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Identification of Barley Varieties Tolerant to Cadmium Toxicity

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Abstract Two successive hydroponic experiments were carried out to identify barley varieties tolerant to Cd toxicity via examining Soil–Plant Analyses Development (SPAD) value, plant height, leaves and tillers per plant, root number and volume, and biomass accumulation. The results showed that SPAD values (chlorophyll meter readings), plant height, leaf number, root number and volume, and biomass accumulation of shoot/root were significantly reduced in the plants grown in 20 μM Cd nutrient solution compared with control, and the uptake and translocation of Zn, Mn, and Cu was also strictly hindered. Furthermore, there was a highly significant difference in the reduction in these growth parameters among varieties, and varieties "Weisuobuzhi" and "Jipi 1" showed the least reduction both in the two experiments, suggesting their high tolerance to Cd toxicity, while "Dong 17" and "Suyinmai 2" with the greatest reduction and the toxicity symptoms appeared rapidly and severely, denoting as Cd-sensitive varieties. Significant variety difference in Cd concentration was also found, with Weisuobuzhi containing the highest and Jipi 1 the lowest Cd concentration in shoots.

Keywords Barley (*Hordeum vulgare*) · Cadmium · Variety · Growth · Metal uptake · Tolerance

Introduction

Cadmium (Cd) is one of the most toxic heavy metals to both of plants and animals. Recently, Cd has become one of the most harmful and widespread pollutants in agricultural soil mainly due to industrial emission, the application of sewage sludge and phosphate fertilizers, and municipal waste disposal containing Cd [1–4]. For example, according to

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recent soil survey done in China, at least 13,330 ha of farmland involved in 11 provinces has been contaminated by varying degrees of Cd [5, 6]. Meanwhile, a number of typical cases about Cd contamination are also available in China, e.g., nearly 2,500 ha of arable land in Zhangtu, Sheyang was highly contaminated with metals, and Cd concentration in the soil/rice grain was as high as 5–7 mg kg^{-1}/1–2 mg kg^{-1}, respectively, both being well above the maximum allowable limit for soils [7] and cereals [8]. Furthermore, Cd, being biologically easily movable, can be readily absorbed by plants and translocated to above-ground tissues, while exceeding certain limit, the elevation of Cd in plant not only affects the crop yield and quality badly but also gives rise to threat on human health via food chain [4, 9–11]. Excess Cd in the diet results in damage to kidney tubules, rhinitis, emphysema, as well as other chronic disorders. Extreme cases of chronic Cd toxicity can result in osteomalacia and bone fractures, as characterized by the disease called Itai-Itai in Japan during the 1950s to 1960s, where local populations were exposed to Cd-contaminated rice. In brief, soil Cd contamination has posed a serious issue to the sustainable agriculture and human health worldwide [1].

Approaches have been sought to prevent Cd accumulation in plants to reduce Cd content in human diets so as to alleviate health risks associated with Cd exposure. One of the best cost-effective and efficient approaches is to develop low Cd accumulation cultivars. To breed crop cultivars with low Cd accumulation, it is important to find out genotypic difference in potential of Cd tolerance/accumulation in existing varieties/lines and their physiological responses to Cd addition. The uptake/tolerance of Cd varies among plant species, and also within a species, for example, soybean [12], maize [13], and lettuce [14]. Our previous study demonstrated the significant genotypic differences in Cd absorption and biomass production response to Cd stress in 11 barley genotypes [15], indicating the possibility to select varieties with low accumulation and highly tolerant to Cd. The present investigation was carried out to select Cd-tolerant and Cd-sensitive varieties, using 105 barley varieties/lines via two successive experiments, through studying the different response to Cd stress of seedling growth and such physiological traits as Soil–Plant Analyses Development (SPAD) value, biomass accumulation, the uptake of Cd and some mineral elements in barley plants.

Materials and Methods

Experiment Design

Cultural Condition

Two successive hydroponic experiments were conducted during 2004–2005 growth season in a greenhouse at Huajiachi Campus, Zhejiang University, Hangzhou, China. The composition of the basic nutrient solution (mg l^{-1}) was: $(NH_4)_2SO_4$ 48.2, $MgSO_4$ 65.9, K_2SO_4 15.9, KNO_3 18.5, $Ca(NO_3)_2$ 59.9, KH_2PO_4 24.8, Fe-citrate 5, $MnCl_2 \cdot 4H_2O$ 0.9, $ZnSO_4 \cdot 7H_2O$ 0.11, $CuSO_4 \cdot 5H_2O$ 0.04, HBO_3 2.9, H_2MoO_4 0.01. The solution pH was adjusted to 5.8±0.1 with NaOH or HCl, as required. The nutrient solution in the growth container was continuously aerated with pumps and renewed once a week, and Cd was also renewed in the exposure solutions. The experiment was laid out as a split-plot design with Cd concentrations as the main plot and variety as the sub-plot with three replicates. Seven individual plants per variety/line per replicate were used.

The Preliminary Selection Experiment

Barley varieties/lines of 105 were used: 88 domestic varieties, including 68 varieties of two-rowed type, 14 varieties of four/six-rowed type, 6 varieties of four/six-rowed and naked type, and 17 varieties collected from abroad, including 6 varieties of two-rowed type, 9 varieties of four/six-rowed type, 2 varieties of four/six-rowed and naked type.

Seeds were surface sterilized in 2% $Ca(OCl)_2$ for 20 min, rinsed in deionized water for seven times and soaked in distilled water for 3 h, and then sowed in 5.5-l container containing 5 l water, covered with a plastic plate with evenly spaced six holes, which was covered with pledget. In each hole, five seeds, being infiltrated with water, were located on the pledget. When the seedlings grew to 2–3 cm high, three seedlings were left in each hole for further growth, the others were removed, and Cd as $CdCl_2$ was added to each container of the basic nutrient solution to form two levels of 0 (control) and 20 μM.

The Secondary Selection Experiment

In the preliminary selection experiment, the ten and five varieties, respectively, were found to be Cd-tolerant and Cd-sensitive varieties. Therefore, they were selected for use in further evaluation of Cd tolerance. The ten tolerant varieties included seven varieties of two-rowed type: Weisuobuzhi, Wx 24, Zhenmingertiao, 99-14, Jipi 1, Harrington, Gebeina; one four-rowed type: Jipi 2; two four/six-rowed naked barley: Wenchengtuzhong barley, Qingyin 3506. The five sensitive varieties included four varieties of two-rowed type: Xiu 97-2, Zheyuan 88-18, Dong 17, Suyinmai 2; one four-rowed type: Nisfa. Seeds were surface sterilized as described above and germinated in sterilized moist quartz sand at 20+1°C. When seedlings grew onto two-leaf stage (10-day-old), the uniform plants were selected and transplanted to 5-l containers containing 4.5 l nutrient solution (14 plants per pot), and the container was covered with a polystyrol plate with seven evenly spaced holes. On the sixth day after transplanting, Cd as $CdCl_2$ was added to each container to form two levels of 0 (control) and 20 μM.

Growth Measurement and Metal Analysis

The Preliminary Selection Experiment

Thirty days after Cd addition, tillers and green leaves were counted, and plant height was measured. Meanwhile, a chlorophyll meter (Minolta SPAD-502) was used to take SPAD values (chlorophyll meter readings) of the fully expanded leaves (the first from the apex) [16]. For the determination of biomass, 12 plants (4 plants of each replicate) of each treatment were uprooted, separated into roots and tops (shoots and leaves), the length and volume of the roots were simultaneously measured, and then dried at 80°C and weighed.

The Secondary Selection Experiment

Plants were harvested after 20 days Cd exposure, and the same measurements of growth parameters as described above were done. For the determination of microelements, the plants were collected and separated into roots and tops (shoots and leaves), after soaking the roots in 20 mM Na_2EDTA for 3 h to eliminate the ions on the surface and then rinsing in deionized water, powdered and weighed, then ashed at 550°C for 12 h. The ash was digested with 5 ml 30% HNO_3, and then diluted using deionized water. Ion concentration, such as Cd, Cu, Fe, Zn, and Mn, was determined using a flame atomic absorption spectrometry (SHIMADZU AA-6300). All data presented are the mean values of three replicates.

Statistics and Calculations

Data are the average of at least three independent replicates. Statistical analysis was carried out by two-way analysis of variance using least significant differences (LSD) to test the difference of the means between varieties or Cd levels by using the Data Processing System Statistical Software Package [17].

Results and Analysis

The Preliminary Selection Experiment

The deteriorate effect of 20 μM Cd stress on barley growth are shown in Fig. 1 and Table 1. Cadmium toxicity markedly inhibited seedling growth; thus, on average of the 105 varieties/lines, SPAD value, plant height, root length and volume, shoot dry weight, and

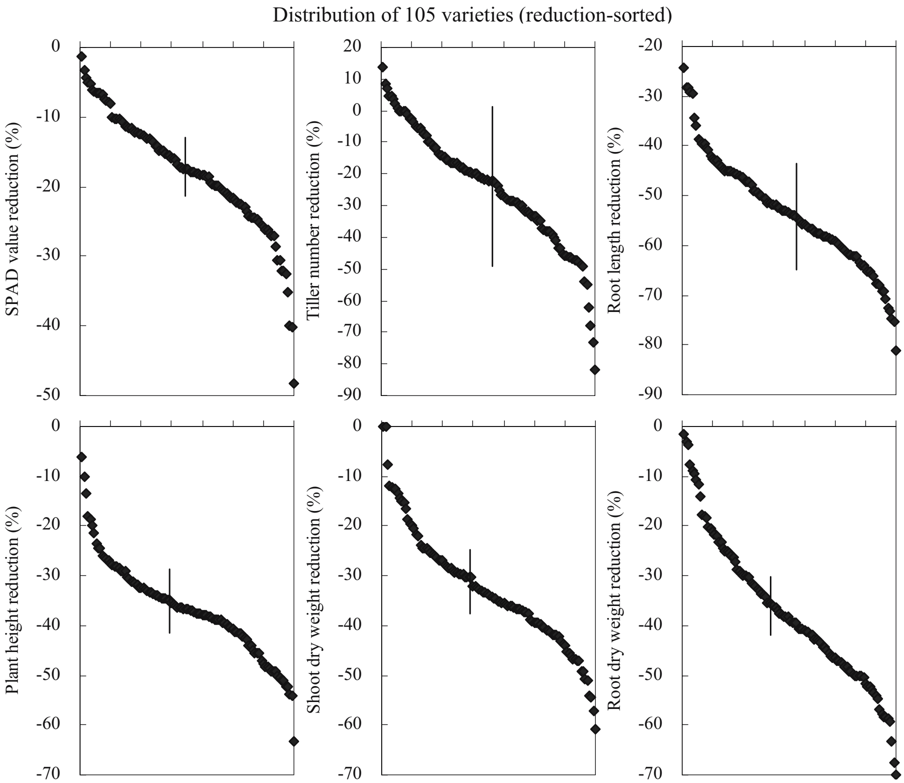

Fig. 1 Differences in growth parameters of the 105 barley varieties after 20 days of Cd exposure in the preliminary selection experiment expressed as the percentage of control (%). *Inset* "|", LSD, with respect to the significance at the 0.05 probability level between varieties

Table 1 Effect of Cd on SPAD Value, Plant Height, Root Length and Volume, and Dry Weight of Shoots and Roots of Barley Seedlings in the Preliminary Selection Experiment Expressed as the Percentage of Control (%)

Reduction percentage	SPAD value	Plant height	Root length	Root volume	Shoot dry weight	Root dry weight
Mean	17.9	36.6	53.4	60.3	32.8	37.3
Min	1.4	6.3	24.2	0.5	0.0	1.4
Max	48.2	63.3	81.2	93.8	60.9	70.0
CV (%)	49.3	22.6	15.7	16.8	35.5	31.0
Between varieties	*	*	*	*	*	*

*Significance at the 0.01 probability level between varieties

root dry weight for the plants exposed to 20 μM Cd reduced by 17.9, 36.6, 53.4, 60.3, 32.8, and 37.3%, respectively, compared with controls. Meanwhile, the time of appearance and severity of Cd toxicity symptoms differed greatly among varieties. Weisuobuzhi, Jipi 1, Wx 24, and Gebeina were the varieties least affected, in terms of SPAD value, yellow necrotic patches, and plant height, whereas four varieties, Dong 17, Suyinmai 2, Xiu 97-2, and Zheyuan 88-18 were the most affected, and Cd toxicity symptoms also appeared rapidly and severely. In addition, there was a largest coefficient of variation (CV) among varieties for the reduction in SPAD value (Table 1), indicating that it is a reasonable trait to be used for revealing variety difference in their response to Cd toxicity. Similar result was also observed in our previous study [15] that SPAD value may be a reasonable indicator for revealing genotypic difference in their response to Cd toxicity, and suggested SPAD value may be used to identify and characterize new metal-tolerant species. As a result, the following formula-based integrated arrangement was adopted to evaluate heavy metal tolerance: integrated score $=$ SPAD value $* \times 0.5 +$ plant height $\times 0.1$ $+$root length $\times 0.1 +$ root volume $\times 0.1 +$ dry weight $\times 0.1 +$ tillers $\times 0.1$ (* absolute values of reduction in growth parameters relative to the controls). Thus, Cd tolerance of barley varieties would be negative to the scores, i.e., the varieties with the lowest and the highest scores were the most tolerant and sensitive, respectively, in the 105 varieties. According to the integrated arrangement, 15 varieties were selected among the 105 varieties, including 10 and 5 varieties, respectively, for Cd-tolerant and Cd-sensitive, i.e., ten tolerant varieties: Weisuobuzhi, Jipi 1, Gebeina, Wx 24, Harrington, Zhenmingertiao, 99-14, Jipi 2, Wenchengtuzhong barley, and Qingyin 3506 (approximately, the corresponding integrated score=17.7, 18.0, 23.9, 26.1, 28.8, 29.1, 29.3, 30.2, 32.0, 32.3, respectively); and five sensitive varieties: Nisfa, Xiu 97-2, Zheyuan 88-18, Dong 17, and Suyinmai 2 (approximately, integrated score=91.7, 98.4, 102.6, 108.5, 113.3, respectively).

The Secondary Selection Experiment

As shown in Table 2, the similar results as that found in preliminary selection experiment were obtained in the secondary selection experiment. Thus, on average of the five Cd-sensitive varieties, much more reductions in SPAD values (even accentuated), root, and shoot dry weight by 26.6, 5.9, and 8.3%, respectively, were observed over the ten tolerant varieties. In addition, Weisuobuzhi was the variety least affected with the least reduction in SPAD values and dry weight, and no visual leaf Cd toxicity symptoms of necrotic patches appeared under 20 μM Cd stress, and then followed by Jipi 1. Whereas Dong 17 and Suyinmai 2 were the most sensitive to Cd toxicity with the largest reduction in these three

Table 2 Shoot and Root Dry Weight and SPAD Value of Different Barley Varieties after 20 Days of Cd Exposure in the Secondary Selection Experiment

Variety	SPAD value		Shoot dry weight (mg plant ^{1})		Root dry weight (mg plant ^{1})	
	Cd treatment (μM)					
	0	20	0	20	0	20
Tolerant varieties (TV)						
Weisuobuzhi	38.3	35.6 (7.0)[1]	122.0	92.1 (24.5)	54.1	43.6 (19.4)
Jipi 1	33.4	26.2 (14.1)	157.8	109.2 (30.8)	70.6	33.6 (52.4)
Zhenmingertiao	36.0	27.6 (23.3)	143.3	83.3 (41.9)	63.3	31.6 (50.1)
Qinying 3506	32.8	25.2 (23.3)	154.6	71.5 (53.8)	52.2	39.3 (24.7)
Jipi 2	39.5	29.0 (26.6)	181.9	102.3 (43.8)	61.9	28.0 (54.8)
99-14	37.9	28.1 (25.9)	196.6	97.2 (50.6)	81.7	50.9 (37.7)
Harrington	40.2	27.2 (32.3)	156.4	71.9 (54.0)	61.7	26.9 (56.4)
Wenchengtuzhong barley	32.9	20.7 (37.1)	155.2	80.2 (48.3)	48.6	22.5 (53.7)
Wx 24	36.5	22.7 (37.8)	144.0	80.0 (44.4)	66.5	34.6 (48.0)
Gebeina	38.1	23.3 (38.7)	165.3	110.1 (33.4)	81.2	54.3 (33.1)
Sensitive varieties (SV)						
Zheyuan 88-18	38.4	25.3 (34.1)	138.1	62.5 (54.7)	47.1	25.8 (45.2)
Nisfa	27.8	16.9 (39.2)	88.9	65.3 (26.5)	30.3	18.0 (40.6)
Xiu 97-2	35.2	14.0 (60.2)	117.7	62.7 (46.7)	53.7	25.2 (53.1)
Dong 17	39.2	12.7 (67.6)	150.8	60.1 (60.1)	51.6	21.0 (59.3)
Suyinmai 2	41.0	10.4 (74.6)	162.3	75.0 (53.8)	67.6	28.3 (58.1)
Mean of the 15 varieties	36.5	23.2 (36.1)	149.0	81.6 (44.5)	59.5	32.2 (45.8)
Mean of the ten TV	36.6	26.8 (28.6)	157.7	89.8 (42.5)	64.2	36.5 (43.0)
Mean of the five SV	36.3	15.9 (55.2)	131.6	65.1 (48.4)	50.1	23.7 (51.3)
LSD 0.05	4.4	4.6 (15.8)	ns[2]	14.6 (20.2)	18.4	8.1 (17.5)

Values within brackets represent the relative reduction in Cd treatment to the control.

ns Nonsignificance at 0.05 probability level

parameters, and the toxicity symptoms appeared rapidly and severely under Cd stress. Accordingly, these four varieties of Weisuobuzhi, Jipi 1 and Dong 17, Suyinmai 2 were selected as Cd-tolerant and Cd-sensitive varieties, respectively, to analyze microelement concentration. As shown in Table 3, significant difference ($P<0.01$) in Cd concentration in roots and shoots was found among the four varieties, but no difference in the ratio of translocation from root to shoot. Cadmium concentration of Weisuobuzhi, one of the two tolerant varieties, had the highest concentrations both in roots and shoots in the four varieties, while the other tolerant variety Jipi 1 being significantly lower.

There was a significant influence of Cd addition on uptake and distribution of Zn, Mn, Cu, and Fe in plants (Table 4). In comparison with the control, Zn, Mn, and Cu concentrations in the plants exposed to 20 μM Cd over the four varieties reduced by 10.6, 20.8, 12.9% in shoot and 17.4, 56.7, and 16.1% in root, respectively. While Fe concentration reduced by 12.3% in shoots but increased by 18.0% in roots. Furthermore, the significant difference was also detected for reduction in Zn, Mn, and Cu concentrations among the four varieties, and in comparison with the two tolerant varieties, the two sensitive varieties showed more reduction in shoot Zn, Cu, Mn, and root Zn and Cu concentrations (approximately 15.0, 8.7, and 11.9%, and 25.2 and 11.6% more reduction vs the two tolerant varieties). Concerning Fe concentration, more reduction of 40.3% in shoot

Table 3 Cd Concentration and Its Root to Shoot Translocation in Different Barley Varieties After 20 Days of Cd Exposure in the Secondary Selection Experiment

Variety	Cd concentration (mg kg ^{1} DW)				Cd translocation	
	Shoot		Root		(root/shoot)	
	Cd treatment (μM)					
	0	20	0	20	0	20
Weisuobuzhi	0.0160	142.56	0.0608	499.91	3.80	3.51
Jipi 1	0.0115	80.46	0.0503	354.99	4.37	4.42
Dong 17	0.0167	111.53	0.0555	356.02	3.32	3.18
Suyinmai 2	0.0163	115.53	0.0589	439.43	3.60	3.83
Mean	0.0143	106.11	0.0539	351.94	3.82	3.38
LSD 0.05	0.0090	19.30	0.0070	71.01	0.55	0.90

Fe concentration of the two sensitive varieties was observed, compared with the two tolerant varieties, although 16.5% higher in the roots.

Discussion

It had been observed that plant Cd content generally reflected the biological availability of this metal in the growth medium. Increased available Cd level in soil caused detrimental effects to plants [18, 19]. The methods used to evaluate heavy metal tolerance were based on root elongation measurement of the plants exposed to high toxic ion level [20]. Root biomass, length and number, and elongating rate of root had all been used as indicators of plant tolerance to heavy metal [21]. Our previous study [15] found that SPAD value may be a reasonable indicator for revealing genotypic difference in their response to Cd toxicity, and suggested SPAD value may be used to identify and characterize new metal-tolerant species. In the present study, we determined SPAD value, biomass, plant height, root length and volume, and significant differences among varieties were observed in these parameters. Furthermore, it was found that SPAD had a greatest coefficient of variation, and moreover

Table 4 Effect of Cd on Microelements of Barley after 20 days of Cd Exposure in the Secondary Selection Experiment Expressed as the Percentage of Control (%)

Variety	Shoot				Root			
	Zn	Cu	Mn	Fe	Zn	Cu	Mn	Fe
Tolerant varieties (TV)								
Weisuobuzhi	4.0	28.5	15.9	12.9	3.8	34.7	65.9	+6.2
Jipi 1	2.1	+11.3	13.8	+28.5	5.8	+14.2	46.0	+13.3
Sensitive varieties (SV)								
Dong 17	18.1	19.2	30.5	25.5	27.2	21.9	52.3	+39.3
Suyinmai 2	18.0	15.3	23.1	39.4	32.8	21.8	62.7	+13.3
Mean of the two TV	3.1	8.6	14.9	7.8	4.8	10.3	56.0	9.8
Mean of the two SV	18.1	17.3	26.8	32.5	30.0	21.9	57.5	26.3
Between variety	**	**	*	**	**	**	**	**

* and **, significant at the 0.05 and 0.01 probability level, respectively, between varieties under Cd treatment

may explain for genotypic/cultivar variation in Cd tolerance. Accordingly, the biggest weight of 0.5 was recorded in the reduction in SPAD value, and the following formula-based integrated arrangement was adopted to evaluate heavy metal tolerance: integrated score = SPAD value $* \times 0.5 +$ plant height $\times 0.1$ +root length $\times 0.1 +$ root volume $\times 0.1 +$ dry weight $\times 0.1 +$ tillers $\times 0.1$ (* absolute values of reduction in growth parameters relative to the controls). Thus, Cd tolerance of barley varieties would be negative to the scores. As a result, ten and five varieties with bottom ten lowest and contrarily top five highest integrated score, respectively, were selected as tolerant and sensitive varieties among 105 varieties in the preliminary selection experiment. Moreover, fairly consistent trends in variety differences response to Cd toxicity were observed in the secondary selection experiment, and that Weisuobuzhi, Jipi 1 and Dong 17, Suyinmai 2, respectively, were selected as Cd-tolerant and Cd-sensitive varieties.

Significant variety difference in Cd concentration was found, with Weisuobuzhi and Jipi 1 containing the highest and the lowest Cd concentration in plant tissues, respectively, despite both showing high Cd tolerance in terms of seedling growth parameters. It may be assumed that Weisuobuzhi, which had higher Cd uptake and translocation with least Cd toxicity symptoms, acts as a type of tissue tolerance but needs further verification. Such tolerance might be due to multiple mechanisms, such as detoxification and sequestration. Zenk [22] reported that metal complexes with phytochelatins, organic acids, and inorganic compounds were responsible for metal tolerance, especially in the case of hyper-accumulators, which would prevent Cd from interfering with more sensitive sites of cellular metabolism. Variety Jipi 1, which had lower shoot Cd and moderate root Cd concentration, apparently prevented the translocation of Cd from root to shoot. In addition, a notable increase in Fe and Cu concentrations was found in shoots of Jipi 1 (Table 4), compared with the other three varieties, indicating that Cu and Fe might play a certain role in preventing Cd translocation. Further studies are needed to better understand the role and the mechanism of inhibited Cd translocation, as high retention of Cd in roots is particularly desirable for cereals where the roots are not utilized, thus reducing Cd burden to animal and human. Another issue is whether the kernel Cd concentration is lower in this plant. Accordingly, kernel Cd content of the four selected varieties grown in different Cd treatments will be determined in our ongoing experiments. Meanwhile, hybrids between Cd-tolerant and Cd-sensitive varieties selected in this study have developed, which will help us to construct doubled-haploid population to further identify/localize Cd tolerance/accumulation relevant gene(s) with the aid of analyzing available simple sequence repeats marks in barley and the establishment of genetic linkage map.

Previous reports considered that there was interaction between Zn and Cd in uptake and distribution in plants [23]. They suggested that Zn might interfere with uptake of Cd and translocation from roots to shoots in young plant. In this study, the two sensitive varieties, Dong 17 and Suyinmai 2, had a more reduction in shoot and root Zn concentrations relative to the two tolerant varieties (Table 4). It may be suggested that more Zn uptake could alleviate Cd toxicity. Ewers et al. [24] found that Zn reduced the bio-availability of Cd in soil. McKenna et al. [25] also reported that Zn interfered with distribution of Cd in lettuce and spinach. However, the reason for this response and relevant mechanism has not been fully understood. Therefore, further studies are needed for making clear of interaction between Cd and Zn in their uptake and translocation by plants before it can be practically applied to alleviate Cd toxicity.

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