Genotypic dependent effect of exogenous glutathione on Cd-induced changes in cadmium and mineral uptake and accumulation in rice seedlings *(Oryza sativa)*

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ABSTRACT

A hydroponic experiment was performed using Cd-sensitive (cv. Xiushui63) and tolerant (cv. Bing97252) rice cultivars to evaluate the difference in their response to Cd toxicity in the presence of exogenous glutathione (GSH). The results showed that Cd stress (5 and 50 μmol/l Cd) decreased plant fresh weight, contents of chlorophyll a, b and carotenoids, with Cd-sensitive genotype being more severely affected. Cd significantly decreased concentration and accumulation of Mn in roots/shoots, and Zn in shoots, but increased Cu concentration in roots/shoots. There was a significantly negative correlation between shoot Zn concentrations and shoot/root Cd concentrations, and between root Cd and Mn concentrations. Exogenous GSH significantly alleviated Cd-induced growth inhibition and markedly reduced Cd uptake in both genotypes. In addition, GSH induced a Cd-dose- and genotype-dependent effects on Cd-induced changes in mineral concentration/accumulation and chlorophyll content in rice seedlings. GSH alleviated Cd-induced decrease in root/shoot Zn and Ca concentrations and accumulation of Xiushui 63, while increased root Ca and Mn concentrations in Bing 97252 under 5 μmol/l Cd stress. In addition, GSH also significantly enhanced chlorophyll a and b contents of Bing 97252 in both 5 and 50 μmol/l Cd, and Xiushui 63 in 50 μmol Cd.

Keywords: cadmium; genotype; glutathione; mineral elements; rice (*Oryza sativa* L.); uptake

Cadmium (Cd) in soil represents a direct contact risk to both human and ecological receptors due to its relatively high toxicity and plant readily uptake (Chen et al. 2007). Recently, Cd has become one of the most harmful and widespread pollutants in agricultural soils mainly due to industrial emission, application of Cd containing phosphate fertilizers, and sewage sludge and municipal waste disposal. According to Japanese nationwide survey on Cd contents in cereals produced from 'non-polluted' area of Japan in 2005, 22 brown rice samples out of 1909 (1.2%), harvested in Japan contained Cd higher than 0.4 mg/kg of Cd. Cadmium principally occurs in the human diet as a result of its uptake and accumulation from soil by crop plants, although the whole pathway of Cd transfer into the food chain is also involved in input from atmosphere, water and aquatic life. For example, rice, a staple crop for Japanese, was estimated to represent 36–50% of the total oral intake of Cd for Japanese population during 1998–2001 (Kikuchi et al. 2008). Moreover, Cd contamination is a non-reversible accumulation process, with the estimated half-life in soil varying between 15 and 1100 years (Kabata-Pendias and Pendias 2001), and high plant-soil mobility to be easily accumulated in plant tissues, while high accumulation of Cd in plants not only deteriorate crop yield and quality, but also gives rise to a threat on human health via food chain. Excess Cd in the diet results in damage to kidney tubules, rhinitis, emphysema as well as other chronic disorders. The most typical case caused by such chronic Cd poisoning is so-called Itai-Itai disease which happened in mid-70's of last century in Japan (Kondo 1996).

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The uptake and distribution of Cd varies widely among plant species and cultivars within a species. It is widely accepted that Cd^{2+} enters root through competition of the transporters of essential metals including Ca, Zn and Mn (Clemens 2006). The interactions of Cd and metal nutrients were reported in some upland crops, such as wheat (Zhang et al. 2002), barley (Wu and Zhang 2002, Wu et al. 2003), tomato (Smith and Brennan 1983), and soybean (Cataldo et al. 1983). However, the interactions between Cd and other nutrients are complicated and quite different with species and genotypes in the same species. The reports available provided the contradictable results. Smith and Brennan (1983) reported a synergistic interaction between Cd and Zn in tomato, while Cataldo et al. (1983) observed antagonistic interaction between Cd and Fe, Zn, Cu, Mn in soybean. Zhang et al. (2002) showed genotype-dependent effects of Cd on Fe, Zn, Cu, Ca and Mg for uptake and translocation in wheat. These conflicting results presumably were due to the differences in the culture methods, species, and conditions such as concentration in medium, growth period and temperature. Therefore, more researches are needed in the interactions between Cd and other elements which could provide clues to explain the nature of Cd accumulation in crops.

Reduced glutathione (γ-Glu-Cys-Gly, GSH), due to its unique redox and nucleophilic properties, is involved in the cellular defense against the toxic action of xenobiotics, oxyradicals, salinity, acidity and as well as metal cations (Wu et al. 2004). It was suggested that GSH acts as a first defense line against metal toxicity through complexing metals before induced synthesis of phytochelatins (PCs) reaches to an effective level (Wu et al. 2004). GSH is also the direct substrate for the synthesis of PCs. Xiang et al. (2001) reported that genetically modified *Arabidopsis* plants with low GSH levels were hypersensitive to Cd due to their limited capacity to produce PCs. Therefore, the question arises whether and/or how external GSH could act as a regulator in preventing Cd stress. The present study was conducted to evaluate the role of external GSH in Cd tolerance, Cd and mineral elements uptake and accumulation using two rice genotypes varying in Cd tolerance, and determine whether there is the difference between rice genotypes differing in Cd tolerance.

MATERIALS AND METHODS

Plant material and experimental design. The hydroponic experiment was carried out in Huajiachi Campus, Zhejiang University, Hangzhou, China. Two *Japonica* rice genotypes were used: Bing97252 and Xiushui63 as relatively Cd-tolerant and sensitive, respectively (Hassan et al. 2005).

Healthy seeds were surface sterilized by soaking in 1.5% H_2O_2 for 30 min, fully rinsed with deionized water. After soaking in deionized water at room temperature for 2 days, the seeds were germinated at 35°C for 1 day. The healthy and germinated seeds were sowed in sterilized sand bed and kept in an incubator at 30°C-day/28°C-night under 80% relative humidity (RH). At the 2nd leaf stage (10 days old), the uniform healthy plants were selected and transplanted to 20-l plastic containers filled up with 20 l basal nutrient solution (mg/l): 114.3 NH_4NO_3, 50.4 $NaH_2PO_4 \cdot 2\ H_2O$, 89.3 K_2SO_4, 110.8 $CaCl_2$, 405 $MgSO_4 \cdot 7\ H_2O$, 1.88 $MnCl_2 \cdot 4\ H_2O$, 0.09 $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$, 1.17 H_3BO_3, 0.05 $ZnSO_4 \cdot 7\ H_2O$, 0.04 $CuSO_4 \cdot 5\ H_2O$, 14.88 Fe-citrate. The container was covered with a polystyrol-plate with 48 evenly spaced holes (2 plants per hole) and placed in a greenhouse. On the 7th day after transplanting, Cd (as $CdCl_2$) and GSH (98%; Roche Nutley, Nutley, New Jersey, USA) were added to the corresponding containers to form 5 treatments: basal nutrient solution (control), 5 μmol/l Cd (Cd1), 5 μmol/l Cd + 50 μnol/l GSH (Cd1 + GSH), 50 μmol/l Cd (Cd2), and 50 μmol/l Cd + 50 μmol/l GSH (Cd2 + GSH). The experiment was laid in a split-plot design with treatment as the main plot and genotype as the sub-plot with 4 replicates. The nutrient solution pH was adjusted to 5.1 by 0.1 mol/l NaOH or HCl, and renewed once a week.

Fresh weight and metal concentration. After 15 days of treatment, plants were uprooted, and the roots were immersed in 20 mmol Na_2-EDTA for 15 min, then separated into roots and shoots (stems and leaves). Plant fresh weight were simultaneously measured, and then dried at 80°C and weighed. Dried shoots and roots were 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. Metal concentration was determined using a flame atomic absorption spectrometry (SHIMADZU AA-6300; Shimadzu Corporation, Japan; Fang et al. 1991).

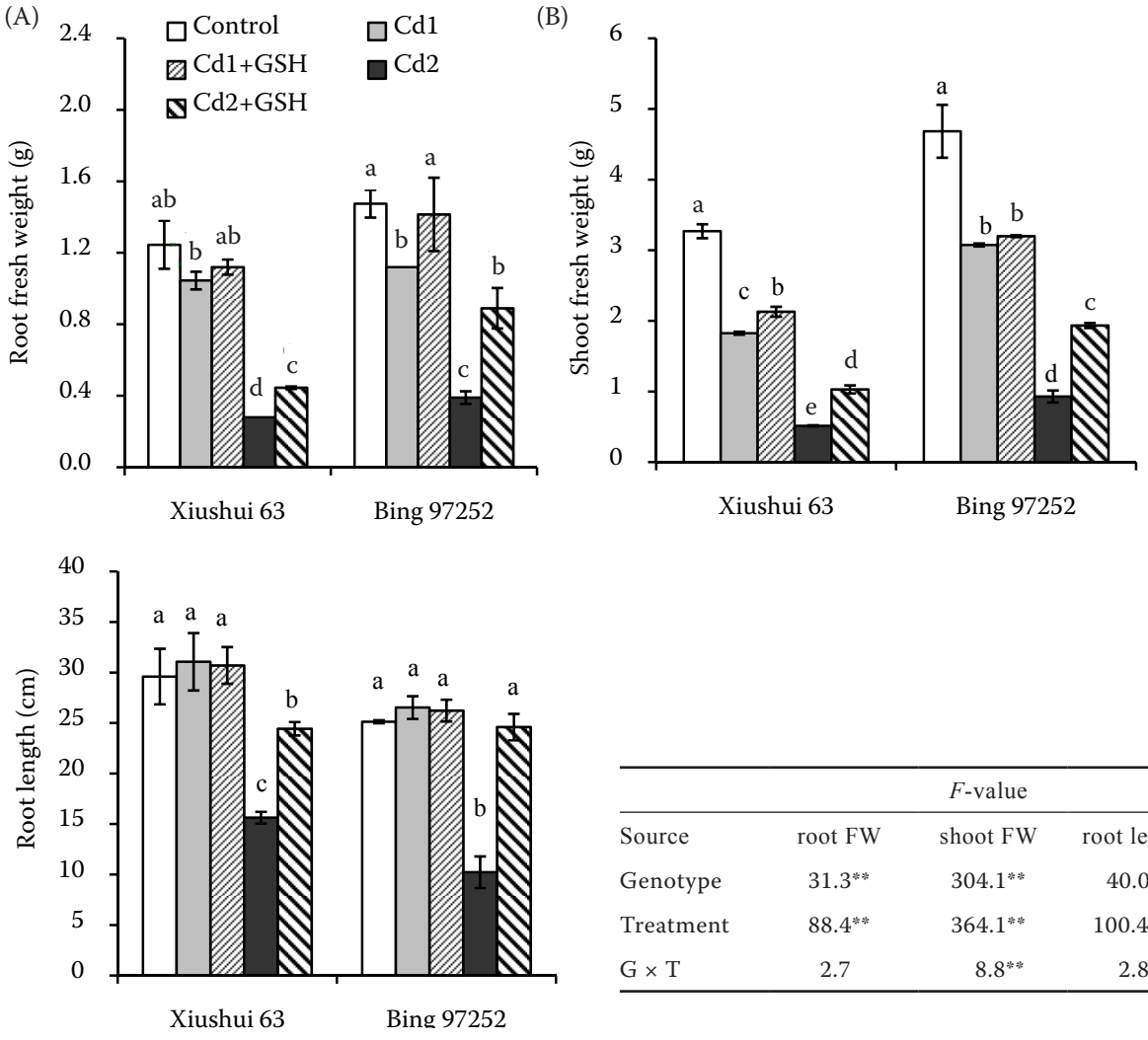
Content of chlorophyll a, b and total carotenoids. Chlorophyll (Chl) a, b and total carotenoids (Cart) content of the 2nd fully expanded leaves was determined according to the method of Chen (1984).

Statistical analyses were performed with Data Processing System (DPS) statistical software pack-

age (Tang and Feng 2002) using two-way ANOVA followed by the Duncan's Multiple Range Test (SSR).

RESULTS

Plant growth. Symptoms of Cd toxicity (5 or 50 µmol/l Cd single treatment) in rice leaves showed brown spots and yellow necrotic patches. As shown in Figure 1, 5 µmol/l Cd (Cd1) significantly reduced the root and shoot fresh weight, but did not affect the root length. Increasing Cd level to 50 µmol/l Cd (Cd2) caused more decrease in the fresh weight and root length. Furthermore, time of appearance and severity of Cd toxicity symptoms significantly differed between genotypes. The tolerant genotype Bing97252 was the genotype less affected, in terms of the above mentioned growth traits and yellow necrotic patches, whereas the sensitive Xiushui63 was more affected and Cd toxicity symptoms also appeared rapidly and severely (c.f. Cd_2 significantly reduced root and shoot weight by 74% and 80% in Bing97252, by 78% and 84% in Xiushui63, compared with the control). To investigate the protective role of GSH, 50 µmol/l GSH was added to the 5, 50 µmol/l Cd medium (Cd1 + GSH, Cd2 + GSH). After 15 days treatment, root length and plant fresh weight increased significantly compared with Cd single treatment in both genotypes (Figure 1), and effectively inhibited the appearance of Cd toxicity of chlorosis or necrosis in the leaves. Moreover, when rice plants treated with GSH in the presence



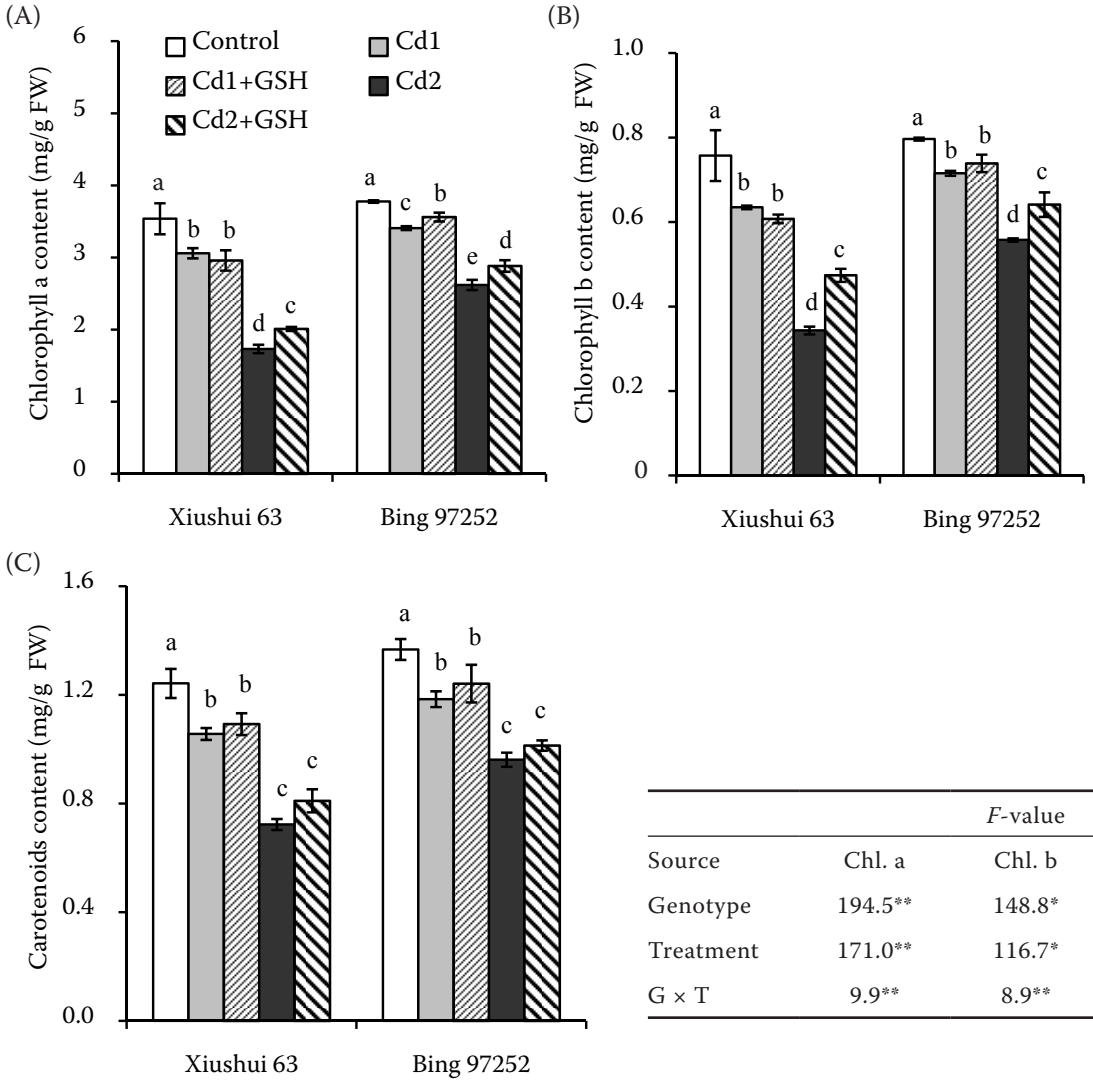
	F-value		
Source	root FW	shoot FW	root length
Genotype	31.3**	304.1**	40.0**
Treatment	88.4**	364.1**	100.4**
G × T	2.7	8.8**	2.8

Figure 1. Effect of Cd on fresh weight and root length of the two rice genotypes and as affected by external GSH. Error bars refer to SD values (n = 6). Treatments refer to control, Cd1, Cd1 + GSH, Cd2 and Cd2 + GSH, which represent the basic nutrition solution (BNS), BNS + 5 µmol/l Cd, BNS + 5 µmol/l Cd + 50 µmol/l GSH, BNS + 50 µmol/l Cd, and BNS + 50 µmol/l Cd + 50 µmol/l GSH, respectively. G × T, interaction between genotype and treatment; * and **significant at 0.05 and 0.01 probability level, respectively. Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype

of 50 µmol/l Cd, Cd-induced growth inhibition was evidently reduced especially in the tolerant genotype Bing97252. e.g. root fresh weight, shoot fresh weight and root length showed 158%, 108% and 140% increase in Bing97252; while 59%, 100% and 56% in Xiushui63, respectively, compared with 50 µmol/l Cd single treatment (Figure 1).

Exposure of the rice plants to 5 and 50 µmol/l Cd for 15 days significantly reduced Chl and Cart contents. Compared to the control, Cd1 treatment induced a significant reduction in the content of Chl a, b and Cart, with 14%, 16% and 15% in Xiushui63; and 10%, 10% and 13% in Bing97252, respectively. The reduction was more intensive by Cd2: in Xiushui63 it was 51%, 55% and 42%, and in Bing97252, 31%, 30% and 30%, when compared with the control (Figure 2). Addition of GSH in 5 µmol/l Cd did not change Chl b content in both genotypes, but significantly increased Chl a content of Bing97252, compared with Cd1. The mitigatory effect of GSH was better on 50 µmol/l Cd-induced inhibition in Chl a and b (c.f. increased by 16%, 38% in Xiushui63, and 10%, 15% in Bing97252 over Cd2, Figure 2).

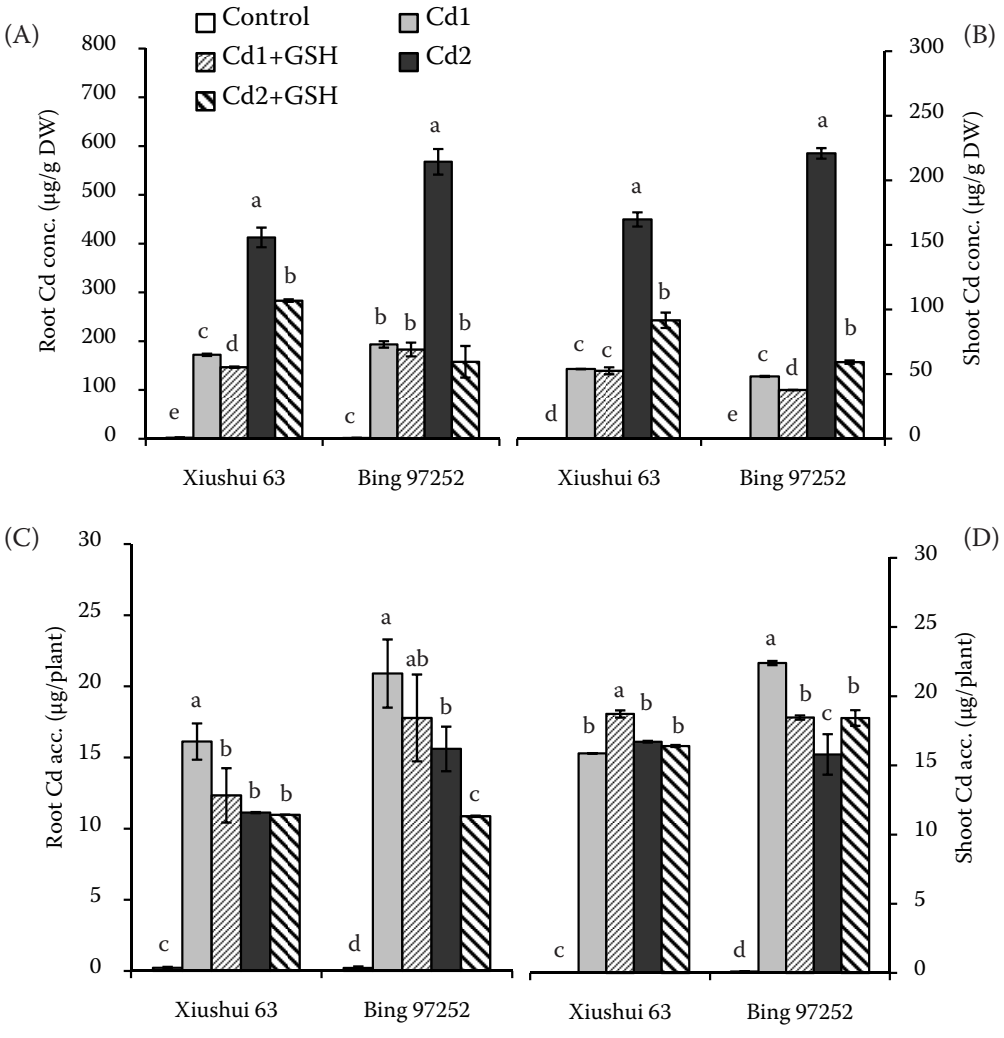
Cadmium concentration and accumulation. Cd concentration in roots and shoots increased with Cd levels in the medium (Figure 3A and B). The Cd concentration was much lower in shoots than in roots in the same treatment. The results of ANOVA showed significant genotypic differences between treatments, interaction of genotype and treatment ($P < 0.05$) for Cd concentrations of shoots and roots.



	F-value		
Source	Chl. a	Chl. b	Cart.
Genotype	194.5**	148.8*	88.2**
Treatment	171.0**	116.7*	88.7**
G × T	9.9**	8.9**	1.5

Figure 2. Effect of Cd on chlorophyll and carotenoids contents of the two rice genotypes and as affected by external GSH. Error bars refer to SD values ($n = 4$). Treatments refer to control, Cd1, Cd1 + GSH, Cd2 and Cd2 + GSH, which represent the basic nutrition solution (BNS), BNS + 5 µmol/l Cd, BNS + 5 µmol/l Cd + 50 µmol GSH, BNS + 50 µmol/l Cd, and BNS + 50 µmol/l Cd + 50 µmol/l GSH, respectively. G × T, interaction between genotype and treatment; * and **significant at 0.05 and 0.01 probability level, respectively. Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype

There were significant genotypic differences for Cd concentration in roots. Cd concentration of Cd1 and Cd1 + GSH treatment in roots of Bing97252 was significantly higher than that of Xiushui63; whereas it was contrary in shoots. Therefore, the ratio of shoot to root Cd concentration was significantly lower in Bing97252. e.g. when treated with 5 μmol/l Cd, the ratio of shoot to root Cd concentration was 0.25 in Bing97252 and 0.31 in Xiushui63. When plants were treated with 50 μmol/l Cd, both root and shoot Cd concentrations of Bing97252 were significantly higher than those of Xiushui63. GSH addition decreased Cd concentration in shoots and roots of both rice genotypes at 50 μmol/l Cd level, roots of Xiushui 63 and shoots of Bing 97252 at 5 μmol/l Cd level. Shoot and root Cd concentrations of Cd2 + GSH treated plants decreased by 46% and 31% in Xiushui63, by 73% and 72% in Bing97252, respectively, compared to Cd2 (Figure 3 A and B).



Source	*F*-value			
	shoot Cd	root Cd	shoot Cd	root Cd
	conc.		acc.	
Genotype	0.1	6.2*	42.9**	18.7**
Treatment	2351.1**	519.3**	996.0**	84.5**
G × T	107.0**	41.6**	35.2**	3.3

Figure 3. Effect of Cd and GSH in nutrient media on Cd concentration and accumulation in roots (A, C) and shoots (B, D) of the two rice genotypes. Error bars refer to SD values (n = 4). Treatments refer to control, Cd1, Cd1 + GSH, Cd2 and Cd2 + GSH, which represent the basic nutrition solution (BNS), BNS + 5 μmol/l Cd, BNS + 5 μmol/l Cd + 50 μmol/l GSH, BNS + 50 μmol/l Cd, and BNS + 50 μmol/l Cd + 50 μmol/l GSH, respectively. G × T, interaction between genotype and treatment; * and **significant at 0.05 and 0.01 probability level, respectively. Different letters indicate significant differences (P < 0.05) among the treatments within each genotype

Table 1. Effect of Cd and GSH in the nutrient media on Ca, Zn, Mn, Cu and Fe concentrations of the two rice genotypes

Treatment	Mineral concentrations (μg/g DW)									
	shoot					root				
	Ca	Zn	Mn	Cu	Fe	Ca	Zn	Mn	Cu	Fe
Bing 97252										
Control	641^{a}	32.2^{a}	335^{a}	11.0^{b}	30.2^{b}	296^{a}	43.9^{a}	11.8^{ab}	16.4^{d}	56^{b}
Cd1	502^{a}	18.4^{b}	115^{b}	9.7^{b}	21.2^{c}	100^{d}	27.7^{c}	8.3^{b}	27.1^{c}	54^{b}
Cd1 + GSH	592^{a}	19.7^{b}	107^{bc}	9.2^{b}	22.2^{c}	211^{b}	28.1^{c}	15.0^{a}	17.16^{d}	62^{b}
Cd2	649^{a}	15.9^{c}	138^{b}	16.0^{a}	66.2^{a}	178^{bc}	37.4^{b}	8.5^{b}	52.1^{b}	130^{a}
Cd2 + GSH	581^{a}	27.3^{ab}	67^{c}	17.2^{a}	14.8^{d}	173^{c}	32.9^{bc}	11.3^{ab}	60.0^{a}	67^{b}
Xiushui 63										
Control	548^{bc}	23.6^{ab}	420^{a}	8.9^{c}	23.7^{b}	223^{b}	22.9^{c}	25.8^{a}	25.2^{c}	51^{c}
Cd1	541^{bc}	18.8^{c}	200^{b}	22.3^{a}	20.2^{c}	99^{e}	21.8^{c}	14.6^{b}	46.1^{b}	44^{d}
Cd1 + GSH	613^{b}	25.3^{a}	165^{c}	15.2^{bc}	22.3^{bc}	168^{c}	30.7^{a}	15.5^{b}	28.2^{c}	44^{d}
Cd2	502^{c}	13.8^{d}	140^{d}	16.4^{ab}	34.1^{a}	148^{d}	26.5^{b}	9.5^{c}	54.9^{b}	101^{a}
Cd2 + GSH	706^{a}	20.9^{bc}	101^{e}	17.1^{ab}	24.8^{b}	269^{a}	30.2^{a}	8.1^{c}	74.7^{a}	79^{b}

Data were means of seven independent replications. Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype. Control, Cd1, Cd1 + GSH, Cd2 and Cd2 + GSH correspond to basic nutrition solution (BNS), BNS + 5 μmol/l Cd, BNS + 5 μmol/l Cd + 50 μmol/l GSH, BNS + 50 μmol/l Cd, and BNS + 50 μmol/l Cd + 50 μmol/l GSH, respectively

Cd accumulation in plants is a function of Cd concentration and dry weight. Significant differences were observed in Cd accumulation ($P < 0.01$) between the two genotypes and among the five treatments (Figure 3). Cd accumulation in Xiushui63 was lower than Bing97252 after 5 μmol/l Cd exposure, whereas there was no significant difference in Cd-translocation from root to shoot between the two genotypes (c.f. 48% in Bing97252 and 50% in Xiushui63, data not shown). When treated with 50 μmol/l Cd, the translocation remained unchanged in Bing97252. However, the ratio of root to the total Cd accumulation reduced to 40% in Xiushui63. Addition of GSH in the presence of Cd improved Cd-translocation in Xiushui63 under 5 μmol/l Cd exposure, and Bing97252 under 50 μmol/l Cd exposure.

Element concentrations and accumulations. Table 1 shows the concentrations of macroelement Ca, and four microelements Zn, Mn, Cu and Fe in shoots and roots of the two rice genotypes with different treatments. The results of ANOVA showed significant differences in the interaction between treatment and genotype for all examined element concentrations. There were highly significant differences between genotypes, but not in shoot Zn and root/shoot Ca (Table 2). In roots, compared with the control, 5 μmol/l Cd (Cd1) induced a significant decrease in the concentration of Ca and Zn in Bing97252 (c.f. 66% and 37%, respec-

Table 2. ANOVA of Cd concentration and accumulation of two rice genotypes at five treatments

Source	*F*-value									
	shoot concentration					root concetration				
	Ca	Zn	Mn	Cu	Fe	Ca	Zn	Mn	Cu	Fe
Genotype	0.3	3.8	86.3**	29.8**	52.3**	4.6	79.6**	41.9**	58.9**	24.7**
Treatment	3.4	15.5**	331.2**	20.0**	193.0**	130.7**	12.8**	40.8**	151.2**	149.7**
G × T	5.1*	4.8*	8.2**	18.5**	74.7**	37.0**	22.3**	26.6**	3.5*	11.9**
	shoot accumulation					root accumulation				
Genotype	186.7**	80.7**	5.1*	56.0**	264.4**	170.0**	195.5**	11.4**	11.0**	60.9**
Treatment	221.6**	91.1**	2109.8**	195.1**	399.6**	443.4**	71.2**	193.4**	42.3**	14.7**
G × T	11.8**	11.7**	6.7**	93.2**	43.0**	38.3**	26.8**	38.4**	23.8**	1.8

G × T – interaction between genotype and treatment; *and **significant at 0.05 and 0.01 probability level, respectively

Table 3. Effect of Cd and GSH in nutrient media on Ca, Zn, Mn, Cu and Fe accumulations of the two rice genotypes

Treatment	Mineral accumulations (μg/plant)					Shoot/root ratio				
	Ca	Zn	Mn	Cu	Fe	Ca	Zn	Mn	Cu	Fe
Bing 97252										
Control	464.1^{a}	27.0^{a}	225.1^{a}	9.4^{a}	27.2^{a}	11.5^{c}	3.9^{a}	149.1^{a}	3.5^{a}	2.9^{a}
Cd1	270.1^{b}	11.3^{b}	54.1^{b}	7.2^{b}	14.8^{c}	26.6^{a}	3.2^{b}	66.6^{b}	1.7^{c}	2.0^{b}
Cd1 + GSH	294.8^{b}	13^{b}	54.3^{b}	6.5^{b}	18.2^{b}	11.0^{c}	2.9^{b}	30.9^{c}	2.3^{b}	1.5^{c}
Cd2	99.2^{d}	7.1^{c}	20.2^{c}	4.0^{c}	9.0^{d}	6.4^{d}	1.8^{c}	66.3^{b}	1.4^{c}	1.0^{d}
Cd2 + GSH	192.8^{c}	10.8^{b}	21.8^{c}	9.5^{a}	9.8^{d}	14.9^{b}	3.7^{a}	26.3^{c}	1.3^{c}	0.9^{d}
Xiushui 63										
Control	290.8^{a}	13.9^{a}	208.4^{a}	7.0^{c}	16.8^{a}	11.8^{b}	5.0^{a}	79.2^{a}	1.7^{a}	2.2^{a}
Cd1	167.9^{c}	7.4^{c}	60.2^{b}	10.6^{a}	9.7^{c}	18.5^{a}	2.9^{b}	45.3^{c}	1.7^{a}	1.6^{b}
Cd1 + GSH	223.0^{b}	11.5^{b}	57.1^{b}	8.7^{b}	11.7^{b}	13.0^{b}	3.0^{b}	37.1^{d}	1.4^{ab}	1.8^{b}
Cd2	53.5^{e}	4.1^{e}	14.0^{c}	3.1^{e}	5.9^{e}	12.4^{b}	1.9^{c}	45.7^{c}	1.1^{bc}	1.0^{c}
Cd2 + GSH	138.3^{d}	5.0^{d}	18.5^{c}	5.5^{d}	7.6^{d}	12.2^{b}	3.2^{b}	60.7^{b}	0.9^{c}	1.5^{b}

Data were means of seven independent replications. Different letters indicate significant differences ($P < 0.05$) among the treatments within each genotype. Control, Cd1, Cd1 + GSH, Cd2 and Cd2 + GSH correspond to basic nutrition solution (BNS), BNS + 5 μmol/l Cd, BNS + 5 μmol/l Cd + 50 μmol/l GSH, BNS + 50 μmol/l Cd, and BNS + 50 μmol/l Cd + 50 μmol/l GSH, respectively

tively), and the concentration of Ca, Mn and Fe in Xiushui63 (c.f. 56%, 43% and 14%). However, Cu concentration of Cd single treatment was even higher than the control. With 50 μmol/l Cd treatment (Cd2), Ca, Zn and Fe concentrations in both genotypes and Cu in Bing97252 were higher than Cd1, and the concentrations of Cu and Fe were even higher than the control. Compared with Cd1, addition of 50 μmol/l GSH in 5 μmol/l Cd (Cd1 + GSH) significantly increased Ca and Mn concentrations by 111% and 81% in Bing97252, and increased Ca and Zn concentrations by 70% and 41% in Xiushui63, respectively. However, Cd1 + GSH decreased Cu concentration, and did not affect Fe concentration in both genotypes. Cd2 + GSH elevated the concentrations of Ca and Zn in Xiushui63, whereas it had no difference in Bing97252, compared with Cd2. Changes induced by Cd2 + GSH in Mn, Cu and Fe were with totally different trends: it did not affect Mn, increased Cu, and decreased Fe.

Concerning shoot mineral concentrations, Cd1 and Cd2 treatments significantly reduced Zn and Mn concentrations in both genotypes, while they had no significant effect on Ca. Shoot Fe concentration was higher in Cd2 but lower in Cd1 when compared to control. External GSH significantly increased shoot Zn concentration of Xiushui63 exposed to 5 μmol/l Cd, and of both genotypes in the presence of 50 μmol/l Cd. However, GSH decreased Mn and Fe concentrations of both genotypes in 50 μmol/l Cd.

The different accumulations of Ca and Zn, Mn, Cu and Fe in two rice genotypes with different treatments are shown in Table 3. There were significant differences between genotypes, treatments, or the interaction between genotype and treatment, except the interaction in root Fe accumulation (Table 2). Cd significantly reduced mineral accumulations in both genotypes except Cu accumulation of Cd1 treatment in Xiushui63. In addition, higher Cd level treatment caused more inhibition in mineral accumulations. Cd1 + GSH increased Ca, Zn, and Fe accumulations in Xiushui63, but only significantly increased Fe accumulation in Bing97252. Cd2 + GSH induced increases in Ca, Zn and Cu accumulations and no difference in Mn accumulation of both genotypes, compared with Cd2 treatment. Moreover, Cd2 + GSH increased Fe accumulation in Xiushui63, but not in Bing97252.

As to ratio of shoot/root compared with control, both 5 and 50 μmol/l Cd decreased the ratios of Zn, Mn, Cu and Fe in Bing97252 and Xiushui63, except the Cu of Cd1 treatment in Xiushui63. The ratio of Ca in Cd1 treatment was even higher than control. Concerning the genotypic differences in shoot/root ratio, Cd decreased less that of Zn and increased more that of Ca in Bing97252 than Xiushui63, compared with control. Cd1 + GSH markedly decreased the ratios, except the ratios of Zn and Cu in both genotypes, and of Fe in Xiushui63, compared to Cd1. Cd2 + GSH increased

Table 4. Relationships between element concentrations in roots and shoots of rice plants

	Shoot metal concentration					Root metal concentration					
	Ca	Zn	Mn	Cu	Fe	Cd	Ca	Zn	Mn	Cu	Fe
SMC											
Cd	0.10	–0.70*	–0.48	0.44	0.78**	0.98**	–0.26	0.13	–0.57	0.60	0.92**
Ca		0.35	–0.08	0.11	0.30	0.12	0.72*	0.59	–0.22	0.27	0.24
Zn			0.41	–0.24	–0.40	–0.76**	0.57	0.51	0.28	–0.35	–0.54
Mn				–0.38	0.00	–0.58	0.38	0.00	0.73*	–0.52	–0.34
Cu					0.07	0.40	–0.35	–0.14	–0.34	0.73*	0.20
Fe						0.74**	0.08	0.44	–0.31	0.16	0.85**
RMC											
Cd							–0.29	0.08	–0.62*	0.58	0.90**
Ca								0.56	0.13	–0.08	0.05
Zn									–0.44	–0.08	0.33
Mn										–0.49	–0.52
Cu											0.52

SMC – shoot metal concentration; RMC – root metal concentration; * and **significant at the 0.05 and 0.01 probability levels, respectively

ratios of Ca and Zn in Bing97252, and Zn, Mn, Fe in Xiushui63, compared to Cd2.

Relationship of cadmium, calcium, zinc, manganese, copper and iron concentrations among different plant organs. Table 4 shows the correlation coefficients between the element concentrations in different organs of rice. Significantly negative correlation was observed between shoot Zn concentration and shoot/root Cd concentration. Similarly, significantly negative correlation was discovered between root Cd concentration and Mn concentration. Yet, significantly positive relationship between Cd and Fe both in shoot and root was found.

DISCUSSION

Cadmium contamination in soil turned into a potential agricultural and environmental issue worldwide (Chen et al. 2007). Correspondingly, it is urgently necessary to develop approaches to prevent the accumulation of Cd in plants so as to alleviate health risks associated with exposure to high Cd content food. Considering large-scale, medium and slightly contaminated farmlands, such approaches as the application of chemical regulators to alleviate Cd toxicity and simultaneously reduce plant Cd uptake would be a cost-effective and practically acceptable strategy for full utilization of natural resource and safe food production. In this work, we observed a genotype and Cd-dose dependent role of exogenous GSH in modulation plant growth, Chl content and mineral uptake and accumulation against Cd stress in the two rice genotypes differing in Cd tolerance. Addition of 50 μmol/l GSH to 50 μmol/l Cd medium (Cd2 + GSH) effectively alleviated Cd-induced growth inhibition and toxicity in both rice genotypes, described as its capability of preventing the inhibition of shoot/root fresh weight, root length and Chl content (Figures 1 and 2). Addition of 50 μmol/l GSH to 5 μmol/l Cd medium (Cd1 + GSH) significantly alleviated the inhibition on root fresh weight of Bing97252, shoot growth of Xiushui63. The results suggested a practical potential for exogenous GSH application as an intervention strategy in mitigating Cd stress in rice plants.

Our previous study suggested that decreasing leaf Chl content was one of the most general toxicity effects of Cd to plants (Wu and Zhang 2002), which was confirmed in the present study. A notable reduction of Chl a, b and Cart content was detected in both genotypes exposed to 5 or 50 μmol/l Cd, with Cd-sensitive genotype being more serious than tolerant one. Cd-induced Chl synthesis inhibition was significantly reverted when rice seedlings were treated with exogenous GSH (Cd1 + GSH, Cd2 + GSH) (Figure 2), but did not affect Cart and Chl. As an indicator of plant health, chlorophyll content was more accurate and sensitive than shoot dry weight and root length. Both Chl and Cart are central parts of energy manifestation of every green plant system. Thus,

any alteration in their levels may cause marked effect on the entire metabolism of plant. Cart protect Chl from photo-oxidative destruction and therefore, a reduction in carotenoids could have a serious consequence on Chl pigments (Abdel-Latif 2008). Van Assche and Clijsters (1990) concluded Cd can alter Chl biosynthesis by inhibiting protochlorophyllide reductase and the photosynthetic electron transport by inhibiting the water-splitting enzyme located at the oxidizing site of photosystem II (PSII). On the other hand, Mn is essential for optimal water-splitting activity (Baszinsky et al. 1980). The negative correlation between concentration of Cd and Mn could partly explain the inhibition of the decrease in Chl contents.

Restricted translocation from root to shoot may result in lower Cd concentration in grains than in roots or leaves (Grant et al. 1998). Florijn and van Beusichem (1993) found internal distribution rather than uptake of Cd caused the genotypic difference in shoot Cd concentration in maize inbred lines. In the present study, higher Cd concentration in roots of the tolerant genotype Bing97252 did not cause higher Cd in shoots. Moreover, Bing97252 had higher root to total ratio of Cd accumulation in Cd2 treatment (Figure 3C and D). It may be suggested that the distribution of Cd in plant may be partly related to Cd tolerance. However, GSH improved the translocation of Cd in Xiushui63 under 5 μmol/l Cd exposure, and Bing97252 under 50 μmol/l Cd exposure (Figure 3C and D). Considering dramatically reduced shoot and root Cd concentration in Cd2 + GSH treatment (Figure 3A and B), exogenous GSH seemed to act by reducing Cd uptake rather than translocation.

There were many researches on metal uptake and accumulation under Cd stress, but the results showed quite different. It is now widely accepted that Cd enters plant root probably through competition with other metals including Zn, Mn, Ca and Fe (Clemens 2006). Wu et al. (2003) reported there was a significantly negative correlation between Zn, Cu, or Mn concentration and Cd concentration in different barley genotypes. In the present study, Cd significantly inhibited the concentration of Mn in plant. Sensitive genotype Xiushui63 was more affected. These results indicate that Cd may compete with Mn transporter systems and keeping the rate of Mn uptake steady may be important for Cd tolerance in rice. Significantly negative correlation was observed between shoot Cd and shoot/root Zn concentration, and between root Cd and Mn concentration. Therefore, excessive Cd accumulation would affect the rate of uptake and distribution of certain nutrients in the plants, and consequently would be responsible for mineral deficiencies/imbalance and depression of the plant growth. The work of Wang et al. (2007) showed increased Fe uptake under Cd stress in maize. Liu et al. (2003) found a significant correlation on root content between Cd and Cu or Fe, in 20 rice cultivars. However, some other researches related to changes of Fe concentrations under Cd stress showed no significant correlation between these two metals (Chaoui et al. 1997). In the present study, there were significantly positive relationships between Cd and Fe both in shoots and roots. The Fe and Cu concentrations in Cd2 treatment were even higher than the control. Addition of GSH in Cd treatments showed a Cd-dose- and genotype-dependent effect on mineral uptake; e.g. external GSH elevated root Ca and Zn concentrations in Xiushui63 in both 5 and 50 μmol/l Cd present solution, but it elevated root Ca and Mn concentrations in Bing97252 only in Cd1. Thus, elevated uptake of Ca and Zn may be one of the mitigatory effects of external GSH in sensitive genotype.

The reduction in accumulation of Ca, Zn, Mn and Fe could be due to the reduced plant growth and impaired penetration of the roots into the medium induced by Cd. Addition of GSH reverted accumulation of Fe in Bing97252 and Cu, Zn, Fe in Xiushui63 with Cd1, and recovered Ca, Zn, Cu, Fe in both genotypes with Cd2 (Table 3). The results showed a better plant growth at exogenous GSH addition. The ratio of accumulation of shoot/root reflects the translocation of elements from roots to shoots. Compared with control, Cd decreased less the shoot/root ratio of Zn and increased more the ratio of Ca in Bing97252 than Xiushui63 (Table 3). It may be assumed that maintaining higher translocation of Zn and Ca is an important way to reduce Cd toxicity. However, the effects of GSH on mineral translocation seemed to be more complex, e.g. Cd1 + GSH markedly reduced the ratio of Fe and enhanced the ratio of Cu in Bing97252, but it had no differences in Xiushui63. When the plants were exposed to Cd, the ratio of Zn was not changed with Cd1 + GSH, compared to Cd1, while the ratio was elevated over Cd2 by Cd2 + GSH. Hence, it may be assumed that the influence of GSH on mineral translocation is variable over the element, genotype and Cd level. It also can be suggested that enhanced Zn translocation may participate in alleviative effect of GSH under higher Cd toxicity in rice.

In conclusion, Cd stress decreased Chl and Cart contents, and affected the rate of uptake and distribution of certain nutrients in rice plants (e.g.

inhibited Mn and Zn uptake and translocation but elevated Cu and Fe content); it also inhibited rice growth, with the Cd-sensitive genotype being much severely affected than the tolerant one. Adverse effects caused by Cd toxicity could be alleviated at different degrees by application of 50 µmol/l GSH. In this experiment, the most pronounced effects of GSH addition to Cd-stressed medium were significantly elevated content of chlorophyll a and b, reduced Cd concentration in roots and shoots of both Cd-sensitive and tolerant genotypes with a much severe response in the presence of 50 µmol/l Cd, and simultaneously improved Zn and Ca uptake and translocation in the sensitive genotype.

REFERENCES

Abdel-Latif (2008): Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivum* seedlings. Australian Journal of Basic and Applied Science, *2*: 57–62.

Baszinsky T., Wajda L., Krol M., Wolinska D., Krupa Z., Tukendorf A. (1980): Photosynthetic activities of cadmium treated tomato plants. Physiologia Plantarum, *48*: 365–370.

Cataldo D.A., Garland T.R., Wildung R.E. (1983): Cadmium uptake kinetics in intact soybean plants. Plant Physiology, *73*: 844–848.

Chaoui A., Ghorbal M.H., Ferjani E.E. (1997): Effects of cadmium-zinc interactions on hydroponically grown bean (*Phaseolus vulgaris* L.). Plant Science, *126*: 21–28.

Chen F.M. (1984): Determining the chlorophyll contents of plant leaves by acetones/ethanol mixture assay. Forest Science Communications, *2*: 4–8.

Chen F., Wu F.B., Dong J., Vincze E., Zhang G.P., Wang F., Huang Y.Z., Wei K. (2007): Cadmium translocation and accumulation in developing barley grains. Planta, *227*: 223–232.

Clemens S. (2006): Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie, *88*: 1707–1719.

Fang Z., Sperling M., Welz B. (1991): Flame atomic absorption spectrometric determination of lead in biological samples using a flow injection system with on-line preconcentration by coprecipitation without filtration. Journal of Analytical Atomic Spectrometry, *6*: 301–306.

Florijn P.J., van Beusichem M.L. (1993): Uptake and distribution of cadmium in maize inbred lines. Plant and Soil, *150*: 25–32.

Grant C.A., Buckley W.T., Bailey L.D., Selles F. (1998): Cadmium accumulation in crops. Canadian Journal of Plant Science, *78*: 1–17.

Hassan M.J., Shao G.S., Zhang G.P. (2005): Influence of cadmium toxicity on antioxidant enzyme activity in rice cultivars with different grain cadmium accumulation. Journal of Plant Nutrition, *28*: 1259–1270.

Kabata-Pendias A., Pendias H. (2001): Trace Elements in Soils and Plants. 3rd Edition. CRC Press Inc, Boca Raton, Florida.

Kikuchi T., Okazaki M., Kimura S.D., Motobayashi T., Baasansuren J., Hattori T., Abe T. (2008): Suppressive effects of magnesium oxide materials on cadmium uptake and accumulation into rice grains: II: Suppression of cadmium uptake and accumulation into rice grains due to application of magnesium oxide materials. Journal of Hazardous Materials, *154*: 294–299.

Kondo K. (1996): Incidence of minamata disease in communities along the Agano river, Niigata, Japan-pattern of the exposure and official diagnosis of patients. Nippon Eisegaku Zasshi, *51*: 599–611.

Liu J.G., Liang J.S., Li K.Q., Zhang Z.J., Yu B.Y., Lu X.L., Yang J.C., Zhu Q.S. (2003): Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. Chemosphere, *52*: 1467–1473.

Smith G.C., Brennan E.G. (1983): Cadmium-zinc interactionship in tomato plants. Phytopathology, *73*: 879–882.

Tang Q.Y., Feng M.G. (2002): DPS Data Processing System for Practical Statistics. Science Press, Beijing.

Van Assche F., Clijsters H. (1990): Effects of metals on enzyme activity in plants. Plant, Cell and Environment, *13*: 195–206.

Wang M., Zou J.H., Duan X.C., Jiang W.S., Liu D.H. (2007): Cadmium accumulation and its effects on metal uptake in maize (*Zea mays* L.). Bioresource Technology, *98*: 82–88.

Wu F.B., Chen F., Wei K., Zhang G.P. (2004): Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere, *57*: 447–454.

Wu F.B., Zhang G.P. (2002): Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedlings. Bulletin of Environmental Contamination and Toxicology, *69*: 219–227.

Wu F.B., Zhang G.P., Yu J.S. (2003): Interaction of cadmium and four microelements for uptake and translocation in different barley genotypes. Communications in Soil Science and Plant Analysis, *34*: 2003–2020.

Xiang C.B., Werner B.L., Christensen E.L.M., Oliver D.J. (2001): The biological functions of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. Plant Physiology, *126*: 564–574.

Zhang G.P., Fukami M., Sekimoto H. (2002): Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. Field Crops Research, *77*: 93–99.

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