggesting that both low-Cd and low-Ca accumulation were influenced by low R/S ratio. The results indicated that R/S ratio was a good indicator to display cultivar difference of Cd accumulation in the Chinese flowering cabbage shoot. The findings provide a possible useful method for rapid screening of low-Cd crop cultivar.

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