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The effect of NaCl on proline accumulation in rice leaves

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Abstract

The regulation of proline accumulation in detached leaves of rice (*Oryza sativa* cv. Taichung Native 1) was investigated. Increasing concentrations of NaCl from 50 to 200 mM progressively increased proline content in detached rice leaves. NaCl induced proline accumulation was mainly due to the effect of both Na^+ and Cl^- ions. Proline accumulation caused by NaCl was related to protein proteolysis, an increase in ornithine-δ-aminotransferase activity, a decrease in proline dehydrogenase activity, a decrease in proline utilisation, and an increase in the content of the precursors of proline biosynthesis, ornithine and arginine. Results also show that proline accumulation caused by NaCl was associated with ammonium ion accumulation.

Abbreviations: DIDS – 4,4′-diisothiocyano-2,2′-disulfonic acid, FW – fresh weight, GS – glutamine synthetase, OAT – ornithine-δ-aminotransferase, P5C – Δ^1-pyrroline-5-carboxylate, P5CR – Δ^1-pyrroline-5-carboxylate reductase, PDH – proline dehydrogenase, RWC – relative water content

Introduction

Plants are exposed to various types of environmental stress. Among these stresses, osmotic stress, in particular that due to drought and salinity, is the most serious problem that limits plant growth and crop productivity in agriculture (Boyer 1982). Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon (La Rosa et al. 1991; Lutts et al. 1999; Madan et al. 1995; Mattioni et al. 1997; Moftah and Michel 1987; Sudhakar et al. 1993; Treichel 1986; Yoshiba et al. 1997). However, the actual role of proline accumulation remains unclear (Rhodes et al. 1999), but it has been speculated that it can serve as an osmotic regulator (Pollard and Wyn Jones 1979), a protector of enzyme denaturation (Paleg et al. 1984), a stabiliser of some macromolecules or molecular assemblies (Schwab and Gaff 1990), a reservoir of nitrogen and carbon sources (Fukutaku and Yamada 1984) or a hydroxyl radical scavenger (Smirnoff and Cumbes 1989). However, some reports indicate no correlation between proline accumulation and stress resistance (Bhaskaran et al. 1985; Garcia et al. 1997; Lutts et al. 1996; Moftah and Michel 1987; Tully et al. 1979).

Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Charest and Phan 1990; Yoshiba et al. 1997). In plants, proline is synthesised from glutamic acid (Glu) via Δ^1-pyrroline-5-carboxylate (P5C) by two enzymes, P5C synthetase and P5C reductase (P5CR, EC 1.5.1.2). It has been shown from labelling experiments that ornithine (Orn) can also serve as a precursor to proline biosynthesis in higher plants (Brown and Fowden 1966; Chiang and Dandekar 1995). The isolation of cDNA encoding ornithine-δ-aminotransferase (OAT, EC 2.6.1.13) in higher plants (Delauney and Verma 1993; Roosens et al. 1998) suggests that OAT participates in proline biosynthesis by producing P5C from ornithine and α-ketoglutarate. Arginine can also contribute to proline biosynthesis, and the pathway from

arginine proceeds via ornithine as a result of catalytic activity of arginase (Brown and Fowden 1966; Lingnowski and Splittstoesser 1971). Proline is metabolised to glutamic acid via P5C by two enzymes, proline dehydrogenase (PDH, EC 1.5.1.2) and P5C dehydrogenase (EC 1.5.1.12) (Yoshiba et al. 1997).

It has been shown that proline accumulation in response to NaCl could be attributed to an increase in P5CR activity (Madan et al. 1995; Mattioni et al. 1997; Sudhakar et al. 1993), an increase in OAT activity (Lutts et al. 1996; Madan et al. 1995) or a decrease in PDH activity (Mattioni et al. 1997; Sudhakar et al. 1993). Recently, we have shown that a decrease in proline utilisation might contribute to dark- and water stress-induced proline accumulation in detached rice leaves (Yang et al. 1999, 2000). However, there is no information concerning the effect of NaCl on proline utilisation. Little is known about whether the contents of three amino acids (glutamic acid, ornithine and arginine) involved in the proline biosynthetic pathway are limiting factors for proline accumulation in plant tissues. In a recent work, Lutts et al. (1999) provided evidence to show that proline accumulation was associated with an increased production of glutamic acid. In this paper, we report the results of investigations into the regulation of proline accumulation in detached rice leaves exposed to NaCl.

Materials and methods

Plant material

Rice (*Oryza sativa* L., cv. Taichung Native 1) was cultured as previously described (Lin et al. 1999). Briefly, rice seedlings were planted on a stainless net floating on half-strength Johnson's modified nutrient solution (Johnson et al. 1957) in a 500 ml beaker. The nutrient solution (pH 4.8) was replaced every three days. Rice plants were grown for 12 days in a greenhouse, where natural light was provided and the temperature was controlled at 30 °C during the day and at 25 °C at night. The apical 3 cm of the third leaves was used for the experiment. A group of 10 segments was floated in a Petri dish containing 10 ml of test solution dissolved in 5 mM sodium phosphate buffer at pH 7.0. Unless otherwise indicated, incubation was carried out at 27 °C for 3 days in the light (40 μmol m^{-2} s^{-1}).

RWC, Na^+ and Cl^-measurements

RWC, defined as water content of leaf tissue as a percentage that of the fully turgid tissue, was determined by the method of Weatherley (1950). For Na^+determination, harvested leaf segments were washed three times (with each one minute) with distilled water, dried at 65 °C for 2 days, extracted in 1 N HCl at room temperature (Hunt 1982) and analysed with a flame photometer (Evans, Electroselenium LTD, England). Chloride was estimated in a separate extract made according to the method described by Hodson et al. (1985) and estimated using an ion meter (Mittler Delta 350, UK). The contents of Na^+ and Cl^- are expressed on the basis of fresh weight (FW).

Determinations of proline and other amino acids

Proline was extracted and its concentration determined by the method of Bates et al. (1973). Leaf segments were homogenised with 3% sulfosalicylic acid and the homogenate was centrifuged at 3,000 *g* for 20 min. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined. Contents of proline are expressed as μmol g^{-1} FW. For determination of glutamic acid, glutamine, arginine, ornithine, and total amino acids, leaf samples were extracted with 2% sulfosalicylic acid and the homogenate was centrifuged at 15,000 *g* for 20 min. The supernatant was used directly for amino acid analysis. Amino acid analysis was carried out by an amino acid analyser (Beckman 6300, California, USA) and contents of amino acids are expressed as nmol g^{-1} FW or μmol g^{-1} FW. For protein determination, leaf segments were homogenised 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). Protein content is expressed as mg g^{-1} FW.

Enzyme assays

A similar extraction procedure was used for P5CR and PDH, which is mainly based on the procedure described by Lutts et al. (1999). Detached rice leaves were homogenised in a prechilled mortar and pestle with 50 mM Tris-HCl buffer (pH 7.4) containing 7 mM $MgCl_2$, 0.6 M KCl and 3 mM EDTA. The homogenate was centrifuged at 15,000 *g* for 20 min. The

supernatant was desalted by a Sephadex G-25 column before the assay of P5CR and PDH. The solution used for extraction of OAT was 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM pyridoxal-5′-phosphate, 1 mM EDTA and 10 mM 2-mercaptoethanol. The extract was centrifuged at 12,000 *g*. The supernatant was desalted by a Sephadex G-25 column before the assay of OAT. All the extraction procedures were conducted at 4 °C.

P5CR was assayed by a NADH dependent P5CR reaction (Madan et al. 1995). The assay mixture contained 0.06 mM NADH, 0.15 mM P5C, 120 mM potassium phosphate buffer, 2 mM dithiothreitol, and the enzyme extract. The reaction was started by the addition of P5C and the decrease in absorbance was followed at 340 nm. P5CR is expressed as units g^{-1} FW (one unit is defined as a decrease in 1 A_{340} per min) or units mg^{-1} protein (one unit is defined as a decrease in 0.01 A_{340} per min). OAT activity was assayed according to Vogel and Kopac (1960). The assay mixture contained 0.2 ml enzyme extract and 0.8 ml 100 mM potassium phosphate buffer (pH 8.0) containing 50 mM L-ornithine, 20 mM α-ketoglutarate and 1 mM pyridoxal-5′-phosphate. The reaction medium was incubated at 37 °C for 30 min. The reaction was stopped by adding 0.5 ml trichloroacetic acid (10%) and the colour was developed by incubating the reaction mixture with 0.5 ml *o*-aminobenzaldehyde (0.5%) in ethanol (95%) for 1 h. After centrifugation at 12,000 *g* for 10 min, the clear supernatant fraction was collected to measure the absorbance at 440 nm. OAT is expressed as units g^{-1} FW (one unit is defined as an increase in 1 A_{440} per h) or units mg^{-1} protein (one unit is defined as an increase in 0.01 A_{440} per h). PDH was assayed by following the NAD^+ reduction at 340 nm in a 0.15 M Na_2CO_3-HCl buffer (pH 10.3) containing 13 mM L-proline and 1.5 mM NAD^+ (Lutts et al. 1999). PDH is expressed as units g^{-1} FW (one unit is defined as an increase in 1 A_{340} per min) or units mg^{-1} protein (one unit is defined as an increase in 0.01 A_{340} per min).

For extraction of GS (EC 6.3.1.2), leaf segments were homogenised with 10 mM Tris-HCl buffer (pH 7.6, containing 1 mM $MgCl_2$, 1 mM EDTA and 1 mM 2-mercaptoethanol) using a prechilled pestle and mortar. The homogenate was centrifuged at 15,000 *g* for 30 min and the resulting supernatant was used for determination in GS activity. 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, 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. GS is expressed as units per g^{-1} FW (1 unit is defined as 1 μmol L-glutamate °C-monohydroxamate formed per min).

Determination of proline utilisation

For proline utilisation, detached rice leaves were pretreated with 50 mM ornithine for 3 h [since addition of ornithine has been observed to be more effective than that of glutamic acid or arginine in increasing proline content in rice leaves (Yang et al. 1999)] to increase the endogenous proline content and then transferred to distilled water and 100 mM NaCl for 8 h in the light. Proline content was then determined. A decline in proline content is considered to imply that proline is utilised (Yang et al. 1999).

Determination of ammonium ions

Ammonium ions were extracted by homogenising leaf segments 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 ammonium as described previously (Lin and Kao 1998).

Experimental design

For all measurements, each treatment was repeated four times. All experiments described here were repeated at least four times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results and discussion

Proline content in detached rice leaves increased with the increase of NaCl concentrations (Figure 1). Proline content increased about 4-fold in detached rice leaves treated with 200 mM NaCl for 3 days in the light (Figures 1, 2 and 3), but there was only a slight increase in proline contents in control leaves (Figure 2). Proline contents in detached leaves treated with 200 mM NaCl increased significantly with the

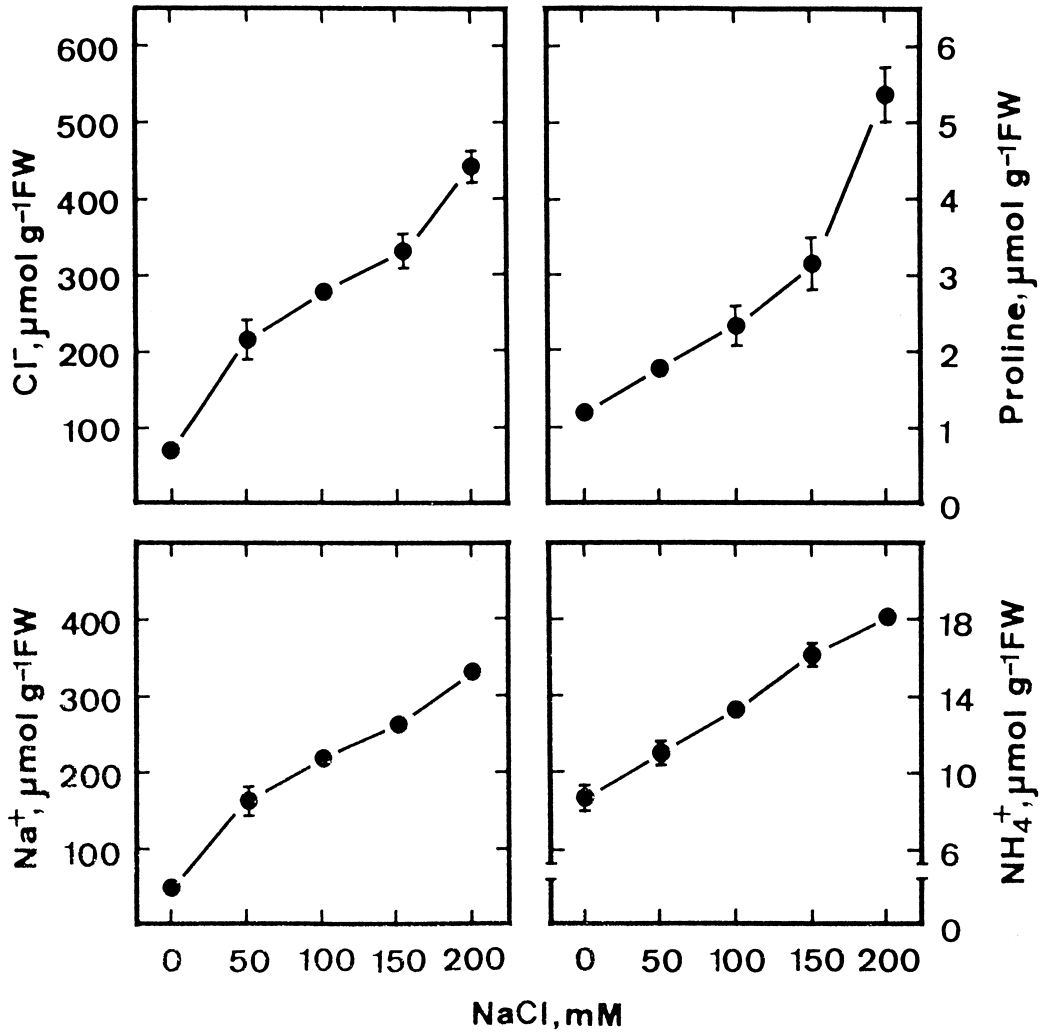

Figure 1. Effect of NaCl on Na^+, Cl^-, proline, and NH_4^+ contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence of NaCl (0–200 mM). Measurements were made after 3 days of treatment in the light. Vertical bars represent standard errors (n = 4).

increase of incubation time (Figure 2). To be sure that the described proline accumulation was related to leaf Na^+ and Cl^- contents, both Na^+ and Cl^- concentrations were determined in detached rice leaves treated with various concentrations of NaCl. Both Na^+ and Cl^- contents in NaCl-treated detached rice leaves increased with increasing concentrations of NaCl (Figure 1) but Na^+ and Cl^- contents remained unchanged in control leaves (Figure 2). However, Na^+ and Cl^- contents in detached rice leaves treated with 200 mM NaCl significantly increased with the increase of incubation time (Figure 2).

The effect of NaCl on proline accumulation in detached rice leaves could be attributed to Na^+, Cl^- or both. Previously, we have reported that NaCl treatment resulted in an accumulation of proline in roots of etiolated rice seedlings (Lin and Kao 1996). We also observed that NaCl-induced proline accumulation in rice roots of etiolated seedlings was mainly due to Na^+, rather than Cl^- (unpublished data). Thus, it is of great interest to know whether proline accumulation caused by NaCl in detached rice leaves is also due to Na^+, rather than Cl^-. To test this possibility, we determined the effect of 4,4′-diisothiocyano-2,2′-disulfonic acid (DIDS), a nonpermeating amino-reactive disulfonic acid known to inhibit the uptake of Cl^- (Lin 1991), on NaCl-induced proline accumulation in detached rice leaves. If Cl^- plays no role in proline accumulation in detached rice leaves treated with NaCl, then addition of DIDS is expected to have

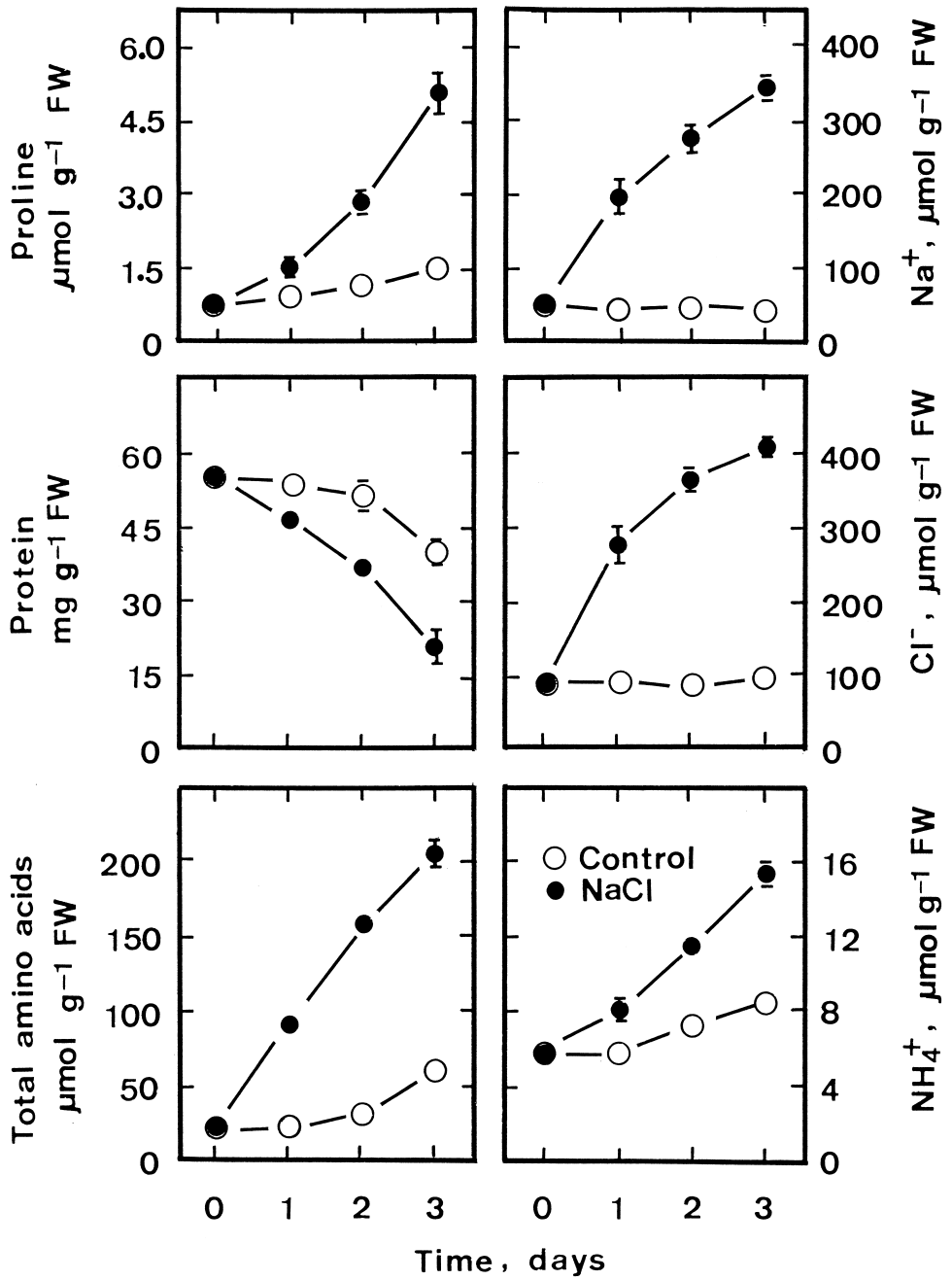

Figure 2. Time courses of the NaCl effect on Na^+, Cl^-, protein, proline, total amino acid, and NH_4^+ contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

no effect on proline accumulation. Results (Figure 3) show that DIDS decreased Cl^- content without affecting Na^+ content, and also decreased proline content in NaCl-treated detached rice leaves. Thus, it seems that both Na^+ and Cl^- are involved in the increase in proline content induced by NaCl in detached rice leaves. No or only slight difference in RWC was observed between NaCl-treated leaves and control leaves (Table 1), suggesting that the osmotic effect is unlikely to be a major factor contributing to the accumulation of proline in detached rice leaves treated with NaCl. This suggestion is supported further by the

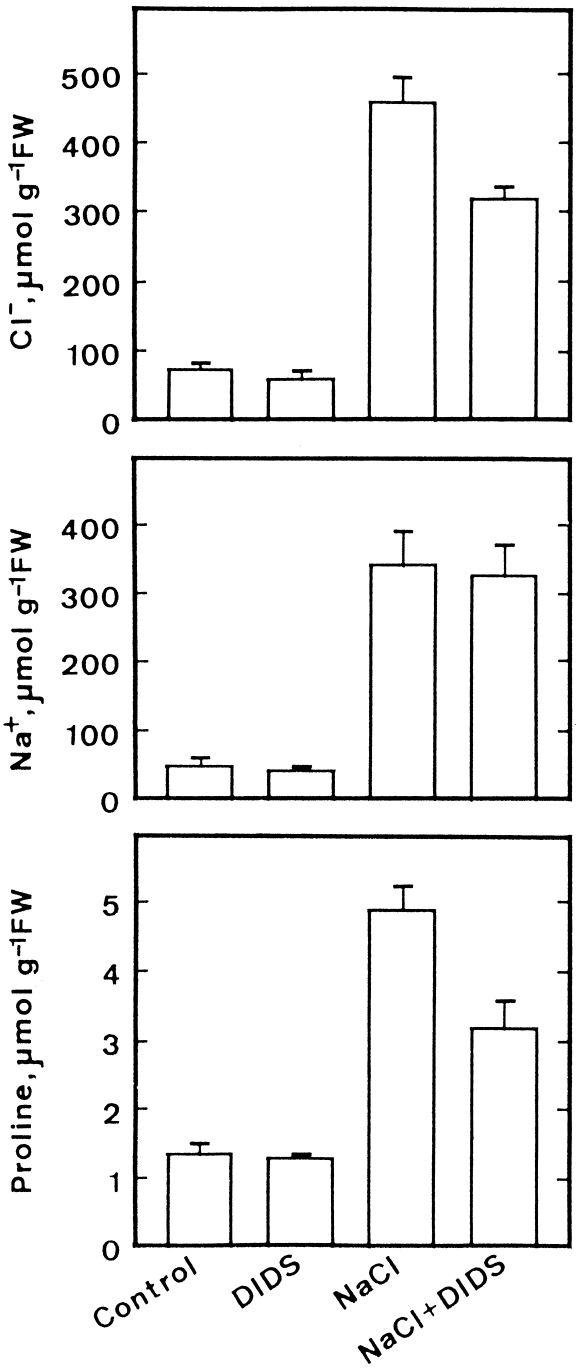

Figure 3. Effect of NaCl and 4,4′-diisothiocyano-2,2′-disulfonic acid (DIDS) on Na^+, Cl^- and proline contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM). Measurements were made after 3 days of treatment in the light. Vertical bars represent standard errors (n = 4).

observations that detached rice leaves treated with sorbitol at the concentration iso-osmotic with 200 mM NaCl had much lower proline contents than those treated with 200 mM NaCl and significant proline accumulation was mainly due to the presence of NaCl (Table 2). The fact that NaCl has no effect on RWC is most likely due to a certain amount of osmotic adjustment caused by the higher contents of Na^+ and Cl^- in NaCl-treated detached rice leaves (Figure 2).

Table 1. Effect of NaCl on relative water content (RWC) in detached rice leaves

Time (days)	RWC (%)	
	Control	NaCl
0	99.8 ± 4.0	
1	98.2 ± 2.7	96.0 ± 2.1
2	98.4 ± 2.7	94.4 ± 2.1
3	96.6 ± 2.9	91.2 ± 3.2

Detached rice leaves were incubated in sodium phosphate buffer (5 mM, pH 7.0) in the presence or absence of NaCl (200 mM). Means ± S.E. (n = 4).

Table 2. Effect of the concentration of NaCl and sorbitol on the proline content in detached rice leaves

Treatment	Proline, μmol g^{-1} FW
Control	1.06 ± 0.03
Sorbitol, 400 mM	1.53 ± 015
Sorbitol, 300 mM + NaCl, 50 mM	1.98 ± 0.05
Sorbitol, 200 mM + NaCl, 100mM	3.40 ± 0.64
NaCl, 200 mM	4.88 ± 0.56

The osmotic potential was kept equivalent to that of medium with 200 mM NaCl by replacing NaCl by sorbitol. Sorbitol and NaCl wer dissolved in sodium phosphate buffer (5 mM, pH 7.0). Proline was determined after 3 days of treatment in the light. Means ± S.E. (n = 4).

The ammonium ions are a central intermediate in the metabolism of nitrogen in plants. Ammonium ions have been shown to accumulate in detached rice leaves subjected to water stress (Lin and Kao 1998). Proline accumulation is often considered to be involved in stress resistance mechanisms, although its precise role continues to be controversial (Hare et al. 1999; Rhodes et al. 1999; Yoshiba et al. 1997). Based on data in the literature, Rabe (1990) postulated that any stress condition causing reduced growth or impaired plant health will result in ammonium ion accumulation early in the stress period and suggested that the detoxification process in which excess ammonium ions in the cells is removed results in the accumulation of nitrogen-containing compounds, such as putrescine and proline. In recent studies, we found that exogenous NH_4Cl and methionine sulfoximine,

which caused an accumulation of ammonium ions in detached rice leaves, increased proline content (Yang and Kao 1999). In the present investigation, we also found that NaCl treatment resulted in an ammonium ion accumulation (Figures 1 and 2).

The decrease in protein content was faster in NaCl-stressed detached rice leaves than in control leaves (Figure 2). Therefore, protein degradation might contribute to NaCl-induced proline accumulation in detached rice leaves. This suggestion is supported further by the observation that total amino acids were higher in detached rice leaves exposed to NaCl than control leaves (Figure 2). However, Lutts et al. (1999) found that proline accumulation in rice (cv. I Kong Pao) plants was not related to proteolysis. It is generally considered that glutamic acid, ornithine and arginine can all contribute to the accumulation of proline (Chiang and Dandekar 1995). The results of Figure 4 show that NaCl treatment resulted in an increase in glutamic acid, ornithine and arginine contents in detached rice leaves. The increase in glutamic acid, ornithine and arginine contents in NaCl-treated rice leaves is most likely to be a result of protein degradation.

Glutamine synthetase (GS) catalyses the conversion of ammonium ion and glutamic acid to glutamine (Ireland and Lea 1999). Figure 5 shows that NaCl markedly decreased GS activity in detached rice leaves. Thus, the possibility that the increase in ammonium ion and glutamic acid contents (Figures 1, 2 and 4) in detached rice leaves treated with NaCl is due to NaCl-inhibited GS activity cannot be excluded.

To determine the role of the biosynthetic pathways for proline accumulation caused by NaCl, the effect of NaCl on OAT and P5CR activities was examined. The results are shown in Figure 6. OAT activity, expressed either on per g FW or per mg protein basis, was higher in NaCl-treated leaves than in control leaves. However, P5CR activity in NaCl-treated leaves was lower than that in control leaves (Figure 6). Thus, the increase in OAT activity may have, to some extent, contributed to the elevated content of proline by NaCl. An increase in OAT activity along with an increase in the content of proline in plants under salt stress has been reported (Lutts et al. 1999; Madan et al. 1995). The results of the present investigation are consistent with those of La Rosa et al. (1991); Lutts et al. (1999), who reported that elevated accumulation of proline under NaCl stress is not due to increase in P5CR. P5C synthetase is also involved in proline biosynthesis (Yoshiba et al. 1997). Thus,

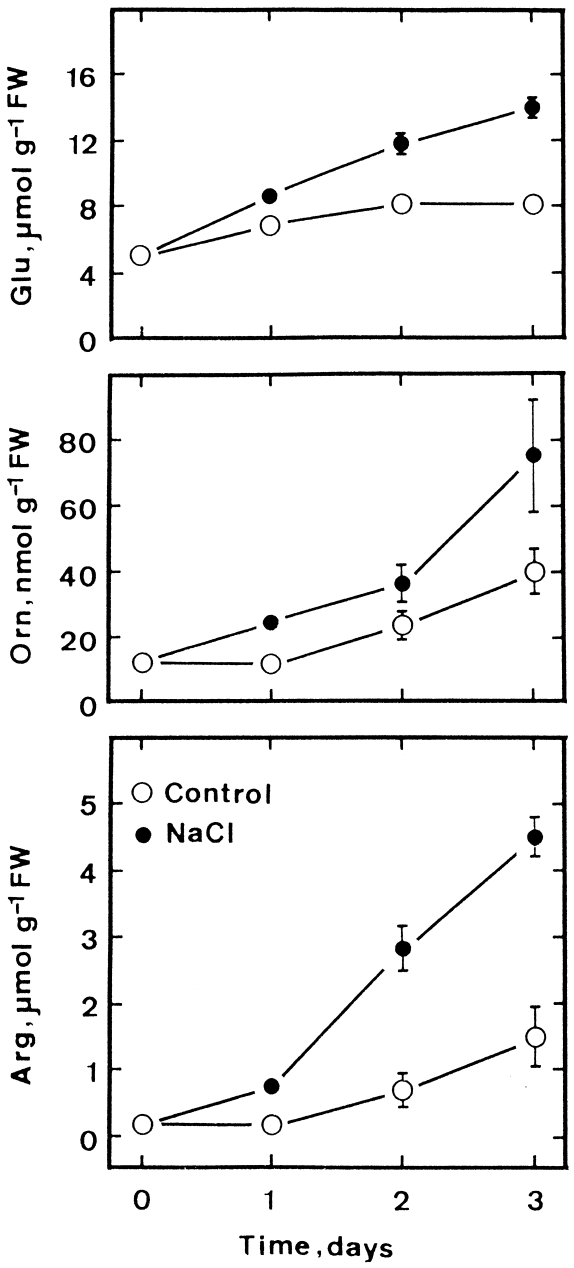

Figure 4. Time courses of the NaCl effect on glutamic acid (Glu), ornithine (Orn), and arginine (Arg) contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

NaCl could also have an effect on this enzyme. However, for unknown reasons, we failed to detect any P5C synthetase activity in crude extracts. Thus, this enzyme was not considered in the present investigation. The enzyme PDH is reported to catalyze proline oxidation (Yoshiba et al. 1997). In the present inves-

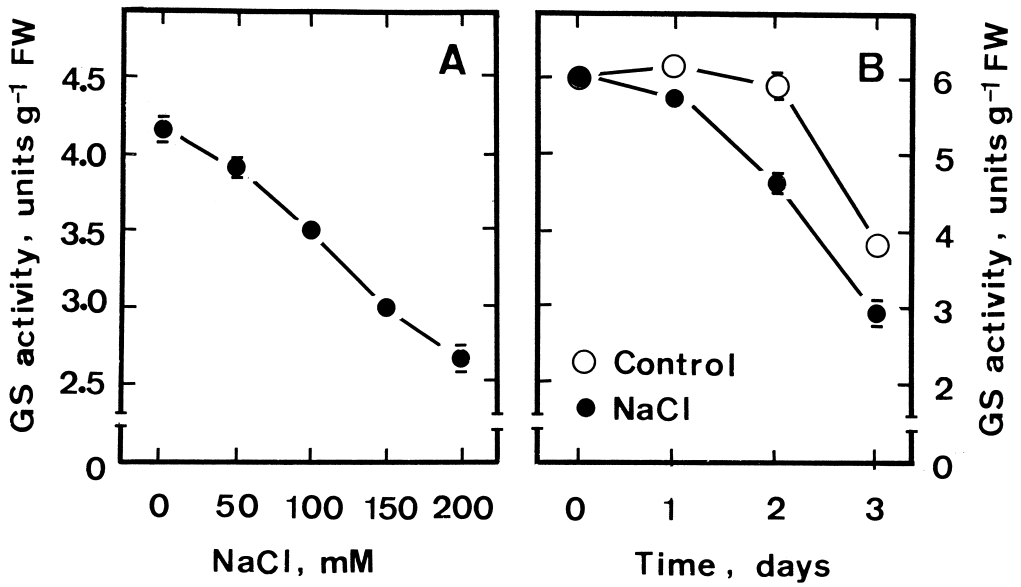

Figure 5. (A)Effect of NaCl concentrations on glutamine synthetase (GS) activity in detached rice leaves; (B) Changes in GS activity in detached rice leaves treated with 200 mM NaCl. GS activity in (A) was determined after 3 days of treatment in the light. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence of absence of NaCl. Vertical bars represent standard errors (n = 4).

tigation, NaCl treatment resulted in a decrease in PDH activity in detached rice leaves (Figure 6). This result suggests that proline oxidation (or degradation) contributes to proline accumulation in detached rice leaves exposed to NaCl. Sudhakar et al. (1993) also reported that PDH was inhibited to a greater extent in green gram seedlings under salt stress.

Recently, we demonstrated that proline in detached rice leaves under dark conditions and exposed to water stress is utilised less than in the control treatment (Yang et al. 1999, 2000). It is possible that less utilisation of proline may also contribute to the accumulation of proline in detached rice leaves caused by NaCl. To test this possibility, detached rice leaves were pretreated with ornithine for 3 h in the light to increase endogenous proline content and then transferred to phosphate buffer in the presence or absence of 200 mM NaCl for 8 h in the light; proline contents were then determined. As indicated in Table 3, the proline content was lower in the absence of NaCl than in the presence of NaCl, suggesting that proline in detached rice leaves treated with NaCl is utilised less than in the absence of NaCl.

Of particular interest in the reported investigation is the finding that both proline and ammonium ion accumulations were observed in detached rice leaves treated with NaCl (Figures 1 and 2). To test the causal relationship between ammonium ion accumulation and proline accumulation in detached rice leaves caused by NaCl, a short term experiment was conducted. The results are shown in Figure 7. The accumulation of ammonium ions caused by NaCl was evident at 4 h after treatment. Proline content decreased slightly in control leaves during the first 10 h of incubation in the light. Significant proline accumulation in detached rice leaves was observed at 8 h after NaCl treatment. It is obvious that ammonium ion accumulation preceded proline accumulation in NaCl-treated detached rice leaves, suggesting that ammonium ion accumulation is involved in regulating proline accumulation during NaCl stress. This suggestion is supported further by the observations that (a) NH_4Cl, similar to NaCl treatment, resulted in an increase in OAT activity and an inhibition in PDH activity in detached rice leaves (Lin and Kao 2001) and (b) proline in NH_4Cl- or methionine sulfoximine-treated rice leaves is less utilised than control leaves (Yang et al. 1999), a finding in the same way that NaCl did (Table 3).

In conclusion, our current results suggest that proline accumulation in detached rice leaves caused by NaCl is related to protein hydrolysis, an increase in OAT activity, a decrease in PDH activity, and a decrease in proline utilisation. Evidence is also provided to show that ammonium accumulation is involved in

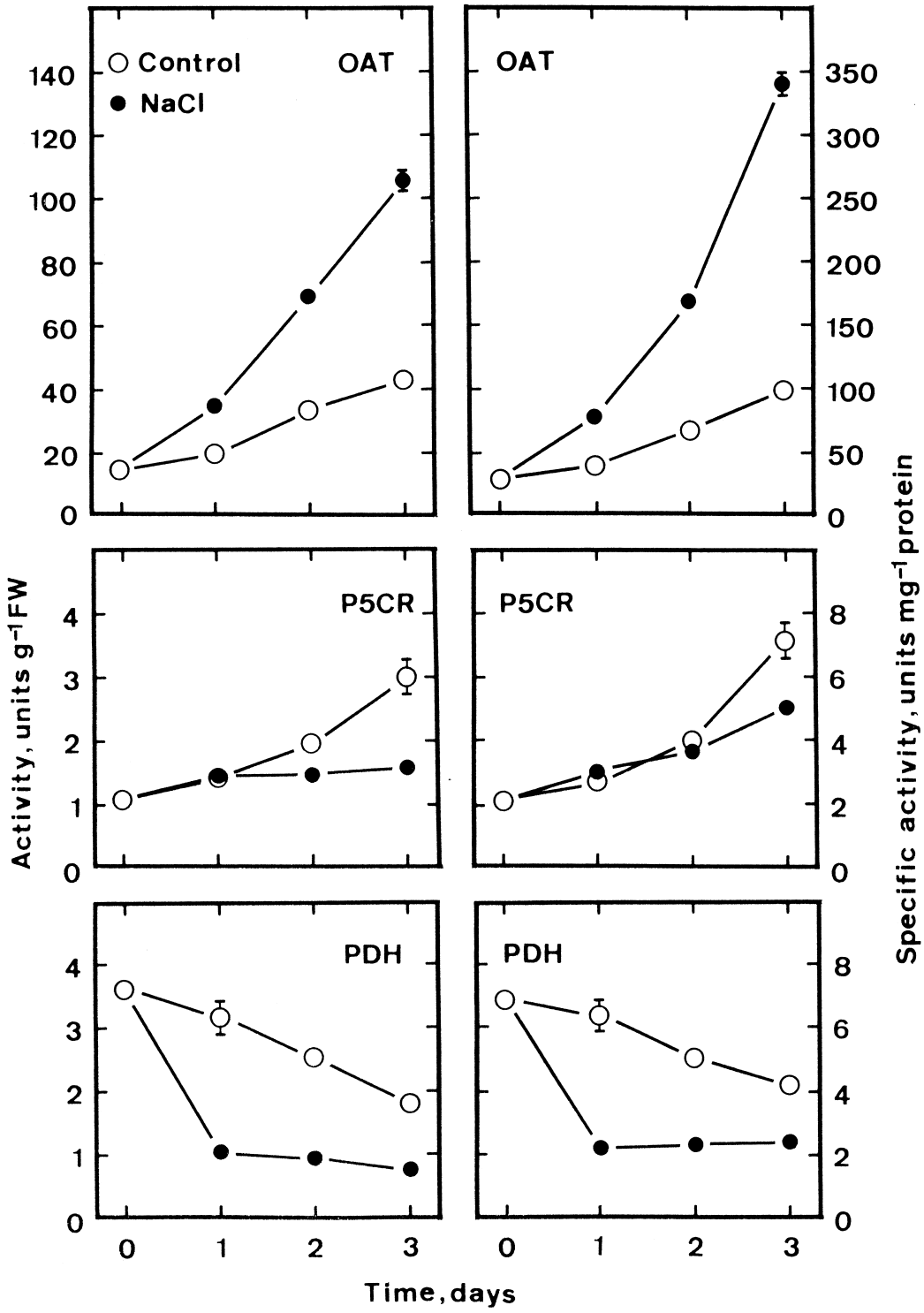

Figure 6. Time courses of the NaCl effect on the activities or specific activities of Δ^1-pyrroline-5-carboxylate reductase (P5CR), ornithine-δ-aminotransferase (OAT), and proline dehydrogenase (PDH) in detached rice leaves. Detached rice leaves were incubated in 50 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

Table 3. Proline content in ornithine-pretreated detached rice leaves incubated in sodium phosphate buffer in the presence or absence of NaCl

Treatment	Proline, μmol g^{-1} FW
H_2O, 3 h	0.77 ± 0.17
Ornithine, 3 h	27.13 ± 0.68
Ornithine, 3 h → Control, 8 h	14.14 ± 1.35
Ornithine, 3 h → NaCl, 8 h	18.27 ± 1.12

Detached rice leaves were pretreated with 50 mM ornnithine for 3 h in the light and then incubated in sodium phosphate buffer (5 mM, pH 7.0) in the presence or absence of NaCl (200 mM) for 8 h in the light. Means ±S.E. (n = 4).

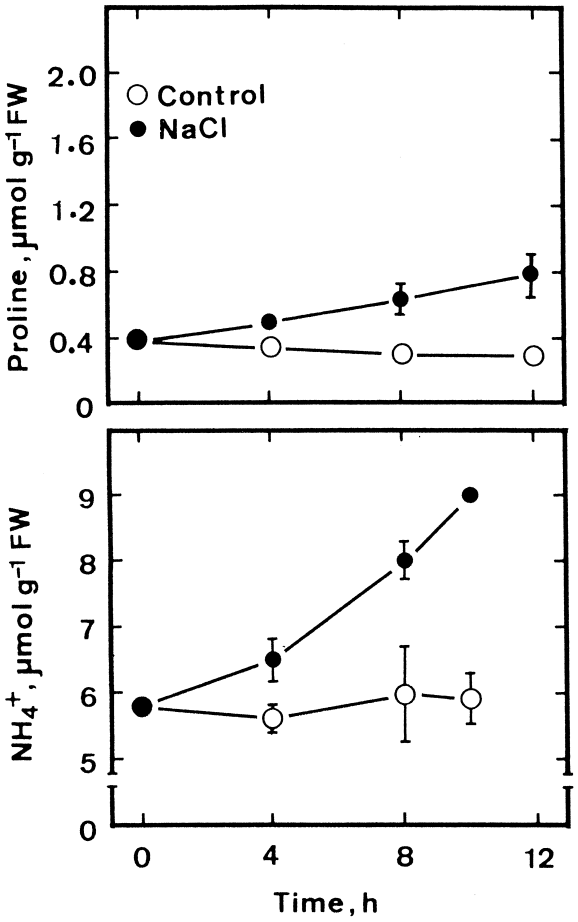

Figure 7. Effect of NaCl on proline and NH_4^+ contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

proline accumulation in detached rice leaves exposed to NaCl.

It has been shown that NaCl-inhibited root growth of etiolated rice seedlings is associated with proline accumulation and exogenously applied proline inhibits root growth (Lin and Kao 1996). The physiological significance of proline accumulation in intact rice leaves is not fully understood. Studying the effect of NaCl stress on enzyme activities involved in proline metabolism in intact rice leaves could provide valuable information on the physiological significance of its accumulation. Regulation of proline accumulation in detached rice leaves under NaCl stress as we reported here is not necessarily similar to that in intact rice leaves. However, the results of the present work do provide some basic information which should be valuable for our future studies.

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