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Ammonium ion, ethylene, and abscisic acid in polyethylene glycol-treated rice leaves

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Abstract

Polyethylene glycol (PEG)-treatment decreased chlorophyll and protein contents and increased NH_4^+ content due to decreased glutamine synthetase activity in detached rice leaves. PEG-treatment also increased abscisic acid (ABA) content and decreased ethylene production. Addition of fluridone, an inhibitor of ABA biosynthesis, reduced ABA content in rice leaves but did not prevent chlorophyll and protein loss in rice leaves induced by PEG. Silver thiosulfate, an inhibitor of ethylene action, was effective in preventing PEG-promoted chlorophyll and protein loss, but had no effect on PEG-induced NH_4^+ accumulation. The current results suggest that NH_4^+ accumulation in rice leaves induced by PEG increases leaf sensitivity to ethylene, which in turn results in an enhancement of chlorophyll and protein loss.

Additional key words: glutamine synthetase, *Oryza sativa*, water stress.

Introduction

Glutamine synthetase (GS, EC 6.3.1.2), the key enzyme in the generally recognized GS/glutamine synthase pathway, plays a crucial role in the assimilation of NH_4^+ (Miflin and Lea 1976). In leaves, water stress is correlated with a decline in the activity of GS (Becker *et al.* 1986, Lin and Kao 1998) which may result in, at least in part, an accumulation of NH_4^+ in leaves. A high content of NH_4^+ is known to have toxic effect on plant cells (Givan 1979). Water stress has been shown to decrease chlorophyll and protein contents (Dwivedi *et al.* 1979, Chen and Kao 1990). We have found that NH_4^+ accumulation was associated with chlorophyll and protein loss in detached rice leaves subjected to water stress caused by dehydration (Lin and Kao 1998).

Ethylene is a potential promoter of chlorophyll and protein loss (Gepstein and Thimann 1981, Kao and Yang 1983). Water stress has been shown to increase ethylene production (Wright 1980, Kimmerer and Kozlowski 1982, Hoffman *et al.* 1983). It has been suggested that abscisic acid (ABA), second to ethylene, is the most effective plant hormone in terms of decreasing chlorophyll and protein contents (Gepstein and Thimann 1980, 1981, Nooden 1988, Creelman 1989). ABA is known to increase rapidly in leaves under water stress (Hsiao 1973). We also reported that water stress resulted in a 25-fold increase in endogenous ABA in detached rice leaves 4 h after treatment (Yang *et al.* 2000). In this communication, we used fluridone, a direct inhibitor of ABA synthesis (Moore and Smith 1985), to reduce leaf ABA content, and silver thiosulfate (STS), an inhibitor of ethylene action (Liu *et al.* 1990), to reduce tissue sensitivity to ethylene, to investigate whether ABA or ethylene mediates NH_4^+ accumulation and chlorophyll and protein loss in rice leaves induced by polyethylene glycol (PEG).

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Abbreviations: ABA - abscisic acid; f.m. - fresh mass; GS - glutamine synthetase; PEG - polyethylene glycol; RWC - relative water content; STS - silver thiosulfate.

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Materials and methods

Rice (*Oryza sativa* L. cv. Taichung Native 1) was cultured on 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 seedlings was used for the experiment. A group of 10 segments floated in a Petri dish containing 10 cm^3 of distilled water served as controls. For induction of water stress, leaf segments were exposed to polyethylene glycol (PEG 6000) solution of osmotic potential -1.5 MPa. All samples were kept at temperature at 27 °C and irradiance of 40 μmol m^{-2} s^{-1} for 4, 8 and 12 h. Relative water content (RWC), defined as water content of leaf tissue as a percentage of that of the fully water saturated tissue, was determined by the method of Weatherley (1950).

Chlorophyll was determined according to Wintermans and De Mots (1965) after extraction in 96 % (v/v) ethanol using spectrophotometer (*U-2000*, *Hitachi*, Tokyo, Japan). For protein determination, 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 supernatant were used for determination of protein by the method of Bradford (1976). For NH_4^+ determination, leaf segments were homogenized 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^+ by the method described previously (Lin and Kao 1996).

For extraction of glutamine synthetase (GS), leaf segments were homogenized with 10 mM Tris-HCl buffer (pH 7.6, containing 1 mM $MgCl_2$, 1 mM EDTA and 10 mM 2-mercaptoethanol) in a chilled pestle and mortar. 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 Oak *et al.* (1980). One unit (U) of GS activity is defined as 1 μmol L-glutamate-γ-monohydroxamate formed per min. ABA was extracted and quantified as described previously (Chen *et al.* 2001). For ethylene production, leaf segments were transferred to the test tubes sealed with serum caps. After 2 h of incubation in the dark at 27 °C, a 1-cm^3 gas sample was withdrawn from the headspace of the test tube. Ethylene was then assayed as described previously (Kao and Yang 1983). In experiment with silver thiosulfate (STS), a stock solution of STS was prepared by mixing equal volumes of 0.01 M $AgNO_3$ and 0.04 M $Na_2S_2O_3$ (Liu *et al.* 1990).

All measurements were expressed on the basis of initial fresh mass (f.m.). All experiments were repeated three times; within each experiment, treatments were replicated 4 times. Similar results and identical trends were obtained in all experiments. The data reported here are from a single experiment.

Results and discussion

PEG is commonly used for induction of a water stress. RWC of detached rice leaves exposed to PEG (-1.5 MPa), under light, decreased considerably during 12-h of incubation (Hsu *et al.* 2003), suggesting that PEG-treatment in our study did indeed cause water stress in detached rice leaves. PEG-treatment resulted in a decrease in chlorophyll and protein contents (Fig. 1).

Ammonium ion content in detached rice leaves treated with PEG was greater than that in control and increased about 2.5-fold in leaves treated PEG for 12 h in the light (Fig. 1). The accumulation of NH_4^+ induced by PEG was evident at 4 h after treatment (Fig. 1). GS is the main enzyme responsible for NH_4^+ assimilation in leaves (Miflin and Lea 1976). We observed that GS activity in control leaves remained unchanged during the entire duration of incubation (Fig. 1). PEG-treated rice leaves had lower GS activity than the control leaves (Fig. 1). The decrease in GS activity caused by PEG was evident at 4 h after treatment in the light (Fig. 1). It appears that PEG-induced NH_4^+ accumulation is attributed to the decrease in GS activity. In leaves, NH_4^+ is produced

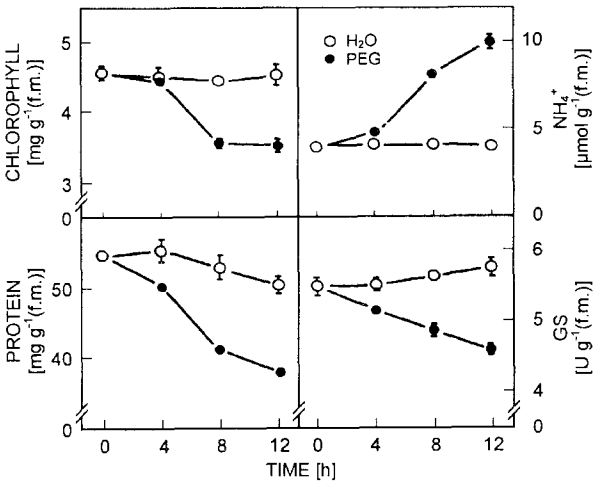

Fig. 1. Changes in chlorophyll, protein and NH_4^+ contents and GS activity of detached rice leaves floated on distilled water (*open circles*) or PEG (*closed circles*) solution in the light. Means ± SD, $n = 4$.

during nitrate reduction, deamination of amino acids or photorespiration (Miflin and Lea 1976). Whether PEG exerts its effect on nitrate reduction, deamination of amino acids or photorespiration in rice leaves remains to be seen.

In previous studies we have shown that detached rice leaves fed with NH_4Cl or methionine sulfoximine, an inhibitor of GS activity (Ronzio *et al.* 1969, Rowe and Meister 1970), resulted in an accumulation of NH_4^+ and a promotion of chlorophyll and protein loss (Chen *et al.* 1997).

It was found that PEG treatment resulted in a significant increase in ABA content in rice leaves (Fig. 2). However, PEG treatment decreased ethylene production in rice leaves (Fig. 2). In order to examine whether promotion of chlorophyll and protein loss by PEG was dependent on the ABA contents, an ABA synthesis inhibitor, fluridone, was used. The results indicate that fluridone treatment reduced PEG-increased ABA content in rice leaves, but did not reduce PEG-decreased chlorophyll and protein contents (Table 1). This reduction in ABA biosynthesis is due to the fluridone effect and this result agrees with Henson (1984), who found that the carotenoid-deficiency induced by norflurazon is connected with ABA-deficiency. It appears that ABA is unlikely to be the hormone regulating PEG-decreased chlorophyll and protein contents in rice leaves.

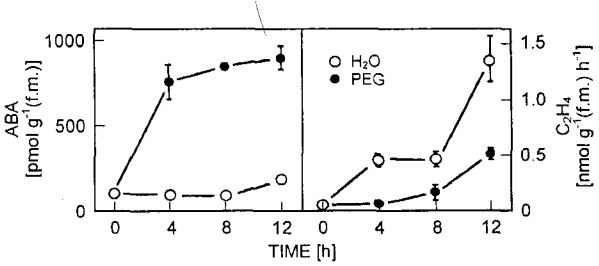

Fig. 2. Changes in ABA content and ethylene production of detached rice leaves floated on distilled water (*open circles*) or PEG (*closed circles*) solution in the light. Means ± SD, $n = 4$.

Table 1. Effect of fluridone (20 μM) on ABA, NH_4^+, chlorophyll and protein contents in detached rice leaves treated with PEG in the light. Detached rice leaves were pretreated with or without fluridone for 3 h in the light and then treated with water or PEG for 12 h. Means ± SD, $n = 4$.

Treatment	ABA [pmol g^{-1}(f.m.)]	NH_4^+ [μmol g^{-1}(f.m.)]	Chlorophyll [mg g^{-1}(f.m.)]	Protein [mg g^{-1}(f.m.)]
$H_2O \rightarrow H_2O$	135.7 ± 3.8	3.57 ± 0.34	4.56 ± 0.08	56.4 ± 1.8
$H_2O \rightarrow$ PEG	800.6 ± 19.1	7.10 ± 0.20	3.59 ± 0.15	43.0 ± 1.3
Fluridone $\rightarrow H_2O$	91.1 ± 4.4	2.94 ± 0.21	4.42 ± 0.15	50.3 ± 2.9
Fluridone $\rightarrow$ PEG	174.5 ± 35.2	7.48 ± 0.23	3.72 ± 0.09	44.8 ± 3.6

Of particular interest is the finding that ethylene production is inhibited by PEG (Fig. 2). This result is unexpected since ethylene production is generally considered to be enhanced by water stress (Wright 1980, Kimmerer and Kozlowski 1982, Hoffman *et al.* 1983). If a change in ethylene production is excluded as an explanation for PEG- or NH_4^+-decreased chlorophyll and protein contents in detached rice leaves, a change in sensitivity to ethylene is an alternate possibility. This possibility was tested by using the inhibitor of ethylene action, STS (Liu *et al.* 1990). STS was found to be effective in preventing PEG-decreased chlorophyll and protein contents, but was ineffective in preventing PEG-induced NH_4^+ accumulation (Table 2). It appears that NH_4^+ accumulation in rice leaves caused by PEG increases sensitivity to ethylene, which in turn results in a decrease in chlorophyll and protein contents.

Table 2. Effect of STS (400 μM) on chlorophyll, protein and NH_4^+ contents in detached rice leaves treated with PEG in the light for 12 h. Means ± SD, $n = 4$.

Treatment	Chlorophyll [mg g^{-1}(f.m.)]	Protein [mg g^{-1}(f.m.)]	NH_4^+ [μmol g^{-1}(f.m.)]
H_2O	4.35 ± 0.27	57.3 ± 2.0	3.42 ± 0.20
PEG	3.42 ± 0.09	35.6 ± 1.6	7.24 ± 0.67
STS	3.97 ± 0.01	65.2 ± 1.7	3.60 ± 0.25
STS + PEG	3.83 ± 0.01	53.5 ± 2.2	6.33 ± 0.28

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