



Does copper reduce cadmium uptake by different rice genotypes?

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Abstract

A hydroponics experiment was conducted to investigate the effect of copper (Cu) on cadmium (Cd), calcium (Ca), iron (Fe), and zinc (Zn) uptake by several rice genotypes. The experiment was carried out as a 2×2×4 factorial with four rice genotypes and two levels of Cu and Cd in nutrient solution. Plants were grown in a growth chamber with controlled environment. The results showed a significant difference between the biomass of different rice genotypes ($P < 0.001$). The Cd and Cu concentration in the solution had no significant effect on the biomass. The addition of Cu significantly decreased Cd uptake by shoots and roots of rice ($P < 0.001$). The Cd concentration did not significantly influence Ca uptake by plants, whereas the Cu concentration did ($P = 0.034$). There was a significant influence of Cd on Fe uptake by shoots and roots ($P < 0.001$, $P = 0.003$, respectively). Zn uptake decreased significantly as the addition of Cd and Cu increased in shoots. We concluded that Cu had significant influence on Cd uptake. The possible mechanisms were discussed.

Key words: cadmium; calcium; copper; food chain; genotypes; zinc

Introduction

Metals are ubiquitous in modern industrialized environment. Some are harmful to human health; others are essential for the human body and may also affect or reduce the toxicity of some toxic metals. When metals are transferred from soil to plants, animals, and/or humans, there is selectivity for some elements and barriers to others in their movement from lower to higher trophic levels within the human food chain (Welch and House, 1984). Cd is one of the most well-known environmental toxicants to humans (WHO, 1992). Cd can be easily uptaken by plants, and its subsequent transfer to humans through food chains has been paid great attention owing to the fact that cereals, potatoes, and vegetables account for 70% of dietary intake (Grawé, 1996). Since the Itai-Itai disease was identified to be related to Cd pollution in Japan, the research has been made considerable advances on controlling Cd pollution associated with assessing and managing acute injury to human health through occupational exposure and disposal of Cd from industrial point sources to local populations through several pathways (Syers and Gochfeld, 2001). Recently, Cd has been the subject of increasing interest with regard to its health effects on the general human population, crop production, and soil quality, owing to its potential toxicity to humans and its relatively high mobility from soil to plant (Jansson, 2002).

The uptake and accumulation of Cd varies greatly be-

tween plant species (Bingham *et al.*, 1975; Kuboi *et al.*, 1986; Welch and Norvell, 1999). Intraspecific variation in Cd concentration has been found in soybean, wheat, maize, sunflower, potatoes, and rice (Eriksson, 1990; Florijn and Van Beusichem, 1993; Grawé, 1996; Li *et al.*, 1997; Wang and Gong, 1996; Wenzel *et al.*, 1996; Zhang *et al.*, 2000). Furthermore, it has been documented that calcium (Ca), iron (Fe), and zinc (Zn) interfere with the Cd absorption in plants (Fox, 1988; Jacobs *et al.*, 1978; McKenna *et al.*, 1992; Reeves, 2001; Reeves and Chaney, 2001). Previous studies have shown that populations with rice as their staple food are more susceptible to Ca, Fe, and Zn deficiency owing to the relatively low densities of these elements in rice grains (Chaney *et al.*, 2001a, 2001b; Reeves, 2001; Reeve and Chaney, 2001, 2002). It has also been observed that these populations seem to be more susceptible to Cd toxicity than those that subsist on more nutritious diets with similar Cd intake (McKenzie-Parnell *et al.*, 1988; Reeves, 2001; Reeves and Chaney, 2001, 2002; Tachechi, 1978). Rice grain is quite low in Fe, Zn, and Ca as compared to soybean and wheat (Wolnik *et al.*, 1983) and much considerably more of grain Zn, Fe, and Ca are removed in the polishing of brown rice (Zhang *et al.*, 1997). Low levels of these elements in food, or marginal nutritional status of the consumer's usual diet may cause increased intestinal absorption and accumulation of Cd (Berglund *et al.*, 1994; Reeves and Chaney, 2001; Stuczynski *et al.*, 2000).

China is one of the largest rice producers and consumers

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in the world, and Cd uptake by rice is therefore a critical issue to ensure food safety. Nevertheless, several studies have indicated that Cd has contaminated large areas of arable land, mainly by smelting industries and sewage irrigation (Cai *et al.*, 1990; Chen *et al.*, 1999; Jin *et al.*, 2002; Nordberg *et al.*, 1997; Zhang *et al.*, 2000). Soil Cd concentration is up to 26 mg/kg and Cd in rice grains is as high as 2.4 mg/kg in some parts of China (Jin *et al.*, 2002); these high Cd concentrations in rice have resulted in human health problems, such as renal dysfunction. Therefore, to reduce Cd accumulation in rice is a critical issue for food security.

There are several different cultivars of rice, some of which produce white grains and others dark grains, and it is generally accepted that the latter contain relatively higher concentrations of Fe, Zn, Ca, and perhaps Cu (Glahn *et al.*, 2002; Lü, 2000; Yu *et al.*, 2000; Yuan *et al.*, 2001). Dark rice is usually recommended for pregnant women to overcome poor Fe nutrition in China. High concentrations of Fe, Zn, Ca, and Cu in dark rice may affect Cd uptake. However, there has been no report on whether there is a difference in Cd uptake by rice cultivars with white or dark grains. Also, there have been some researches on the interaction between Cd and Zn uptake by plants, but few studies on the interaction of Cd and Cu. The purposes of this study were: (1) to investigate the effect of Cu on Cd uptake by several rice genotypes; (2) to investigate the effect of Cd on Ca, Fe, and Zn uptake by the genotypes; and (3) to identify whether the dark rice genotypes have low Cd accumulation. In this case, a hydroponic experiment was conducted in a plant growth chamber.

1 Materials and methods

1.1 Solution composition, seeds of rice genotype, and plant growth conditions

The experiment was designed as a 2×2×4 factorial with four rice genotypes and two levels of Cu and Cd in nutrient solution. Cd was added as CdCl₂ at a concentration of 5 and 10 µmol/L in the nutrient solution. Cu was added as CuSO₄ at a concentration of 0 and 2 µmol/L in total nutrient solution. Each Cd-Cu treatment in various genotypes had three replicates and one PVC bucket with 550 ml nutrient solution for one seedling plant. The basal solution selected from the international institute for rice was employed in this study (Table 1). The solution pH was buffered at 6±0.2.

Rice seeds (four genotypes), two producing dark grain

(Jilinheino marked as A and Tianjin1032 marked as B) and the other two producing white grain (Jiyoul marked as C and Zhongyou307 marked as D), were sterilized with 10% H₂O₂ followed by thoroughly washing with deionized water. The seeds were then germinated in perlite saturated with deionized water. Two weeks after germination, the seedlings were carefully washed to remove adhering perlite particles and transferred into 550 ml PVC buckets with basal nutrient solution and cultured for two weeks; the solution was completely changed once a week. Cu treatment (0 and 2 µmol/L Cu in total solution, respectively) was performed in the third week; Cd was added in the nutrient solution with 5 and 10 µmol/L for 10 d from the 4th week. All plants were grown in a growth chamber with controlled environment (temperature maintained at 30°C/25°C (day/night)), relative humidity 50%/65% (day/night), light period 14 h/10 h (day/night), and the light intensity was 280 µmol/(m²·s)). After 10 d growth in nutrient solution with Cd treatment, all plants were harvested and the experiment was terminated. Each plant was separated into root and shoots and the root was rinsed with deionized water and blotted with paper towels for harvest.

1.2 Plant tissue analysis

Plants were oven-dried at 70°C to constant weight and dry weights were recorded. Plant materials were cut with stainless steel scissors followed by ground in an earthen bowl. Samples from shoots (0.5 g) and roots (whole root 0.25 g) were digested in a polyvinyl-fluoride crucible with 3 ml of concentrated nitric acid (HNO₃). The suspension was left at room temperature for 2 h before the sealed vessel was fitted into a high-pressure metal cylinder. These were heated in an oven at 100°C for 1 h followed by heating at 160°C for 5 h. After digestion, the vessels (open at this stage) were put on a hot plate at 105°C to evaporate excessive acid until about 0.5 ml solution remained. The residual samples were then diluted with deionized water to 50 ml and stored at 4°C prior to analysis.

Concentrations of Cd, Fe, Ca, Cu, and Zn in shoots and roots were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 2000 DV, USA). Samples of tea and soil with certified concentration of minerals (GBW07605, GBW07401, respectively) were included to assure the accuracy of measurement.

1.3 Statistical analysis

Analysis of variance was employed with SPSS to test the significant effect of Cu and Cd treatments on biomass and the Cd, Ca, Fe, Cu, and Zn uptake by rice. The significant level was set at 0.05.

2 Results

2.1 Plant biomass

There are significant differences in the biomass of roots and shoots between rice genotypes ($P < 0.001$, Table 2).

Table 1 Constituent of the nutrient solutions

Compound	Concentration (µmol/L)	Compound	Concentration (µmol/L)
Fe-EDTA	25	NaCl	50
ZnSO ₄	0.5	NH ₄ NO ₃	2.52
CuSO ₄	0.5	K ₂ SO ₄	1
MnSO ₄	2.5	CaCl ₂	2
H ₃ BO ₃	5.0	MgSO ₄	0.8
Na ₂ MoO ₄	0.25	KH ₂ PO ₄	0.6
CoSO ₄	0.1		

Table 2 Biomass of shoots and roots

Treatment	Biomass of shoots (g)				Biomass of roots (g)			
	A	B	C	D	A	B	C	D
Cd5Cu0	0.605±0.096	0.755±0.042	0.421±0.016	0.296±0.013	0.235±0.04	0.327±0.019	0.190±0.048	0.100±0.005
Cd5Cu2	0.629±0.089	0.77±0.062	0.345±0.027	0.375±0.003	0.221±0.056	0.276±0.022	0.120±0.010	0.119±0.004
Cd10Cu0	0.742±0.017	0.813±0.006	0.44±0.037	0.413±0.019	0.314±0.019	0.356±0.008	0.133±0.016	0.136±0.006
Cd10Cu2	0.733±0.011	0.872±0.029	0.422±0.117	0.306±0.018	0.312±0.006	0.321±0.018	0.147±0.045	0.103±0.001
Analysis of variance								
Genotype	$P < 0.001$				$P < 0.001$			
Cd	$P = 0.011$				$P = 0.033$			
Cd×Cu	NS				NS			

A: Jilinheino; B: Tianjin1032; C: Jiyou1; D: Zhongyou307. Cd5: 5 $\mu\text{mol/L}$ Cd; Cd10: 10 $\mu\text{mol/L}$ Cd; Cu0: 0 $\mu\text{mol/L}$ Cu; Cu2: 2 $\mu\text{mol/L}$ Cu; NS: no significance.

The biomass in dark rice was considerably higher than in white rice. The significant differences of biomass for various rice genotypes between different treatments were also observed in shoots ($P = 0.011$) and in roots ($P = 0.033$). However, the addition of Cu did not influence the biomass in genotypes significantly; no interaction between Cd and Cu was found for biomass.

2.2 Cd and Cu uptake by shoots and roots

Cd uptake by shoots and roots is shown in Figs.1 and 2. With zero Cu treatment, the Cd accumulation in

both shoots and roots was significantly different between the genotypes ($P < 0.001$). Addition of Cd into solution increased the Cd concentrations in shoots and roots of all cultivars significantly ($P < 0.001$). The Cd concentrations in roots were considerably higher than that in shoots. There was a trend of Cd concentrations in shoots from genotype A to D, which was genotype $A > B > C > D$ (Fig.1a); however, there was no such trend in roots (Fig.1b).

Addition of Cu into the solution decreased the Cd concentrations in the shoots of dark rice genotypes ($P < 0.001$) but not in white rice (Fig.2). The interaction

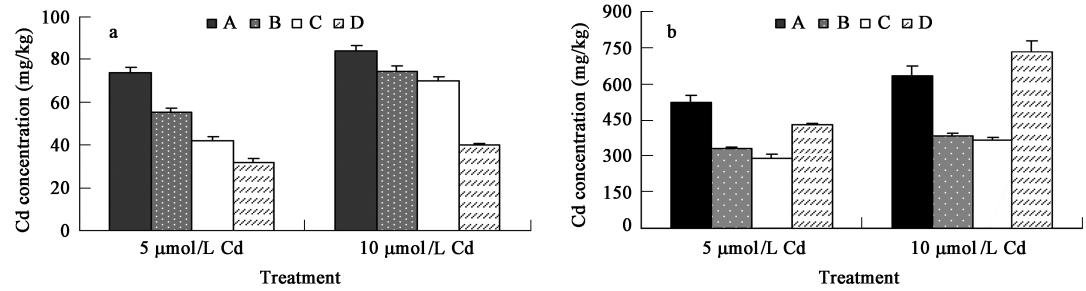


Fig. 1 Cd uptake by shoots (a) and roots (b) with zero Cu treatment. A, B, C, D are the same as in Table 2.

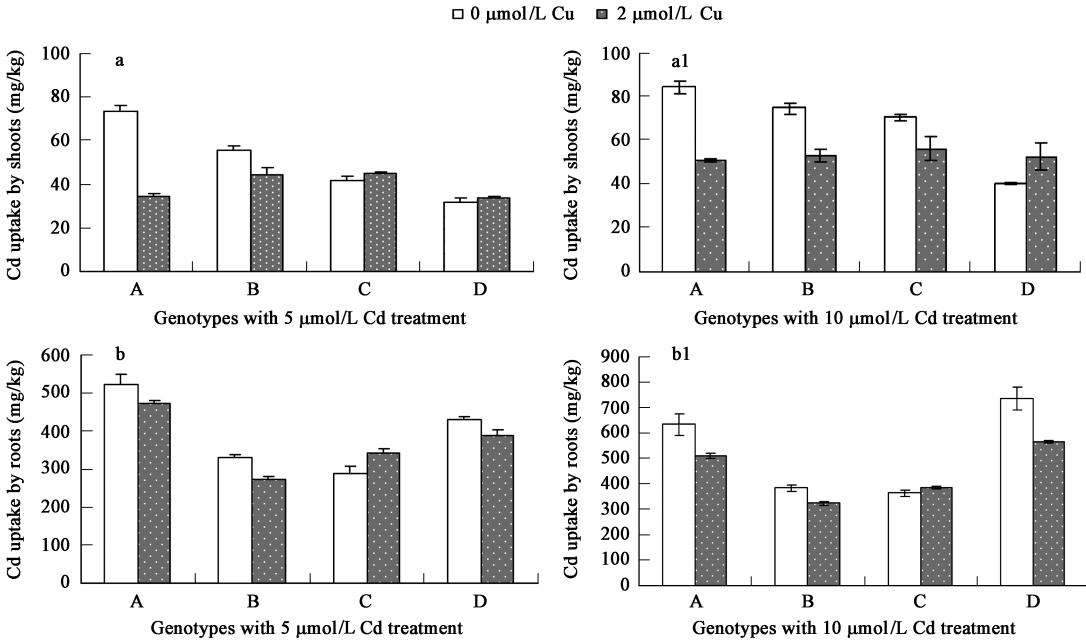


Fig. 2 Cd uptake by shoots (a, a1) and roots (b, b1) with 5 and 10 $\mu\text{mol/L}$ Cd treatment in dark rice (a) and white rice (b). A, B, C, D are the same as in Table 2.

between the Cu treatment and genotypes was observed in the Cd uptake by shoots and roots ($P < 0.001$).

2.3 Cu uptake by shoots and roots

There was a significant difference for the Cu concentration of shoots and roots varying in genotypes ($P = 0.004$, $P < 0.001$). Addition of Cd into solution did not significantly influence Cu uptake by genotypes. Adding Cu into solution increased the Cu concentration in shoots and roots (Table 3).

2.4 Ca uptake by shoots and roots

Ca uptake by shoots and roots varied with genotypes significantly ($P < 0.001$). Ca concentrations were higher in shoots than in roots. There was no statistical effect on Ca uptake by shoots and roots by adding Cd into solution. Addition of Cu into solution could significantly influence Ca uptake by shoot ($P = 0.034$) but not in roots (Table 4).

2.5 Fe uptake by shoots and roots

Fe uptake was significantly different between genotypes in shoots and roots ($P < 0.001$) as shown in Table 5. Fe uptake significantly decreased with the addition of Cd increasing except for Jilinheino in shoots ($P < 0.001$) and roots ($P = 0.003$). Cu treatment did not significantly influence the Fe uptake by shoots but significantly influenced the Fe uptake by roots ($P < 0.001$). An interaction of Cd and Cu treatment on Fe uptake existed in shoots ($P = 0.005$) but there was no interaction in roots. There were interactions of Cd treatment and genotypes, Cu treatment

and genotypes, and Cd and Cu treatment and genotypes on Fe uptake in shoots and roots.

2.6 Zn uptake by shoots and roots

Addition of Cd decreased the Zn concentration in shoots in genotype Jilinheino, Tianjin1032, and Zhongyou 307 ($P = 0.045$); however, it had no significant effect in roots (Table 6). There was a significant difference in Zn uptake by shoots and roots ($P < 0.01$). Addition of Cu also had a significant effect on Zn uptake by shoots and roots ($P < 0.001$). An interaction of Cu treatment and genotypes was observed in shoot and roots.

3 Discussion

3.1 Cadmium uptake

One of the main aim of this study was to identify whether dark rice can accumulate lower Cd than white rice. Our data showed there was no significant difference of Cd uptake between genotypes in dark and white rice as shown in Figs.1 and 2. This result was consistent with our previous experiment (Cui *et al.*, 2004). Generally, dark rice grain contains more nutrient elements such as Fe, Ca, and Zn (Glahn *et al.*, 2002; Yu *et al.*, 2000; Yuan *et al.*, 2001) and it has been known that the presence of these elements inhibited Cd absorption (Mckenna *et al.*, 1992; Reeves and Chaney, 2001, 2002). However, our results seemed to be reverse. It was apparent that a certain unknown factor affected their inhibition on Cd uptake. Crop genotype is one factor to affect grain Cd accumulation. The results in

Table 3 Cu concentrations in genotypes

Treatment	Cu concentration in shoots (mg/kg)				Cu concentration in roots (mg/kg)			
	A	B	C	D	A	B	C	D
Cd5Cu0	6.33±0.52	6.00±0.42	6.40±0.31	6.87±0.24	11.67±1.02	7.13±0.29	10.58±0.89	14.41±0.79
Cd5Cu2	26.33±0.64	29.27±0.13	22.80±0.72	26.13±1.84	172.44±6.87	114.53±15.80	132.33±12.17	132.13±6.34
Cd10Cu0	6.47±0.47	5.67±0.24	5.73±0.07	7.00±0.42	9.67±1.17	6.73±0.18	10.80±0.83	16.17±1.01
Cd10Cu2	27.73±1.16	30.27±2.21	30.52±2.46	18.93±1.07	184.27±8.70	146.22±8.15	137.78±6.45	83.00±1.76
Analysis of variance								
Genotype	$P = 0.004$				$P < 0.001$			
Cd	NS				NS			
Cu	$P < 0.001$				$P < 0.001$			
Cd×Cu	NS				NS			
Cd×Genotype	$P < 0.001$				$P = 0.001$			
Cu×Genotype	$P < 0.001$				$P < 0.001$			
Cd×Cu×Genotype	$P < 0.001$				$P < 0.001$			

Table 4 Ca concentrations in genotypes

Treatment	Ca concentration in shoots (mg/kg)				Ca concentration in roots (mg/kg)			
	A	B	C	D	A	B	C	D
Cd5Cu0	2.91±0.55	3.39±0.05	2.84±0.11	2.32±0.01	1.37±0.10	0.95±0.01	1.10±0.03	0.75±0.15
Cd5Cu2	2.39±0.08	3.63±0.03	2.52±0.13	2.14±0.10	1.15±0.08	1.08±0.04	1.03±0.05	1.15±0.14
Cd10Cu0	2.19±0.09	3.31±0.10	2.98±0.15	2.19±0.05	0.99±0.05	0.88±0.04	1.26±0.11	1.31±0.04
Cd10Cu2	2.45±0.06	3.15±0.18	2.46±0.15	2.45±0.20	1.29±0.09	0.85±0.06	1.28±0.07	1.20±0.06
Analysis of variance								
Genotype	$P < 0.001$				$P < 0.001$			
Cd	NS				NS			
Cu	$P = 0.034$				NS			
Cd×Cu	NS				NS			
Cd×Genotype	NS				$P < 0.001$			
Cu×Genotype	NS				NS			
Cd×Cu×Genotype	NS				$P < 0.001$			

Table 5 Fe concentrations in genotypes

Treatment	Fe concentration in shoots (mg/kg)				Fe concentration in roots (mg/kg)			
	A	B	C	D	A	B	C	D
Cd5Cu0	81.47±12.97	113.60±3.69	112.67±10.44	136.40±1.73	167.89±15.58	85.07±0.57	145.69±21.51	226.73±31.78
Cd5Cu2	250.53±22.23	70.27±4.74	73.67±6.87	106.73±1.56	117.00±7.77	101.27±6.27	137.80±4.75	192.96±17.49
Cd10Cu0	109.93±4.97	85.67±4.14	85.20±7.08	115.60±4.40	117.67±9.53	87.07±2.14	234.93±26.07	328.33±18.78
Cd10Cu2	93.40±2.91	82.46±3.38	79.42±13.94	95.20±3.14	133.44±6.30	94.11±13.24	127.78±6.11	247.00±7.47
Analysis of variance								
Genotype	$P < 0.001$				$P < 0.001$			
Cd	$P < 0.001$				$P = 0.003$			
Cu	NS				$P < 0.001$			
Cd×Cu	$P = 0.005$				NS			
Cd×Genotype	$P < 0.001$				$P < 0.001$			
Cu×Genotype	$P < 0.001$				$P = 0.006$			
Cd×Cu×Genotype	$P < 0.001$				$P = 0.004$			

Table 6 Zn concentrations in genotype

Treatment	Zn concentration in shoots (mg/kg)				Zn concentration in roots (mg/kg)			
	A	B	C	D	A	B	C	D
Cd5Cu0	48.73±3.67	30.67±1.16	41.33±4.04	39.87±2.26	44.78±0.87	25.53±1.92	50.44±3.85	46.63±10.02
Cd5Cu2	28.07±1.35	27.53±0.58	26.20±3.08	35.00±2.31	33.33±0.67	29.47±4.32	25.56±3.44	40.67±4.44
Cd10Cu0	39.27±7.66	26.40±1.10	50.28±5.59	30.07±0.74	31.78±1.37	24.20±0.46	72.67±7.77	52.83±7.80
Cd10Cu2	26.33±1.35	23.40±1.21	25.83±4.04	28.33±3.05	37.36±3.74	26.00±2.50	24.78±1.79	51.67±6.36
Analysis of variance								
Genotype	$P = 0.002$				$P < 0.001$			
Cd	$P = 0.045$				NS			
Cu	$P < 0.001$				$P < 0.001$			
Cd×Cu	NS				NS			
Cd×Genotype	NS				NS			
Cu×Genotype	$P < 0.001$				$P < 0.001$			
Cd×Cu×Genotype	NS				$P = 0.038$			

this study indicated that accumulation of Cd in shoots and roots varied with genotypes.

Jarvis *et al.* (1976) reported that roots Cd account for 34%–97% of the total Cd in plant. This study showed that the Cd concentrations were considerably higher in roots than in shoots. Root Cd may account for more than 80% of the total Cd in plants. Moreover, a more interesting finding was that the Cd concentration decreased from Jilinheino (A) to Zhongyou 307 (D) in shoots with zero Cu added. The rank of genotypes for Cd concentration was $A > B > C > D$. However, the situation was different in roots (Fig.1b). These results suggested that genotypic variation in Cd distribution may be related to the structural and physiological differences located in either shoots or roots. Moreover, the addition of Cu significantly reduced Cd accumulation in dark rice but not in white rice, especially in shoots. Therefore, the previous rank of Cd concentration in shoots disappeared. This may suggest that the mechanism of response to Cu and the translocation of Cd also varied with genotypes. Owing to the genotypic differences, it seems difficult to select the genotypes with low Cd grain from seedling performance. Further study on the molecular level of genetic character should be conducted.

3.2 Nutrient elements uptake

There are still some challenges for the effects of Cd addition on nutrient elements uptake (Jalil *et al.*, 1994; Vassilev *et al.*, 2002; Zhang *et al.*, 2002). Our present study

showed that the effect of Cd on nutrient elements uptake and accumulation by plants varied with plant section, the elements, and genotypes (Tables 3–6), which is consistent with the results reported by Zhang *et al.* (2002). With zero Cu treatment, Fe concentrations tended to decrease in shoots but the different change in roots varied with genotypes as the Cd addition increased (Table 5); increase of Cd addition tended to decrease the Ca concentration in dark rice and increase in white rice in shoots but did not affect that in roots considerably (Table 4); increase of Cd addition reduced Zn uptake in shoots and in roots (Table 6). With 2 $\mu\text{mol/L}$ Cu treatments, Fe accumulations in shoots and roots were seldom affected by increasing Cd addition; the changes of Ca and Zn concentration both in shoots and in roots varied with genotypes for the addition of Cd. These results may suggest that the ability of accumulation and translocation in plants depended on the genetic character in genotypes. We also found that the addition of Cu affected the Fe, Ca, and Zn accumulation in shoots and roots. These may be partly explained by the fact that plants take up ions as chemical equivalents and the effect on any one may influence the concentrations of the others (Vassilev *et al.*, 2002).

In summary, Cu significantly reduced Cd accumulation and translocation in dark rice but not in white rice; the effects of Cd and Cu on Ca, Fe, and Zn uptake by genotypes varied with genotypes; Cd accumulations were not significantly different between dark and white rice.

4 Conclusions

Cd uptake by rice depends on the genotypes but not on the color. Cu significantly reduced the Cd accumulation and translocation in dark rice. The effects of Cd and Cu on Ca, Fe, and Zn uptake by genotypes varied with genotypes. Furthermore, the current experiment was carried out only for the vegetative stage; therefore, there is still uncertainty whether the differences observed at the vegetative stage will result in similar difference in the grains.

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