Bot. Bull. Acad. Sin. (2004) **45:** 41-48

Phosphinothricin tolerance in rice (*Oryza sativa* L.) seedlings is associated with elevated abscisic acid in the leaves

Yi Ting Hsu and Ching Huei Kao*

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

(Received March 18, 2003; Accepted August 29, 2003)

Abstract. Phosphinothricin (PPT, known as glufosinate) is a nonselective herbicide that is a potent inhibitor of glutamine synthetase (GS). PPT-tolerant and PPT-sensitive rice cultivars were used to study the role of abscisic acid (ABA) in PPT tolerance. ABA content was determined by enzyme-linked immunosorbent assay. PPT toxicity was evaluated by the decrease in chlorophyll and protein contents in the leaves. On treatment with PPT, ABA content significantly increased in the leaves of the PPT-tolerant cultivars (cv. Tainung 67, TNG67) but not in the PPT-sensitive cultivar (cv. Taichung Native 1, TN1). The reduction of transpiration rate of TN1 seedlings caused by PPT was less than that of TNG67. Pretreatment with ABA enhanced PPT tolerance and reduced PPT-induced NH_4^+ accumulation in leaves of TN1. Exogenous application of the ABA biosynthesis inhibitor, fluridone, decreased PPT tolerance and increased NH_4^+ content in the leaves of TNG 67. Fluridone effect on PPT toxicity of TNG67 seedlings was reversed by the application of ABA. Evidence is presented to show that PPT-induced NH_4^+ accumulation, rather than depletion of glutamine, is the mechanism that regulates PPT-induced toxicity in TN1 leaves. In conclusion, the increase in endogenous ABA content in the leaves is closely related to PPT tolerance of rice seedlings.

Keywords: Abscisic acid; Ammonium ion toxicity; Glutamine synthetase; Phosphinothricin tolerance; Rice.

Abbreviations: ABA, abscisic acid; **ELISA**, enzyme-linked immunosorbent assay; **f.wt**, fresh weight; **GS**, glutamine synthetase; **PPT**, phosphinothricin; **TN1**, Taichung Native 1; **TNG67**, Tainung 67.

Introduction

Glutamine synthetase (GS, EC 6.3.1.2) is the initial enzyme in the pathway that assimilates inorganic nitrogen into organic compounds (Lea and Miflin, 1974). It is an important enzyme in nitrogen metabolism in that, in addition to assimilating NH_4^+ produced by nitrite reductase, reassimilates NH_4^+ released as a result of photorespiration and the breakdown of proteins and nitrogen transport compounds (Miflin and Habash, 2002). Phosphinothricin [PPT, 2-amino-4-(methylphosphinyl)-butanoic acid, known as glufossinate] is a nonselective herbicide that is a potent inhibitor of GS (Leason et al., 1982; Ridley and McNally, 1985). Inhibition of GS activity by PPT leads to a rapid accumulation of NH_4^+ under conditions in which nitrite is being photosynthetically reduced and/or conditions which support photorespiration (Tachinbana et al., 1986; Wild et al., 1987; Sauer et al., 1987; Wendler et al., 1990). A high level of NH_4^+ is known to have a toxic effect on plant cells (Givan, 1979). Thus, PPT toxicity may be a direct result of NH_4^+ accumulation.

The plant hormone abscisic acid (ABA) is a sesquiterpenoid synthesized from xanthophylls (Taylor et al., 2000; Seo and Koshiba, 2002) and appears to influence several physiological and developmental events (Zeevaart and Creelman, 1988; Kende and Zeevaart, 1997). The level of ABA in plants increases upon their exposure to environmental stress (Zeevaart and Creelman, 1988; Xu et al., 1995). ABA content has been correlated with increased tolerance to freezing (Guy, 1990), chilling (Lee et al., 1993), drought (Zeevaart and Creelman, 1988), salt (LaRose et al., 1987), and cadmium toxicity (Hsu and Kao, 2003).

It has been shown that ABA accumulation is a common effect of using auxin herbicides on susceptible plants (Grossmann et al., 1996; Grossmann, 2000; Hansen and Grossmann, 2000). Recently, we demonstrated that PPT treatment resulted in an increase in ABA level in detached rice leaves (Tsai et al., 2002). However, no correlation between ABA and PPT toxicity or PPT tolerance in detached rice leaves could be established (Tsai et al., 2002). Our preliminary observation showed that rice seedlings of cultivar Tainung 67 (TNG67) are more tolerant to PPT than those of cultivar Taichung Native 1 (TN1) when PPT was added directly to the culture solution. It appears that these two cultivars of rice seedlings with different tolerance to PPT provide a useful system to study mechanism of PPT tolerance of rice plants. In this investigation, we examine the role of ABA in PPT tolerance of rice seedlings.

Materials and Methods

Plant Cultivation and Treatment

Two rice (*Oryza sativa* L.) cultivars, an Indica type cultivar TN1 and a Japonica cultivar, TNG67, obtained from

*Corresponding author. Tel: 886-2-23698159; Fax: 886-2-23620879; E-mail: kaoch@ntu.edu.tw

Taiwan Agricultural Research Institute, Taichung, Taiwan, were used in this study. Seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in a Petri dish on wetted filter papers at 37 °C in the dark. After 48 h incubation, uniformly germinated seeds were selected and cultivated in a 250 ml beaker containing half-strength Kimura B solution as described previously (Chu and Lee, 1989). The concentrations of N, P, K, S, Ca and Mg in half-strength Kimura B solution are 11.5, 2.9, 7.2, 15.0, 7.4 and 8.7 ppm, respectively. The hydroponically cultivated seedlings were grown in a Phytotron with natural light at 30 °C day (12 h)/25 °C night (12 h) and 90% relative humidity. Twelve–day-old seedlings with three leaves were used in all experiments.

For PPT, ABA, and fluridone treatments, the chemicals were added directly to the culture solution during the experiment.

Determinations of Chlorophyll, Protein, NH_4^+, and Glutamine

Chlorophyll was determined according to Wintermans and De Mots (1965) after extraction in 96% (v/v) ethanol. For protein extraction, leaf segments were homogenized in 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). Ammonium ion was extracted by homogenizing leaf samples in 0.3 mM sulphuric acid (pH 3.5). The homogenate was centrifuged for 10 min at 39,000 *g* and the supernatant was used for determination of NH_4^+ as described by Lin and Kao (1996). For determination of glutamine, leaf samples were extracted with 2% sulfosalicylic acid, and the homogenates were centrifuged at 15,000 *g* for 20 min. The supernatant was used directly for amino acid analysis (amino acid analyzer, Beckmann 6300, Palo Alto, USA). All measurements are expressed on the basis of f. wt.

GS Activity

For extraction of GS, leaf segments were homogenized with 10 mM Tris-HCl buffer (PH 7.6, containing 1 mM $MgCl_2$, 1 mM EDTA, and 1 mM 2-mercaptoethanol) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000 *g* for 30 min, and the resulting supernatant was used for determination of GS activity. The whole extraction procedure was carried out at 4°C. GS was assayed by the method of Oaks et al. (1980). The reaction mixture contained in a final volume of 1 ml was 80 µmol Tris-HCl buffer, 40 µmol L-glutamic acid, 8 µmol ATP, 24 µmol $MgSO_4$, and 16 µmol NH_2OH; the final pH was 8.0. The reaction was started by addition of the enzyme extract and after incubation for 30 min at 30°C, it was stopped by adding 2 ml 2.5% (w/v) $FeCl_3$ and 5% (w/v) trichloroacetic acid in 1.5 M HCl. After centrifugation the absorbance of the supernatant was read at 540 nm. One unit of GS activity was defined as 1 µmol L-glutamate γ-monohydroxamate formed per min. GS activity is expressed on the basis of f. wt.

ABA Determination

For extraction of ABA, leaves were homogenized with a mortar and pestle 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 polyvinylprrolidone column and C18 cartridges. The elutes 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). ABA immunoassay detection kit (PGR-1) was purchased from Sigma Chemical Co. (St Louis, MO, USA) and is specific for (+)-ABA. By evaluating ^{3}H-ABA recovery, ABA loss was less than 3% by the method described here. ABA content is expressed on the basis of f. wt.

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 seedlings basis.

Experimental Design

In our investigation, the seedlings were grown for 12 days in a greenhouse, where natural light was provided. The growth of rice seedlings is very sensitive to light and varies with different light intensities. Experiments were carried out at different times of the year. Thus, the absolute levels of each measurement varied among experiments because of seasonal effects. However, the patterns of response to PPT were reproducible. For all measurements, each treatment was performed four times. All experiments described here were performed three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results

Evaluation of PPT Tolerance

When rice seedlings were treated with 10 mM PPT for 7 days, leaf chlorosis was observed in TN1 seedlings, but not in TNG67 seedlings (Figure 1). In short term (3 days) experiments, chlorosis was first observed in the second leaf of TN1 seedlings. Thus, PPT toxicity in the second leaf was assessed by a decrease in chlorophyll and protein contents. Increasing concentration of PTT from 3 to 10 µM progressively decreased chlorophyll and protein contents in leaves of TN1 seedlings (Figure 2). However, the decrease in chlorophyll and protein contents in leaves of TNG67 seedlings was not observed (Figure 2). PPT at 10 µM seems to be the optimum concentration in inducing toxicity. Figure 3 also shows PPT (10 µM) had no effect on chlorophyll and protein contents in leaves of TNG67 seedlings, but significantly decreased chlorophyll and protein contents in leaves of TN1 seedlings. All these results suggest that TNG67 seedlings are apparently more tolerant to PPT than TN1 seedlings.

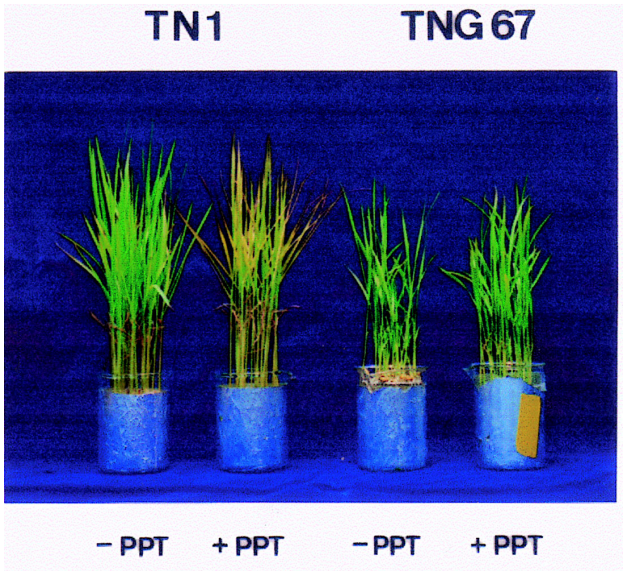

Figure 1. Effect of phosphinothricin (PPT, 10 µM) on leaf chlorosis of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings. Picture was taken after 7 days of treatment.

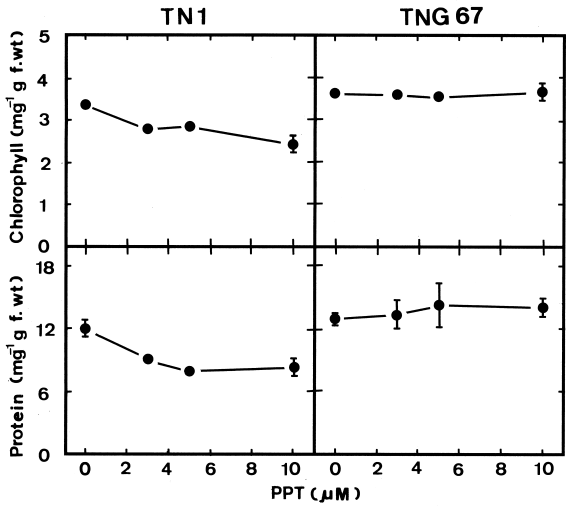

Figure 2. Effect of phosphinothricin (PPT, 10 µM) concentrations on chlorophyll and protein contents in the second leaf rice seedlings. Chlorophyll and protein contents were measured after 2 days of treatments. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

Table 1. Effect of PPT (10 µM) on transpiration rate of rice seedlings.

Cultivar	PPT treatment	Transpiration rate (g H_2O day^{-1} $seedlings^{-1}$)
TN1	–	0.89 ± 0.02
	+	0.46 ± 0.03
TNG67	–	0.70 ± 0.05
	+	0.10 ± 0.01

Transpiration rate was measured after 2 days of treatment. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

Ammonium Ion Content and GS Activity

Under PPT treatment NH_4^+ accumulated significantly in leaves of TN1 seedlings (Figure 3). However, none accumulated in the second leaf of TNG67 seedlings treated with PPT. GS is the primary enzyme responsible for NH_4^+ assimilation in plants (Lea and Miflin, 1974; Miflin and Habash, 2002). We observed that GS activity in the second leaf of TN1 treated with PPT decreased compared with that given no PPT treatment (Figure 3). In contrast, PPT had no effect on the GS activity of the second leaf of TNG67 seedlings (Figure 3).

The decrease in GS activity by PPT in the second leaf possibly results from the direct effect of PPT. This possibility was tested by mixing enzyme extract of the second leaf with various concentrations of PPT. GS activity was then assayed 30 min after mixing. It was found that GS activity was directly inhibited by PPT in both TNG67 and TN1 leaf extracts (Figure 4). Thus, the lack of inhibition of GS activity in the second leaf of TNG67 seedlings is mainly due to the lack of increase in PPT content. Current results also suggest that PPT-induced NH_4^+ accumulation in the second leaf of TN1 seedlings is attributable to the decrease in GS activity.

Transpiration Rate

The transpiration rate of TN1 seedlings was observed to be higher than that of TNG67 seedlings (Table 1). PPT treatment decreased this rate in both TN1 and TNG 67 seedlings (Table 1). However, the decrease was less pronounced in the former (Table 1).

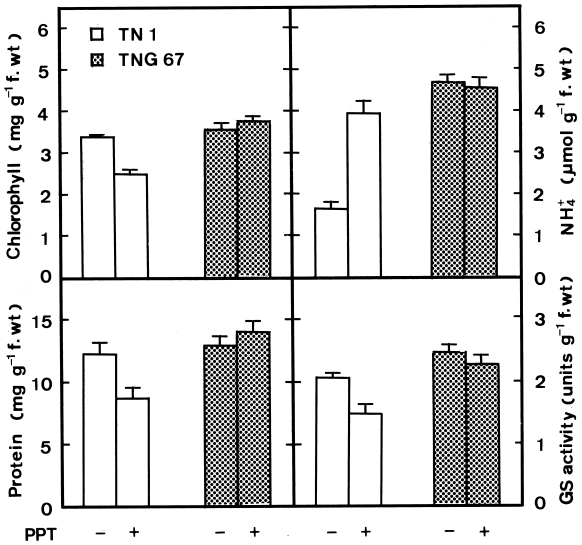

Figure 3. Effect of phosphinothricin (PPT, 10 µM) on the contents of chlorophyll, protein, NH_4^+ and the activity of glutamine synthetase (GS) in the second leaf of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings. All measurements were made after 2 days of treatment. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

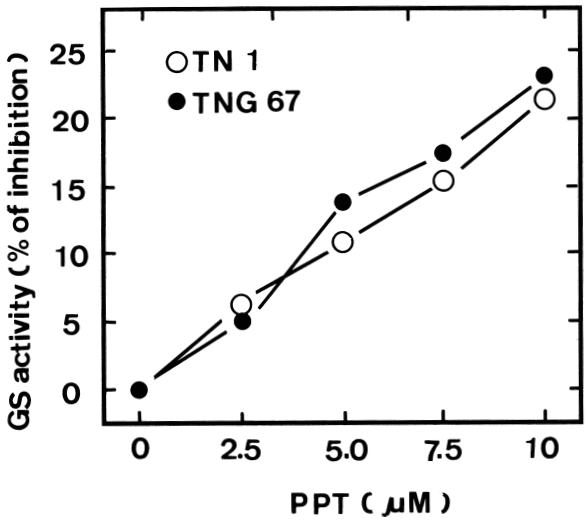

Figure 4. Effect of phosphinothricin (PPT) on the activity of GS extracted from rice leaves. Extracts were prepared from the second leaf of 12-day-old Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings. Assays were carried out 30 min after the addition of various concentrations of PPT. Each treatment was performed four times. The data reported here were from one of three independent experiments.

Glutamine Content

An inhibition of GS activity in the second leaf of TN1 seedlings may also result in a decrease in glutamine. Depletion of glutamine could be another reason for PPT-induced toxicity of TN1 seedlings. To test this possibility, we measured the content of glutamine in the second leaf of rice seedlings either untreated or treated with PPT. Contrary to our expectation, PPT-treated second leaf of TN1 seedlings had more glutamine than control leaves (Figure 5). Higher glutamine content in the second leaf of TNG67 seedlings was observed only at 3 days after PPT treatment (Figure 5).

ABA Content

Changes in endogenous ABA in leaves of seedlings treated with or without PPT are shown in Figure 6. In TN1 seedlings, no differences in ABA content were observed between PPT-treated leaves and controls. However, ABA content in PPT-treated TNG67 leaves was observed to be higher than in controls after 2 days of treatment. It is evident that ABA accumulates greater in TNG67 seedlings than in TN1 seedlings in response to PPT.

Pretreatment with ABA

If ABA plays an important role in PPT tolerance, then pretreatment of TN1 seedlings with ABA could be expected to reduce PPT toxicity, PPT-induced NH_4^+ accumulation, and PPT-decreased GS activity in TN1 leaves. Since some chlorosis was observed in the second leaf of TN1 treated with ABA (5 μM) for 2 days, PPT toxicity was evaluated using the third leaf. ABA pretreatment reduced PPT toxicity in the third leaf of TN1 seedlings (Figure 7). However, ABA pretreatment resulted in no further enhancement of PPT tolerance in TNG67 leaves (Figure 7), indicating that the amount of ABA in PPT-treated TNG67 leaves is sufficient to exert its effect on PPT tolerance. As expected, ABA pretreatment reduced PPT-induced NH_4^+ accumulation and PPT-decreased GS activity in the third leaf of TN1 seedlings (Figure 8).

Fluridone Treatment

The role of ABA in PPT tolerance was further tested using an inhibitor of ABA biosynthesis fluridone, which blocks the conversion of phytoene to phytofluene in carotenoid biosynthesis pathway (Kowalczyk-Schröder and Sandmann, 1992). Fluridone was observed to inhibit the

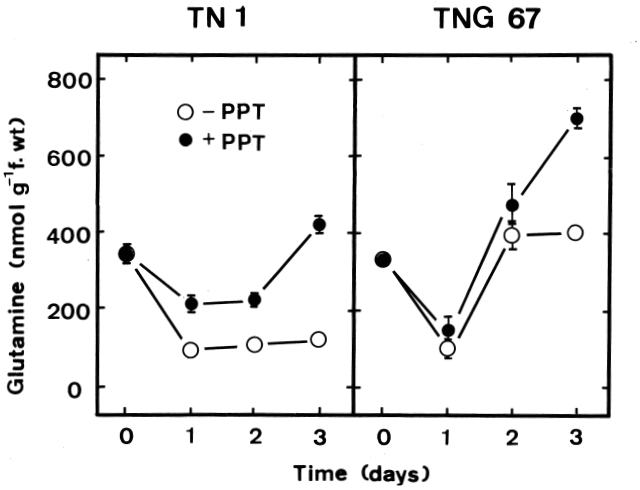

Figure 5. Changes in glutamine content in the second leaf of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings either untreated or treated with phosphinothricin (PPT, 10 μM). Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

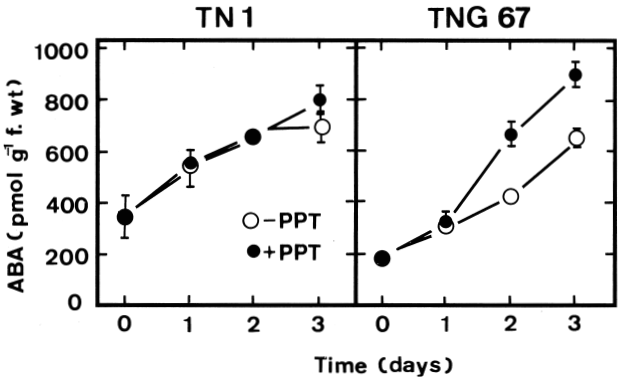

Figure 6. Changes in ABA content in the second leaf of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings either untreated or treated with phosphinothricin (PPT, 10 μM). Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

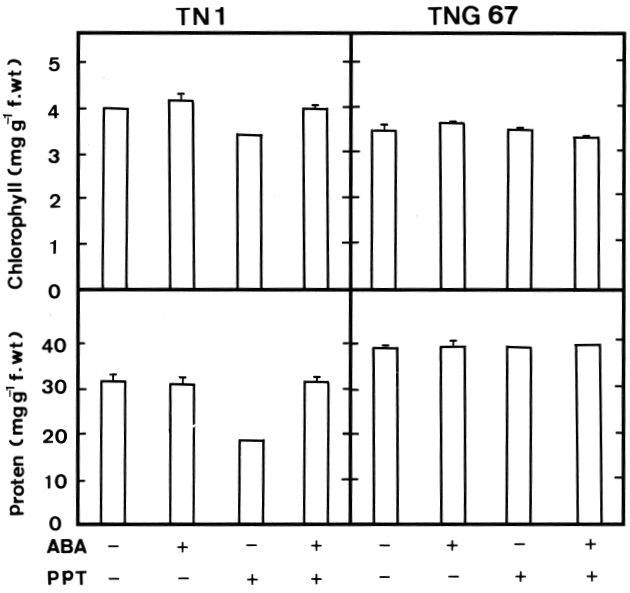

Figure 7. Effect of ABA-pretreatment on the contents of chlorophyll and protein in the third leaf of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings either untreated or treated with phosphinothricin (PPT, 10 µM). Rice seedlings were pretreated with ABA (5 µM) for 2 days and then either untreated or treated with PPT for 2 days. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

increase in ABA content (Table 2) and enhance PPT toxicity (Figure 9), NH_4^+ accumulation, and the decrease in GS activity (Figure 10) in leaves of TNG67. Figure 9 also shows that the effect of fluridone on PPT toxicity in leaves of TNG67 seedlings can be rescued by the application of 2 µM ABA. These results further strengthened the role of ABA on PPT tolerance of rice seedlings. Fluridone treatment, however, did not enhance PPT toxicity in leaves of TN1 seedlings (Figure 9).

Discussion

Our study indicates that ABA is involved in the PPT tolerance of rice seedlings. This conclusion was based on the observation that: (a) ABA accumulated more in TNG67 seedlings, a PPT tolerant cultivar, than in TN1 seedlings, a PPT sensitive cultivar, in response to PPT (Figure 6); (b) exogenous application of ABA increased the PPT tolerance of TN1 seedlings (Figure 7); (c) fluridone treatment reduced ABA content (Table 2), as well as PPT tolerance of TNG67 seedlings (Figure 9); (d) the effect of fluridone on the PPT toxicity of TNG67 seedlings can be reversed by the application of ABA (Figure 9). The results suggest that the regulation of endogenous ABA biosynthesis by PPT, applied directly to culture solution, is causally correlated to the PPT tolerance of rice seedlings. Since fluridone is an inhibitor of ABA biosynthesis through carotenoid pathway (Kowalczyk-Schröder and Sandmann, 1992), the effects of this inhibitor on TNG67

Table 2. Effect of fluridone (Flu, 0.2 mM) on the content of ABA in the second leaf Tainung 67 rice seedlings either untreated or treated with phosphinothricin (PPT, 10 µM).

Treatment		ABA (pmol g^{-1} f. wt)
PPT	Flu	
–	–	423.7 ± 28.0
–	+	347.4 ± 42.3
+	–	663.1 ± 60.2
+	+	307.3 ± 53.9

ABA content was measured after 2 days of treatment. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

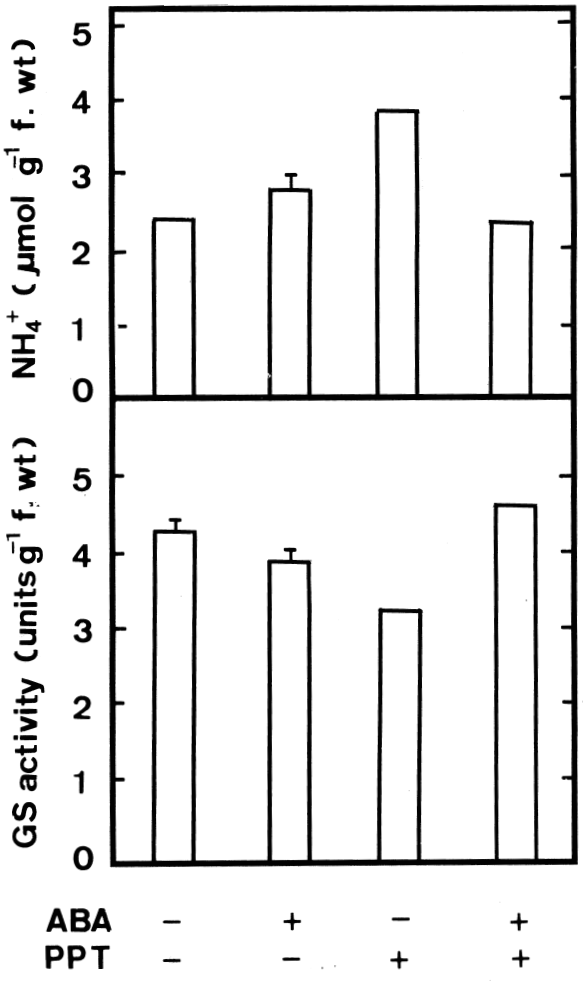

Figure 8. Effect of ABA-pretreatment on the content of NH_4^+ and the activity of GS in the third leaf of Taichung Native 1 rice seedlings either untreated or treated with phosphinothricin (PPT, 10 µM). Rice seedlings were pretreated or treated with ABA (5 µM) for 2 days and then either untreated or treated with PPT for 2 days. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

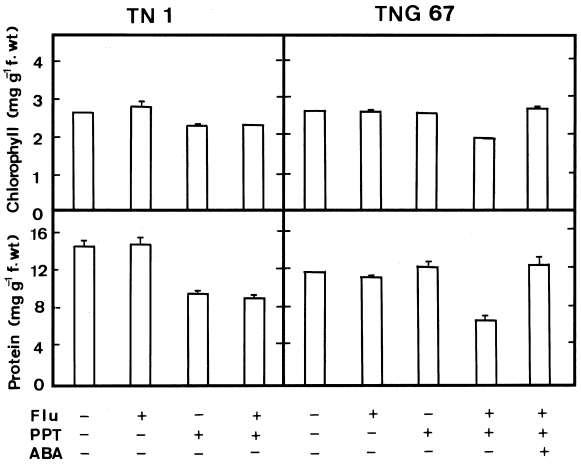

Figure 9. Effect of fluridone (Flu, 0.2 mM) or the content of chlorophyll and protein contents in the second leaf of Taichung Native 1 (TN1) and Tainung 67 (TNG67) rice seedlings either untreated or treated with phosphinothricin (PPT, 10 μM) or ABA (2 μM). Chlorophyll and protein contents were measured after 2 days of treatment. Data are means (± SE) of four replicates of single typical experiment. Three series of independent experiments were carried out giving reproducible results.

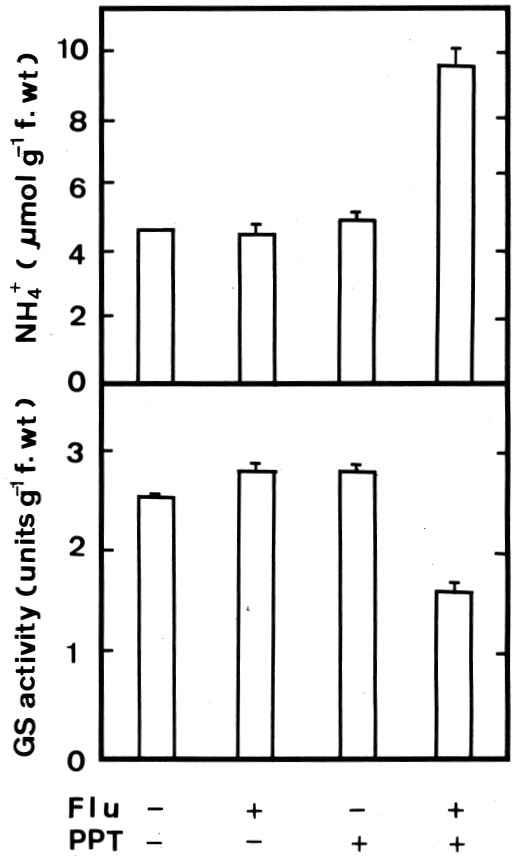

Figure 10. Effect of fluridone (Flu, 0.2 mM) on the content of NH_4^+ and the activity of GS in the second leaf of Tainung 67 rice seedlings either untreated or treated with phosphinothricin (PPT, 10 μM). All measurements were made after 2 days of treatment. Data are means (± SE) of four replicates of single typical experiment. Three series of independent experiments were carried out giving reproducible results.

may imply that the ABA biosynthetic pathway in response to PPT appears to be the same as that established under stress conditions (Zeevaart and Creelman, 1988; Seo and Koshiba, 2002). In addition, the defect of ABA accumulation in TN1 seedlings may account for the PPT intolerance of the cultivar.

In previous work, we observed that PPT at a concentration of 50 μM increased ABA content in detached rice leaves of TN1 (Tsai et al., 2002). In the present investigation, no accumulation of ABA was observed in leaves of TN1 seedlings treated with 10 μM PPT (Figure 6). When detached rice leaves of TN1 were treated with 10 μM PPT, no ABA accumulation was observed (data not shown). It appears that PPT concentration in leaves of TN1 seedlings treated with 10 μM PPT is high enough to cause toxicity, but not to cause ABA accumulation. It has been shown that roots possess the ability to synthesize ABA (Davies and Zhang, 1991). Thus, the possibility that PPT is unable to trigger the ABA biosynthetic pathway in TN1 roots cannot be excluded.

It has been shown that ABA accumulation is a common effect of auxin herbicides in susceptible plants (Grossmann et al., 1996; Grossmann, 2000; Hansen and Grossmann, 2000). PPT, a GS inhibiter, is widely used as a nonselective herbicide. Here, we demonstrate, for the first time, that ABA accumulation was observed only in PPT tolerant rice seedlings.

It is generally considered that inhibition of GS by PPT leads to the accumulation of NH_4^+ and depletion of glutamine in treated tissue (Lea et al., 1984; Hurst et al., 1993; Downs et al., 1994). PPT-induced NH_4^+ accumulation appears to be the mechanism that regulates PPT-induced toxicity in TN1 leaves. This conclusion is based on the observations that: (a) PPT toxicity is associated with the increased NH_4^+ level in TN1 leaves (Figure 3), (b) exogenous application of ABA decreased PPT-induced NH_4^+ accumulation and PPT-induced toxicity as well in TN1 leaves (Figures 7 and 8), and (c) PPT toxicity cannot be explained by the depletion of glutamine content in TN1 leaves (Figure 5).

TNG67 seedlings have significantly smaller leaves and less stomata per unit leaf area than TN1 seedlings (Figure 1, Lin et al., 2001), which may lead to lower transpiration rates in TNG67 leaves (Table 1). We also observed that PPT decreased the transpiration rate of both cultivars (Table 1). PPT treatment reduced transpiration rate in TN1 and TNG67 to about 48% and 85% of the control value, respectively (Table 1). Thus, the reduction of transpiration rate of TN1 seedlings caused by PPT, which are unable to accumulate ABA, was less than that of TNG67 seedlings, which accumulate ABA, and consequently may have a higher PPT content. In this study, we are unable to provide direct evidence that TNG67 leaves have less PPT than TN1 leaves. However, the facts that PPT directly inhibits GS activity extracted from TNG67 leaves (Figure 4) and that PPT fails to inhibit GS activity in the second leaf of TNG67 (Figure 3) provide circumstantial evidence that TNG67 leaves may have a negligible amount of PPT.

It appears that an increase in endogenous ABA content is closely related to the PPT tolerance of rice seedlings. ABA may exert its regulatory effect on transpiration rate, which reduces the translocation of PPT to the shoot.

There are reports that plants are able to reduce the toxic effect of the PPT by metabolizing PPT to stable and inactive compounds like 4-methylphosphinico-2-hydroxy-butanoic acid and its derivatives by non-specific actylation reactions (Dröge-Laser et al., 1994; Jansen and Schimidt, 2000; Müller et al., 2001). It is not known whether ABA is effective in metabolizing PPT in TNG67 leaves. This would be an interesting direction for our future study.

Acknowledgments. This work was financially supported by Grant NSC 91-2313-B002-364 from the National Science Council of the Republic of China.

Literature Cited

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248-254.

Chen, L.M., C.C. Lin, and C.H. Kao. 2000. Copper toxicity in rice seedlings: Changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. Bot. Bull. Acad. Sin. **41:** 99-103.

Chu, C. and T.-M. Lee. 1989. The relationship between ethylene biosynthesis and chilling tolerance in seedlings of rice (*Oryza sativa*). Bot. Bull. Acad. Sin. **30:** 263-273

Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42:** 55-76.

Downs, C.G., M.C. Christey, D. Maddocks, J.F. Seelye and D. G. Stevenson. 1994. Hairy root of *Brassica napus*: I. Applied glutamine overcomes the effect of phosphinothricin treatment. Plant Cell Rep. **14:** 37-40.

Dröge-Laser, W., U. Siemeling, A. Pühler and I. Brver. 1994. The metabolites of herbicides L-phosphinithricin (glufosinate). Plant Physiol. **105:** 159-166.

Givan, C.V. 1979. Metabolic detoxification of ammonium in tissues of higher plants. Phytochemistry **18:** 375-382.

Greger, M. and M. Johansson. 1992. Cadmium effect on leaf transpiration of sugar beet (*Beta vulgaris*). Physiol. Plant. **86:** 465-473.

Grossmann, K. 2000. Mode of action of auxin hericides: a new ending to long, drawn out story. Trends Plant Sci. **5:** 506-508.

Grossmann, K., F. Scheltrup, J. Kwiatowski, and G. Caspar. 1996. Induction of abscisic acid is a common effect of auxin hericides in susceptible plants. J. Plant Physiol. **149:** 475-478.

Guy, C.L. 1990. Cold acclimation and freezing stress tolerance: Role of protein metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. **41:** 187-223.

Hansen, K. and K. Grossmann. 2000. Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiol. **124:** 1437-1448.

Hsu, Y.T. and C.H. Kao. 2003. Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa*) seedlings. Plant Cell Environ. **26:** 867-874.

Hurst, P.L., G.A. King, and W.M. Borst. 1993. Postharvest inhibition of glutamine synthetase activity with phosphinothricin reduces the shelf-life of asparagus. Postharvest Biol. Technol. **3:** 327-334.

Jasen, C. and B. Schimidt. 2000. Glufosinate metabolism in excised shoots and leaves of twenty plant species. Weed Sci. **48:** 319-326.

Kende, H. and J.A.D. Zeeveaart. 1997. The five classical plant hormones. Plant Cell **9:** 1197-1210.

Kowalczyk-Schröder, S. and G. Sadmann. 1992. Interaction of fluridone with phytoene clesaturase of *Aphanocapse*. Pestic. Biochem. Physiol. **42:** 7-12.

LaRosa, P.C., P.M. Hasegawa, D. Rhodes, J.M. Clithero, A. Watad, and R. Bressan. 1987. Abscisic aicd stimulated osmotic adjustment and its involvement in adaptation of tobacco cells to NaCl. Plant Physiol. **85:** 174-181.

Lea, P.J. and B.J. Miflim. 1974. Alternative route for nitrogen assimilation in higher plants. Nature **21:** 614-616.

Lea, P.J., K.W. Joy, J.L. Ramos, and M.G. Guerrero. 1984. The action of 2-amino-4-(methylphosphiny)-butanoic acid (phospinothricin) and its 2-oxo-derivative on the metabolism of cyanobacteria and higher plants. Phytochemistry **23:** 1-6.

Leason, M., D. Cunliffe, D. Parkin, P.J. Lea, and B.J. Miflin. 1982. Inhibition of pea leaf glutamine synthetase by glutamate analogues. Phytochemistry **21:** 855-857.

Lee, T.-M., H.-S. Lur, and C. Chu. 1993. Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings. I. Endogenous abscisic acid levels. Plant Cell Environ. **16:** 481-490.

Lin, C.C. and C.H. Kao. 1996. Disturbed ammonium assimilation is associated with growth inhibition of roots in rice seedlings caused by NaCl. Plant Growth Regul. **18:** 133-138.

Lin, D.-I., H.-S. Lur, and C. Chu. 2001. Effect of abscisic acid on ozone tolerance of rice (*Oryza sativa* L.) seedlings. Plant Growth Regul. **35:** 395-300.

Miflin, B.J. and D.Z. Habash. 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J. Exp. Bot. **53:** 979-987.

Müller, B.P., A. Eumdick, I. Schuphan, and B. Schmidt. 2001. Metabolism of the herbicide glufosinate-ammonium in plant cell culture of transgenic (rhizomania-resistant) and non-transgenic sugar beet (*Beta vulgaris*), carrot (*Daucus carota*) purple foxglove (*Digitalis purpurea*) and apple (*Datura stramonium*). Pest Management Sci. **57:** 46-56.

Oaks, A., J. Stulen, K. Jones, M.J. Winspear, and I.L. Booesel. 1980. Enzymes of nitrogen assimilation in maize roots. Planta **148:** 477-487.

Ridley, S.M. and S.F. McNally. 1995. Effect of phosphinothricin on the isozymes of glutamine synthetase isolated from plant species which exhibit varing degrees of susceptibility to the herbicide. Plant Sci. **39:** 31-36.

Sauer, H., A. Wild, and W. Ruhle. 1987. The effect of phosphinothricin (glufosinate) on photosynthesis II. The causes of inhibition of photosynthesis. Z. Naturforsch. **42c:** 270-278.

Seo, M. and T. Koshiba. 2002. Complex regulation of ABA biosynthesis. Trends Plant Sci. **7:** 41-48.

Tachibana, K., T. Watanabe, Y. Seikizawa, and T. Takematsu. 1986. Accumulation of ammonium in plants treated with bialaphos. J. Exp. Bot. **51:** 1563-1574.

Taylor, I.B., A. Burbidge, and A.J. Thompson. 2000. Control of abscisic acid synthesis. J. Exp. Bot. **51:** 1563-1574.

Tsai, Y.-C., C.T. Chen, Y.T. Hsu, and C.H. Kao. 2002. The toxicity induced by phosphinothricin and methionine sulfoximine in rice leaves is mediated through ethylene but not abscisic acid. Plant Grown Regul. **37:** 167-176.

Walker-Simmons, M. 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol. **84:** 61-66.

Wendler, C.M., M. Barniske, and A. Wild. 1990. Effect of phosphinithricin (glufosinate) on photosynthesis and photorespiration of C_3 and C_4 plants. Photosyn. Res. **24:** 55-61.

Wild, A., H. Sauer, and W. Ruble. 1987. The effect of phosphinothricin (glufosinate) on photosynthesis I. Inhibition of photosynthesis and accumulation of ammonia. Z. Naturforsch. **42c:** 263-269.

Wintermans, J.F.G.M. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophyll *a* and *b* and their pheophytins in ethanol. Biochem. Biophys. Acta **109:** 448-453.

Xu, Q., R.L. Henry, J.A. Guikena, and G.M. Paulsen. 1995. Association of high-temperature injury with increased sensitivity of photosynthesis to abscisic acid in wheat. Environ. Exp. Bot. **35:** 441-454.

Zeevaart, J.A.D. and R.A. Creelman. 1988. Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39:** 439-473.

水稻幼苗葉片之脫落酸與對 phosphinothricin 之耐性間之關係

許奕婷　高景輝

國立台灣大學農藝系

Phosphinothricin（PPT，或稱為 glufosinate）是一種非選擇性殺草劑，可抑制 glutamine synthetase 之活性。本研究係利用耐與不耐 PPT之水稻品種，來探討脫落酸與 PPT耐性間之關係。脫落酸是利用酵素連結免疫法來測定，PPT 之毒害以葉綠素與蛋白質含量降低來代表。處理 PPT，則耐 PPT 之台農 67 號水稻幼苗葉片其脫落酸含量明顯累積，但對 PPT 敏感之台中在來 1 號水稻幼苗葉片，則不會累積脫落酸。PPT 處理降低台中在來 1 號幼苗之蒸散速率小於台農 67 號幼苗。台中在來 1 號前處理脫落酸，可增加對 PPT 之耐性以及降低 PPT 所誘導的銨離子累積。以脫落酸合成之抑制劑 fluridone 處理台農 67 號幼苗，則可以降低對 PPT之耐性與增加銨離子含量。Fluridone 之效果又可被脫落酸處理所克服。我們的結果顯示 PPT所引起之毒害是由PPT引起銨離子累積，而不是 glutamine 含量降低所造成。本研究之結果指出，內生脫落酸之含量與水稻幼苗對 PPT之耐性有關。

關鍵詞：脫落酸；銨離子；Glutamine synthetase；Phosphinothricin 耐性；水稻。