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Genotypic and environmental variation in cadmium, chromium, arsenic, nickel, and lead concentrations in rice grains*

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Abstract: Genotypic and environmental variation in Cd, Cr, As, Ni and Pb concentrations of grains, and the relationships between these heavy metals and Fe, Zn were investigated using 9 rice genotypes grown in 6 locations for two successive years. Significant genotypic variation was detected in the five heavy metal concentrations in grains, indicating the possibility to reduce the concentration of these heavy metals in grains through breeding approach. The environmental effect varied with metal, with Pb and Ni having greater variation than the other three metals. There was significant genotype-environment (location) interaction of the concentrations of all five heavy metals in grains, suggesting the importance of cultivar choice in producing rice with low heavy metal concentrations in grains for a given location. Correlation analysis showed that Cd and As, Cr and Ni, and As and Pb concentrations in rice grains were closely associated, and that Ni concentration in grains was negatively correlated with Zn concentration.

Key words: Rice (*Oryza sativa* L.), Heavy metals, Genotype, Environment, Grain
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INTRODUCTION

Heavy metals are found ubiquitously in both polluted and unpolluted soils. Although these heavy metals occur naturally in the Earth's crust, they tend to be concentrated in agricultural soil because of irrational application of commercial fertilizers, manures and sewage sludge containing heavy metals and of contamination caused by mining and industry (Gimeno-García *et al*., 1996; Grant *et al*., 1998; McLaughlin *et al*., 1999). All heavy metals are toxic at higher concentrations (Marschner, 1995; McLaughlin *et al*., 1999). Heavy metals are toxic to higher plants by causing oxidative stress, displacing other essential metals in plant pigments or enzymes, leading to disruption of function of these molecules and of many metabolic processes, and finally reducing growth and yield (Rulkens *et al*., 1998; Seregin and Ivanov, 2001; Verma and Dubey, 2001; Zhang *et al*., 2002; Wang *et al*., 2003). Moreover, toxic heavy metals enter the food chain due to uptake and accumulation by crops, posing a potential threat to human health (Jackson and Alloway, 1992; Brzóska and Moniuszko-Jakoniuk, 2001; Sponza and Karaoğlu, 2002). Among the heavy metals, cadmium (Cd), arsenic (As), chromium (Cr), nickel (Ni) and lead (Pb) are commonly considered as toxic to both plants and humans. For instance, in Japan, Cd contamination of rice led to renal impairment and bone disease in an exposed population. It is necessary to decrease toxic heavy metal accumulation in cereals for food production, particularly in rice, which is one of the most

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frequently consumed cereals worldwide.

Heavy metal accumulation in crops is a function of complex interaction among soil, plant and environmental factors. It has been well documented that the contents of heavy metals in crop plants are closely associated with their levels in soil. Moreover, the uptake and accumulation of heavy metal by plants are largely dependent on the available rather than total level of a metal in soil (Dudka *et al*., 1996; Garrett *et al*., 1998; Norvell *et al*., 2000; Moral *et al*., 2002). The uptake of some heavy metals varies greatly among plant species (Sarić, 1983). In rice, a wide difference exists among genotypes in their ability to accumulate Cd in grains (Morishita *et al*., 1987; Arao and Ae, 2003; Liu *et al*., 2003), indicating the potential possibility of reducing grain Cd accumulation by means of genetic improvement. Breeding for low Cd accumulating cultivars has been undertaken in sunflower and durum wheat (Li *et al*., 1997; Penner *et al*., 1995). Meanwhile, environmental factors which may alter the availability of heavy metals in soil and the metabolic pattern of crop plants, are also the cause of variation of heavy metal accumulation in crops (Garrett *et al*., 1998; Moral *et al*., 2002; Nan *et al*., 2002; Norvell *et al*., 2000; Wu *et al*., 2002). Thus in moderately contaminated soils, heavy metal accumulation in crops could be reduced by using alternative cultivars with lower accumulation or by improving agronomic practices, such as water and fertilizer management, which lower the availability of heavy metals in the rhizosphere (McLaughlin *et al*., 1999; Melamed *et al*., 2003). These practices will depend on understanding genetic and environmental variation in heavy metal concentrations of crops. However, little is known about genotypic and environmental variation in toxic heavy metals, including Cd, Cr, Ni, Pb and As.

The objectives of the present study were to determine: (1) genotypic and environmental effects on the concentrations of the toxic heavy metals, Cd, As, Cr, Ni and Pb in rice grain; (2) the correlation among these toxic heavy metal and two micro-nutrient (Fe and Zn) concentrations in rice grains.

MATERIALS AND METHODS

Planting and sampling

During the rice growing season (May to November) in two successive years, 2002 and 2003, nine *Japonica* rice genotypes, namely Jiahua 1, Xiushui 110, Xiushui 63, Xiushui 11, Guan 95, Xiushui 213, ZH9820, Xiushui 50 and Chunjiang 101, differing in the grain concentration of heavy metals, according to a previous survey (data unpublished), were grown at six locations differing in heavy metal content in soil (Table 1), in Jiaxing, China (31°15′ N, 121°18′ E). In each location, the genotypes were grown in adjacent 1.8 m×5.0 m plots in a paddy field. The experiment was arranged in a randomized complete block design

Table 1 Soil characteristics for six locations

Location	Year	Soil pH	DTPA-extractable content in soil (mg/kg)				
			Cd	Cr	As	Ni	Pb
Hehua	2002	6.31	0.045	0.561	0.156	0.045	3.548
	2003	6.23	0.048	0.578	0.160	0.048	3.593
Yuxin	2002	6.31	0.031	0.554	0.158	0.033	2.932
	2003	6.25	0.035	0.561	0.159	0.032	2.900
Buyun	2002	4.60	0.036	0.867	0.156	0.027	3.548
	2003	4.90	0.045	0.879	0.157	0.027	3.545
Daqiao	2002	5.38	0.094	0.767	0.218	0.053	2.996
	2003	5.24	0.102	0.787	0.234	0.057	3.000
Shuangqiao	2002	5.81	0.062	0.883	0.130	0.045	2.283
	2003	5.78	0.065	0.888	0.140	0.049	2.100
Wangjangjin	2002	5.94	0.109	0.895	0.198	0.076	2.836
	2003	5.23	0.123	0.895	0.179	0.078	2.345
Mean			0.066	0.760	0.170	0.048	2.969
Max./Min. ratio			3.97	1.62	1.80	2.89	1.71

with three replicates. Field management followed standard local practice.

When the rice matured, both plants and soil were sampled randomly, with each rice sample consisting of 30~40 panicles, taken from five hills, and each about 1 kg soil sample was taken from the 0~15 cm soil layer.

Heavy metal analysis

The grain samples were dried in an oven at 70 °C for 48 h, dehulled and then milled. The milled rice was dried at 80 °C for 24 h, ground with a stainless steel grinder and passed through a 100-mesh sieve. The rice powder was ashed in a muffle furnace at 550 °C for 10 h, and dissolved by adding 2 ml of 1:1 (v/v) hydrochloric acid. After being air-dried and crushed, the soil samples were thoroughly mixed, and passed through a 2-mm polyethylene sieve. A pH meter was used to determine soil pH in a mixture of soil and deionized water (1:2.5, w/v). Heavy metals in the soil were extracted with 0.005 mol/L DTPA (diethylene triamine pentaacetic acid) according to the method of Lindsay and Norvell (1978). The contents of Cd, As, Cr, Ni, Pb and Zn, Fe in both rice grains and soils were simultaneously determined by ICP-AES (IRIS/AP, Inductively coupled plasma atomic emission spectroscope, TJA, USA). All values reported in this work are the mean of at least three independent measurements.

Statistics

All data were subjected to analysis of variance (ANOVA). Mean values of heavy metal concentrations in grains and soils were compared by the LSD method. Analysis of correlations among heavy metal concentrations in rice grain was performed using a statistical soft DPS (data processing system) developed by Tang and Feng (2002).

RESULTS AND ANALYSIS

Heavy metal concentrations in grains and soils

The pH of the soils at six locations ranged from 4.60 to 6.31, mean of 5.67. The results indicated that weakly acidic soils were predominant locally. The DTPA extracted-metal contents, which are commonly recognized as available for the uptake by plants, also differed greatly among the soils. Among the five heavy metals in this study, Cd showed the largest relative difference; presented as the ratio of the maximum/minimum concentration, with 3.97 fold and Ni ranked the second with a relative difference of 2.89 fold among the locations. In contrast, the other three elements, Cr, As and Pb had smaller differences, less than two fold. The difference in heavy metal content among locations with the same kind of soil could be due to different contamination.

A large difference in grain metal concentration was found among locations and genotypes, but not between years, although slightly higher in 2003 than in 2002 except for Pb (Tables 2 and 3). In terms of location, Pb had the largest relative difference with more than 21.1-fold, ranging from 0.013 mg/kg in Yuxin to 0.281 mg/kg in Daqiao, on average of the nine genotypes, and As had the smallest relative difference with less than 1.8-fold, ranging from 0.054 mg/kg in Buyun to 0.094 mg/kg in Daqiao. The relative difference among locations for other elements ranged from 1.9- to 2.9-fold. In terms of genotype, Cd showed the largest relative difference, being 7.4-fold averaged over the six locations, ranging from 0.025 mg/kg for Chunjiang 101 to 0.185 mg/kg for Xiushui 110. There were 3.4-, 3.1- and 2.7-fold differences in Cr, Pb and As concentrations, respectively, ranging from 0.29 mg/kg of ZH9820 to 0.98 mg/kg of Xiushui 50 for Cr, from 0.037 mg/kg of Xiushui 50 to 0.114 mg/kg of Xiushui 11 for Pb, and from 0.034 mg/kg of Chunjiang 101 to 0.090 mg/kg of Jiahua 1 for As, respectively. Ni had smallest relative difference among genotypes with less than 1.5-fold, although the difference was also significant. In the present study, approximately 34.72%, 10.19% and 10.18% of rice samples had the Cd, Cr and Pb concentration beyond the AMV (allowable maximum value) (Codex Alimentarius Commission, 2000), respectively, while no sample had Ni and As concentrations higher than the corresponding AMV, indicating that the major issue in safe rice production locally is the risk posed by Cd, Cr and Pb contamination.

Genetic and environmental effect on concentrations of the heavy metals in rice grain

The effects of location, genotype and interaction of location by genotype on the five heavy metal concentrations in rice grain were all highly significant

(Table 3), but no significant effect was found for year and interactions of year and location or genotype and interaction of location by genotype. The contribution of each factor or interaction between factors to the total variation (sum of square, *SS*) varied greatly in heavy metals. For Cd, genotype was the largest, being 44.79% of total *SS*, followed by interaction of genotype by location, being 41.70% of total *SS*, while all other factors and interactions only occupied 13.51% of total *SS*, indicating the predominance of genotype in affecting variation of grain Cd concentration. For Cr, As and Ni, the interaction of genotype by location was the largest, being 46.22%, 52.37% and 49.78% of total *SS*, respectively, and followed by genotype for Cr and As, and by location for Ni, while all other interactions had little contribution to the total *SS*. For Pb, the largest contributor of the total *SS* was location, being 51.27%, and interaction of location by genotype ranked second, being 40.77% of the total *SS*. With the similar to the other four metals, other interactions showed little effect on the variation of grain Pb concentration.

Table 2 Concentration (mg/kg) of five toxic heavy metals in grains of nine rice genotypes grown at six locations for two years

	Effect	Cd	Cr	As	Ni	Pb
Year	2002	0.090 a	0.47 a	0.072 a	0.52 a	0.079 a
	2003	0.092 a	0.48 a	0.077 a	0.54 a	0.075 a
Location	Hehua	0.086 b	0.22 e	0.082 ab	0.39 d	0.044 bc
	Yuxin	0.078 c	0.45 c	0.069 bc	0.68 b	0.013 d
	Buyun	0.061 d	0.60 a	0.054 c	0.74 a	0.028 cd
	Daqiao	0.115 a	0.64 a	0.094 a	0.63 c	0.281 a
	Shuangqiao	0.094 b	0.38 d	0.070 bc	0.40 d	0.057 b
	Wangjangjin	0.113 a	0.53 b	0.078 ab	0.32 e	0.038 bc
Genotype	Jiahua 1	0.082 d	0.42 c	0.090 a	0.55 b	0.079 c
	Xiushui 110	0.185 a	0.48 b	0.088 ab	0.43 f	0.100 ab
	Xiushui 11	0.093 c	0.41 cd	0.083 ab	0.49 cd	0.114 a
	Xiushui 63	0.097 bc	0.37 cd	0.075 bc	0.48 de	0.042 de
	Guan 95	0.101 b	0.49 b	0.063 cd	0.62 a	0.099 b
	Xiushui 213	0.099 bc	0.36 d	0.089 ab	0.60 a	0.055 d
	ZH9820	0.068 e	0.29 e	0.089 a	0.62 a	0.111 ab
	Xiushui 50	0.069 e	0.98 a	0.059 d	0.44 ef	0.037 e
	Chunjiang 101	0.025 f	0.47 b	0.034 e	0.53 bc	0.056 d

Note: The values within a column followed by different letters are significantly different at 95% probability

Table 3 Analysis of variance of grain heavy metal concentration in nine rice genotypes grown at six locations

Sources of variation	*df*	Cd		Cr		As		Ni		Pb	
		SS	*F* value	*SS*	*F* value	*SS*	*F* value	*SS*	*F* value	*SS*	*F* value
Year (Y)	1	0.0004	1.87	0.0034	0.08	0.0013	239.98	0.0184	0.35	0.0013	0.93
Error *a*	1	0.0002		0.0449		0.0000		0.0531		0.0014	
Location (L)	5	0.0790	66.41**	4.2745	86.86**	0.0332	6.75**	5.4143	124.29**	1.8386	269.24**
Y×L	5	0.0004	0.30	0.0663	1.35	0.0006	0.12	0.0576	1.32	0.0036	0.53
Error *b*	10	0.0024		0.0984		0.0098		0.0871		0.0137	
Genotype (G)	8	0.3485	237.23**	7.6782	119.04**	0.0716	14.01**	1.0478	23.17**	0.1746	33.49**
Y×G	8	0.0007	0.49	0.0737	1.14	0.0005	0.11	0.0287	0.63	0.0021	0.41
L×G	40	0.3245	44.18**	11.5180	35.72**	0.1998	7.82**	7.4364	32.88**	1.4622	56.10**
Y×L×G	40	0.0043	0.59	0.3874	1.20	0.0034	0.13	0.2535	1.12	0.0260	1.00
Error *c*	96	0.0176		0.7740		0.0613		0.5427		0.0626	
Total	214	0.778		24.919		0.382		14.940		3.586	

Note: *SS:* Sum of square; ** represents significant and highly significant at 99% probability

Correlations among the concentrations of five toxic heavy metals and Fe, Zn in rice grain

The correlation among five toxic heavy metals and Zn, Fe concentrations, is shown in Table 4. A significant positive correlation was found between Cd and As, Cr and Ni, As and Pb or Zn, and Fe and Zn. In contrast, there was a significant negative association between Zn and Ni. These results indicate that high Cd and As concentrations would likely happen simultaneously, and the same thing could occur for Cr and Ni, As and Pb or Zn as well as grain Zn and Fe. Moreover, the results indicated that grain Zn concentration would be lower in grain with higher Ni concentration.

DISCUSSION

It becomes increasingly concern whether the safe agricultural product with lower heavy metal content can be produced in the slightly and moderately contaminated soils in light of extensive occurrence of the contamination. Development of the cultivars with lower toxic heavy metal accumulation in edible parts and meanwhile with high tolerance is commonly considered as the most effective approach for solving the issue. The existence of genetic difference in heavy metal uptake and accumulation, as well as tolerance has been found in diverse crop plants, including rice (Aniol and Gustafson, 1990; Yang *et al*., 2000; Zhang *et al*., 2000; Arao and Ae, 2003; Liu *et al*., 2003), indicating the possibility of developing the reasonable cultivars suitable for planting in the contaminated soil. However, most researches on the genetic difference in heavy metal uptake and accumulation are conducted in the pot or hydroponic solution, few investigations are done in the field. In the present research, we planted 9 rice genotypes in 6 locations with different soil DTPA-extracted heavy metal content, and found a huge difference in grain heavy metal concentration among the cultivars, in particular for Cd. Except for Pb, the genotypic effect on the variation of other four heavy metal content is much larger than the environmental effect. The results confirmed the possibility of reducing Cd, Cr, As and Ni concentration in rice grains through using low-accumulation cultivars. On the other hand, Pb concentration in grains is mainly dependent on the environment (soil condition). Thus the effective strategies of reducing Pb uptake and accumulation should be agronomic measurements, which may cause reduced Pb bio-availability in soil or uptake by the plants. Moreover, this study found a highly significant interaction between cultivar (genotype) and location (environment) in all the 5 heavy metal concentration of grains, suggesting the importance of using proper cultivars in a given environment. Although the mechanism of the interaction remains to be clarified, it may be assumed that the changeable growth and metabolic properties of rice plants, in particular of their roots under different environmental conditions could be relevant.

Interactions among the co-existed elements present at root surface and within the plants also affect their uptake and accumulation in plants (Fox, 1974; Anderson *et al*., 1992; Nan *et al*., 2002). In the current study, interaction between heavy metals and Fe or Zn, two important nutrients for human health, was detected. It was shown that Cd and As concentrations tended to change simultaneously in rice grains, and that the same tendency could be found for Cr and Ni, and As and Pb. In contrast, Zn concentra-

Table 4 Correlations among concentrations of toxic heavy metal and two nutrients in rice grains

	r						
	Cd	Cr	As	Ni	Pb	Fe	Zn
Cd	1						
Cr	0.1665	1					
As	0.4162**	−0.0823	1				
Ni	0.0320	0.2008*	0.0089	1			
Pb	0.1613	0.0672	0.1967*	0.0384	1		
Fe	0.1761	0.1460	0.0512	−0.0477	0.0869	1	
Zn	0.1655	0.0234	0.2763**	−0.2241*	0.0154	0.5202**	1

* and ** represent significant and highly significant at 95% and 99% probability, respectively

tion in grains showed significant negative correlation with Ni concentration. Much research had been conducted on the interaction of Cd and Zn in uptake and accumulation in plant because of their normally accompanying pollution in soil (Nan *et al*., 2002). Some reports concluded that Cd addition decreased the Zn concentration in corn (Root *et al*., 1975) and barley (Wu and Zhang, 2002). However, there were also contrasting reports on the relationship between Cd and Zn. For instance, Smilde *et al*.(1992), Moraghan (1993) and Nan *et al*.(2002) reported that Zn and Cd concentration in plants was synergistic. Clarke *et al*.(1997) found that the near-isogenic high/low Cd lines of durum wheat did not differ in grain Zn concentrations. This study did not find a distinct relationship between Cd and Zn concentrations in rice grains. The difference between the present results and those of others may be ascribed to the experimental conditions. It can be suggested that the interaction between heavy metals is more complex in the field than in the hydroponic solution and other elements. Chemical and physical properties of soil supposedly affect in the diverse patterns the bio-availability and uptake of both Zn and Cd, thus interfering with the interaction of Zn and Cd in their bio-availability and uptake. More researches should be done to gain understanding of the relationships between mineral nutrients and toxic heavy metals in their availability in soil and accumulation in plants, in order to develop rice cultivars with a balanced nutrient component and lower toxic heavy metal concentration.

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