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BRIEF COMMUNICATION

Proline accumulation induced by phosphinothricin in rice leaves

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Abstract

The effect phosphinothricin (PPT), an inhibitor of glutamine synthetase (GS), on proline accumulation in detached rice leaves was investigated. During 12 h incubation, PPT inhibited GS activity and induced accumulation of NH_4^+, and accumulation of proline in the light but not in darkness. Proline accumulation caused by PPT in the light was related to protein hydrolysis, and increase in the contents of precursors of proline, ornithine and arginine. Abscisic acid accumulation was not required for proline accumulation in PPT-treated rice leaves.

Additional key words: abscisic acid, arginine, glutamic acid, ornithine, ornithine-δ-aminotransferase, *Oryza sativa*, proline dehydrogenase, Δ^1-pyroline-5-carboxylate reductase.

In plants, proline is synthesized from glutamic acid via Δ^1-pyrroline-5-carboxylate (P5C) by two enzymes, P5C synthetase and P5C reductase (P5CR, EC 1.5.1.2) (Delauney and Verma 1993, Yoshiba *et al.* 1997). Plants also synthesize proline from ornithine, by ornithine δ-aminotransferase (OAT, EC 2.6.1.13) (Delauney and Verma 1993). Arginine can also contribute to proline biosynthesis, and the pathway form arginine proceeds via ornithine as a result of catalytic activity of arginase (Brown and Fowden 1966, Lingnowski and Splittstoesser 1971). On the other hand, the content of proline also depends on its degradation, which is catalysed by the enzyme proline dehydrogenase (PDH, EC 1.5.99.8) (Yoshiba *et al.* 1997). An increase in protein hydrolysis has been suggested to be another source of proline accumulation in plants. Proline accumulation can be induced by abscisic acid (ABA) in plant tissues (Aspinall and Paleg 1981). However, the lack of proline accumulation in response to ABA treatment has also been reported in plant (Aspinall and Paleg 1981, McDonell *et al.* 1983).

Phosphinothricin [PPT, 2-amino-4-(methylphosphinyl)-butanoic acid, known as glufosinate] is a non-selective herbicide that is a potent inhibitor of glutamine synthetase in plants (Leason *et al.* 1982). It is generally accepted that glutamine synthetase is the primary enzyme responsible for assimilating NH_4^+ in plants (Miflin and Lea 1976) and therefore PPT treatment induces an accumulation of NH_4^+ (Tachibana *et al.* 1986, Wild *et al.* 1987). In recent studies, we found that proline accumulation was associated with NH_4^+ accumulation in rice leaves (Yang and Kao 2000) and exogenous NH_4Cl, which caused an accumulation of NH_4^+ in detached rice leaves, increased proline content (Lin and Kao 2001). This paper reports the results of an investigation into the regulation of proline accumulation in detached rice leaves exposed to PPT.

Rice (*Oryza sativa* L. cv. Taichung Native 1) was cultured in a stainless net floating on half strength Johnson's modified nutrient solution (pH 4.2) in a 500-cm^3 beaker (Lin *et al.* 1999). The nutrient solution was replaced every 3 d. Rice plants were grown for 12 d in a greenhouse, under natural light and the day/night temperature of 30/25 °C. The apical 3 cm of the third leaf of 12-d-old seedings was used for the experiment. A group of 10 segments floated in a Petri dish containing

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Abbreviations: ABA - abscisic acid; f.m. - fresh mass; GS - glutamine synthetase; OAT - ornithinine-δ-aminotransferase; P5C - Δ^1-pyrroline-5-carboxylate; P5CR - Δ^1-pyrroline-5-carboxylate reductase; PDH - proline dehydrogenase; PPT - phosphinothricin.

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Table 3. The contents of glutamic acid, glutamine, arginine, and ornithine in detached rice leaves floating on water (H_2O) or PPT (50 µM) solution for 4, 8, and 12 h under light. Means ± SE, *n* = 4.

Time [h]	Glutamic acid [µmol g^{-1}(f. m.)]		Glutamine [µmol g^{-1}(f. m.)]		Arginine [µmol g^{-1}(f. m.)]		Ornithine [nmol g^{-1}(f. m.)]	
	H_2O	PPT	H_2O	PPT	H_2O	PPT	H_2O	PPT
0	5.38 ± 0.11		2.90 ± 0.12		0.24 ± 0.01		50.2 ± 2.4	
4	5.31 ± 0.11	2.69 ± 0.13	0.74 ± 0.06	0.53 ± 0.02	0.08 ± 0.01	0.18 ± 0.01	22.2 ± 1.4	41.1 ± 3.1
8	4.93 ± 0.13	1.33 ± 0.29	0.29 ± 0.02	0.16 ± 0.04	0.05 ± 0.02	0.25 ± 0.01	21.6 ± 2.0	209.7 ± 9.5
12	2.97 ± 0.34	0.97 ± 0.15	0.23 ± 0.04	0.14 ± 0.03	0.04 ± 0.01	0.26 ± 0.02	21.1 ± 5.1	265.2 ± 32.9

Table 4. The activities of P5CR, OAT, and PDH in detached rice leaves floating on water (H_2O) or PPT (50 µM) solution for 4, 8, and 12 h under light. Means ± SE, *n* = 4.

Time [h]	P5CR [U mg^{-1}(protein)]		OAT [U mg^{-1}(protein)]		PDH [U mg^{-1}(protein)]	
	H_2O	PPT	H_2O	PPT	H_2O	PPT
0	0.29 ± 0.06		0.27 ± 0.02		0.50 ± 0.03	
4	0.33 ± 0.08	0.33 ± 0.07	0.27 ± 0.02	0.29 ± 0.01	0.54 ± 0.02	0.57 ± 0.01
8	0.21 ± 0.01	0.26 ± 0.07	0.30 ± 0.01	0.32 ± 0.05	0.57 ± 0.01	0.57 ± 0.01
12	0.34 ± 0.05	0.32 ± 0.07	0.33 ± 0.04	0.36 ± 0.07	0.72 ± 0.03	0.65 ± 0.02

as a consequence of GS inhibition (Lea *et al.* 1984, Wendler and Wild 1990, Wendler *et al.* 1990, Hurst *et al.* 1993, Downs *et al.* 1994). PPT-treatment also reduced glutamic acid content, suggesting that glutamic acid was converted to proline in detached rice leaves exposed to PPT (Table 3).

To determine the role of the biosynthetic pathways for proline accumulation caused by PPT treatment, the effect of PPT on OAT and P5CR activities was examined. It was observed that PPT had no effect on OAT and P5CR activities in detached rice leaves under light (Table 4). It appears that OAT and P5CR are not limiting and not involved in PPT-dependent regulation of proline synthesis in the light. The PDH is reported to catalyse proline oxidation (Yoshiba *et al.* 1997). In the present investigation, PPT treatment resulted in a decrease in PDH activity in detached rice leaves at longer treatment (12 h) (Table 4). This result suggests that proline oxidation (or degradation) contributes to proline accumulation in detached rice leaves only at 12 h of PPT treatment.

Under both light and dark conditions, PPT treatment resulted in an increase in ABA content in detached rice leaves (Table 5), indicating that ABA accumulation caused by PPT is not light-dependent. This result suggests that ABA is not a mediator of proline accumulation induced by PPT. This conclusion is supported further by the observation that proline accumulation occurs at 4 h after PPT treatment (Table 1), which is prior to the accumulation of ABA (8 h after PPT treatment) in detached rice leaves under light conditions (Table 5).

Table 5. The content of ABA in detached rice leaves floating on water (H_2O) or PPT (50 µM) solution for 4, 8, and 12 h under light and dark conditions. Means ± SE, *n* = 4.

Time [h]	ABA [pmol g^{-1}(f. m.)]			
	light		dark	
	H_2O	PPT	H_2O	PPT
0	45.1 ± 8.1		45.1 ± 8.1	
4	45.5 ± 2.9	61.6 ± 12.1	61.5 ± 4.2	117.6 ± 19.5
8	46.4 ±. 7.4	911.0 ± 61.5	50.5 ± 8.2	123.6 ± 9.2
12	78.0 ± 15.6	808.7 ± 56.7	53.8 ± 8.1	93.6 ± 19.2

In conclusion, our results suggest that NH_4^+ and proline accumulations in detached rice leaves in response to PPT treatment are light dependent, which provides further evidence that NH_4^+ accumulation is linked to proline accumulation. PPT-induced proline accumulation in the light is related to protein hydrolysis, bringing about an increase in the contents of ornithine and arginine. Evidence is also provided to show that ABA is not required for proline accumulation in PPT-treated rice leaves under light conditions.