Cd uptake in rice cultivars treated with organic acids and EDTA

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Abstract

A pot experiment was conducted to examine the activity of antioxidant enzymes, the content of malondialdehyde (MDA), proline and protein, and Cd uptake in different rice cultivars exposed to Cd (0, 1 and 5 mg/kg) in the presence of organic acids and ethylenediamine tetraacetic acid (EDTA). The results showed the increase in activity of dismutase (SOD), contents of proline and protein but a decline in activities of peroxidase (POD) and catalase (CAT), and MDA content for cultivars Xiushui63 and IIyou527. The resistance to Cd was higher in Xiushui63 than that in IIyou527 under the same Cd treatment. Cadmium contents in grain, straw and roots of both cultivars were markedly reduced in the presence of organic acids and EDTA. Grain Cd contents was the highest for plants treated with organic acids, followed by organic acids + 1/2EDTA, and the lowest with EDTA; Cd contents in straw and root were the lowest for plants treated with organic acids, followed by organic acids + 1/2EDTA, and the highest with EDTA treatment when exposed to Cd.

Key words: organic acid; EDTA; antioxidant enzymes; Cd uptake; rice

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Introduction

Cadmium is a toxic metal to humans, animals and plants (Wagner, 1993). Although plants do not require Cd for growth and reproduction, the bioaccumulation index of Cd in plants is high and may exceed that of many essential elements (Kabata-Pendias and Pendias, 2001). In addition, Cd may pose a risk to human and animal health because of the transfer of a high level of Cd from agricultural soils to the human food chain (Jackson and Alloway, 1992). Therefore, Cd is one of the most important metals to consider in terms of food-chain contamination.

Cadmium toxicity gives rise to damage in higher plants through interference in many molecular and physiological processes. It can damage the photosynthetic apparatus (Siedlecka and Baszynski, 1993), induce cellular oxidative damage or lipid peroxidation (Chaoui et al., 1997; Chien et al., 2002), inhibit or stimulate the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Kuo and Kao, 2004; Liu, 2005), and activate proline and malondialdehyde (MDA) content (Sun et al., 2004; Tang et al., 2005). Genotypic differences in resistance to oxidative stress were found when plants were exposed to Cd (Li et al., 2004).

Genotypic variations of Cd absorption in food crops grown in Cd contaminated soil have also been observed (Oliver et al., 1995). The uptake and translocation of Cd in plants varied greatly not only between species but also between cultivars within the same species (McLaughlin et al., 1994; Yang et al., 1995). These differences could provide a long term effective and economical means of reducing Cd contamination in crops. Paddy rice is one of the most important crops in the world, especially in Asia. The amount of Cd that enters the human diet from a crop depends on the amount of Cd accumulated in the edible parts. The translocation of Cd within the rice plant, especially into grain, is very important for human Cd intake through diet.

The availability of metals in soil to plants is one of the major factors that influence metal uptake by plants. Phytoavailability of metals is controlled by the chemical characteristics of the metal considered, rhizosphere soil properties, and the specific characteristics of plant species, cultivars, or populations. Ethylenediamine tetraacetic acid (EDTA) is a chemical extractant widely used to remove heavy metals from the soil via chelation, especially when the metal bioavailability is low (Salt et al., 1998). Organic acids are negative anions under most soil conditions, which allow them to react strongly with metal ions in soil (Jones et al., 1996; Gao et al., 2002). The interactions of organic acids with metals in the soil-plant system may affect the solubility and phytoavailability of metals in soil and their transport in plants. Some organic acids, such as citric, malic, and oxalic, have been reported to be potential metal chelators (Naidu and Harter, 1998; Gao et al., 2002). Several studies have shown that rice cultivars

vary significantly in Cd uptake and accumulation (Liu et al., 2003, 2005). However, limited information is available on the mechanism of this variation when organic acids and EDTA are supplied to rice cultivars. To understand these mechanisms, we studied the relationship between plant Cd uptake and organic acids and EDTA for different rice cultivars. The effect of organic acids and EDTA on antioxidant systems in different rice cultivars exposed to Cd was also investigated.

1 Materials and methods

1.1 Plant material, soil and Cd treatments

The experiment was carried out in a glasshouse from March to August 2005. After germination, rice seedlings of Xiushui63 and IIyou527 were grown in a seedbed until 3-leaf stage with a height of 8–10 cm. Then the plants were transplanted into plastic pots (25 cm in depth, 60 cm in diameter) filled with 5.0 kg soil. Two seedlings were grown in each pot.

The soil was characterized with pH 6.5, organic matter of 10.4 g/kg, total N of 0.53 g/kg, and available N 78.5 mg/kg, P 12.3 mg/kg and K 97.0 mg/kg, and total Cd of 0.52 mg/kg. The available Cd was below the detection level (< 0.01). Soil (1.5 kg) was thoroughly mixed with 100 mg/kg P of KH_2PO_4, 150 mg/kg K of KCl and 150 mg/kg N of urea. Three levels of Cd (0, 1 and 5 mg/kg) as $CdCl_2$ were applied in combination with organic acids (1.0 mmol/L citric acid, 3.0 mmol/L oxalic acid) and EDTA (2 mmol/L Na_2-EDTA). The experimental design was completely randomized, and each treatment was replicated three times. After 8 weeks, the youngest and the second youngest fully opened leaves of one pot in each treatment were cut for the analysis of activities of antioxidant enzymes, concentrations of proline, glutathione (GSH), water-soluble proteins and MDA. All remaining plants were harvested at maturity, and dried at 60°C. Biomass accumulation and concentrations of Cd in straw, root and grain were determined.

1.2 Protein and enzyme analyses

Tissue samples were homogenized in ice-cold deoxygenated 20 mmol/L Tris-HCl buffer (pH 7.4, 1:9, *W/V*) and centrifuged at 3000 $\times g$ for 10 min. Aliquots of 100 μL were used for enzyme activity measurement. The activity of CAT was measured using a microtiter plate assay (Hansen et al., 2006). The activity of POD was determined according to Nakano and Asada (1981). The activity of SOD was determined by the method of Minami and Yoshikawa (1979) with 50 mmol/L Tris-Ca-codylic sodium salt buffer at pH 8.2 and containing 0.1 mmol/L EDTA. Total water-soluble proteins in samples were measured by Bradford assay (Bradford, 1976).

1.3 Proline analysis

Proline was extracted and determined by the method of Bates et al. (1973). Leaf segments were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged at 3000 $\times g$ for 10 min. After acetic acid and acid ninhydrin were added, the supernatant was boiled for 1 hr and the A520 was determined.

1.4 Malondialdehyde analysis

The MDA assay was based on the condensation of one molecule malondialdehyde with two molecules of thiobarbituric acid (TBA) in the presence of reduced reagent volumes to increase sensitivity, generating a chromogen with UV absorbance. The TBA + MDA complex was analyzed essentially as described by Bird et al. (1983). Aliquots of the TBA + MDA samples were injected into a 5-mm Supelcosil LC-18 reversed phase column (30 mm × 4.6 mm). The mobile phase consisted of 15% methanol in double-distilled water degassed by filtering through a 0.5-μm filter (Millipore, USA). The flow rate was 2 mL/min. The MDA + TBA standards were prepared using tetraethoxypropane. The absorption spectra of standards and samples were identical with a characteristic peak at 540 nm.

1.5 Analyses of Cd concentration

Dried plant samples were ground and digested with concentrated HNO_3-$HClO_4$ (4:1, *V/V*) (Allen, 1989). Cadmium concentrations in the solutions were analyzed using a flame atomic absorption spectrometer (SIMMA 6000, PerkinElmer, USA). Soil pH (soil: water of 1:2.5 of *m/v*) was measured with a digital acidometer (pHSJ-5, Shanghai Precision & Scientific Instrument Co., Ltd., China).

1.6 Statistical analysis

The effects of Cd, organic acids and EDTA on plant dry weight, activities of antioxidant enzymes, concentrations of proline and MDA, concentrations and accumulation of Cd in straw, roots and grain were subjected to a three-way analysis of variance (ANOVA, i.e., cultivars, chelators and Cd levels), followed by the least significant difference test ($p = 0.05$). The statistical analysis was performed using the SPSS 12.0 software.

2 Results

2.1 Activities of antioxidant enzymes

Significant differences in the activities of SOD, POD and CAT were observed in the two cultivars as well as in Cd and chelator treatments (Table 1). The activities of SOD increased at 1 mg/kg Cd, but decreased at 5 mg/kg Cd. After adding organic acids (O) and EDTA (E), the activities of SOD in treatment of Cd1+E for Xiushui63 and all treatments of Cd+O and/or E for IIyou527 increased compared to the Cd-only treatments.

The activities of POD and CAT in both cultivars decreased with an increase of soil Cd. After adding the organic acids and EDTA, the activities of POD and CAT further decreased compared to Cd-only treatments, except for Cd1+O in which the activity of CAT increased by 0.5%. Moreover, the activities of POD and CAT were lower in IIyou527 than that in Xiushui63 under the same

Table 1   Activities of POD, SOD and CAT, concentration of MDA in two rice cultivars and probability (p) by three-way ANOVA (cultivars, chelators and Cd levels)

Treatment	SOD (U/g)		POD (U/(g·min))		CAT (U/(g·min))		MDA (µmol/g)	
	IIyou527	Xiushui63	IIyou527	Xiushui63	IIyou527	Xiushui63	IIyou527	Xiushui63
Cd0	63.01	72.44	124.16	182.33	205.36	227.96	6.49	6.99
Cd1	64.31	74.41	111.29	177.83	175.68	227.44	7.16	8.53
Cd1+E	67.99	75.39	83.14	120.08	148.94	163.88	6.52	7.13
Cd1+O	67.33	73.40	68.24	105.43	176.52	203.88	6.92	7.89
Cd1+O+1/2E	65.30	60.46	87.57	139.69	169.79	192.31	6.57	8.56
Cd5	45.52	54.63	90.77	114.31	134.69	142.59	8.87	9.90
Cd5+E	57.36	64.66	62.61	82.14	102.04	113.73	7.52	8.85
Cd5+O	51.76	59.56	57.25	75.21	123.16	135.54	7.11	9.54
Cd5+O+1/2E	56.65	56.08	81.55	93.97	112.20	117.82	7.37	9.18
Probability (p)								
Cd	< 0.001		< 0.001		< 0.001		< 0.001	
Cultivar	< 0.001		< 0.001		< 0.001		< 0.001	
Chelator	0.016		< 0.001		< 0.001		< 0.001	
Cd × chelator	0002		0.014		0.573		0.094	
Cd × cultivar	0.983		< 0.001		0.002		0.327	
Chelator × cultivar	0.033		0.021		0.067		0.306	
Cd × chelator × cultivar	0.582		0.187		0.041		0.136	

Cd0, Cd1 and Cd5 represent Cd levels of 0, 1 and 5 mg/kg. E: 2.0 mmol/L EDTA; O: a combination of organic acids (1.0 mmol/L citric acid, 3.0 mmol/L oxalic acid); O+1/2E: a combination of organic acids (1.0 mmol/L citric acid, 3.0 mmol/L oxalic acid) and 1/2EDTA (1.0 mmol/L).

treatments.

2.2 MDA contents

MDA contents in the two cultivars significantly increased with an increase of soil Cd in the absence of organic acids and EDTA, but decreased after adding organic acids and EDTA (except for Cd1+O+1/2E for Xiushui 63, in which the MDA content increased by 0.4%) compared to the Cd contaminated soil (Table 1). The content of MDA in Xiushui63 was higher than that in IIyou527 at the same treatment, and was the highest in Cd5 treatment of Xiushui63, followed by Cd5+O treatment of Xiushui63.

## 2.3 Contents of proline and proteins

Proline contents in the shoots of two rice cultivar seedlings exposed to Cd, organic acids and EDTA are shown in Fig. 1. Proline content in both cultivars significantly increased with the decrease of soil Cd in the absence of organic acids and EDTA. Compared to the Cd-only soil, the addition of organic acids and EDTA significantly increased proline content in both cultivars in the order of EDTA > organic acids + 1/2EDTA > organic acids. Cultivar IIyou527 had a higher proline content than Xiushui63 under the same treatments.

Total soluble protein in the seedlings of two rice cultivars decreased with the increase of soil Cd level in the absence of organic acids and EDTA (Fig. 2). Addition of organic acids and EDTA increase total soluble protein at two soil Cd levels. Cultivar IIyou527 had higher contents of total soluble protein than Xiushui63 with the same treatment.

2.4 Cadmium accumulation in grain, straw and roots

Cadmium accumulation in grain, straw and roots in both cultivars increased with the increase of soil Cd level (Table 2). After adding organic acids and EDTA, grain Cd concentration respectively decreased by 9.0% to 49.3% at 1 mg/kg Cd and by 12.7% to 28.5% at 5 mg/kg Cd for IIyou527, and respectively decreased by 16.5% to 30.6%
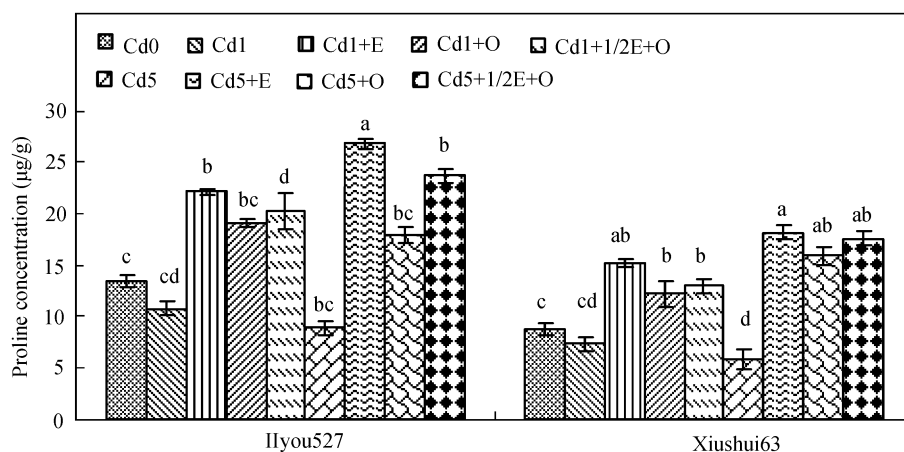
Fig. 1   Effect of organic acids and EDTA on the concentrations of proline in two rice cultivars. Cd0, Cd1, Cd5, E, O and O+1/2E have the same meaning as in Table 1. Vertical bars represent standard error. Means with different letters indicate significant difference at $p < 0.05$.
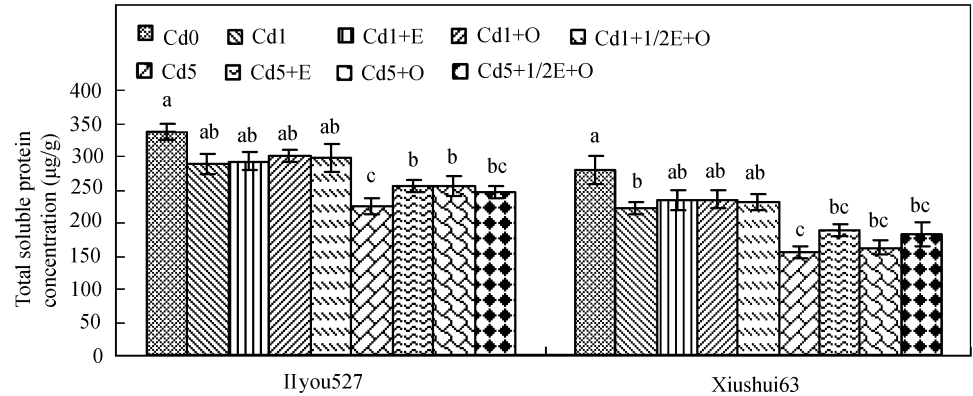
Fig. 2 Effect of organic acids and EDTA on the concentrations of protein in two rice cultivars. Cd0, Cd1, Cd5, E, O, and O+1/2E have the same meaning as in Table 1. Vertical bars represent ± standard error. Means with different letters indicate significant difference at $p < 0.05$.

at 1 mg/kg Cd and 4.3% to 19.1% at 5 mg/kg Cd for Xiushui63. Grain Cd concentration was in the order of Cd contaminated soil > Cd+O > Cd+O+1/2E > Cd+E. The highest grain Cd concentration was 2.77 mg/kg in the Cd5 treatment of Xiushui63, followed by 2.21 mg/kg in the Cd5 treatment of IIyou527. Grain Cd concentration

was greater in Xiushui63 than IIyou527 for all treatments in the absence and presence of EDTA and organic acids. The lowest grain Cd concentration was 0.34 mg/kg in the Cd1+E treatment of IIyou527.

Cadmium concentrations in straws and roots were greater in Xiushui63 than in IIyou527 at all treatments

Table 2 Cadmium concentration and accumulation in grain, straw and roots and probability by three-way ANOVA

Treatment	Cd concentration in grain (mg/kg)		Cd concentration in straw (mg/kg)		Cd concentration in root (mg/kg)	
	IIyou527	Xiushui63	IIyou527	Xiushui63	IIyou527	Xiushui63
Cd0	0	0	0	0	0	0
Cd1	0.67	0.85	4.67	7.07	22.01	29.05
Cd1+E	0.34	0.59	3.43	4.37	16.85	17.12
Cd1+O	0.61	0.71	3.86	6.35	14.78	27.60
Cd1+O+1/2E	0.45	0.68	3.77	4.98	14.93	19.40
Cd5	2.21	2.77	10.79	14.46	108.17	115.89
Cd5+E	1.58	2.24	7.44	10.14	49.42	96.01
Cd5+O	1.93	2.65	10.23	13.70	97.76	98.88
Cd5+O+1/2E	1.84	2.25	8.14	9.70	73.70	78.81
Probability (*P*)						
Cd	< 0.001		< 0.001		< 0.001	
Cultivar	< 0.001		< 0.001		0.047	
Chelator	< 0.001		< 0.001		< 0.001	
Cd × chelator	0.073		0.072		0.007	
Cd × cultivar	0.001		0.009		0.610	
Chelator × cultivar	0.851		0.180		0.586	
Cd × chelator × cultivar	0.935		0.873		0.489	

Treatment	Cd accumulation in grain (μg/plant)		Cd accumulation in straw (μg/plant)		Cd accumulation in root (μg/plant)	
	IIyou527	Xiushui63	IIyou527	Xiushui63	IIyou527	Xiushui63
Cd0	0	0	0	0	0	0
Cd1	18.76	13.01	109.3	270.1	128.3	275.5
Cd1+E	9.38	9.44	71.7	154.7	87.8	151.0
Cd1+O	18.48	11.72	78.46	203.8	75.6	221.4
Cd1+O+1/2E	11.93	11.42	73.9	171.8	73.0	169.4
Cd5	52.16	26.87	247.1	371.6	608.5	967.7
Cd5+E	37.45	27.33	121.3	335.6	206.3	802.4
Cd5+O	48.64	43.20	190.3	395.9	464.4	734.9
Cd5+O+1/2E	44.71	33.30	185.6	392.9	421.3	778.3
Probability (*P*)						
Cd	< 0.001		< 0.001		< 0.001	
Cultivar	< 0.001		< 0.001		< 0.001	
Chelator	0.181		< 0.001		< 0.001	
Cd × chelator	0.288		0.162		0.001	
Cd × cultivar	< 0.001		< 0.001		< 0.001	
Chelator × cultivar	0.004		0.474		0.968	
Cd × chelator × cultivar	0.046		< 0.001		0.245	

The same as Table 1.

in the absence and presence of EDTA and organic acids. Straw Cd concentration respectively decreased by 17.3% to 26.6% at 1 mg/kg Cd by 5.2% to 31.0% at 5 mg/kg Cd for IIyou527, and respectively decreased by 10.2% to 38.2% at l mg/kg Cd and 5.3% to 29.9% at 5 mg/kg Cd for Xiushui63 in the presence of organic acids and EDTA. Straw Cd concentration, respectively was in the order of Cd contaminated soil > Cd+O > Cd+O+1/2E > Cd+E. The root Cd concentration respectively decreased by 23.4% to 32.8% at l mg/kg Cd and by 9.6% to 54.3% at 5 mg/kg Cd for IIyou527, and respectively decreased by 5.0% to 41.1% at l mg/kg Cd and by 17.2% to 32.0% at 5 mg/kg Cd for Xiushui63 in the presence of organic acids and EDTA. Cadmium concentration in the plant tissues was in the order of root > straw > grain.

3 Discussion

Cadmium is known to increase the production of H_2O_2 (Schützendübel et al., 2001; Kuo and Kao, 2004) and induce oxidative stress in plants (Chien et al., 2002; Schützendübel and Polle, 2002). The present study showed that the activities of SOD for both cultivars increased at low soil Cd levels (Table 1). The increase of SOD activity may contribute to Cd-induced phytotoxicity (Hou and Kao, 2007), and act as an adaptive response of plants exposed to heavy metals (Camp, 1996). However, we found that the SOD activity decreased at high soil Cd level, suggesting that the high soil Cd level might damage the antioxidant defense by reducing the activity of SOD in rice leaves. The decline of CAT and POD activities with increase in soil Cd levels occurred in both cultivars. It seems that antioxidant enzymes (such as, SOD, POD and CAT) may work in a cooperative way to minimise the oxidative stress. Optimal protection is only achieved at an appropriate balance between the enzymes (Cho and Seo, 2005; Bagnyukova et al., 2006; Guo et al., 2007). In this study, the activities of SOD, POD and CAT differed between rice cultivars after exposure to Cd, mostly consistent with previous studies (Cho and Sohn, 2004; Naser et al., 2008). With the addition of organic acids and EDTA, the activity of SOD generally increased compared to the Cd contaminated treatment. The organic acids – salicylic acid (SA) pretreatment significantly increased the SOD activity compared with the corresponding Cd treatment (Guo et al., 2007). In our study, a general decline of POD and CAT activities was found in both rice cultivars under organic acids and EDTA supply, supporting the view that optimal protection is only achieved at an appropriate balance between antioxidant enzymes. The inhibition on POD and CAT activity by SA and an increase in H_2O_2 level were also reported by Chen et al. (1993) and Guo et al. (2007).

Oxidative stress due to the existence of the toxic metals can be demonstrated by MDA content, which is considered to be a general indicator of lipid peroxidation (Chaoui et al., 1997). In the present study, significant increases in MDA content were observed in both cultivars after the plants were exposed to Cd (Table 1), indicating that Cd toxicity in detached rice leaves was linked to lipid peroxidation. After adding organic acids and EDTA, the MDA content generally decreased compared to the Cd contaminated treatment. This suggests that organic acids and EDTA can reduce Cd-induced oxidative stress due to the decline of bioavailability of Cd in soil and Cd uptake and accumulation by plants. It was reported that organic acids and EDTA were associated with decreasing heavy metal-induced membrane deterioration in rice (Mishra and Choudhuri, 1999; Guo et al., 2007). We found a lower content of MDA in IIyou527 than in Xiushui63 under the same Cd treatment. Indicating that lipid peroxidation was higher in Xiushui63 than that in IIyou527, possibly due to a higher uptake of Cd in Xiushui63 than in IIyou527. Proline decline (Fig. 1) and protein loss (Fig. 2) with increased Cd levels in the Cd-treated rice leaves also suggested a strong induction of oxidative stress. The content of proline and protein of both cultivars increased after adding organic acids and EDTA due to decreased Cd-induced oxidative stress and toxicity in rice leaves. Organic acids, and organic acids + 1/2 EDTA treatments had higher yields in both cultivars than EDTA treatments due to the harmful side effects of EDTA on plant growth and yield, as previously described by Robinson et al. (2000).

Cadmium concentrations of grain, straw and root of both cultivars significantly increased with the increase in Cd levels (Table 2). At 5 mg/kg Cd, Cd concentrations in grain were 2.21 mg/kg in IIyou527 and 2.77 mg/kg in Xiushui63, which are far higher than the FAO/WHO quality requirements ($\leqslant$ 0.40 mg/kg Cd). As we known, highly polluted soils containing over 100 mg/kg Cd have been reported in China, France and some other countries (Alloway and Steinnes, 1999; Kabata-Pendias and Pendias, 2001; Wang et al., 2001). The Cd concentration of grain in those areas may be higher than that in our experiment and pose a threat to human and animal health in terms of food chain contamination. Cadmium contents of straw and grains were higher in Xiushui63 than that in IIyou527 fot all treatments, probably due to higher uptake and translocation of Cd from roots to straw and grains in Xiushui63 comparing to in IIyou527. The mechanisms controlling these processes may be related to genotypic tolerance to heavy metals. Genotypic variations of Cd tolerance in rice have been reported by Wu et al. (1999) and Liu et al. (2005).

Some studies have reported that Cd enters grain in two ways: firstly, Cd is absorbed by roots and transferred from roots to shoot, and secondly, Cd can directly transfer from roots to grains at the period of anthesis. Only 30% of Cd in grains came from the accumulation before booting stage, whereas close to 70% of Cd in grains came from the accumulation after booting stage (Zhang et al., 1999). In this study, Cd concentrations of grain, straw and roots in both rice cultivars decreased after adding organic acids and EDTA, probably because organic acids and EDTA decreased the solubility and bioavailability of Cd in the soil and the potential for plant uptake (Jiang et al., 2003). However, Zhang et al. (1999) thought that the decline of Cd in grain after adding organic acids and EDTA was caused by the inhibition of root growth. The Cd contents of straw

and roots for both cultivars were in the order of EDTA > organic acids + 1/2EDTA > organic acids, whereas the Cd content of grain was in the order of organic acids > organic acids + 1/2EDTA > EDTA. These results suggest that EDTA can inhibit Cd translocation from straw and roots to grain, but organic acids can decrease Cd contents in straw and roots only. The treatment of organic acids + 1/2EDTA not only decreased Cd translocation from straw and roots to grain, but also decreased Cd contents of straw and roots. Significant difference in Cd contents and accumulations were observed in the two cultivars in all treatments.

4 Conclusions

Addition of organic acids and EDTA to Cd contaminated soil generally increased the activity of SOD, but decreased the activities of POD and CAT, compared to the Cd-only treatment. The content of MDA was also decreased in the presence of organic acids and EDTA. Comparing two cultivars, the content of MDA was lower in IIyou527 than that in Xiushui63 under all the treatments. The content of proline and protein in both cultivars increased in the presence of organic acids and EDTA. Cadmium contents of grain, straw and roots of both cultivars significantly increased with the increase of Cd levels. Adding organic acids and EDTA may decrease Cd concentrations of grain, straw and roots in both rice cultivars.

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