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Abscisic acid accumulation and cadmium tolerance in rice seedlings

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Rice (*Oryza sativa* L.) seeds were soaked for 18 h in distilled water in the absence (–PBZ) or presence (+PBZ, a triazole) of 100 mg l^{-1} paclobutrazol and then air dried. These air-dried seeds were germinated in the dark and then cultivated in a Phytotron. Twelve-day-old –PBZ and +PBZ seedlings were treated or not with $CdCl_2$. Cd toxicity was judged by the decrease in biomass production, decrease in chlorophyll and protein content, increase in NH_4^+ content and induction of oxidative stress. The results indicated that PBZ applied to seeds was able to protect rice seedlings from Cd toxicity. On treatment with $CdCl_2$, the abscisic acid (ABA) content increased in +PBZ leaves, but not in –PBZ leaves. The decrease in the transpiration rate of –PBZ seedlings by $CdCl_2$ was less than that of +PBZ seedlings. Exogenous application of the ABA biosynthesis inhibitor, fluridone (Flu), reduced ABA accumulation, increased the transpiration rate and Cd content, and decreased the Cd tolerance of +PBZ seedlings. The effects of Flu on the Cd toxicity, transpiration rate and Cd content were reversed by the application of ABA. It seems that the PBZ-induced Cd tolerance of rice seedlings is mediated through an accumulation of ABA.

Introduction

Paclobutrazol [PBZ, (2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazolyl)-pentan-3-ol], a member of the triazole plant growth regulator group, is an inhibitor of gibberellin (GA) biosynthesis (Graebe 1987, Rademacher et al. 1987, Davis and Curry 1991) and a retardant of shoot growth (Cox and Keever 1988, Davis et al. 1988, Davis and Curry 1991). The primary mode of action of PBZ is inhibition of *ent*-kaurene oxidase to *ent*-kaurenoic acid in the early pathway of GA biosynthesis (Graebe 1987, Rademacher et al. 1987). PBZ has been used to provide plant protection against environmental stresses, such as chilling (Whitaker and Wang 1987, Lurie et al. 1994, Pinhero and Fletcher 1994, Pinhero et al. 1997), heat (Kraus and Fletcher 1994, Pinhero and Fletcher 1994, Kraus et al. 1995, Gilley and Fletcher 1998, Vettakkorumakankav et al. 1999), flooding (Webb and Fletcher 1996), salt (Abou El-Khashab et al. 1997) and gaseous sulphur dioxide (Lee et al. 1985).

Cadmium (Cd) is a heavy metal that is toxic to humans, animals and plants, and is a widespread pollutant with a long biological half-life (Wagner 1993). This metal enters the environment mainly from industrial processes and phosphate fertilizers and is transferred to animals and humans through the food chain (Wagner 1993). Taken up in excess by plants, Cd directly or indirectly inhibits physiological processes, such as

Abbreviations – ABA, abscisic acid; AOS, active oxygen species; APX, ascorbate peroxidase; ASC, ascorbate; CAT, catalase; d. wt., dry weight; ELISA, enzyme-linked immunosorbent assay; Flu, fluridone; f. wt., initial fresh weight; GA, gibberellin; GR, glutathione reductase; GS, glutamine synthetase; GSH, reduced glutathione; MDA, malondialdehyde; PAL, phenylalanine ammonia-lyase; PBZ, paclobutrazol; POX, peroxidase; SOD, superoxide dismutase; TN1, Taichung Native 1.

respiration, photosynthesis, cell elongation, plant–water relationships, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (Sanitá di Toppi and Gabbrielli 1999).

In Taiwan, inappropriate disposal of industrial waste has given rise to widespread Cd contamination of irrigated water (higher than 10 mg l^{-1}). Thus, there is an urgent need to study the mechanism of Cd tolerance of rice plants.

It has been demonstrated that uniconazole, another potent member of the triazole family, induces Cd tolerance in wheat (Singh 1993). However, this is the only report describing the protective effect of triazole against Cd toxicity. Here, we show that PBZ protects rice seedlings from Cd toxicity, confirming the ability of PBZ to induce stress tolerance in plants.

In higher plants, abscisic acid (ABA) is a well-known stress hormone leading to the induction of various protective reactions, which are adaptations for coping with abiotic environmental stresses, such as ozone (Lin et al. 2001), freezing (Guy 1990), chilling (Lee et al. 1993), drought (Zeevaart and Creelman 1988) and salt (La Rosa et al. 1987). Recently, a role of ABA in the Cd tolerance of rice seedlings has been demonstrated (Hsu and Kao 2003a, 2003b, Kuo and Kao 2004). We found that rice seedlings of cultivar Tainung 67 were more tolerant to Cd than were those of cultivar Taichung Native 1 (TN1), and the increase in endogenous ABA content was closely related to the Cd tolerance of rice seedlings (Hsu and Kao 2003a). It is not known whether PBZ-induced Cd tolerance of rice seedlings is mediated through ABA accumulation. Thus, we also investigated the role of ABA in the PBZ-induced Cd tolerance of TN1 rice seedlings.

Materials and methods

Plant material and treatment

Rice (*Oryza sativa* L., cv. TN1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were soaked for 18 h at room temperature in 100 mg l^{-1} PBZ or in distilled water (–PBZ), as described by Fletcher and Hosfsta (1990), and then were air dried for 5 days. These air-dried seeds were then germinated in Petri dishes with wet filter papers at 37°C in the dark. After 48 h of incubation, uniformly germinated seeds were selected and cultivated in a 250 ml beaker containing half-strength Kimura B solution including the following macro- and microelements: 182.3 μ*M* $(NH_4)_2SO_4$, 91.6 μ*M* KNO_3, 273.9 μ*M* $MgSO_4{\cdot}7H_2O$, 91.1 μ*M* KH_2PO_4, 182.5 μ*M* $Ca(NO_3)_2$, 30.6 μ*M* Fe-citrate, 0.25 μ*M* H_3BO_3, 0.2 μ*M* $MnSO_4{\cdot}H_2O$, 0.2 μ*M* $ZnSO_4.7H_2O$, 0.05 μ*M* $CuSO_4{\cdot}5H_2O$ and 0.07 μ*M* H_2MoO_4 (Chu and Lee 1989). The hydroponically cultivated seedlings were grown 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. Twelve-day-old seedlings with three leaves were used in all experiments.

For Cd, ABA or fluridone (Flu) treatment, 12-day-old seedlings were grown in basic nutrient solution plus 1.5 m*M* $CdCl_2$, 5 μ*M* ABA or 0.2 m*M* Flu.

Growth analysis

At the end of treatment, the seedlings were divided into their separate parts (shoot, adventitious roots and primary roots). The length of the shoot and primary roots and the fresh weight (f. wt.) of the shoot and roots (adventitious roots plus primary roots) were then measured. For dry weight (d. wt.) estimation, the shoot and roots were dried at 65°C for 48 h.

Cd determination

For the determination of Cd, leaves were dried at 65°C for 48 h. Dried material was ashed at 550°C for 20 h. 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 spectrophotomer (Model AA-6800, Shimadzu, Kyoto, Japan). The amount of Cd was expressed on a d. wt. basis.

Determination of chlorophyll, protein, NH_4^+, malondialdehyde (MDA), ascorbate (ASC) and reduced glutathione (GSH)

The chlorophyll content was determined according to Wintermans and De Mots (1965) after extraction in 96% (v/v) ethanol. For protein determination, leaves were homogenized in a 50 m*M* sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17 600 *g* for 20 min, and the supernatants were used for determination by the method of Bradford (1976). NH_4^+ was measured in the crude extract by the Berthelot reaction, modified according to Weatherburn (1967). The detailed procedure has been described previously (Lin and Kao 1996). MDA, routinely used as an indicator of lipid peroxidation, was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer (1968). ASC in 5% (w/v) trichloroacetic acid

and GSH in 3% sulphosalicylic acid extract were determined as described by Laws et al. (1983) and Smith (1985), respectively.

Enzyme extraction and assays

For the extraction of enzymes, leaf tissues were homogenized with 0.1 *M* sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12 000 *g* for 20 min and the resulting supernatant was used for the determination of the enzyme activity. The whole extraction procedure was carried out at 4°C. Superoxide dismutase (SOD) was determined according to Paoletti et al. (1986). One unit of SOD was defined as the amount of enzyme that inhibited by 50% the rate of NADH oxidation observed in a blank sample. Peroxidase (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 definedas the amount of enzyme that caused the formation of 1 μmol tetraguaiacol per minute. Glutamine synthetase (GS) was assayed by the method of Oaks et al. (1980). One unit of GS was defined as the amount of enzyme that caused the formation of 1 μmol L-glutamate γ-monohydroxamate per minute. Phenylalanine ammonia-lyase (PAL) was extracted and determined according to Hyodo and Fujinami (1989). The activity was calculated using the extinction coefficient (9500 M^{-1} cm^{-1} at 290 nm) for *trans*-cinnamate. One unit of PAL was defined as the amount of enzyme that caused the formation of 1 μmol *trans*-cinnamate per hour.

ABA determination

For the extraction of ABA, leaves were homogenized with a pestle and mortar in extraction solution (80% methanol containing 2% glacial acetic acid). To remove plant pigments and other non-polar compounds which could interfere in the immunoassay, extracts were first passed through a polyvinylpyrrolidone column and C18 cartridges. The eluates were concentrated to dryness by vacuum evaporation and resuspended in Tris-buffered saline before enzyme-linked immunosorbent assay (ELISA). ABA was quantified by ELISA (Walker-Simmons 1987). The ABA immunoassay detection kit (PGR-1) was purchased from Sigma Chemical Co. (St. Louis, MO) and was specific for (+)-ABA. By evaluating ^{3}H-ABA recovery, ABA loss was less than 3% by the method described here.

Transpiration rate

The transpiration rate was measured according to Greger and Johansson (1992). The transpiration rate was calculated from the water loss during each interval and converted to a per day per seedling basis.

Expression of data and statistical analysis

In the present study, the third leaves of rice seedlings were used to determine chlorophyll, protein, NH_4^+, MDA, ASC, GSH and ABA. As the f. wt. of –PBZ leaves was no different from that of +PBZ leaves, data were expressed on the basis of initial fresh weight (f. wt.). Statistical differences between measurements ($n = 4$) for different treatments or for different times were analysed following the LSD test.

Results

Growth analysis

PBZ-treated rice seedlings exhibited typical characteristics of triazole treatment, such as reduced shoot length and f. wt. and enhanced primary root length (Table 1; Pinhero and Fletcher 1994). Shoot d. wt., root d. wt. and root f. wt. of PBZ-treated rice seedlings were not significantly different from those of the controls (–PBZ) (Table 1). Less adventitious roots were visually observed in +PBZ than in –PBZ seedlings. Both adventitious roots and primary roots were used to determine the f. wt. and d. wt. of roots. This may explain why the d. wt. and f. wt. of +PBZ roots were not significantly different from those of –PBZ roots.

Evaluation of Cd toxicity

Biomass production

Cd is readily taken up by rice seedlings, leading to growth reduction (Chen and Kao 1995). Thus, in the

Table 1. Growth analysis of rice seedlings 12 days after planting. Seeds were soaked for 18 h in water (–PBZ) or PBZ (+PBZ) and dried. The seedlings were cultivated for 12 days in a Phytotron with natural sunlight at 30°C (day)/25°C (night) and 90% relative humidity. Significant difference at $^{a}P < 0.01$ and $^{b}P < 0.05$, respectively. PBZ, paclobutrazol.

Parameter	–PBZ	+PBZ
Shoot length (cm)	13.7 ± 0.3	9.4 ± 0.1[a]
Root length (cm)	7.9 ± 0.2	13.2 ± 0.3[a]
Shoot fresh weight (mg seedling^{-1})	53.6 ± 3.1	44.0 ± 1.7[b]
Shoot dry weight (mg seedling^{-1})	12.7 ± 0.2	12.9 ± 0.1
Root fresh weight (mg seedling^{-1})	47.1 ± 3.9	40.5 ± 3.2
Root dry weight (mg seedling^{-1})	6.1 ± 0.4	6.7 ± 0.2

present study, Cd toxicity was first evaluated by biomass production (shoot and root d. wt.). The effect of $CdCl_2$ concentration on shoot and root d. wt. of rice seedlings is presented in Fig. 1. In –PBZ seedlings, the shoot d. wt. was decreased by 0.5 m*M* $CdCl_2$ and no further decrease was observed at 1 and 1.5 m*M* (Fig. 1A). However, $CdCl_2$ (0.5 – 1.5 m*M*) had no effect on the shoot d. wt. of +PBZ seedlings (Fig. 1B). Increasing concentrations of $CdCl_2$ from 0.5 to 1.5 m*M* progressively decreased the root d. wt. of –PBZ seedlings, but had no effect on the root d. wt. of +PBZ rice seedlings. Fig. 2 shows the time courses of biomass production of –PBZ and +PBZ seedlings in the presence or absence of 1.5 m*M* $CdCl_2$. The d. wt. of the shoot and roots of –PBZ seedlings treated with $CdCl_2$ was significantly lower than that of the –PBZ seedlings not treated with $CdCl_2$. However, the d. wt. of the shoot and roots of +PBZ seedlings was only slightly affected by $CdCl_2$. All of these results suggest that +PBZ seedlings are Cd tolerant.

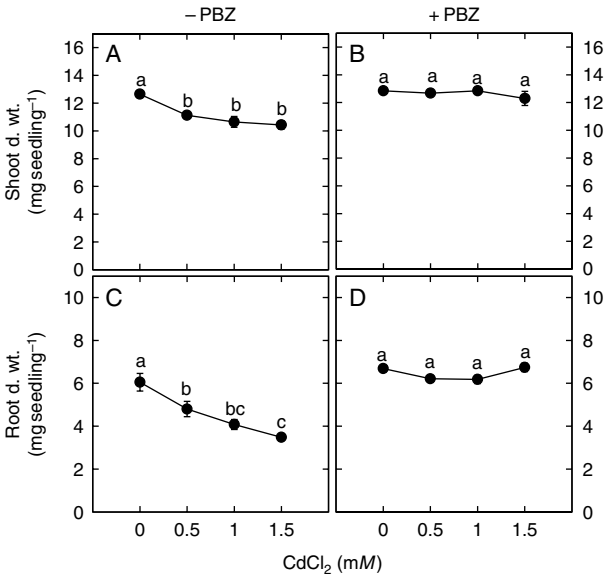

Fig. 1. Effect of $CdCl_2$ concentration on the dry weight (d. wt.) of shoot (A, B) and roots (C, D) of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol). The d. wt. of shoot and roots were measured 2 days after treatment. Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$.

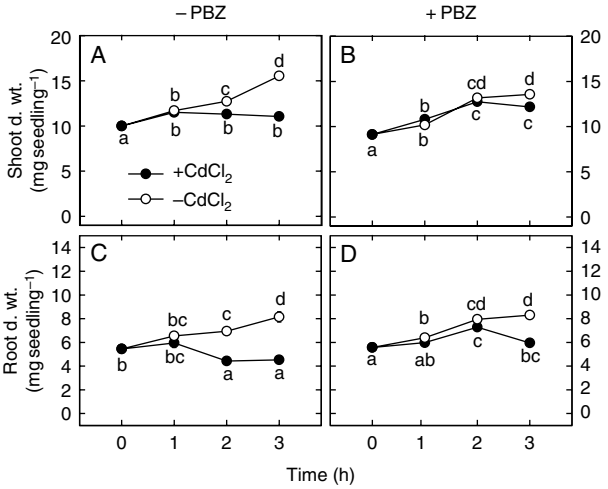

Fig. 2. Changes in the dry weight (d. wt.) of shoot (A, B) and roots (C, D) of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol) in the presence or absence of $CdCl_2$ (1.5 m*M*). Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$.

Chlorophyll and protein loss

In plants, the most general symptom of Cd toxicity is chlorosis (Das et al. 1997). In previous work, we have shown that rice seedlings treated with $CdCl_2$ at high (0.5 – 1.5 m*M*) and low (10–50 μ*M*) concentrations show chlorosis and protein loss (Hsu and Kao 2003a). However, a longer period (more than 6 days) was required to show chlorosis when rice seedlings were treated with low concentrations of $CdCl_2$ (Hsu and Kao 2003a). When seedlings were treated with 0.5 m*M* $CdCl_2$, chlorosis was first shown in the second leaves, but not the third leaves, of rice seedlings in a short-term experiment (3 days) (Hsu and Kao 2004). In order to show chlorosis in the third leaves in a short-term experiment, 1.5 m*M* $CdCl_2$ was required. Thus, in the present study, Cd toxicity in the third leaves exposed to 1.5 m*M* $CdCl_2$ was assessed by the decrease in chlorophyll and protein content. A marked decrease in chlorophyll and protein was observed in –PBZ leaves after Cd treatment (Fig. 3A,C). In contrast, only a slight decrease in chlorophyll and protein content caused by $CdCl_2$ was observed in +PBZ leaves (Fig. 3B,D).

NH_4^+ accumulation

NH_4^+ is a central intermediate of nitrogen metabolism in plants (Miflin and Lea 1976), but a high content of NH_4^+ is known to have toxic effects on plant cells (Givan 1979). Recent studies have demonstrated that NH_4^+ accumulation in the leaves of rice seedlings is linked to Cd toxicity (Hsu and Kao 2003b). In this study, we showed that, on treatment with $CdCl_2$, the NH_4^+ content increased markedly in –PBZ leaves (Fig. 4A), but only slightly in +PBZ leaves (Fig. 4B). These results suggest that PBZ protects rice seedlings from NH_4^+ accumulation caused by Cd toxicity.

GS is the key enzyme in NH_4^+ assimilation and catalyses the ATP-dependent condensation of NH_4^+

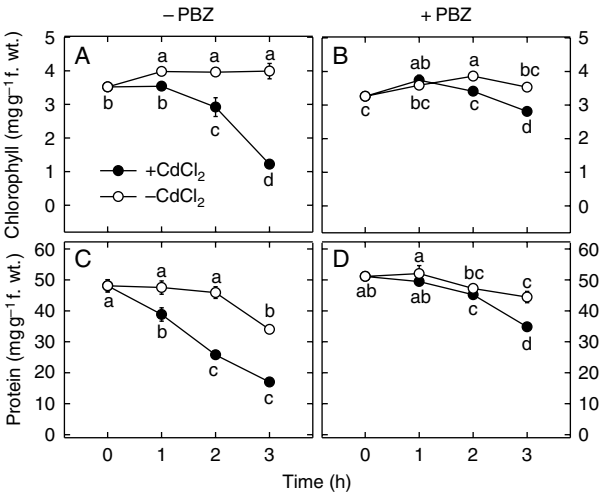

Fig. 3. Changes in the contents of chlorophyll (A, B) and protein (C, D) in the third leaves of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol) in the presence or absence of $CdCl_2$ (1.5 m*M*). Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. f. wt., fresh weight.

with glutamate to produce glutamine (Miflin and Lea 1976). PAL catalyses the elimination of NH_4^+ from phenylalanine and produces *trans*-cinnamate (Hahlbrock and Grisebach 1979). NH_4^+ released from PAL reaction has been shown to be trapped in glutamine molecules by the action of GS (Razel et al. 1996, Van Heerden et al. 1996). Sakurai et al. (2001) have provided evidence to show that GS is partly coupled to the reaction of PAL in developing rice leaves. Previous

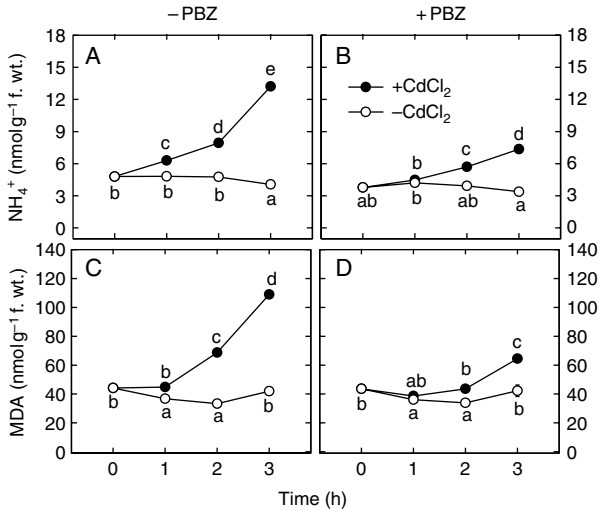

Fig. 4. Changes in the contents of NH_4^+ (A, B) and malondialdehyde (MDA) (C, D) of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol) in the presence or absence of $CdCl_2$ (1.5 m*M*). Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. f. wt., fresh weight.

work has indicated that PAL and GS are the enzymes responsible for NH_4^+ accumulation in rice leaves caused by Cd toxicity (Hsu and Kao 2003b). In this study, we demonstrated that the increase in PAL specific activity and the decrease in GS activity caused by $CdCl_2$ were more pronounced in –PBZ than in +PBZ leaves (Fig. 5A,B). These results further support the conclusion that PBZ-treated seedlings are Cd tolerant.

NH_4^+ can also be produced during nitrate reduction and photorespiration (Miflin and Lea 1976). It is not known whether NH_4^+ accumulation in rice leaves caused by Cd toxicity is mediated via the promotion of nitrate reduction and photorespiration. Further research in this area is likely to be highly rewarding.

Oxidative stress

Unlike Cu and Fe, Cd is not a redox metal, and therefore cannot catalyse Fenton-type reactions yielding active oxygen species (AOS). However, Cd can induce oxidative stress indirectly by producing a disturbance in chloroplasts. Thus, Cd produces the degradation of chlorophyll and carotenoids, as well as an inhibition of their biosynthesis (Bazzaz et al. 1974), which can produce disturbances in the electron transport rates of PSI and PSII, leading to the generation of AOS. In a

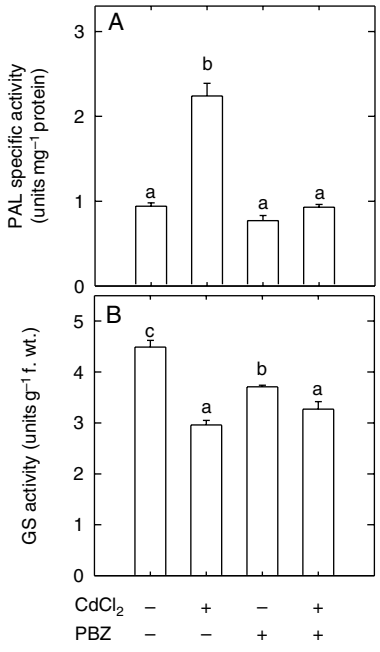

Fig. 5. Effect of $CdCl_2$ on phenylalanine ammonia-lyase (PAL) specific activity (A) and glutamine synthetase (GS) activity (B) in the third leaves of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol). Rice seedlings were either untreated or treated with $CdCl_2$ (1.5 m*M*) for 2 days. Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. f. wt., fresh weight.

recent review, Schützendübel and Polle (2002) suggested that the depletion of GSH was a critical step in Cd-induced AOS generation.

In previous work, it has been demonstrated that Cd can induce oxidative stress in rice leaves, characterized by an increase in the content of MDA (an indicator of lipid peroxidation) (Hsu and Kao 2004, Kuo and Kao 2004). In the present study, we observed that the increase in the content of MDA (Fig. 4) caused by $CdCl_2$ was more pronounced in –PBZ leaves (Fig. 4C) than in +PBZ leaves (Fig. 4D).

The striking increase in lipid peroxidation seen in –PBZ leaves treated with $CdCl_2$ (Fig. 4C) may reflect changes in the activities of antioxidant enzymes and contents of antioxidants. Previously, it has been observed that increases in SOD and POX specific activities in rice seedlings take place prior to the occurrence of Cd toxicity (decrease in protein content) (Kuo and Kao 2004). In this study, we showed that the increase in the specific activities of SOD and PO, caused by $CdCl_2$, was more pronounced in –PBZ leaves than in +PBZ leaves (Fig. 6A,B). ASC is a major antioxidant in photosynthetic and non-photosynthetic tissues, which reacts directly with AOS and is utilized as a substrate for APX-catalysed H_2O_2 detoxification (Noctor and Foyer 1998). GSH is involved in ASC regeneration and also functions as a direct scavenger of AOS (Noctor and Foyer 1998). It is clear from Figs 6C and 6D that the decrease in ASC and GSH contents caused by $CdCl_2$ was greater in –PBZ leaves than in +PBZ leaves. However, the depletion of ASC and GSH caused by $CdCl_2$ could not be prevented completely in +PBZ leaves, suggesting that there was still some oxidative stress in +PBZ seedlings when exposed to $CdCl_2$. All of the results presented here indicate that PBZ can be used to protect rice seedlings from oxidative stress caused by Cd toxicity.

ABA accumulation in +PBZ leaves

Previously, we have shown that an increase in endogenous ABA content is closely related to the Cd tolerance of rice seedlings (Hsu and Kao 2003a). In this study, we showed that $CdCl_2$ treatment resulted in an increase in endogenous ABA in +PBZ leaves, but not in –PBZ leaves (Table 2), suggesting that ABA may play a role in Cd tolerance.

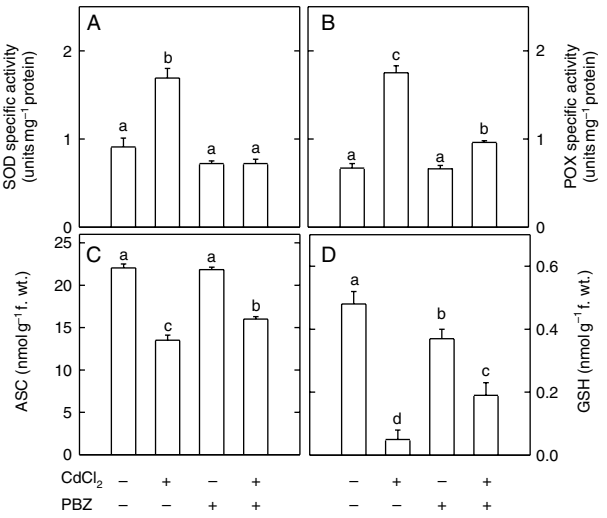

Fig. 6. Effect of $CdCl_2$ on the specific activities of superoxide dismutase (SOD) (A) and peroxidase (POX) (B) and the contents of ascorbate (ASC) (C) and reduced glutathione (GSH) (D) in the third leaves of –PBZ and +PBZ rice seedlings (PBZ, paclobutrazol). Rice seedlings were either untreated or treated with $CdCl_2$ (1.5 m*M*) for 2 days. Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. f. wt., fresh weight.

Flu treatment

The role of ABA in PBZ-induced Cd tolerance was tested by using an inhibitor of ABA biosynthesis, Flu, which blocks the conversion of phytoene to phytofluene in the carotenoid biosynthesis pathway (Kowalczyk-Schröder and Sandmann 1992). Flu was observed to inhibit the increase in ABA content (Fig. 7E) and to enhance Cd toxicity (as judged by biomass production and the contents of chlorophyll and protein) in +PBZ seedlings (Fig. 7A – D). The effect of Flu on Cd toxicity in +PBZ seedlings was reversed by the application of

Table 2. Effect of $CdCl_2$ on abscisic acid (ABA) content, transpiration rate and Cd content in the third leaves of –PBZ and +PBZ rice seedlings. Rice seedlings were either untreated or treated with $CdCl_2$ (1.5 m*M*) for 2 days. Values with the same letter are not significantly different at $P < 0.05$. d. wt., dry weight; f. wt., fresh weight; PBZ, paclobutrazol.

Treatment		ABA content (pmol g^{-1} f. wt.)	Transpiration rate (mg H_2O seedling^{-1} day^{-1})	Cd content (μg g^{-1} d. wt.)
–PBZ	– $CdCl_2$	334.8 ± 21.0 (b)	566 ± 39.0 (d)	4.15 ± 0.34 (a)
	+ $CdCl_2$	337.6 ± 20.7 (b)	145 ± 9.1 (b)	33.68 ± 1.24 (c)
+PBZ	– $CdCl_2$	255.1 ± 27.6 (a)	456 ± 20.5 (c)	5.33 ± 0.84 (a)
	+ $CdCl_2$	328.1 ± 19.5 (b)	55 ± 6.1 (a)	20.13 ± 0.71 (b)

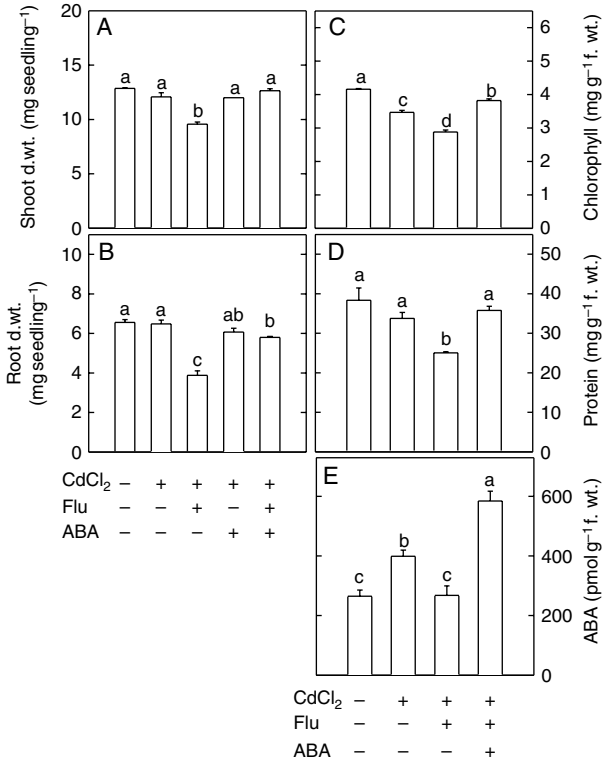

Fig. 7. Effect of fluridone (Flu, 0.2 m*M*) and abscisic acid (ABA) (5 μ*M*) on the dry weight (d. wt.) of shoot (A) and root (B) and the contents of chlorophyll (C), protein (D) and ABA (E) in the third leaves of +PBZ rice seedlings (PBZ, paclobutrazol) treated or not with $CdCl_2$ (1.5 m*M*). All measurements were made 2 days after treatment. Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. f. wt., fresh weight.

ABA (Fig. 7A – D). It should be noted that $CdCl_2$ +ABA treatment resulted in a similar biomass production as $CdCl_2$ treatment (Fig. 7A,B). These results suggest that the ABA content in the leaves of +PBZ seedlings treated with $CdCl_2$ is sufficient to exert an effect on biomass production.

Cd content

Table 2 shows the effect of $CdCl_2$ on the Cd content in –PBZ and +PBZ leaves. The Cd content in +PBZ leaves increased about four-fold after Cd treatment (Table 2). However, an eight-fold increase in Cd content in Cd-treated –PBZ leaves was observed (Table 2). Flu treatment caused an increase in Cd content in the leaves of +PBZ seedlings exposed to $CdCl_2$ (Fig. 8A). The effect of Flu on the Cd content in Cd-treated leaves of +PBZ seedlings was reversed by the application of ABA (Fig. 8A).

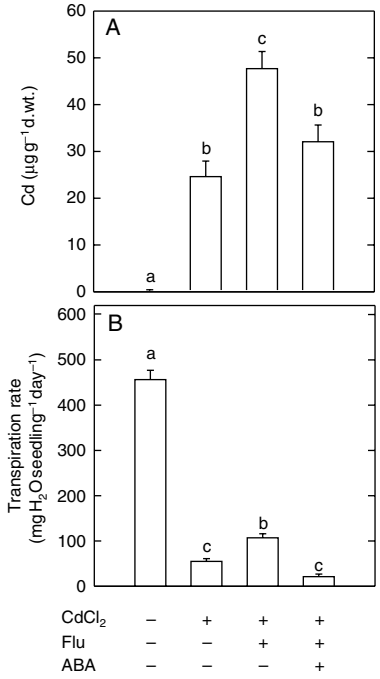

Fig. 8. Effect of fluridone (Flu, 0.2 m*M*) and abscisic acid (ABA) (5 μ*M*) on the Cd content (A) in the third leaves and the transpiration rate (B) of +PBZ rice seedlings (PBZ, paclobutrazol) treated or not with $CdCl_2$ (1.5 m*M*). The Cd content was measured 2 days after treatment, and the transpiration rate was measured 1 day after treatment. Bars indicate the standard error ($n = 4$). Values with the same letter are not significantly different at $P < 0.05$. d. wt., dry weight.

Transpiration rate

In the absence of $CdCl_2$, the transpiration rate of –PBZ seedlings was observed to be higher than that of +PBZ seedlings (Table 2). Cd treatment decreased the transpiration rate in both –PBZ and +PBZ seedlings (Table 2). However, the decrease in the transpiration rate in response to $CdCl_2$ was less pronounced in –PBZ seedlings than in +PBZ seedlings (Table 2). Flu treatment resulted in an increase in the transpiration rate in +PBZ seedlings treated with $CdCl_2$ (Fig. 8B). The effect of Flu on the transpiration rate in +PBZ seedlings treated with $CdCl_2$ was reversed by ABA application (Fig. 8B).

Discussion

Cd causes biomass reduction (Chen and Kao 1995), chlorophyll and protein loss (Hsu and Kao 2003a), NH_4^+ accumulation (Hsu and Kao 2003b) and oxidative stress (Kuo and Kao 2004) in rice seedlings. In the present study, we evaluated Cd toxicity by the decrease in biomass production, decrease in chlorophyll and protein content, increase in NH_4^+ content and

induction of oxidative stress. On the basis of these criteria, we demonstrated that PBZ applied to seeds was able to protect rice seedlings from Cd toxicity. The protective effect of uniconazole, a member of the triazole family, against Cd stress has also been described previously (Singh 1993).

The present study indicated that ABA was involved in the Cd tolerance of +PBZ seedlings. This conclusion was based on the following observations: (1) the increase in the endogenous ABA content in response to Cd in +PBZ leaves was more pronounced than that in –PBZ leaves (Table 2); (2) Flu treatment led to a decrease in the ABA content, as well as Cd tolerance, of +PBZ seedlings (Fig. 7); and (3) the effect of Flu on the Cd toxicity of +PBZ seedlings was reversed by the application of ABA (Fig. 7). These results suggest that the regulation of endogenous ABA biosynthesis under Cd stress is correlated with the tolerance of +PBZ seedlings. As Flu is an inhibitor of ABA biosynthesis through the carotenoid pathway (Kowalczyk-Schröder and Sandmann 1992), the effects of this inhibitor on +PBZ leaves may imply that the ABA biosynthesis pathway in response to Cd appears to be the same as that established in other stress conditions (Zeevaart and Creelman 1988, Seo and Koshiba 2002). In addition, the defect in ABA accumulation in –PBZ leaves may account for the Cd intolerance of –PBZ seedlings.

Plants have a range of potential mechanisms at the cellular level that may be involved in the detoxification of, and thus tolerance to, heavy metals. These all appear to be involved primarily in avoiding the build-up of toxic concentrations at sensitive sites within the cell, and thus preventing damaging effects (Hall 2002). In this context, reduced translocation of Cd to the shoot appears to be a possible mechanism of Cd tolerance in the shoot. Cd translocation to the shoot has been suggested to be driven by transpiration (Salt et al. 1995). Cd has been shown to decrease the transpiration rate in several plants (Bazzaz et al. 1974, Kirkham 1978, Lamoreaux and Chaney 1978, Hagemeyer et al. 1986, Schlegel et al. 1987, Hsu and Kao 2003a). Here, we also observed that Cd treatment decreased the transpiration rate of –PBZ and +PBZ seedlings (Table 2). Cd treatment decreased the transpiration rate in –PBZ and +PBZ seedlings to about 74% and 88% of the control value, respectively (Table 2). Thus, the decrease in the transpiration rate of –PBZ seedlings (which were unable to accumulate ABA) caused by Cd was less than that in +PBZ seedlings (which accumulated ABA), and consequently resulted in a higher Cd content in –PBZ than in +PBZ seedlings (Table 2). The effect of Flu on +PBZ seedlings indicated that not only was ABA biosynthesis blocked, but the transpiration rate and Cd content were increased (Figs 7E and 8). Furthermore, the effect of Flu on the transpiration rate and Cd content of +PBZ seedlings was reversed by the application of ABA (Fig. 8). It appears that the increase in endogenous ABA content is closely related to the Cd tolerance of +PBZ seedlings. ABA may exert its regulatory effect on the transpiration rate, decreasing the translocation of Cd to the shoot.

Stress-tolerant plants often grown more slowly than stress-intolerant plants. It has been hypothesized that +PBZ plants show a better quality of growth than –PBZ plants under stress conditions as a result of the slower growth rate or metabolism of the former (Abou El-Khashab et al. 1997). Thus, the possibility that ABA may also exert its effect on the metabolism of +PBZ seedlings cannot be excluded.

PBZ inhibits GA biosynthesis (Graebe 1987, Rademacher et al. 1987). It has been shown that the application of GA counters both the growth inhibitory and stress-protective effects of triazoles (Guoping 1997, Vettakkorumakankav et al. 1999, Sarkar et al. 2004). An interesting question then arises: is a decrease in both endogenous GA content and shoot length essential to enhance Cd tolerance in rice seedlings? Future work will focus on this question by examining the Cd tolerance in GA-responsive and GA non-responsive dwarf mutants of rice.

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