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ORIGINAL PAPER

Cadmium-induced oxidative damage in rice leaves is reduced by polyamines

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Abstract The protective effect of polyamines against Cd toxicity of rice (*Oryza sativa*) leaves was investigated. Cd toxicity to rice leaves was determined by the decrease in protein content. $CdCl_2$ treatment results in (1) increased Cd content, (2) induction of Cd toxicity, (3) increase in H_2O_2 and malondialdehyde (MDA) contents, (4) decrease in ascorbic acid (ASC) and reduced glutathione (GSH) contents, and (5) increase in the activities of antioxidative enzymes (superoxide dismutase, glutathione reductase, ascorbate peroxidase, catalase, and peroxidase). Spermidine (Spd) and spermine (Spm), but not putrescine (Put), were effective in reducing $CdCl_2$-induced toxicity. Spd and Spm prevented $CdCl_2$-induced increase in the contents of H_2O_2 and MDA, decrease in the contents of ASC and GSH, and increase in the activities of antioxidative enzymes. Spd and Spm pretreatments resulted in a decrease in Cd content when compared with H_2O pretreatment, indicating that Spd and Spm may reduce the uptake of Cd. Results of the present study suggest that Spd and Spm are able to protect Cd-induced oxidative damage and this protection is most likely related to the avoidance of H_2O_2 generation and the reduction of Cd uptake.

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Keywords Cadmium · Oxidative stress · Putrescine · Rice · Spermidine · Spermine

Abbreviations

APX	Ascorbate peroxidase
ASC	Ascorbic aicd
CAT	Catalase
DAB	3,3′-Diaminobenzidine
DHA	Dehydroascorbate
DW	Dry weight
FW	Fresh weight
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
POX	Peroxidase
Put	Putrescine
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Spd	Spermidine
Spm	Spermine

Introduction

Cadmium (Cd), a heavy metal toxic to humans, animals, and plants, is a widespread pollutant with a long biological half-life (Wagner 1993). Cd is readily taken up by plants, leading to toxic symptoms such as growth reduction (Chen and

Kao 1995). Cd damages the photosynthetic apparatus (Krupa 1988; Siedlecka and Baszynski 1993), lowers chlorophyll (Stobart et al. 1985; Larsson et al. 1998), and alters proline and polyamine contents (Sharma and Dietz 2006).

Oxygen is essential for the existence of aerobic life, but toxic reactive oxygen species (ROS), which include the superoxide anion O_2^-, hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2), are generated in all aerobic cells during metabolic processes (Asada 1999; Foyer et al. 1994, 1997). Initially, ROS were only regarded as damaging to cells (Apel and Hirt 2004). More recently, ROS emerged as ubiquitous signaling molecules participating in the recognition of and the response to stress factors (Foyer and Noctor 2005).

Injury caused by these ROS, known as oxidative stress, is one of the major damaging factors in plants exposed to environmental stress. Plants cope with oxidative stress by using antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), catalase (CAT), and the low molecular weight antioxidants, ascorbic acid (ASC) and glutathione (GSH) (Asada 1999; Noctor and Foyer 1998). Three lines of evidence indicate that one mechanism of Cd toxicity is related to oxidative stress in plant cells. First, Cd can promote the generation of ROS (Kuo and Kao 2004; Olmos et al. 2003; Piqueras et al. 1999; Romero-Puertas et al. 2003, 2004; Sandalio et al. 2001; Schützendübel et al. 2001; Shah et al. 2001). Second, Cd can inhibit or stimulate the activities of antioxidant enzymes (Chaoui et al. 1997; Dixit et al. 2001; Gallego et al. 1996; Innelli et al. 2002; Kuo and Kao 2004; León et al. 2002; Shah et al. 2001; Shaw 1995). Third, treatment with Cd results in cellular oxidative damage or lipid peroxidation (Chaoui et al. 1997; Chien et al. 2002; Dixit et al. 2001; Gallego et al. 1996; Kuo and Kao 2004; Lozano-Rodríguez et al. 1997; Shah et al. 2001; Shaw 1995).

The polyamines putrescine (Put), spermidine (Spd), and spermine (Spm) are polycationic cellular molecules and are present in all living organisms. Experimental evidence now indicates that polyamines are involved in a number of cellular and molecular processes in plants (Bouchereau et al. 1999; Wallace et al. 2003). The levels of polyamines in plants are altered in response to heavy metals (Sharma and Dietz 2006). Weinstein et al. (1986) showed an up to 10-fold increase in Put content with a marginal rise in Spd and Spm contents in Cd-treated oat seedlings and detached oat leaves. Similar results were obtained in Cd-treated detached rice leaves (Hou and Kao 1993). It has been shown that polyamines are able to protect against oxidative damage caused by paraquat (Benavides et al. 2000; Chang and Kao 1997; Kurepa et al. 1998; Minton et al. 1990), acid rain (Velikova et al. 2000) and heavy metals such as Cd and Cu (Groppa et al. 2001). Borrell et al. (1997) demonstrated that polyamines inhibited lipid peroxidation in senescing oat leaves. Evidence has been provided to show that polyamines are effective radical scavengers in a number of chemical and in vitro enzyme systems (Drolet et al. 1986) and that the reduction in polyamine content in leaves of *Glycyrrhiza inflata* under osmotic stress promoted the increase in the production of ROS (Li and Wang 2004). A close interrelationship between polyamines and oxidative stress was documented by the finding that leaf necrosis caused by ozone in tomato plants could be suppressed by an exogenous supply of polyamines (Ormrod and Beckerson 1986). However, Bors et al. (1989) claimed that the scavenging of radicals by polyamines cannot explain the protection against ozone damage observed after exogenous application. Recently, Tang et al. (2004) demonstrated that exogenously added polyamines recover browning tissues into normal callus cultures of Virginia pine by decreasing oxidative damage. In the present study, we investigated the effect of polyamines on Cd toxicity of rice leaves, and we observed that oxidative damage caused by $CdCl_2$ is reduced by Spd and Spm.

Materials and methods

Plant material

Rice (*Oryza sativa* L., cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively seeds with

distilled water. These seeds were then germinated in Petri dishes with wetted filter paper at 37°C under dark conditions. After 48 h incubation, uniformly germinated seeds were selected and cultivated in a 500 ml beaker containing half-strength Kimura B solution as described previously (Hsu and Kao 2005). The hydroponically cultivated seedlings were grown for 12 days in a Phytotron (Agricultural Experimental station, National Taiwan University, Taipei, Taiwan) with natural sunlight at 30°C day/25°C night and 90% relative humidity. The apical 3 cm of the third leaf was used in all experiments. Detached rice leaves were pretreated with distilled water or polyamines for 6 h at 27°C in darkness and then transferred to distilled water or 5 mM $CdCl_2$ for 4, 8, 12, and 18 h at 27°C in the light (40 μmol m^{-2} s^{-1}).

Determination of protein, H_2O_2, lipid peroxidation, GSH, oxidized glutathione (GSSG), ASC, dehydroascorbate (DHA), and Cd

For protein determination, leaf segments were homogenized in a 50 mM sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17,600 × *g* for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976) and antioxidative enzyme activities. The H_2O_2 content was measured colorimetrically as described by Jana and Choudhuri (1982). H_2O_2 was extracted by homogenizing leaf tissue with phosphate buffer (50 mM, pH 6.5) containing 1 mM hydroxylamine. The homogenate was centrifuged at 6,000 × *g* for 25 min. To determine H_2O_2 content, the extracted solution was mixed with 0.1% titanium chloride in 20% (v/v) H_2SO_4. The mixture was then centrifuged at 6,000 × *g* for 15 min. The absorbance was measured at 410 nm. Using this method, we obtained that absorbance increased linearly with the amount of H_2O_2 and addition of H_2O_2 to leaf extracts resulted in the predicted increase of absorbance, i.e. added H_2O_2 was fully recovered (data not shown). The H_2O_2 content in leaf extracts was calculated using the extinction coefficient of 0.28 μmol^{-1} cm^{-1}. In some experiments, H_2O_2 was also visually detected in the leaves by using 3,3-diaminobenzidine (DAB) as substrate (Orozco-Cárdenas and Ryan 1999). Detached rice leaves were supplied through the cut ends with DAB (1 mg ml^{-1}) solution for 24 h under light at 27°C. Leaves were them decolorized in boiling ethanol (95%) for 0.5 h. This treatment decolorized the leaves except for the brown polymerization product produced by DAB with H_2O_2. After cooling, the leaves were extracted at room temperature with fresh ethanol. The H_2O_2 staining was repeated four times with similar results.

MDA, routinely used as an indicator of lipid peroxidation, was extracted with 5% (w/v) trichloroacetic acid and determined by the thiobarbituric acid reaction as described by Heath and Packer (1968). GSH and GSSG in 3% sulfosalicylic acid extract and ASC and DHA in 5% (w/v) trichloracetic acid extract were determined as described previously (Hsu and Kao 2005). For determination of Cd, leaves were dried at 65°C for 48 h and the dried material ashed at 550°C for 4 days. The ash residue was incubated with 31% HNO_3 and 17.5% H_2O_2 at 72°C for 2 h, and dissolved in distilled water. Cd was then quantified using an atomic absorption spectrophotometer (Model AA-6800, Shimadzu, Kyoto, Japan).

Polyamine determination

Leaf tissues were homogenized with 5 ml of 5% (w/v) perchloric acid. Polyamine contents were determined using high performance liquid chromatography (Waters 484, Milford, USA) after benzoylation as described previously (Chen and Kao 1991).

Enzyme extraction and assays

For extraction of enzymes, leaf tissues were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. For analysis of APX activity, 2 mM ASC was added to the extraction buffer. The homogenate was centrifuged at 12,000 × *g* for 20 min and the resulting supernatant was used for determination of enzyme activity. The whole extraction procedure was carried out at 4°C. SOD was determined according to Paoletti et al. (1986). One unit of SOD was defined as the amount of enzyme that

inhibits by 50% the rate of NADH oxidation observed in blank sample. POX activity was measured using a modification of the procedure of MacAdam et al. (1992). The activity was calculated using the extinction coefficient (26.2 mM^{-1} cm^{-1} at 470 nm) for tetraguaiacol. One unit of POX was defined as the amount of enzyme that caused the formation of 1 μmol tetraguaiacol per min. CAT activity was assayed according to Kato and Shimizu (1987). One unit of CAT was defined as the amount of enzyme which degraded 1 μmol H_2O_2 per min. APX activity was determined according to Nakano and Asda (1981). One unit of activity for APX was defined as the amount of enzyme that degraded 1 μmol of ASC per min. GR was determined by the method of Foster and Hess (1980). One unit of GR was defined as the amount of enzyme that decreased 1 A_{340} per min.

Statistical analysis

Statistical differences between measurements ($n = 4$) on different treatments or on different times were analyzed following the Duncan's multiple range test or Student's t-test.

Results

Cd promotes protein loss

In plants, the most general symptom of Cd toxicity is chlorosis (Das et al. 1997). In rice, we have shown that detached leaves and seedlings treated with $CdCl_2$ show chlorosis and protein loss (Chien and Kao 2000; Hsu and Kao 2003, 2005). In the present study, Cd toxicity in detached rice leaves caused by excess Cd was assessed by a decrease in protein content. Increasing concentration of $CdCl_2$ from 0.1 to 5 mM progressively decreased protein content in detached rice leaves in the light and no further decrease was observed at 10 mM $CdCl_2$ (data not shown). Thus, 5 mM $CdCl_2$ was used in the present investigation. The promotion of the loss of protein by $CdCl_2$ was evident 8 h after treatment (Fig. 1A). Cd concentration in the control leaves remained unchanged during 18 h of incubation (Fig. 1C). However, Cd concentration in $CdCl_2$-treated leaves increased with increasing duration of incubation (Fig. 1C). The increase in Cd concentration in $CdCl_2$-treated leaves was evident 4 h after treatment (Fig. 1C).

Cd induces oxidative stress

MDA content in $CdCl_2$-treated detached rice leaves was observed to be greater than that in water-treated controls at 8 h after treatment (Fig. 1B). This showed that Cd toxicity in detached rice leaves was linked to lipid peroxidation. Lipid peroxidation is caused by ROS (Thompson et al. 1987). $CdCl_2$ treatment also

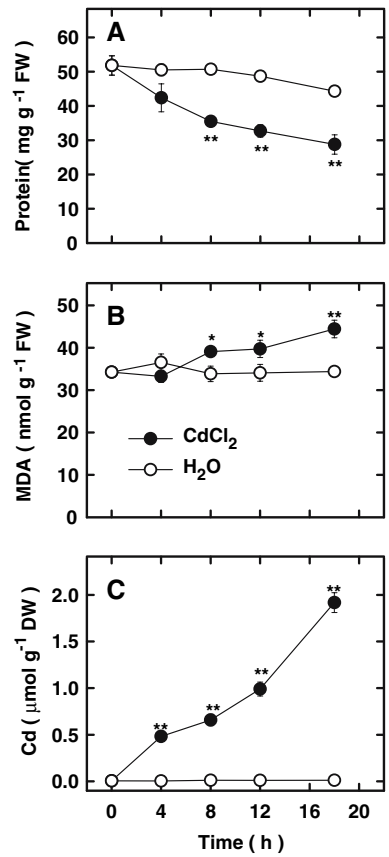

Fig. 1 Changes in the contents of protein (A), MDA (B), and Cd (C) in rice leaves treated with $CdCl_2$. Detached rice leaves were pretreated with H_2O for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 4, 8, 12, and 18 h in the light. * and ** represent values that are significantly different between H_2O and $CdCl_2$ treatment at $P < 0.05$ and $P < 0.01$, respectively

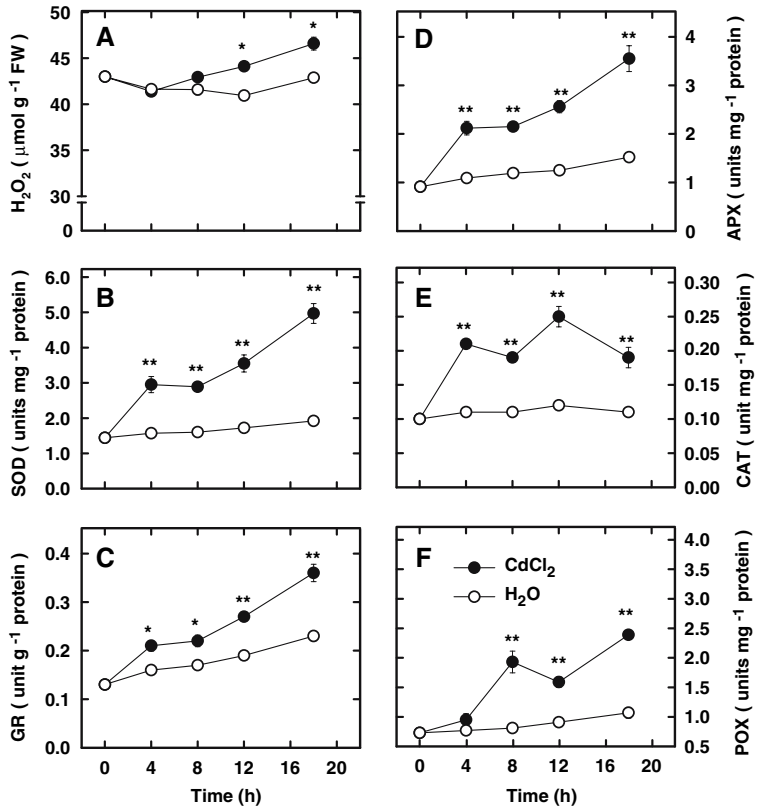

Fig. 2 Changes in the contents of H_2O_2 (A) and the activities of SOD (B), GR (C), APX (D), CAT (E), and POX (F) in rice leaves treated with $CdCl_2$. Detached rice leaves were pretreated with H_2O for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 4, 8, 12, and 18 h in the light. * and ** represent values that are significantly different between H_2O and $CdCl_2$ treatment at $P < 0.05$ and $P < 0.01$, respectively

caused an increase in H_2O_2 content (Fig. 2A). To verify in situ the increase in H_2O_2 in leaves treated with $CdCl_2$, a histochemical method with DAB that is based on the formation by H_2O_2 of brown polymerization product was used. The development of DAB-H_2O_2 reaction product in H_2O- and $CdCl_2$-treated leaves is shown in Fig. 3. It is clear that the DAB-H_2O_2 reaction product was observed after H_2O_2 and $CdCl_2$ treatments. All these results support the involvement of ROS as the chemical species inducing Cd toxicity in rice leaves.

$CdCl_2$-treated rice leaves had higher activities of SOD, GR, APX, and CAT than the controls at 4 h after treatment (Figs. 2B–E). Higher activities of POX were observed at 8 h after treatment (Fig. 2F). GSH, GSSG, and ASC contents were observed to be lower than the controls at 4 h after treatment (Figs. 4A–C). However, DHA content in Cd-treated leaves was observed to be higher than the contents at 18 h after treatment (Fig. 4D). The increased activities of antioxidative enzymes

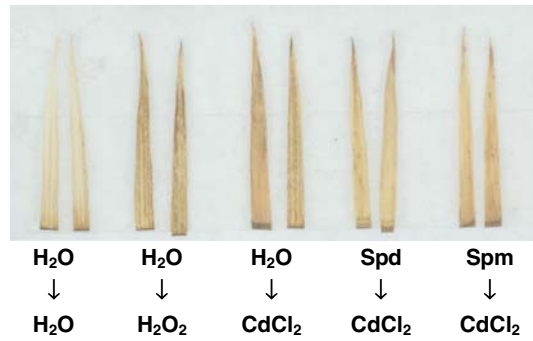

Fig. 3 Histochemical detection of H_2O_2 with DAB staining in rice leaves. Detached leaves were pretreated with H_2O, Spd, and Spm, respectively, for 6 h in the dark, and then treated with either H_2O, H_2O_2, or $CdCl_2$ for 18 h in the light. The concentrations of Spd, Spm, H_2O_2, and $CdCl_2$ were 5, 5, 1, and 5 mM, respectively

and the decreased contents of ASC and GSH in response to $CdCl_2$ are further suggestive of strong induction of oxidative stress.

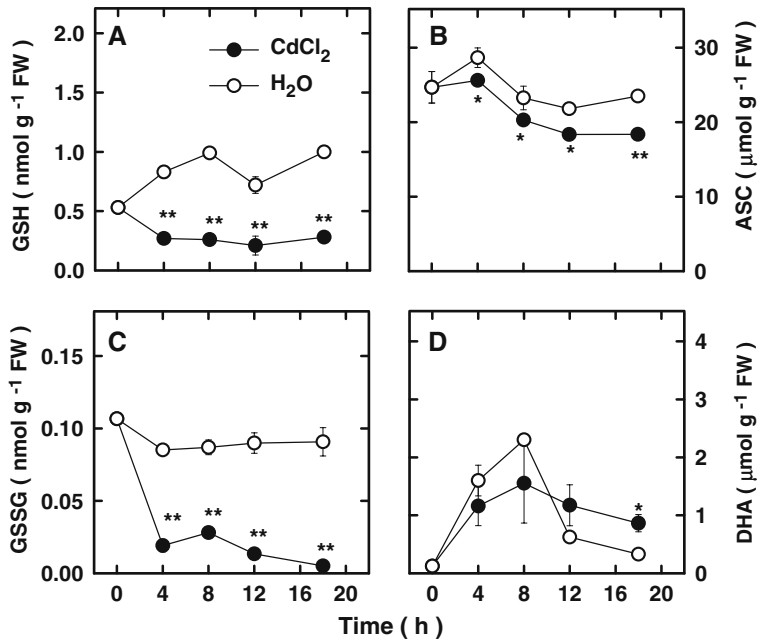

Fig. 4 Changes in contents of GSH (A) and ASC (B) in rice leaves treated with $CdCl_2$. Detached rice leaves were pretreated with H_2O for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 4, 8, 12, and 18 h in the light. * and ** represent values that are significantly different between H_2O and $CdCl_2$ treatment at $P < 0.05$ and $P < 0.01$, respectively

Spd and Spm reduce Cd-induced oxidative damage

To test if polyamines could reduce the toxicity caused by $CdCl_2$, as judged by the changes in protein levels, detached rice leaves were pretreated with either water or polyamines for 6 h in the dark and then transferred to either water or $CdCl_2$ for 18 h in the light. Spd and Spm, but not Put, pretreatments reduced Cd toxicity (Fig. 5). We also observed that Spd and Spm were effective in reducing Cd-induced lipid peroxidation (Fig. 6A) and H_2O_2 production (Figs. 3, 6B), Cd-increased antioxidative enzyme activities (Fig. 7), and Cd-decreased ASC and GSH contents (Fig. 8). Furthermore, detached rice leaves pretreated with Spd or Spm for 6 h in the dark had higher endogenous levels of Spd and Spm, and Spm, respectively, than those pretreated with water (Table 1). However, Put pretreatment had no effect on endogenous levels of Spd and Spm (Table 1).

Spd and Spm inhibit the uptake of Cd

To test if endogenous Spd and Spm affect Cd uptake, Cd content in detached rice leaves pretreated with Spd or Spm followed by treatment of $CdCl_2$ was determined. It was observed that Spd and Spm pretreatments resulted in a decrease (about 27%) in Cd content when compared with H_2O pretreatment (Fig. 9).

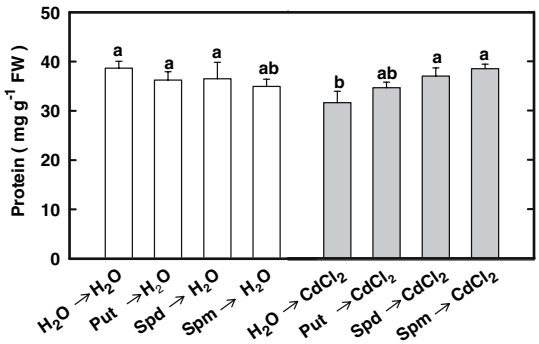

Fig. 5 Effect of pretreatments with polyamines on the content of protein in detached rice leaves in the presence or absence of $CdCl_2$. Detached rice leaves were pretreated with H_2O, 5 mM Put, 5 mM Spd, and 5 mM Spm, respectively, for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 18 h in the light. Values with the same letter are not significantly different at $P < 0.05$

Discussion

It has been shown that Cd increased ethylene production in detached rice leaves (Hou and Kao 1993). Here, we show that Cd induced H_2O_2

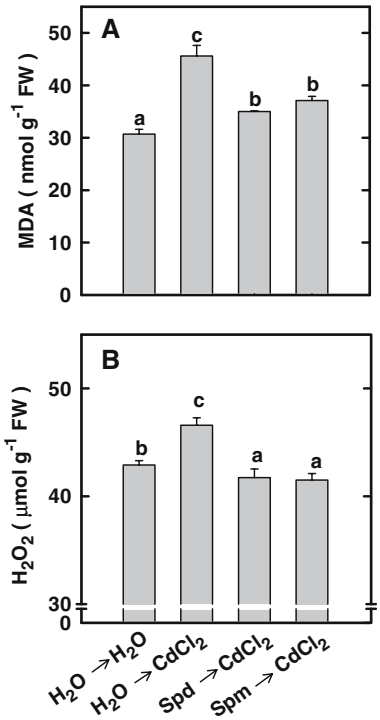

Fig. 6 Effect of pretreatments with Spd and Spm on the contents of MDA (A) and H_2O_2 (B) in detached rice leaves in the presence or absence of $CdCl_2$. Detached rice leaves were pretreated with H_2O, 5 mM Spd, and 5 mM Spm, respectively, for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 18 h in the light. Values with the same letter are not significantly different at $P < 0.05$

production in rice leaves (Fig. 2A, 3). Wounding is known to induce ethylene production (Yu and Yang 1980) and H_2O_2 generation (Orozco-Cárdenas et al. 2001). Ethylene biosynthesis shares a common precursor with Spd and Spm, thus wounding may induce a direct modification of the synthesis of Spd and Spm. When detached rice leaves are used to study H_2O_2, ethylene, polyamines, and senescence, wounding is always a problem. However, in the present study, each long and narrow rice leaf was cut transversely; thus, the area of wounding was very small. Therefore, H_2O_2 and ethylene production of detached leaves induced by Cd is unlikely to be complicated by the wounding effect.

Cd is known to increase the production of H_2O_2 (Kuo and Kao 2004; Schützendübel et al. 2001; Olmos et al. 2003) and induce lipid peroxidation (Chien et al. 2002; Gallego et al. 1996; Kuo and Kao 2004). These results suggest that Cd treatment causes an oxidative stress in plants. Our results not only have shown that $CdCl_2$ increased the content of H_2O_2 (Figs. 2A, 3) and the activities of SOD, APX, GR, CAT, and POX (Figs. 2B–F), but also demonstrated that caused a decrease in GSH and ASC contents (Fig. 4). Meanwhile, protein loss (Fig. 1A) and lipid peroxidation (Fig. 1B) were observed in $CdCl_2$-treated rice leaves. All these results suggest that $CdCl_2$ causes an oxidative stress and that $CdCl_2$-induced toxicity in rice leaves is mediated through oxidative stress.

GSH functions as a direct antioxidant of ROS and is involved in the generation of ASC, which is utilized as a substrate for APX (Noctor and Foyer 1998). In the present study, we observed that the decrease in GSH content is one of the earliest steps in oxidative stress induced by $CdCl_2$ in rice leaves, which occurred at 4 h after treatment (Fig. 4B). It may be suspected that the decrease in GSH may favor the accumulation of ROS in Cd-treated rice leaves. In a review, Schützendübel and Polle (2002) also suggest that the depletion of GSH is apparently a critical step in Cd toxicity.

Cd induced a significant accumulation of H_2O_2 in rice leaves (Figs. 2A, 3). Accumulation of H_2O_2 has also been observed in Cd-treated pine and pea roots, pea leaves, and tobacco cells (Olmos et al. 2003; Romero-Puertas et al. 2003; 2004; Schützendübel et al. 2001). There are reports showing that NADPH oxidase was possibly involved in Cd-induced H_2O_2 production in pea leaves and tobacco cells (Olmos et al. 2003; Romero-Puertas et al. 2004). Our unpublished observations indicate that diphenyleneiodonium chloride and imidazole, inhibitors of NADPH oxidase, prevented Cd-induced H_2O_2 production in rice leaves.

Data from the present study indicate that Cd-induced oxidative damage in rice leaves is reduced by Spd and Spm. This conclusion is based on the observations that pretreatment with Spd and Spm prevented Cd-induced loss of protein (Fig. 5), increase in the contents of MDA (Fig. 6) and H_2O_2 (Figs. 3, 6B), decrease in the content of ASC and GSH (Fig. 8), and increase in the

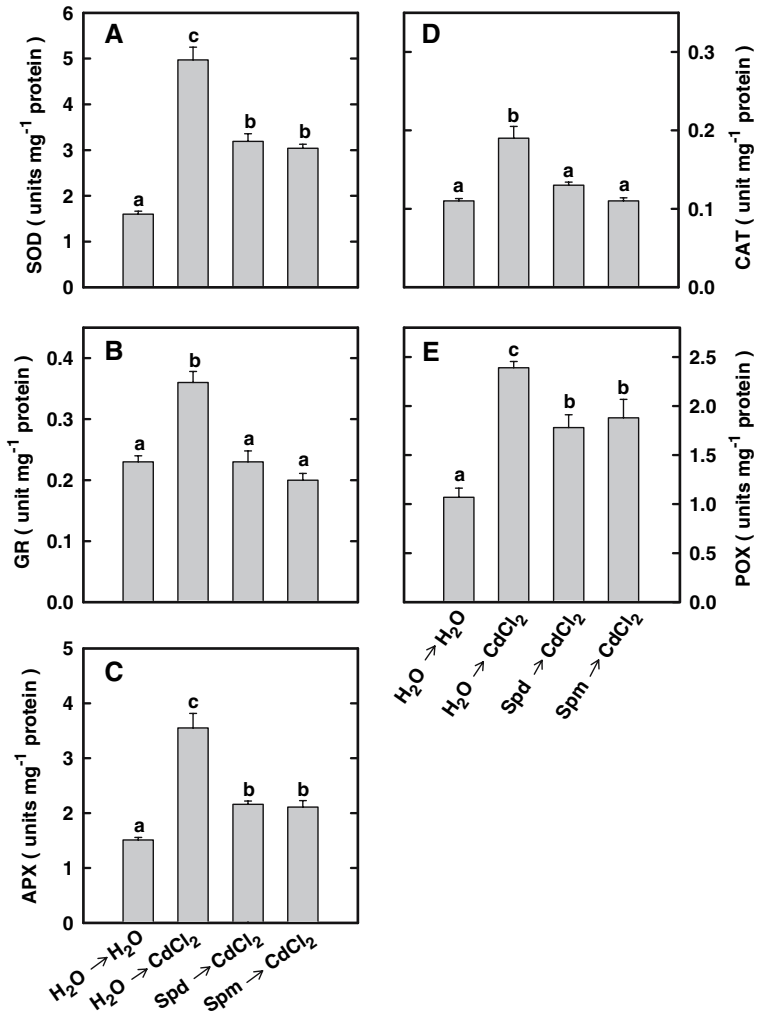

Fig. 7 Effect of pretreatments with Spd and Spm on the activities of antioxidative enzymes [SOD (A), GR (B), APX (C), CAT (D), and POX (E) in detached rice leaves in the presence or absence of $CdCl_2$. Detached rice leaves were pretreated with H_2O, 5 mM Spd, and 5 mM Spm, respectively, for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 18 h in the light. Values with the same letter are not significantly different at $P < 0.05$

activities of antioxidative enzymes (Fig. 7). Groppa et al. (2001) also demonstrated that Spm and Spd were effective in reducing Cd-caused lipid peroxidation in sunflower leaf discs. It is generally accepted that polyamines are highly protonated at physiological pH, which favors electrostatic binding of polyamines to negatively charged components of membranes, leading to membrane stabilization through ionic interactions (Slocum et al. 1984). The more pronounced protective effect of Spd and Spm could be accounted for by its longer chain and greater number of positive charges, which allows membrane stabilizing ability.

The decrease in ASC and GSH contents in rice leaves treated with $CdCl_2$ suggests that ASC and GSH contents may be regulated by the synthesis and oxidation. GSH is the precursor of phytochelatins, cysteine-rich peptides, synthesized via phytochelatin synthase (Cobbett and Goldsbrough 2002). A severe depletion of GSH is a common response to Cd caused by an increased consumption of GSH for phytochelatin production (Schützendübel and Polle 2002). Thus, the sharp decline in GSH content in $CdCl_2$-treated rice leaves may also be due to phytochelatin biosynthesis.

The present results indicated that Spd and Spm reduced Cd-decreased ASC and GSH contents (Fig. 8). Theses observations suggest that the capacity of Spd and Spm to scavenge H_2O_2 might

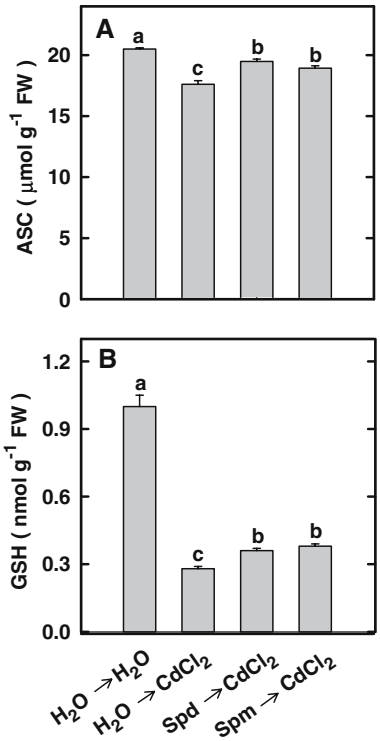

Fig. 8 Effect of pretreatments with Spd and Spm on the contents of ASC (A) and GSH (B) in detached rice leaves in the presence or absence of $CdCl_2$. Detached rice leaves were pretreated with H_2O, 5 mM Spd, and 5 mM Spm, respectively, for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 18 h in the light. Values with the same letter are not significantly different at $P < 0.05$

Table 1 Effect of polyamines on the contents of endogenous polyamines in detached rice leaves

Treatment	Put (nmol g^{-1} FW)	Spd (nmol g^{-1} FW)	Spm (nmol g^{-1} FW)
H_2O	182.9 ± 9.6^b	121.83 ± 6.6^b	89.9 ± 8.9^c
Put	222.8 ± 3.0^a	142.8 ± 7.0^b	74.1 ± 2.2^c
Spd	149.9 ± 5.8^c	168.91 ± 5.5^a	184.1 ± 15.1^b
Spm	145.9 ± 13.7^c	136.38 ± 11.5^b	341.1 ± 33.9^a

Detached rice leaves were treated with either water, Put, Spd, or Spm (5 mM) for 6 h in dark. Values with the same letter in each column are not significantly different at $P < 0.05$

increase in rice leaves that were pretreated with Spd or Spm followed by treatment of $CdCl_2$ (Fig. 6B).

In considering a possible mechanism for the reduction of Cd-induced oxidative damage by

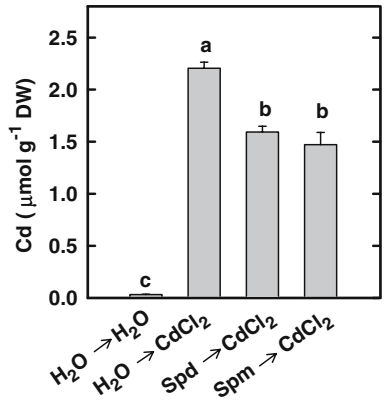

Fig. 9 Effect of pretreatments with Spd and Spm on the content of Cd in detached rice leaves in the presence or absence of $CdCl_2$. Detached rice leaves were pretreated with H_2O, 5 mM Spd, and 5 mM Spm, respectively, for 6 h in the dark and then treated with H_2O or 5 mM $CdCl_2$ for 18 h in the light. Values with the same letter are not significantly different at $P < 0.05$

polyamines, we speculated that Spd and Spm might inhibit Cd uptake from the medium. Here, we show that Cd content in detached rice leaves pretreated with Spd and Spm followed by treatment of $CdCl_2$ was lower that those pretreated with H_2O. Our findings suggest that increase in endogenous Spd or Spm may block, though slightly (27%), the uptake of Cd (Fig. 9). In the present study, pretreatment of detached rice leaves with exogenous Spd or Spm was found to reverse almost completely the Cd-induced H_2O_2 generation and lipid peroxidation (Fig. 6). It appears that Spd and Spm were able to protect Cd-induced oxidative damage and this protection was related to the avoidance of H_2O_2 generation and the reduction of Cd uptake.

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