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Cadmium uptake by different rice genotypes that produce white or dark grains

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Abstract: A pot experiment was conducted to investigate cadmium(Cd) uptake by different rice cultivars that produce white or dark grains. Four cultivars with white grains (hereafter, white rice) and five cultivars with dark colors (hereafter dark rice) were selected for this experiment. Three levels of soil Cd concentrations, background(0), 5 and 10 mg/kg, were used. After harvest, plant biomass, tissue concentrations of Cd, Ca, Fe, Cu and Zn were analyzed. The results showed that Cd concentrations are significantly different between different genotypes, but when comparing the Cd concentrations for the two groups, no significant difference was found. For other divalent cations, Ca concentrations in dark rice were higher than those in white ones ($P < 0.001$ for shoots, $P = 0.037$ for roots); Fe concentrations in dark rice were also higher than those in white ones($P = 0.001$ either in shoot or root); Zn concentrations in shoot of dark rice were higher than those in white ones, but no significant difference in roots. The total molar concentrations of divalent cations in dark rice were also significantly higher than in white rice. The potential benefit of higher Ca and Fe concentrations in dark rice and similar Cd concentrations in both groups is also discussed in this paper.

Keywords: cadmium; nutrient deficiency; plant nutrition; rice; dark grain; soil contamination

Introduction

Cadmium(Cd) is a non-essential trace metal that has adverse effects on plants, animals and humans. Environmental contamination with Cd due to anthropogenic activities, such as mining, industry, agriculture and waste disposal has been increasing since the beginning of the 20th century (Alloway, 1995). Research 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 has made considerable advances (Syers, 2001) since itai-itai disease was identified to be related to Cd pollution in Japan. 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, due to its potential toxicity to humans and its relatively high mobility from soil to plant(Jansson, 2002). Cadmium can be easily taken up by plants, and its subsequent transfer to humans through food chains has been given great attention owing to the fact that cereals, potatoes and vegetables account for 70% of dietary intake (Grawé, 1996).

The uptake and accumulation of Cd varies greatly between plant species (Welch, 1999; Kuboi, 1986; Bingham, 1975). Intraspecific variation in Cd concentration has been found in soybean, wheat, maize, sunflower, potatoes and rice(Wang, 1996; Eriksson, 1990; Florijin, 1993; Li, 1997; Wenzel, 1996; Zhang, 2000; Grawé, 2001). Furthermore, it has been documented that calcium (Ca), iron(Fe) and zinc(Zn) interfere with Cd absorption in plants (Jacobs, 1978; Fox, 1988; McKenna, 1992; Reeves, 2001a; 2001b). Previous studies have shown that populations with rice as their staple food are more susceptible to Ca, Fe and Zn deficiency due to relatively low densities of these elements in rice grains(Reeve, 2001a; 2001b; 2002; Chaney, 2001a; 2001b). 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 equivalent Cd intake (Tachechi, 1978; McKenzie-Parnell, 1988; Reeves, 2001a; 2001b; 2002).

China is one of the biggest rice producers and consumers in the world, and Cd uptake by rice is therefore a critical issue to ensure food safety there. Nevertheless, several studies have indicated that large areas of arable land have been contaminated by Cd, mainly by smelting industries and sewage irrigation(Cai, 1990; Nordberg, 1997; Chen, 1999; Jin, 2002). Soil Cd concentration is up to 26 mg/kg in some parts of China, and Cd in rice grains is as high as 2.4 mg/kg (Jin, 2002); these high Cd concentrations in rice have resulted in human health problems, such as renal dysfunction. There are many different cultivars of rice, some of which produce white grains and dark grains, and it is generally accepted that the latter contain relatively higher concentrations of Fe, Zn, Ca and perhaps Cu(Glahn, 2002; Yu, 2002; Lü, 2000; Yuan, 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. The purpose of this study was to determine the Cd uptake and accumulation

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by different rice genotypes that produce white or dark grains during vegetative growth in a pot experiment. In addition, uptake of calcium, iron, zinc and copper was also measured.

1 Materials and methods

1.1 Soil

A loamy soil(0—25 cm) was collected from Huairou, a suburb of Beijing. The soil sample was air-dried and passed through a 2-mm mesh before chemical analysis and potting. Soil basic properties(Table 1) were determined according to the methods recommended by the Chinese Society of Soil Science(Lu, 1999). Soil pH was measured in a 1:1 soil / water suspension with a combination electrode. Available N was extracted using 2 mol/L KCl solution; available P was extracted using 0.5 mol/L $NaHCO_3$ and available K was extracted using neutral 1 mol/L ammonium acetate. Cation exchange capacity(CEC) was measured using the method of displacing exchangeable cations on soil particles with NH_4^+ followed by NH_4^+ determination. The soil was uniformly supplied with N, P and K fertilizer at amounts equal to 200 mgN(urea)/kg soil, 133 mgP_2O_5/ kg soil as $CaHPO_4$ and 133 mgK_2O/kg soil as K_2SO_4. There were three Cd treatments: CK(0 Cd), Treatment 1(5 mgCd/kg soil) and Treatment 2 (10 mgCd/kg soil). The soils were amended by cadmium in form of $CdCl_2 \cdot 5H_2O$. The treated soil was thoroughly mixed and put into PVC pots(1 kg/pot) respectively. There were 3 replicates per treatment.

Table 1 Selected chemical properties of soil used in the experiment

pH	OM, %	Available N, mg/kg	Available P, mg/kg	Available K, mg/kg	CEC, cmol/kg	Total Zn, mg/kg	Total Cd, mg/kg
7.4	1.35	92.5	11.2	67.5	10.0	154.9	0.17

1.2 Plants

Selected seeds of 9 different rice genotypes as Table 2(4 with white grain and 5 with dark grain) were sterilized in 10% H_2O_2 for 10 min followed by thorough washing with deionized water. Seeds were then germinated in moist perlite, and allowed to grow to their four-leaf stage under controlled environment (day/night light period 14/10 h, day/night temperature 25/18℃ and relative humidity 40%/60% day/night). Seedlings were carefully washed to remove adhering perlite particles and transplanted to PVC pots(one plant each pot). Soil in each pot was kept submerged using deionized water. The growth temperature was controlled at 25/30℃ day/night and relative humidity 30%/80% day/night, with ambient light intensity in the greenhouse. Plants were harvested 12 weeks after transplanting. At harvest, plant roots were washed in tap water to remove soil particles, followed by final washing using deionized water. Plants were divided into roots and shoots before oven-dry at 70℃ for 72 h and dry weights were recorded. Dried samples were ground for later analysis.

Table 2 Selected rice in different genotype used in the experiment

Code	Cultivar name	Grain color	Source
1	Lüyuanzhan1	White	South of Agricultural University
2	Lühuangzhan	White	South of Agricultural University
3	Zhongyou 307	White	Jilin Academy of Agricultural Sciences
4	Jiyou 1	White	Jilin Academy of Agricultural Sciences
5	Zhinuo	Dark	Chinese Academy of Agricultural Sciences
6	Jilin Heinuo	Dark	Chinese Academy of Agricultural Sciences
7	Tianjin 1032	Dark	Chinese Academy of Agricultural Sciences
8	Shangzhuangbuxienuo	Dark	Chinese Academy of Agricultural Sciences
9	Duanshuxiejing	Dark	Chinese Academy of Agricultural Sciences

1.3 Plant analysis

Samples from shoots(0.5 g) and roots(0.25 g) were digested in a polyvinyl-fluoride crucible with 4 ml of concentrated nitric acid(HNO_3). The suspension was left at room temperature for two hours before the sealed vessel was fitted into a high-pressure metal cylinder. These were heated in an oven at 100℃ for 1 h followed by heating at 170℃ for 5 h. After digestion, vessels(open at this stage) were put on a hot plate at 105℃ to evaporate excessive acid until about 0.5 ml remained. The residual samples were then diluted with de-ionized water to 50 ml and stored at 4℃ prior for analysis.

Concentrations of Cd, Zn, Ca, Cu, and Fe in shoots and roots were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7500 USA) with an auto-sampler (Chen, 2002). Interference corrections were made for ^{114}Cd with ^{115}Cd, and for ^{66}Zn, ^{57}Fe, ^{65}Cu and ^{43}Ca with ^{72}Ge. Samples of tea and soil with certified concentration of minerals (GBW07605, GBW07401 respectively) were included to assure the accuracy of measurement, and all values were within the acceptable range.

2 Results

2.1 Plant biomass

There are significant differences in biomass of roots and shoots between rice cultivars(Table 3). Cultivar 5 had the lowest biomass for both root and shoot irrespective of treatments. The addition of 5 mgCd/kg only marginally affected plant biomass in most cultivars; while the addition of 10 mgCd/kg significantly reduced the biomass of both shoots and roots, except for cultivars 2 and 3. Further analysis showed that the shoot biomass of white rice is significant higher than that of dark rice($P = 0.004$) but no statistical difference in root biomass between the two groups.

Table 3 Biomass of shoots and roots of rice genotypes, with different additions of cadmium

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	7.34 ± 0.55	5.78 ± 0.32	5.69 ± 0.55	3.43 ± 0.61	1.59 ± 0.10	1.68 ± 0.28
2	5.87 ± 0.89	6.31 ± 0.31	5.27 ± 0.56	1.61 ± 0.37	1.72 ± 0.18	1.44 ± 0.30
3	7.91 ± 0.13	9.50 ± 0.56	8.06 ± 0.95	2.65 ± 0.23	2.57 ± 0.16	2.06 ± 0.58
4	9.21 ± 0.30	9.36 ± 0.63	7.46 ± 0.20	3.41 ± 0.06	3.23 ± 0.31	2.39 ± 0.08
5	3.75 ± 0.26	3.02 ± 0.60	2.50 ± 0.53	1.40 ± 0.21	0.64 ± 0.16	0.54 ± 0.13
6	7.01 ± 0.13	5.77 ± 0.35	5.90 ± 0.11	2.84 ± 0.13	2.29 ± 0.22	2.42 ± 0.12
7	7.16 ± 1.06	6.91 ± 0.86	6.21 ± 0.60	3.84 ± 1.52	2.17 ± 0.51	2.30 ± 0.31
8	6.45 ± 0.35	5.75 ± 1.19	5.85 ± 0.70	1.88 ± 0.10	1.85 ± 0.63	1.77 ± 0.33
9	5.60 ± 0.19	4.85 ± 1.14	3.39 ± 1.01	1.54 ± 0.07	1.50 ± 0.43	0.91 ± 0.24
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P = 0.002$			$P < 0.001$	
G × Cd		$P = 0.680$			$P = 0.560$	

2.2 Uptake of cadmium

Addition of Cd to soil increased Cd concentrations in roots and shoots of all cultivars significantly (Table 4). Cd concentrations in both shoots and roots were significantly different between the genotypes ($P < 0.001$). Cd concentrations in roots were much higher than in shoots. Cd concentrations in different cultivars responded differently to increasing soil Cd concentrations. For example, at 5 mgCd/kg, cultivars 1 and 3 had the lowest shoot Cd concentrations but cultivar 6 had the lowest Cd concentration with 10 mgCd/kg added. The lowest Cd concentrations in roots with 5 mgCd/kg added were in cultivars 1 and 7, and the lowest concentration with 10 mgCd/kg added was in cultivar 7. However when comparing Cd concentrations in white rice with those in dark ones, no significant difference was found between two groups.

Table 4 Cd concentration in different rice genotypes (mg/kg)

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	0.11 ± 0.01	2.33 ± 0.11	5.41 ± 0.08	1.04 ± 0.04	34.17 ± 0.56	85.24 ± 3.06
2	0.15 ± 0.01	4.95 ± 0.31	6.11 ± 0.23	0.59 ± 0.02	89.00 ± 3.96	123.23 ± 11.86
3	0.05 ± 0.00	2.37 ± 0.03	5.77 ± 0.22	0.49 ± 0.02	54.52 ± 2.56	143.41 ± 7.69
4	0.07 ± 0.00	3.35 ± 0.11	6.37 ± 0.16	0.55 ± 0.03	65.35 ± 1.49	129.19 ± 9.91
5	0.05 ± 0.00	2.98 ± 0.07	7.68 ± 0.17	0.42 ± 0.01	45.74 ± 1.08	110.30 ± 7.10
6	0.09 ± 0.00	2.60 ± 0.03	3.43 ± 0.23	0.49 ± 0.00	41.41 ± 0.43	82.77 ± 1.38
7	0.05 ± 0.00	2.69 ± 0.19	5.83 ± 0.42	0.44 ± 0.02	36.17 ± 1.32	57.17 ± 0.36
8	0.04 ± 0.00	4.50 ± 0.06	5.03 ± 0.22	0.89 ± 0.04	38.98 ± 4.21	88.61 ± 3.12
9	0.14 ± 0.01	4.54 ± 0.21	7.89 ± 0.31	0.96 ± 0.01	100.68 ± 9.90	129.11 ± 6.83
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P < 0.001$			$P < 0.001$	
G × Cd		$P < 0.001$			$P < 0.001$	

Total Cd uptake also increased significantly with increasing Cd additions (Table 5), and differed between the cultivars except for cultivar 9 which reduced slightly with 10 mgCd/kg. Except for cultivars 2 and 9, increasing Cd addition from 5 to 10 mg/kg approximately doubled total Cd uptake. The percentages of total Cd uptake translocated to shoots were relatively low (Fig. 1) and differed between cultivars. At the background level of soil Cd (0.17 mg/kg), the percentages translocated ranged from 15% to 50%. With the addition of Cd, the percentages translocated were reduced and there were only small changes in percentages translocated to shoots between the two levels of Cd additions, except for cultivars 6 and 8 (decrease) and 9 (increase).

Table 5 Total uptake of Cd by different rice genotypes (μg/pot)

	Total Cd uptake		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg
1	4.41 ± 0.77	67.87 ± 3.53	173.01 ± 23.34
2	1.89 ± 0.44	182.67 ± 11.45	208.93 ± 39.33
3	1.73 ± 0.08	161.71 ± 3.96	343.26 ± 95.27
4	2.57 ± 0.09	241.73 ± 20.33	355.51 ± 27.65
5	0.77 ± 0.10	37.75 ± 8.42	76.60 ± 14.21
6	1.98 ± 0.03	109.76 ± 8.97	221.13 ± 13.08
7	1.96 ± 0.61	97.92 ± 22.83	167.33 ± 17.95
8	1.96 ± 0.15	93.31 ± 23.62	187.97 ± 36.10
9	2.23 ± 0.03	181.51 ± 60.60	142.71 ± 36.35
	Analysis of variance		
Genotype (G)		$P < 0.001$	
Cd		$P < 0.001$	
G × Cd		$P < 0.001$	

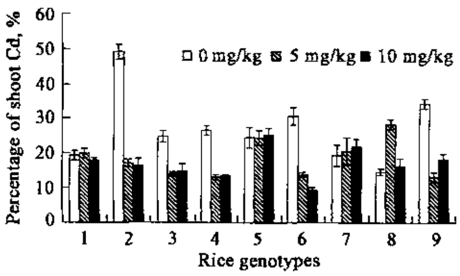

Fig.1 Percentages of total Cd translocated to shoots at different soil Cd concentrations
Error bars are S.E. values

2.3 Uptake of calcium, iron, zinc and copper

Calcium concentrations in shoot ranged from 0.40 to 0.94 gCa/100g rice plants for all cultivars with different treatments (Table 6). There were significant differences in Ca concentration in shoots, but no significant differences between Cd treatments. Ca concentrations in roots were generally lower than in shoots, and different cultivars responded differently to Cd additions. For cultivars 1, 2, 5, 6, 8 and 9, Cd additions slightly increased Ca concentration in roots; for cultivars 3 and 4, Cd additions had no effect, and for cultivar 7 addition of 10 mg/kg slightly reduced the root Cd concentration. Further analysis was performed on white and dark rice genotype the significant difference was found between these two rice genotypes ($P < 0.001$, $P = 0.037$ respectively for shoot and root).

Table 6 Ca concentration in different rice genotypes (g/100g)

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	0.64 ± 0.01	0.62 ± 0.02	0.70 ± 0.05	0.42 ± 0.01	0.52 ± 0.02	0.56 ± 0.02
2	0.61 ± 0.03	0.62 ± 0.03	0.70 ± 0.03	0.42 ± 0.02	0.54 ± 0.01	0.59 ± 0.01
3	0.54 ± 0.01	0.39 ± 0.02	0.42 ± 0.04	0.43 ± 0.01	0.43 ± 0.01	0.42 ± 0.01
4	0.52 ± 0.02	0.52 ± 0.03	0.51 ± 0.02	0.43 ± 0.01	0.51 ± 0.01	0.48 ± 0.02
5	0.78 ± 0.04	0.79 ± 0.02	0.71 ± 0.02	0.58 ± 0.02	0.71 ± 0.07	0.55 ± 0.01
6	0.79 ± 0.01	0.61 ± 0.03	0.62 ± 0.05	0.48 ± 0.01	0.49 ± 0.02	0.60 ± 0.03
7	0.86 ± 0.01	0.86 ± 0.02	0.94 ± 0.03	0.54 ± 0.00	0.53 ± 0.05	0.41 ± 0.02
8	0.93 ± 0.07	0.90 ± 0.04	0.83 ± 0.02	0.47 ± 0.03	0.57 ± 0.05	0.60 ± 0.09
9	0.67 ± 0.01	0.7 ± 0.06	0.63 ± 0.05	0.40 ± 0.02	0.60 ± 0.03	0.58 ± 0.03
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P = 0.104$			$P < 0.001$	
G × Cd		$P = 0.001$			$P = 0.004$	

Iron concentrations in shoots differed significantly between cultivars (Table 7). Although ANOVA analysis showed that Cd treatments had significant effects on Fe concentration in shoots, the effect was not consistent among cultivars. With 5 mg/kg Cd, Fe concentrations decreased slightly in shoots of cultivars 2, 4 and 9. Fe concentrations in roots were much higher than in shoots (Table 7), and there were significant differences between cultivars. Increasing Cd addition enhanced Fe concentrations in roots except for cultivars 1, 5 and 7. Further analysis showed that significant difference ($P < 0.01$) exists between the two groups of rice cultivars.

Table 7 Iron concentration in different rice genotypes (g/kg)

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	0.27 ± 0.01	0.25 ± 0.01	0.22 ± 0.02	7.01 ± 0.34	8.36 ± 1.16	7.78 ± 0.81
2	0.21 ± 0.02	0.17 ± 0.01	0.21 ± 0.02	7.20 ± 0.34	10.43 ± 0.42	10.77 ± 0.32
3	0.17 ± 0.01	0.16 ± 0.01	0.22 ± 0.01	7.41 ± 0.52	8.24 ± 0.54	8.52 ± 0.64
4	0.23 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	5.36 ± 0.26	6.37 ± 0.59	7.20 ± 0.28
5	0.25 ± 0.02	0.28 ± 0.03	0.25 ± 0.02	14.67 ± 1.24	15.10 ± 0.68	11.65 ± 2.56
6	0.26 ± 0.01	0.25 ± 0.02	0.25 ± 0.01	11.33 ± 0.28	11.00 ± 0.95	13.68 ± 0.43
7	0.25 ± 0.03	0.23 ± 0.03	0.26 ± 0.01	10.79 ± 0.79	10.00 ± 0.56	9.12 ± 0.45
8	0.24 ± 0.02	0.26 ± 0.02	0.24 ± 0.01	9.04 ± 0.55	9.52 ± 1.36	12.89 ± 1.94
9	0.35 ± 0.01	0.28 ± 0.01	0.33 ± 0.01	7.93 ± 0.19	8.59 ± 0.94	10.45 ± 2.29
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P = 0.013$			$P < 0.030$	
G × Cd		$P = 0.017$			$P = 0.046$	

Zinc concentrations in shoots differed significantly between cultivars and treatments (Table 8). Zn concentrations in shoots tended to decrease with increasing Cd added except for cultivars 2, 3 and 4. Zn concentrations in roots were generally higher than in shoots (Table 8). Significant differences between white and dark genotypes were observed for shoots ($P < 0.01$) but not for roots.

Table 8 Zinc concentrations in different rice genotypes(mg/kg)

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	17.08 ± 1.41	17.34 ± 0.69	14.44 ± 1.48	20.09 ± 1.26	17.30 ± 0.37	29.89 ± 2.93
2	16.96 ± 0.46	18.38 ± 0.69	15.29 ± 1.37	16.98 ± 1.01	20.42 ± 0.08	17.01 ± 0.63
3	12.34 ± 1.53	14.43 ± 0.34	20.93 ± 5.37	16.51 ± 0.16	17.22 ± 0.16	23.60 ± 0.58
4	14.34 ± 1.24	12.36 ± 0.10	13.00 ± 0.24	13.90 ± 0.34	18.15 ± 0.34	17.97 ± 0.80
5	21.80 ± 1.22	20.53 ± 0.74	16.28 ± 0.45	17.89 ± 0.75	18.20 ± 0.75	16.87 ± 2.02
6	19.12 ± 0.97	17.73 ± 1.37	12.64 ± 0.08	20.02 ± 0.67	33.47 ± 0.67	23.66 ± 1.22
7	19.36 ± 0.62	15.97 ± 0.48	16.22 ± 0.49	18.05 ± 0.82	18.31 ± 0.82	19.98 ± 0.71
8	21.44 ± 1.23	17.37 ± 0.96	15.44 ± 0.24	15.37 ± 0.11	16.65 ± 0.11	16.58 ± 0.06
9	22.20 ± 1.59	18.31 ± 1.66	19.12 ± 1.04	19.47 ± 1.21	23.69 ± 0.12	23.65 ± 0.79
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P = 0.004$			$P < 0.001$	
G × Cd		$P < 0.001$			$P < 0.001$	

Copper concentrations in shoots differed significantly between cultivars and increases of Cd adding tended to decrease Cu concentration (Table 9). Cu concentrations in roots were higher than in shoots. Cu concentrations in roots increased with increasing Cd additions in white rice but reverse situations were found in dark. However no statistical differences were found between the two groups of rice genotypes.

Table 9 Copper concentrations in different rice genotypes (mg/kg)

	Cd additions					
	Shoot			Root		
Genotypes	0 mg/kg	5 mg/kg	10 mg/kg	0 mg/kg	5 mg/kg	10 mg/kg
1	6.35 ± 0.24	5.36 ± 0.54	4.87 ± 0.39	9.45 ± 0.33	8.04 ± 0.48	9.41 ± 0.74
2	7.11 ± 0.46	6.02 ± 0.65	5.04 ± 0.47	8.35 ± 0.80	12.57 ± 0.40	10.27 ± 0.46
3	6.73 ± 0.20	5.04 ± 0.14	5.99 ± 0.15	8.79 ± 0.49	8.90 ± 0.42	12.34 ± 0.29
4	6.64 ± 0.54	5.87 ± 0.24	5.57 ± 0.18	8.40 ± 0.40	10.11 ± 0.63	11.29 ± 0.60
5	6.31 ± 0.46	7.61 ± 0.21	5.72 ± 0.27	8.71 ± 0.60	11.78 ± 0.68	10.52 ± 0.11
6	6.59 ± 0.20	7.52 ± 0.18	5.29 ± 0.33	7.88 ± 0.44	10.87 ± 0.23	9.57 ± 0.72
7	6.61 ± 0.09	5.44 ± 0.26	5.36 ± 0.26	7.03 ± 0.26	9.33 ± 1.61	7.44 ± 0.61
8	5.96 ± 0.52	6.49 ± 0.39	5.29 ± 0.68	9.17 ± 0.44	9.63 ± 1.02	8.84 ± 0.24
9	9.79 ± 0.73	8.21 ± 0.21	7.95 ± 0.01	12.04 ± 0.46	16.75 ± 0.81	18.87 ± 3.33
	Analysis of variance					
Genotype (G)		$P < 0.001$			$P < 0.001$	
Cd		$P < 0.001$			$P < 0.001$	
G × Cd		$P = 0.002$			$P = 0.003$	

Total molar concentrations (TMC) of Ca, Fe, Zn and Cu are shown in Fig. 2. For cultivars 1, 2, 4 and 5, Cd addition had no effect on TMC; for cultivar 7 TMC at 10 mgCd/kg was slightly higher than the other two treatments; for the other cultivars, TMC at 10 mgCd/kg were slightly lower than the other two treatments. Furthermore, the total molar concentrations in dark rice were significantly higher than that in white rice ($P < 0.001$).

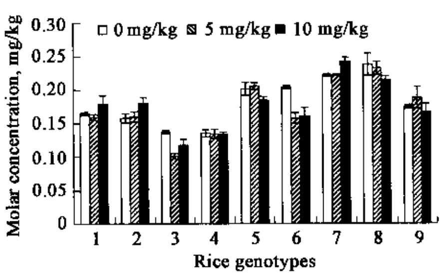

Fig.2 Total molar concentration of Ca, Cd, Cu, Fe and Zn in different rice genotypes
1—4, white rice; 5—9, dark rice; error bars are *S.E.* value

3 Discussion

One of the agronomic measures to reduce the health risk of soil contamination with Cd is to breed cultivars with reduced accumulation of Cd and increase divalent elements such as Ca, Fe, Zn in the grains (McLaughlin, 1999). The present study demonstrated that genotypic variation in the uptake and translocation of Cd. However, there are no significant differences in Cd concentrations between the two groups of rice genotypes. Total molar concentrations of other divalent elements (Fe, Ca, Zn and Cu) differed significantly between the two groups. This situation may be explored to reduce the health risk of Cd accumulation in rice. It has been shown that marginal deficiencies of Fe, Zn and Ca in diets could enhance the health risk of food-derived Cd (Reeves, 2001a; 2001b; 2002). Rice is known to contain relatively low densities of Fe, Zn and Ca compared with other cereal crops, such as maize, wheat and beans (Reeves, 2002), therefore rice is considered as a Cd-risk crop. In order to reduce the health risks associated with soil contamination with Cd, while attempts should be made to reduce the uptake by and translocation within rice plants, it may be equally important to increase the densities of Fe, Zn and Ca in grains so as to reduce Cd bioavailability in food. In China, there are numerous rice genotypes that produce dark-colored grains, and it is generally known that dark rice often contain relatively higher densities of Fe, Zn and Ca (Lü, 2000). Our study confirmed that the total molar concentrations of Fe, Zn, Ca and Cu in shoots of rice genotypes producing dark-colored rice were significantly higher ($P < 0.001$) than those producing white grains. Despite the higher molar concentrations of Fe, Zn, Ca and Cu in shoots of genotypes producing dark-colored grains, there was no significant difference in concentrations of Cd in shoots between the two types of rice genotypes. This may potentially result in higher molar ratios of Cd: (Fe + Zn + Ca + Cu) in grains with dark color than in white grains, which is conducive to minimize the toxicity of Cd in rice grains.

The actual antagonistic effect of Fe, Zn, Ca and Cu in rice grain may depend on the bioavailability of these elements in these two groups of rice. Using a limited number of cultivars with dark grains, Glahn *et al*. (Glahn, 2002) demonstrated that the bioavailability of Fe was markedly lower than that in white grains. This result may discount the actual beneficial effect of increased Fe, Zn, Ca and Cu in dark-colored grains on reducing Cd toxicity, while more genotypes remain to be tested systematically. However, it is unknown if Cd in dark-colored is also less bioavailable than in white rice grains. If this is the case, planting rice cultivars producing dark-colored grains may be useful in reducing the health risk of soils with low or moderate Cd contamination. Given that fact that Cd contamination is a widespread problem in areas with mining and smelting activities in China, further investigation on the Cd behavior in soil-rice-grain-human with emphasis on biotic factors, such as antagonistic effects of Fe, Zn, Ca and Cu, bioavailability of Cd and other minerals in rice genotypes different grain colors is warranted. 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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