Effect of Exogenous Glutathione and Selenium on Cadmium-Induced Changes in Cadmium and Mineral Concentrations and Antioxidative Metabolism in Maize Seedlings

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A hydroponic experiment was carried out to study the modulation of exogenous reduced glutathione and selenium in antioxidant defense system and microelement uptake against cadmium-toxicity in maize seedlings. The results showed that 50 µM cadmium increased accumulation of H_2O_2 and malondialdehyde but reduced plant height, root length, chlorophyll content (SPAD value) and biomass. Antioxidant enzyme activities of root/leaf peroxidase and leaf superoxide dismutase increased significantly under cadmium stress, while leaf catalase decreased significantly. Moreover, cadmium-stress reduced manganese concentrations both in shoots and roots. Significantly negative correlation was discovered between manganese and cadmium concentration in different plant organs. Addition of 2.5 µM selenium or pretreated with 100 µM glutathione for 24 h significantly alleviated cadmium-induced growth inhibition and dramatically diminished leaf H_2O_2 and root malondialdehyde accumulation. Se addition or glutathione pretreatment significantly decreased cadmium concentration in roots/shoots, increased root iron level, while glutathione reduced zinc uptake. Furthermore, GSH/Se counteracted cadmium-induced alterations of certain antioxidant enzymes, *e.g.* brought root/leaf peroxidase and leaf superoxide dismutase activities down towards to the control level, but elevated root ascorbate peroxidase activity. These data suggest that reduced cadmium concentration and diminished cadmium-induced H_2O_2 and malondialdehyde accumulation in plants could be principal protective mechanism for the exogenous glutathione/selenium against cadmium toxicity.

Key Words: Antioxidative metabolism, Maize (*Zea mays* L.), Cadmium, Glutathione, Microelement, Selenium, Stress mitigation.

INTRODUCTION

Cadmium, one of the most aggressive heavy metals, is easily taken up by plants and translocated to above-ground tissues and then becomes a potential threat to human health *via* food chain[1,2]. Such a situation did occur in the 1950s and 1960s in Japan where cadmium contamination of rice fields led to renal impairment and bone disease in exposed populations. In China, cadmium has posed a serious problem for food production and at least 13,330 ha of farmland involved in 11 provinces is contaminated by varying degrees of cadmium[3]. In brief, cadmium contamination in soil has turned into a potential agricultural and environmental issue worldwide[4-6]. Correspondingly it is urgently necessary to develop approaches to prevent its accumulation in plants so as to alleviate health risks associated with exposure to highly cadmium-contented food.

Reduced glutathione (γ-Glu-Cys-Gly, GSH), a naturally occurring tripeptide, is a constituent of the cellular defense mechanism of the body against various exogenous as well as endogenously produced xenobiotics. Zhu *et al.*[7] found that the increased capacity of glutathione (GSH) synthesis enhanced cadmium tolerance and accumulation in Indian mustard. Studies about the effect of intracellular GSH on heavy metal metabolism and toxicity indicate that GSH serves in bioreductive reactions as an important defense line against reactive oxygen species to protect cells from oxidative stress damage[8] and modify metal toxicity by altering the rates of metal uptake and elimination[9] and by chelating metal ions in cells[10]. Selenium was recognized as one of the indispensable essential trace elements for human and animal by the world health organization in 1973. Humans and animals require Se for the function of a number of Se-dependent enzymes, also known as selenoproteins for which a metabolic function has not yet been discovered. Gromer *et al.*[11] suggested that the selective advantage of selenoenzymes is a broader range of substrates and a broader range of micro environmental conditions in which enzyme activity is possible. Concerning selenium-

dependent enzymes, Se is an essential component of various enzymes involved in defense mechanism for biological membrane systems in animal bodies from various oxidative processes[12,13]. Within this context, Se has been classified as an antioxidant and/or a heavy metal antagonist[14], for providing protection from the toxic effects of heavy metals and other substances. The protective effect of Se against mercury toxicity in different biological systems of animals and aquatic organisms appears to have been the most studied[15,16]. Beneficial effects of Se in exposure to cadmium and silver have also been observed in mice and human[17,18] although a full mechanistic explanation has not been realized. Gotsis[19] reported that similar antagonism on the cell growth of the planktonic alga *Dunaliella minuta* Lerche was observed in combinations of Se/Hg and Se/Cu for cultures with and without prior exposure to either metal. However, so far there is little study on the effect of extracellular Se on responses to cadmium stress in plants. Thus a precise knowledge is required about the physiological processes responsible for the role of external Se on heavy metal stress, in order to test the hypothesis that external Se could act as a regulator or antioxidant intervention strategy in preventing oxidative stress in responses to cadmium stress and to get a better understanding of how plants adjust to an adverse environment.

The present study demonstrated at a physiological level the role of GSH and Se in promoting adaptive responses in antioxidant systems, mineral nutrients uptake and distribution of maize seedlings to cope with cadmium stress, where Se was administered along with the cadmium, but GSH was pretreatment for 24 h before cadmium stress. Hence, we conducted a hydroponic experiment to investigate effects of GSH and Se addition on the changes of mineral nutrients content, antioxidant defense system, H_2O_2 and lipid peroxidation level under cadmium stress.

EXPERIMENTAL

Plant material and experimental design: The hydroponic experiment was carried out on Huajiachi Campus, Zhejiang University, Hangzhou, China, using a common maize variety Nongda 108. Healthy maize seeds were surface sterilized by soaking in 2 % H_2O_2 for 0.5 h, rinsed with distilled water for seven times and then germinated in sterilized moist sand in an incubator at 20 ℃. At the 2nd leaf stage (10 days old), the uniform healthy plants were selected and transplanted to 5 L containers filled up 4.5-L basal nutrient solution (mg L^{-1}): $(NH_4)_2SO_4$, 48.2; $MgSO_4$, 65.9; K_2SO_4, 15.9; KNO_3, 18.5; $Ca(NO_3)_2$, 59.9; KH_2PO_4, 24.8; Fe-citrate, 5; $MnCl_2 \cdot 4H_2O$, 0.9; $ZnSO_4 \cdot 7H_2O$, 0.11; $CuSO_4 \cdot 5H_2O$, 0.04; HBO_3, 2.9; H_2MoO_4, 0.01. The solution pH was adjusted to 5.8 ± 0.1 with NaOH or HCl. The container was covered with a polystyrol-plate with 7 evenly spaced holes (1 plant per hole) and placed in a greenhouse. On the 7th day after transplanting, cadmium (as $CdCl_2$), Se (as Na_2SeO_3) and GSH were added to the corresponding containers to form 6 treatments: (1) control, basal nutrient solution (BNS); (2) Se, BNS + 2.5 µM Se; (3) GSH, BNS + 100 µM GSH, GSH was added on the 6th day after transplanting and on the second day (7th day after transplanting, *i.e.* after 24 h pretreated with GSH) replaced with basal nutrient solution;

(4) cadmium, BNS + 50 µM Cd; (5) Cd + Se, BNS + 50 µM Cd + 2.5µM Se; and (6) Cd + GSH, BNS + 24 h pretreated with 100 µM GSH + 50 µM Cd, 100 µM GSH was added on the day before cadmium treatment and on the second day replaced with 50 µM cadmium. There were 3 replicates for each treatment. The nutrient solution was continuously aerated with pumps and renewed in 5 days interval.

Sampling and statistic analysis: Plant samples were collected after 5 days cadmium exposure. SPAD value of the 2nd fully expanded leaves was determined using SPAD-502 chlorophyll meter. The fresh plant samples were directly used for the determination of antioxidative enzyme activities. Meanwhile, histochemistry staining of H_2O_2 in leaves was performed according to Romero-Puertas[20]. Each measurement was replicated 3 times. Statistical analyses were performed with data processing system statistical software package using ANOVA followed by the Duncan's multiple range test (SSR) to evaluate significant treatment effects at significance level of P ≤ 0.05.

Measurements of plant height, root length, biomass and microelements concentration: After 5 days treatment, plants were uprooted and separated into roots and leaves, the roots were soaked with 20 mM Na_2EDTA for 1 h to eliminate the ions on the surface and then rinsing in deionized water, plant height, root length and fresh weights were simultaneously measured, then dried at 80 ℃ and weighed. Dried shoots and roots were powdered and weighted, then ashed at 550 ℃ for 12 h. The ash was digested with 5 mL 30 % HNO_3 and then diluted using deionized water. Cadmium and other elements concentration were determined using a flame atomic absorption spectrometry (SHIMADZU AA-6300).

Histochemical staining of H_2O_2 and determination of lipid peroxidation: Histochemistry staining of H_2O_2 were performed as described by Romero-Puertas *et al.*[20] with minor modification. Leaves were vacuum-infiltrated with 10 mM potassium phosphate buffer (PBK, pH 6.5) containing 1 % diaminobenzidine for 5 min and then incubated at 25 ℃ for 12 h in dark. Leaves were illuminated until appearance of brown spots characteristic of the reaction of diaminobenzidine with H_2O_2.

The level of lipid peroxidation was quantified as malondialdehyde content according to Wu *et al.*[21]. Plant tissues were homogenized and extracted in 10 mL of 0.5 % thiobarbituric acidmade in 5 % trichloroacetic acid. Extract was heated at 95 ℃ for 0.5 h and then quickly cooled with ice. After centrifugation at 10000 g for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was calculated using an extinction coefficient of 155 mM cm^{-1}.

Assay of enzyme activities: For the determination of enzyme activities, plant tissue was homogenized in 8 mL 50 mM sodium phosphate buffer (PBS, pH 7.8) using a prechilled mortar and pestle, then centrifuged at 10000 g for 15 min at 4 ℃. The supernatant was used for enzyme activity assay[21]. Superoxide dismutase, peroxidase and catalase activities were determined according to Zhang[22]. For the analysis of ascorbate peroxidase, ascorbate (AsA) was used as the substrate and the decrease in ascorbate concentration followed as a decline in

optical density at 290 nm and the activity was calculated using the extinction coefficient 2.8 (mM cm⁻¹) for ascorbate[23].

RESULTS AND DISCUSSION

Exogenous Se and GSH pretreatment alleviate cadmium toxicity and reduce cadmium concentration in maize seedlings: As compared with the control, maize plants after 5 d exposure to 50 μM cadmium significantly decreased plant height, root length and biomass and showed obvious symptoms of cadmium toxicity: leaf chlorosis and necrosis, brown roots and growth inhibition (Fig. 1). Pretreated with 100 μM GSH for 24 h before 50 μM cadmium stress (Cd + GSH) significantly alleviated cadmium-induced growth inhibition and the symptoms of chlorosis and necrosis on leaves. Glutathione pretreatment (Cd + GSH) suppressed cadmium uptake and leaf and root cadmium concentrations reduced by 61.1 % and 29.0 % when compared with those in cadmium alone treatment (Table-1). Similar alleviation trend was found in the Cd + Se treatment (addition of 2.5 μM Se in 50 μM cadmium culture medium), *i.e.* after 5 d Cd + Se treatment, plant height, root length and dry weight increased significantly by 14.3, 36.8 and 50.2 % compared with cadmium alone treatment (Fig. 1) and cadmium concentration reduced by 39.9 %, 17.9 % in leaves and roots respectively (Table-1). Furthermore, Se addition effectively inhibited the appearance of cadmium toxicity of chlorosis or necrosis in the leaves. No significant differences were observed between GSH pretreatment or Se addition (without cadmium) and control.
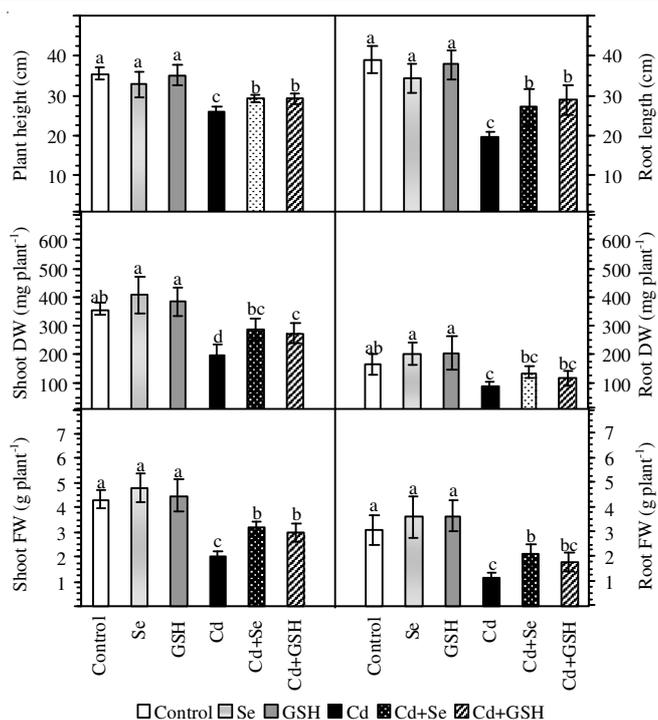

Fig. 1. Effect of external glutathione (GSH) pretreatment and selenium (Se) supply on growth of maize seedlings exposed to Cd for 5 days. Control, Cd, Se, GSH, Cd + GSH and Cd + Se correspond to basic nutrition solution (BNS), BNS + 50 μM Cd, BNS +2.5 μM Se, BNS + 100 μM GSH (24 h pretreatment) and BNS + 50 μM Cd + 100 μM GSH (24 h pretreatment before Cd exposure), BNS + 50 μM Cd + 2.5 μM Se respectively. Error bars represent SD values (n = 3). Means with the same letters are not significantly different at P = 0.05

Determination of SPAD values of the fully expanded leaves of maize plants after 5 d cadmium exposure was shown in Fig. 2. The SPAD values of 50 μM cadmium treatment greatly decreased by 60.8 % relative to those of control, while addition of Se and GSH induced significant increase of 43.4 % and 52.5 %, respectively, over the cadmium alone treatment. Meanwhile no significant effects of Se and GSH alone (in the absence of cadmium) on SPAD value was observed when compared with the control.

TABLE-1
EFFECT OF GSH PRETREATMENT AND SE SUPPLY
ON CADMIUM CONCENTRATIONS (MG KG⁻¹ DW)
IN LEAVES AND ROOTS OF MAIZE SEEDLINGS
EXPOSED TO CADMIUM FOR 5 DAYS

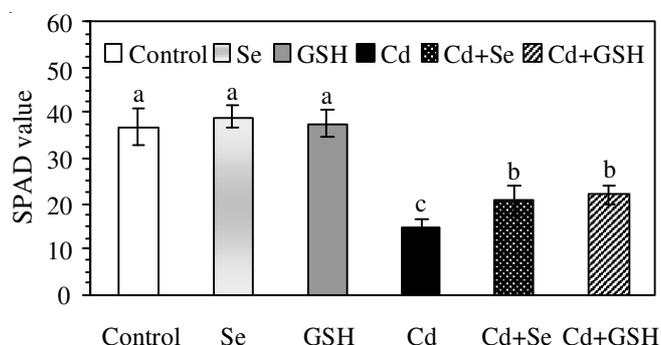|	Control	Se	GSH	Cd	Cd + Se	Cd + GSH
Shoot	0.7d	0.4d	1.0d	183.2a	110.1b	71.2c
Root	2.9d	3.3d	2.8d	532.7a	437.2b	378.3c
Means with the same letters are not significantly different at P = 0.05						

Fig. 2. Effect of GSH pretreatment and Se supply on SPAD value in leaves of maize seedlings exposed to Cd for 5 days. Control, Cd, Se, GSH, Cd + GSH and Cd + Se correspond to basic nutrition solution (BNS), BNS + 50 μM Cd, BNS + 2.5 μM Se, BNS + 100 μM GSH (24 h pretreatment) and BNS + 50 μM Cd + 100 μM GSH (24 h pretreatment before Cd exposure), BNS + 50 μM Cd + 2.5 μM Se respectively. Error bars represent SD values (n = 3). Means with the same letters are not significantly different at P = 0.05

GSH and Se alleviate cadmium-induced overproduction of H_2O_2 and lipid peroxidation: A significant increase in the accumulation of H_2O_2 (dark spots) was observed in the leaves of maize after 5 days cadmium exposure (Fig. 3C) and H_2O_2 scattered in the whole leaf. Glutathione pretreatment and Se addition in 50 µM cadmium solution markedly diminished cadmium-induced H_2O_2 accumulation in leaves.

Cadmium stress increased malondialdehyde accumulation by 37.8 % and 129.9 % in the leaves and roots respectively compared with those in controls. Glutathione pretreatment decreased cadmium-induced malondialdehyde accumulation down towards control level (*c.f.* decreased by 36.2 % and 62.8 % in leaves and roots, respectively relative to cadmium alone treatment). Se addition effectively inhibited the cadmium-induced malondialdehyde accumulation in roots (*c.f.* 53.5 % lower than the cadmium alone treatment), but no significant effect on leaf malondialdehyde content. In addition, no significant change in malondialdehyde content was found in seedlings pretreated with GSH or Se addition (without cadmium) in comparison with controls (Fig. 3A).
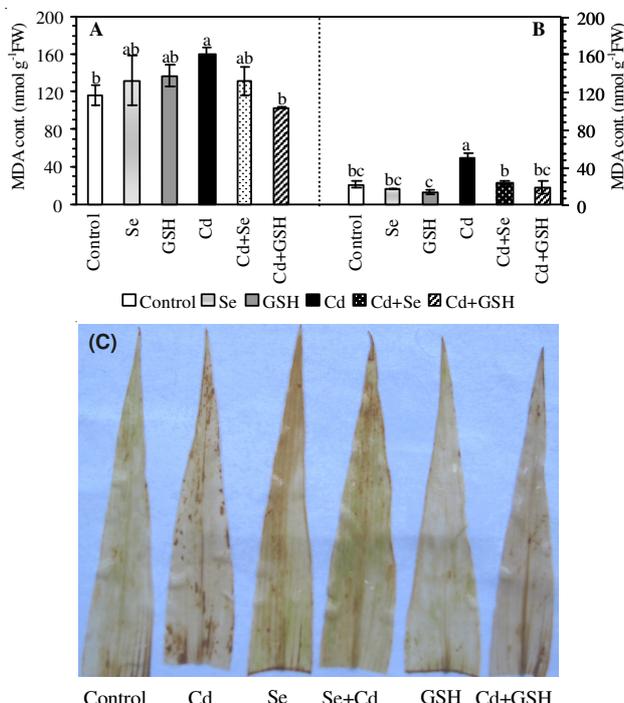

Fig. 3.   Effect of GSH pretreatment and Se supply on MDA content in leaves (A) and roots (B) and histochemical detection of H_2O_2 in leaves (C) of maize seedlings exposed to Cd for 5 days. Control, Cd, Se, GSH, Cd + GSH and Cd + Se correspond to basic nutrition solution (BNS), BNS + 50 µM Cd, BNS + 2.5 µM Se, BNS +100 µM GSH (24 h pretreatment) and BNS + 50 µM Cd +100 µM GSH (24 h pretreatment before Cd exposure), BNS + 50 µM Cd + 2.5 µM Se respectively. Error bars represent SD values (n = 3). Means with the same letters are not significantly different at P = 0.05

Response of antioxidant enzymes to cadmium, Se and GSH addition: Maize plants treated with 50 µM cadmium showed significant increase in peroxidase activity, being 327 % in leaves and 85.4 % in roots higher than those in the controls (Fig. 4). Both of GSH pretreatment and Se addition lowered cadmium-mediated increase of peroxidase activity in maize seedlings, giving readings 34.5 %, 36.5 % in leaves

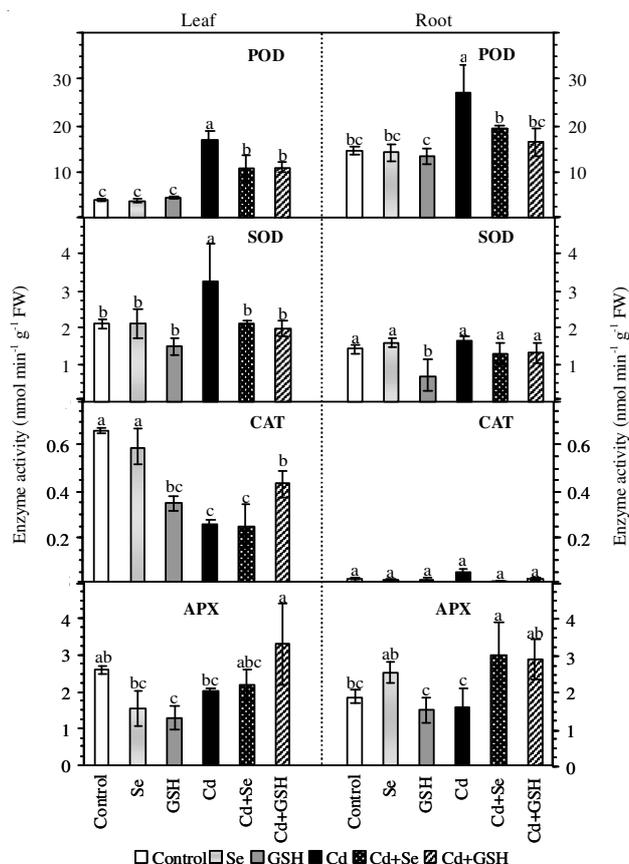and 39.9 %, 28.5 % in roots lower than that of cadmium alone treatment.

Fig. 4.   Effect of GSH pretreatment and Se supply on MDA, POD, SOD, CAT and ascorbate peroxidase activities in leaves (left) and roots (right) of maize seedlings exposed to Cd for 5 days. Control, Cd, Se, GSH, Cd + GSH and Cd + Se correspond to basic nutrition solution (BNS), BNS + 50 µM Cd, BNS + 2.5 µM Se, BNS +100 µM GSH (24 h pretreatment) and BNS + 50 µM Cd + 100 µM GSH (24 h pretreatment before Cd exposure), BNS + 50 µM Cd + 2.5 µM Se respectively. Error bars represent SD values (n = 3). Means with the same letters are not significantly different at P = 0.05

The effect of cadmium on superoxide dismutase activity showed significant difference in leaves and roots (Fig. 4). Cadmium stress induced substantial increase by 55.4 % in leaf superoxide dismutase activity over the control, GSH pretreatment and Se addition noticeably brought superoxide dismutase activity back to the control level: markedly down-regulated cadmium-dependent marked increase and the activity almost returned to the control level. However, cadmium stress and Cd + GSH/Cd + Se induced no significant effects on root superoxide dismutase activity compared with control (Fig. 4).

Cadmium stress resulted in a significant reduction in leaf catalase activity (49.2 % reduction relative to control). Pretreatment of GSH greatly alleviated the decline in catalase activity caused by cadmium toxicity and the activity in Cd + GSH treatment increased by 44.2 % when compared with cadmium treatment (Fig. 4). Addition of Se had no effect on catalase activity and there was no significant difference in root catalase activity among the six treatments.

The activity of ascorbate peroxidase in maize seedlings was shown in Fig. 4. No significant difference in ascorbate

peroxidase activity was observed between cadmium alone treatment and control. Glutathione pretreatment alone significantly decreased leaf ascorbate peroxidase activity. Cd + GSH markedly increased ascorbate peroxidase activity in leaves/roots, while Cd + Se only improved root ascorbate peroxidase activity compared with cadmium alone treatment and even higher than control.

Effects of GSH pretreatment and Se addition on micronutrient concentration under cadmium stress in maize seedlings: Effects of GSH pretreatment and Se addition on cadmium concentration were shown in Table-1. Addition of Se (Cd + Se) significantly decreased cadmium levels by 39.9 % and 17.9 % in leaves and roots, respectively, compared with cadmium alone treatment. Similar decreasing trend was found in the Cd + GSH treatment: decreased by 61.1 % and 29.0 % in leaves and in roots respectively. The trends of Cd + Se and Cd + GSH lowered cadmium concentration was much more pronounced in leaves than that in roots (Table-1).

Concerning Zn, Fe, Cu and Mn concentrations (Fig. 5), cadmium stress markedly reduced leaf/root Mn and root Fe concentrations (58.6, 91.2 and 23.1 % reduction relative to the control), while no significantly effect on Cu, Zn and leaf Fe concentrations.
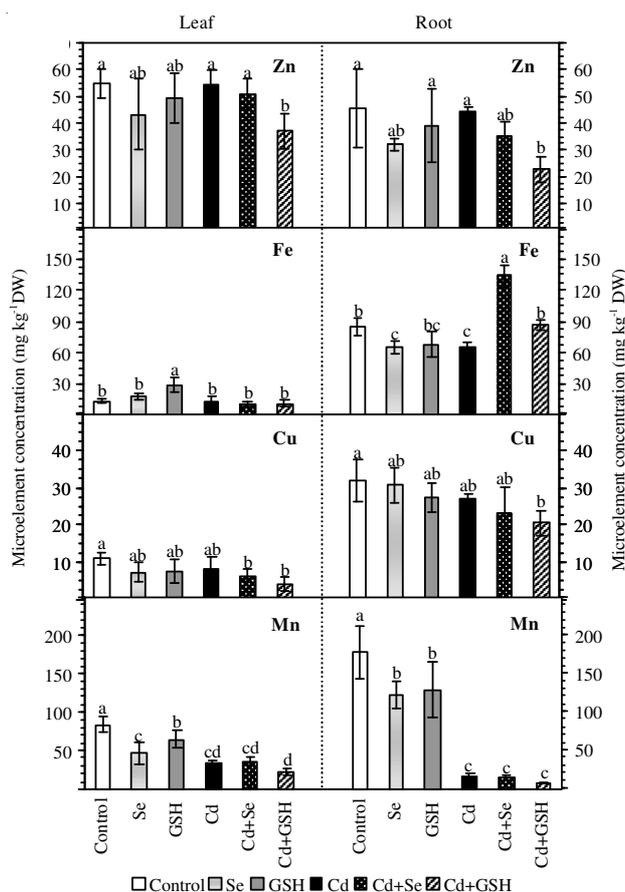

Fig. 5. Effect of GSH pretreatment and Se supply on micronutrient element concentrations in leaves (left) and roots (right) of maize seedlings exposed to Cd for 5 days. Control, Cd, Se, GSH, Cd + GSH and Cd + Se correspond to basic nutrition solution (BNS), BNS + 50 µM Cd, BNS + 2.5 µM Se, BNS + 100 µM GSH (24 h pretreatment) and BNS + 50 µM Cd + 100 µM GSH (24 h pretreatment before Cd exposure), BNS + 50 µM Cd + 2.5 µM Se respectively. Error bars represent SD values (n = 3). Means with the same letters are not significantly different at P = 0.05

Glutathione pretreatment (Cd + GSH) decreased Zn concentration by 31.9 % in leaves and 48.4 % in roots respectively compared with cadmium alone treatment, while no significant difference was found between Cd + Se and cadmium alone treatments. Cd+GSH/Cd+Se significantly increased cadmium-induced reduction in root Fe levels (33.8 %, 106.4 % higher than cadmium alone treatment), but no effect on leaf Fe and leaf/root Mn and Cu levels.

Correlations between cadmium and four microelements: The relationship between cadmium and Fe, Zn, Cu, Mn was shown in Table-2. Significantly negative correlation was discovered between manganese and cadmium concentration in the different plant organs. The significant positive correlation occurred between Zn, Mn and Cu both in leaves and in roots. In addition, Cu, Fe was negative correlated with cadmium, but did not reach the significant level.

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 cadmium transfer into the food chain is also involved in input from atmosphere, water and aquatic life. Moreover, cadmium contamination is a non-reversible accumulation process, with the estimated half-life in soil varying between 15 and 1100 years[24] and high plant-soil mobility to be easily accumulated in plant tissues, while high accumulation of cadmium in plants not only deteriorates crop yield and quality, but also gives rise to a threat on human health *via* food chain. Excess cadmium in the diet results in damage to kidney tubules, rhinitis, emphysema as well as other chronic disorders. Correspondingly it is urgently necessary to develop approaches to prevent the accumulation of cadmium in plants so as to alleviate health risks associated with exposure to highly cadmium-contaminated food. Considering large-scale medium/slightly contaminated farmlands such approaches as the application of chemical regulators to alleviate cadmium toxicity and simultaneously reduce plant cadmium uptake would be a cost-effective and practically acceptable strategy for full utilization of natural resource and safe food production. In our previous work, we described that exogenous GSH significantly alleviated cadmium-toxicity in barley seedlings and decreased cadmium concentrations in all plant tissues[8]. The present study demonstrated that addition of 2.5 µM Se (Cd+Se treatment) or pretreated with 100 µM GSH for 24 h (Cd + GSH) significantly alleviated cadmium-induced growth inhibition, diminished leaf H_2O_2 and root malondialdehyde accumulation and dramatically reduced shoot and root cadmium concentration (Table-1). The results suggested a practical potential for exogenous GSH/Se application as an intervention strategy in mitigating cadmium stress and reducing cadmium uptake and translocation in maize plants.

The interactions of cadmium and metal nutrients have been reported in some upland crops, such as wheat[25], barley[26,6], tomato[27] and soybean[28]. However, the interactions between cadmium and other nutrients are complicated and quite different with species. Our previous study found significantly negative correlation between Zn, Cu, or Mn concentration and cadmium concentration in barley plants[6]. The same effects on Mn were observed in the present experiment. Maize plants exposed to 50 µM cadmium significantly reduced Mn concentration by 58.6 %, 91.2 % in leaves and roots, respectively, compared

TABLE-2
CORRELATIONS BETWEEN CADMIUM AND FOUR MICRONUTRIENT ELEMENTS CONCENTRATIONS
IN LEAVES AND ROOTS FOR MAIZE SEEDLINGS EXPOSED TO CADMIUM FOR 5 DAYS

	Shoot mental concentration (SMC)					Root mental concentration (RMC)				
	Cd	Zn	Fe	Cu	Mn	Cd	Zn	Fe	Cu	Mn
SMC										
Cd	1.00	0.24	-0.54	-0.26	-0.65	0.96**	0.09	0.23	-0.48	-0.83*
Zn		1.00	0.06	0.81*	0.55	0.02	0.96**	0.06	0.51	0.27
Fe			1.00	0.17	0.46	-0.64	0.18	-0.49	0.32	0.52
Cu				1.00	0.87*	-0.48	0.90**	-0.24	0.87**	0.74
Mn					1.00	-0.78*	0.67	-0.23	0.80*	0.93**
RMC										
Cd						1.00	-0.16	0.39	-0.70	-0.94**
Zn							1.00	-0.18	0.68	0.45
Fe								1.00	-0.50	-0.43
Cu									1.00	0.85*
Mn										1.00

*and **, significantly at 0.05 and 0.01 probability levels, respectively

with control (Fig. 5). Significantly negative correlation was discovered between Mn and cadmium concentration in different plant organs (Table-2). This result indicate that cadmium may compete with manganese transporter systems and keeping rate of manganese uptake steady may be important for cadmium tolerance in maize. Therefore, excessive cadmium accumulation would affect the rate of manganese uptake and distribution in the plants and consequently would be responsible for manganese deficiencies/mineral imbalance and depression of the plant growth. The work of Wang and his coworkers showed increased Fe uptake under cadmium stress in maize[29]. Liu et al.[30] found significant correlation on root content between cadmium and Cu or Fe in rice cultivars. However, some other researches related to changes of Fe concentrations under cadmium stress, showed not significant correlation between these two metals[31]. In the present study, cadmium significantly reduced root Fe concentration but no effect on shoot concentration. Addition of Se or pretreatment with GSH elevated root Fe concentration in 50 µM cadmium present solution. Thus, elevated uptake of Fe may be one of the mitigatory effect of external GSH/Se in maize plants. In addition, significantly positive correlations between Zn, Cu and Mn were observed in this study. The results highlight the possibility of developing maize cultivars/lines with moderately high Mn, Cu and Fe levels.

Conclusion

The present study demonstrated that cadmium-induced oxidative stress being visualized by the increased H_2O_2 and malondialdehyde accumulation in leaves and roots of maize plants exposed to 50 µM cadmium over the control (Fig. 3), in agreement with the results reported by Chen et al.[8], also by Hegedüs[32], May and Leaver[33] and Zhang et al.[34]. To protect oxidative stress, plants evolutionally developed enzymatic and non-enzymatic reactive oxygen species scavenging systems to maintain cellular redox state[35]. In this study, as an adaptive mechanism peroxidase and superoxide dismutase activities were correspondingly induced to cope with reactive oxygen species (Fig. 4), while leaf catalase activity decreased in the plants exposed to 50 µM cadmium. Selenium addition or GSH pretreatment successfully decreased cadmium-induced reactive oxygen species and malondialdehyde accumulation, significantly counteracted cadmium-induced alterations of certain antioxidant enzymes, e.g. brought root/leaf peroxidase and leaf superoxide dismutase activities down towards to the control level, but elevated root ascorbate peroxidase activity. Meanwhile, elevated catalase and ascorbate peroxidase activities in leaves were also observed in the plants under Cd + GSH treatment. These results provide evidence that GSH/ Se facilitates substantial protection against oxidative injury in responses to cadmium stress. Taken together, reduced cadmium uptake and translocation and the protection against oxidative injury could be principal protective mechanism for the exogenous GSH/Se in alleviation cadmium toxicity. The results also suggested a practical potential for GSH/Se as a potent antioxidant in plants and that its action may, at least in part, be explained by their ability to directly and indirectly scavenge reactive oxygen species.

REFERENCES

1. J. Dong, W.H. Mao, G.P. Zhang, F.B. Wu and Y. Cai, *Plant Soil Environ.*, **53**, 193 (2007).
2. K. Smeets, J. Ruytinx, B. Semane, F.V. Belleghem, T. Remans, S.V. Sanden, J. Vangronsveld and A. Cuypers, *Environ. Exp. Bot.*, **63**, 1 (2008).
3. J.B. Zhang and W.N. Huang, *Acta Ecol. Sin.*, **20**, 514 (2000).
4. F.Y. Wang, C.S. Zhang and X.F. Xu, *Asian J. Chem.*, **24**, 657 (2012).
5. F. Chen, J. Dong, F. Wang, F.B. Wu, G.P. Zhang, G.M. Li, Z.F. Chen, J. Chen and K. Wei, *Chemosphere*, **67**, 2082 (2007).
6. F.B. Wu, G.P. Zhang and J.S. Yu, *Commun. Soil Sci. Plant Nutr.*, **34**, 2003 (2003).
7. Y.L. Zhu, E.A.H. Pilon-smits, A.S. Tarun, S.U. Weber, L. Jouanin, N. Terry, *Plant Physiol.*, **121**, 1169 (1999).
8. F. Chen, F. Wang, F.B. Wu, W.H. Mao, G.P. Zhang and M.X. Zhou, *Plant Physiol. Biochem.*, **48**, 663 (2010).
9. Y.J. Kang and M.D. Enger, *Cell Biol. Toxicol.*, **3**, 347 (1987).
10. P.L. Lima, J.C. Benassi, R.C. Pedrosa, J.D. Magro, T.B. Oliveira and D.W. Filho, *Arch. Environ. Contam. Toxicol.*, **50**, 23 (2006).
11. S. Gromer, L. Johansson, H. Bauer, L.D. Arscott, S. Rauch, D.P. Ballou, C.H. Williams Jr., R.H. Schirmer and E.S. Arnér, *Proc. Nat. Acad. Sci. USA*, **100**, 12618 (2003).
12. L.R. McDowell, Minerals in Animal and Human Nutrition, University of Florida, Gainesville, edn. 2, pp. 235-276 (2003).

13. E.J. Underwood and N.F. Suttle, The Mineral Nutrition of Livestock, Midlothian, UK, edn. 3, pp. 283-392 (1999).
14. K.O. Cafferky, D.D. Richardson and J.A. Caruso, *Spectroscopy*, **21**, 18 (2006).
15. K.T. Suzuki and Y. Ogra, *Phosphorus Silicon Sulfur Rel. Elem.,* **171**, 135 (2001).
16. S. Mounicou, M. Shah, J. Meija, J.A. Caruso, A.P. Vonderheide and J. Shann, *J. Anal. Atom Spectrom.*, **21**, 404 (2006).
17. L.B. Nehru and M.P. Bansal, *J. Appl. Toxicol.*, **17**, 81 (1997).
18. G. Drasch, J. Schopfer and G.N. Schrauzer, *Biol. Trace Elem. Res.*, **103**, 103 (2005).
19. O. Gotsis, *Mar Biol.*, **71**, 217 (1982).
20. M.C. Romero-Puertas, M. Rodriguez-Serrano, F.J. Corpas, M. Gomez, L.A. Del Rio and L.M. Sandalio, *Plant Cell Environ.*, **27**, 1122 (2004).
21. F.B. Wu, G.P. Zhang and P. Dominy, *Environ, Exp, Bot.*, **50**, 67 (2003).
22. X.Z. Zhang, Research Methodology of Crop Physiology, Agriculture Press, Beijing, pp. 208-211 (1992).
23. H.H. Ruan, W.B. Shen, M.B. Ye and L.L. Xu, *Chinese Sci. Bull.*, **46**, 1993 (2001).
24. A. Kabata-Pendias and H. Pendias, Trace Elements in Soils and Plants, CRC Press Inc. Boca Raton, Florida, edn. 3 (2001).
25. G.P. Zhang, M. Fukami and H. Sekimoto, *Field Crops. Res.*, **77**, 93 (2002).
26. F.B. Wu, G.P. Zhang, *Bull. Environ. Contam. Toxicol.*, **69**, 219 (2002).
27. G.C. Smith and E.G. Brennan, *Phytopathology*, **73**, 879 (1983).
28. D.A. Cataldo, T.R. Garland and R.E. Wildung, *Plant Physiol.*, **73**, 844 (1983).
29. M. Wang, J.H. Zou, X.C. Duan, W.S. Jiang and D.H. Liu, *Bioresour. Technol.*, **98**, 82 (2007).
30. J.G. Liu, J.S. Liang, K.Q. Li, Z.J. Zhang, B.Y. Yu, X.L. Lu, J.C. Yang and Q.S. Zhu, *Chemosphere*, **52**, 1467 (2003).
31. A. Chaoui, M.H. Ghorbal and E.E. Ferjani, *Plant Sci.*, **126**, 21 (1997).
32. A. Hegedüs, S. Erdei and G. Horváth, *Plant Sci.*, **160**, 1085 (2001).
33. M.J. May and C.J. Leaver, *Plant Physiol.*, **103**, 621 (1993).
34. H. Zhang, W. Xu, J. Guo, Z. He and M. Ma, *Plant Sci.*, **169**, 1059 (2005).
35. R.D. Baisak, P.B.B. Rana and M.K. Acharya, *Plant Cell Physiol.*, **35**, 489 (1994).