Physiological responses of maize to elemental sulphur and cadmium stress

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ABSTRACT

The physiological response to application of elemental sulphur (S) and cadmium (Cd) of maize (*Zea mays* L.) grown for 60 days in pot soil was studied. The S was added into the soil with 2 rates (0 and 50 mmol/kg) and Cd was added in solution in 4 rates (0, 20, 50, 100 mg/kg). All the S and Cd were added before planting. Shoot biomass decreased with the application of Cd to the soil whether S was applied or not. The application of S and Cd to soil led to an increasing accumulation of Cd in the shoots of maize. The concentration of chlorophyll was reduced significantly in Cd-treated plants with or without supplementary S. The content of malondialdehyde (MDA) was increased significantly in treatments with S and Cd, compared to the control. The activity of peroxidases (POD) was increased but catalase (CAT) was decreased in plants treated with Cd, again with or without S, in comparison with control. POD and CAT activities decreased in all the Cd treated plants with S, as compared to the plants without S. The results suggest that Cd reduces the crop growth, concentration of chlorophyll and activity of CAT, but increases the content of MDA and activity of POD. S supplies decrease the content of MDA, activities of POD and CAT, as compared to zero S supplies at the same rate of Cd application.

Keywords: maize; elemental sulphur; cadmium, chlorophyll; lipid peroxidation; antioxidant enzymes

Elemental sulphur (S) is used as a fertilizer and has been reported to increase the solubility of cadmium (Cd) in soils and to enhance plant uptake of Cd (Tichý et al. 1997, Kayser et al. 2000, Cui et al. 2004). Toxic level of Cd can result from environmental pollution due to mining, smelting and dumping of municipal sewage wastes, manufacturing processes and disposal of used batteries. High concentration of Cd in the soil may inhibit seed germination and exert a wide range of adverse effects on the growth and metabolism of plants (Mohan and Hosetti 1997, Hegedüs et al. 2001).

Plants exposed to Cd stress invariably show marked alterations in electron transport in both chloroplasts and mitochondria (Prasad et al. 2001, Shah et al. 2001, Zhang et al. 2005). This results in the production of active oxygen species (AOS) such as superoxide radicals (O_2^-), single oxygen ($^1O_2$), hydroxyl radical (OH) and peroxide (H_2O_2), which affect various cellular processes mostly concerned with the functioning of membrane systems. Plants have evolved protective mechanisms to eliminate or ameliorate these damages, such as altering of antioxidant enzyme activities and antioxidant levels and enhancement of lipid peroxidation (Prasad et al. 2001, Shah et al. 2001).

Effects of Cd stress on plant growth and development were reported in several studies (Mohan and Hosetti 1997, Hegedüs et al. 2001, Prasad et al. 2001, Shah et al. 2001). However, investigations on the combined effects of elemental S with Cd on antioxidative processes are rare. The main objective

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of the present study was therefore to investigate the physiological responses of maize to elevated soil concentrations of elemental S combined with different levels of Cd.

MATERIAL AND METHODS

Soil. The top 20 cm of soil was collected from a profile in Qinghe, Beijing, China. The soil was air-dried, ground and sieved by passing through a 2-mm sieve. Selected physicochemical properties were as follows: pH (in 0.01M $CaCl_2$) 7.25; organic mater content 12 g/kg; total Cd 0.8 mg/kg and total S 210 mg/kg.

Experimental design and treatments. The experiment involved an application of elemental S and Cd (as $CdCl_2$) to the soil. The S (solid) and Cd (in solution) were added at two rates (0 and 50 mmol/kg S) and four rates (0, 20, 50 and 100 mg/kg Cd), respectively. These amendments were made prior to planting. Thus, there were eight treatments in triplicate, giving a total of 24 pots. Basal fertilizers were applied at the rates of 120 mg N/kg (NH_4NO_3), 80 mg P/kg (KH_2PO_4), and 120 mg K/kg (KCl and KH_2PO_4) and thoroughly mixed with the soil. Each pot received 1 kg of amended soil, which was allowed to equilibrate for 14 days before the seeds were sown. Six seeds of maize (*Zea mays* L.) were sown in each pot and thinned to three seedlings per pot one week after germination. The experiment was carried out in a glasshouse with a 14 h (26°C)/10 h (13°C) day/night cycle. Soil water content was adjusted regularly by weight equivalent to about 60% of water holding capacity and the plants grew for 60 days.

Chemical analysis of plants and soil. Soil samples were digested with a mixture of concentrated HNO_3:$HClO_4$:HF (3:1:1) and total Cd was determined by atomic absorption spectrophotometry (AAS). The harvested plant samples were rinsed with deionized water, oven dried at 70°C for 48 h, ground with an agate mill, digested with a mixture of concentrated HNO_3:$HClO_4$:HF (3:1:1), and total Cd was analyzed as described above. Soil total S was determined on air-dried soil by oxidation with $Mg(NO_3)_2$ followed by a turbidimetric method (Nanjing Agricultural University 1986).

Chlorophyll. Chlorophylls *a* and *b* were determined in 96% ethanol extracts of 0.2-g aliquots of fresh leaf tissue as described by Zhou (2001a).

Lipid peroxidation. Lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) by a modification of the method of Zhou (2001b). About 1g fresh tissues was ground in 10 ml 10% trichloracetic acid (TCA) using a mortar and pestle. The homogenate was centrifuged at 10 000 rpm for 20 min. The reaction mixture containing 2 ml extract and 2 ml thiobarbituric acid (TBA) was heated at 95°C for 30 min, quickly cooled on ice and then centrifuged again at 10 000 rpm for 20 min. The absorbance of the supernatant was determined at 532 nm (A_{532}), 600 nm (A_{600}) and 450 nm (A_{450}), respectively with a UV/VIS spectrophotometer. The MDA content was calculated by the equation: C (MDA content) = 6.45 (A_{532}–A_{600}) – 0.56 A_{450}.

Antioxidant enzyme assays. Activities of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) were assayed in fresh leaf tissue extracts by UV/VIS spectrophotometry using a modification of the method of Zhou (2001b) and Zhang (1990). Briefly, the samples were prepared for catalase analysis by homogenization of fresh tissue with a mortar and pestle in a buffer solution (5 ml/g fresh weight) containing 0.2M NaH_2PO_4/Na_2HPO_4 (pH 7.8). After the homogenate was centrifuged at 10 000 rpm for 20 min at 4°C, the supernatant was used immediately to determined catalase activity by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 0.3 ml 0.1M H_2O_2, 1.5 ml 0.2M NaH_2PO_4/Na_2HPO_4 buffer solution (pH 7.8), 1 ml deionized water and 1 ml enzyme extract. Catalase activity was expressed as μmol of H_2O_2 decomposed per min per gram of fresh weight (μmol/min/g FW). The samples were prepared for peroxidase analysis by homogenization of fresh tissue with a mortar and pestle in a solution (5 ml/g fresh weight) containing 0.02M KH_2PO_4. After the homogenate was centrifuged at 10 000 rpm for 20 min at 4°C, the supernatant was used immediately for the indirect determination of peroxidase activity by monitoring the decrease in absorbance at 470 nm of a reaction mixture containing 3 ml 0.1M mixture solution (0.1M NaH_2PO_4/Na_2HPO_4 buffer (pH 6.0), 0.56mM guaiacol and 0.38mM H_2O_2) and 0.2 ml enzyme extract diluted 5 times. The kinetics of the reaction were measured as Δ_{OD} at 470 nm and expressed as units Δ_{OD} 470 nm per min per gram of fresh weight (Δ_{OD}/min/g FW).

Statistical analysis. All data were subjected to ANOVA and subsequently to Duncan's multiple range tests by application of SAS package (version 8.1, SAS Inc., Cary, NC, USA).

RESULTS

Shoot biomass. Shoot biomass decreased significantly with Cd added. Application of elemental S had no significant effect on shoot biomass (Figure 1).

Shoot concentrations and uptake of Cd and S. Shoot Cd concentration and uptake increased significantly with Cd increase in soil. Elemental S was also shown to significantly increase shoot Cd concentration and uptake. The highest shoot Cd concentration and uptake occurred in the treatment of 50 mmol/kg S combined with 100 mg/kg Cd (Tables 1 and 2).

Chlorophyll concentration in leaves of maize. S application had no significant effect on chloro-

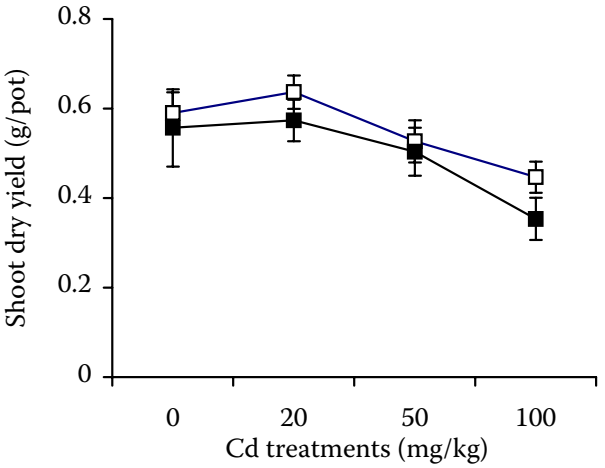

Figure 1. Shoot dry yields (g/plant) of maize at different cadmium treatments (□ 0 mmol/kg S, ■ 50 mmol/kg S)

Table 1. Mean shoot cadmium concentration (mg/kg) of maize with and without addition of sulphur to the soil together with cadmium or controls with no cadmium application

S rate (mmol/kg)	Cd treatments (mg/kg soil)			
	0	20	50	100
0	ND[a]	3.68 ± 0.32	7.88 ± 0.80	23.47 ± 2.22
50	ND	16.7 ± 1.62	42.40 ± 1.56	85.80 ± 6.88
Significance[b] of				
Sulphur			***	
Cd			***	
S × Cd			***	

[a]not determined because shoot Cd concentrations were below the detection limit; [b]by analysis of variance; ***$P < 0.001$

Table 2. Mean shoot cadmium uptake (µg/pot) of maize with and without addition of sulphur to the soil together with cadmium or controls with no cadmium application

S rate (mmol/kg)	Cd treatments (mg/kg soil)			
	0	20	50	100
0	ND[a]	2.34 ± 0.23	4.22 ± 0.79	10.38 ± 0.74
50	ND	9.67 ± 1.45	21.51 ± 3.08	30.90 ± 6.00
Significance[b] of				
Sulphur			***	
Cd			***	
S × Cd			*	

[a]not determined because shoot Cd concentrations were below the detection limit; [b]by analysis of variance; ***$P < 0.001$; *$P < 0.05$

Table 3. Chlorophyll concentration (mg/g fresh weight) in leaves of maize with and without addition of sulphur to the soil together with cadmium or controls with no cadmium application

S rate (mmol/kg)	Cd treatments (mg/kg soil)			
	0	20	50	100
0	2.90 ± 0.29	2.37 ± 0.24	1.66 ± 0.16	1.45 ± 0.13
50	2.72 ± 0.29	2.06 ± 0.13	1.38 ± 0.07	1.21 ± 0.07
Significance[a] of				
Sulphur		NS		
Cd		***		
S × metal		NS		

[a]by analysis of variance; ***$P < 0.001$; NS – not significant

phyll concentration in the leaves. Cd lowered leaf chlorophyll content significantly whether or not S was applied to the soil (Table 3).

Leaf lipid peroxidation. Leaf malondialdehyde content increased in all S and Cd treatments compared to the unamended control, especially

Table 4. Mean malondialdehyde (MDA, nmol/g) content and activities of two antioxidant enzymes (peroxidase, units Δ_{OD} at 47 nm/min/mg; catalase, μmol H_2O_2 decomposed min/mg) in leaves of maize with and without addition of sulphur to the soil together with cadmium or controls with no cadmium application (all fresh weight basis)

S rate (mmol/kg)	Cd treatments (mg/kg soil)			
	0	20	50	100
Malondialdehyde				
0	7.11 ± 0.52	7.19 ± 0.48	13.01 ± 0.94	15.83 ± 1.25
50	6.57 ± 0.54	8.29 ± 0.64	15.20 ± 0.95	17.83 ± 1.40
Significance[a] of				
Sulphur		NS		
Cd		***		
S × Cd		NS		
Peroxidase				
0	25.73 ± 1.59	31.97 ± 1.44	34.47 ± 1.39	39.83 ± 2.58
50	26.63 ± 1.61	33.20 ± 2.31	37.40 ± 1.67	40.83 ± 3.96
Significance[a] of				
Sulphur		NS		
Cd		***		
S × Cd		NS		
Catalase				
0	4.09 ± 0.33	3.14 ± 0.25	2.60 ± 0.08	2.25 ± 0.26
50	3.83 ± 0.32	2.42 ± 0.19	2.08 ± 0.39	1.72 ± 0.26
Significance[a] of				
Sulphur		*		
Cd		***		
S × Cd		NS		

[a]by analysis of variance; ***$P < 0.001$; *$P < 0.05$; NS – not significant

without S application. Application of S together with Cd gave a lower MDA content than addition of Cd only (Table 4).

Antioxidant enzyme activity. Leaf peroxidase activity increased significantly in Cd treatments compared with unamended controls and increased, even if not significantly, in all the Cd treatments with supplementary S compared to the Cd addition only (Table 4). Leaf catalase activity decreased significantly in Cd treatments compared with unamended controls, and decreased (not significantly) in all the Cd treatments with supplementary S compared to the Cd addition only (Table 4).

DISCUSSION

As expected, application of Cd to the soil led to a higher concentration of the metal in the shoots of maize, which is in agreement with other studies (Mohan and Hosetti 1997, Hegedüs et al. 2001). Supplementation of Cd contaminated soils with S showed an influence of the concentrations of Cd in plants (Tichý et al. 1997, Kayser et al. 2000, Cui et al. 2004). In the present study, application of S together with Cd increased shoot Cd concentration and uptake and this corresponds well with the results of Kayser et al. (2000) and Tichý et al. (1997).

Based on the leaf chlorophyll and malondialdehyde levels and activities of peroxidase and catalase in the present study, leaf chlorophyll decreased with the Cd addition, and this is also in accordance with earlier studies (Stobart et al. 1985, Padmaja et al. 1990, Böddi et al. 1995). There are several key steps in the biosynthesis and organization of chlorophyll and the following were identified as sensitive targets of Cd: synthesis of δ-aminolevulinic acid (Stobart et al. 1985), aminolevulinic acid dehydratase (Padmaja et al. 1990), protochlorophyll reductase (Stobart et al. 1985, Böddi et al. 1995) and integration of chlorophyll molecules into pigment protein complexes (Horvath et al. 1996). Thus, the inhibition of these processes of chlorophyll biosynthesis and chlorophyll organization by Cd would explain the observed decrease in leaf chlorophyll concentration.

Sulphur is also an essential nutrient for normal plant growth. However, higher S application rates may induce adverse effects on plant growth. Bussotti et al. (2003) reported that the concentration of S in *Quercus pubescens* leaves correlated positively with chlorophyll concentration. In the present experiment, the increase of S application decreased the chlorophyll concentration, indicating that chlorophyll concentration is influenced by factors such as plant species, Cd and its concentration, and the concentration of S in soils.

An enhanced content of malondialdehyde in leaves of maize exposed to Cd without supplemental S in the present study indicates that the metal may have caused a membrane damage. There have been numerous reports on increasing malondialdehyde content induced by cadmium stress (Shah et al. 2001). However, unlike our study, the content of malondialdehyde did not changed in maize root under Cd stress (Astolfi et al. 2005). Interestingly, the malondialdehyde content increased with S application combined with metal addition. It indicated that though sulphur is an important essential element in the growth of plants, the excess supply would bring a negative effect. Therefore, the direction of the plant response depends on the plant species, the metal used for the treatment and the intensity of the stress.

Peroxidases exist in both the cytosol and the cell wall and decompose H_2O_2 to H_2O and O_2; they have been reported to be involved in several physiological and biochemical processes such as cell growth and expansion, differentiation and development, auxin catabolism, lignification, as well as abiotic and biotic stress responses. Increases in peroxidase activity under Cd stress were observed in our study. The results agree well with the result of some researchers, who observed increases in peroxidase under Cd stress in barley, sunflower cotyledons, cattail and reed (Gallego et al. 1999, Hegedüs et al. 2001, Fediuc and Erdei 2002). Peroxidase activity increased under the heavy metal stress when combined with S compared to the treatments without supplemental S. It indicated that the excess supply S would bring a negative effect. Thus, a peroxidase activity depends on plant species, the metal concentration and elemental S (Schützendübe and Polle 2002).

Catalase is a universally present oxidoreductase that decomposes H_2O_2 to H_2O and O_2 and it is one of the key enzymes involved in removal of toxic peroxides. A decrease was observed in catalase activity under Cd stress in the present study. Similar declines in catalase activity were reported under under Cd stress in rice, cabbage, bean, carrot, radish and pea (Chaoui et al. 1997, Sandalio et al. 2001, Shah et al. 2001, Pandey and Sharma 2002). However, unlike our study, increased

catalase activity was also observed in sunflower cotyledons and barley (Patra and Panda 1998, Gallego et al. 1999) under Cd stress. There are few reports on the effects of Cd combined with elemental S on catalase activity. In the present study, excessive elemental S led to a decline in shoot biomass and the catalase activity decreased with an S application of 50 mmol/kg. This indicates that excess S supply can give negative effects. Thus, the direction of the plant response depends on the plant species, the metal concentration and the intensity of the stress.

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