# 行政院國家科學委員會補助專題研究計畫 ☑成果報告 □期中進度報告

## 氯化鈉對水稻幼苗生理作用影響之研究 [1/3]
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計畫主持人：高景輝

共同主持人：

計畫參與人員：


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執行單位：台大農藝系


中　華　民　國　93　年　8　月　10　日

# 行政院國家科學委員會專題研究計畫成果報告

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## 一、中文摘要

本研究以台中在來一號水稻為材料，探討 (一) 氯化鈉抑制水稻幼苗根生長之部位，(二) $Na^+$ 與 $Cl^-$ 對氯化鈉抑制水稻幼苗根生長之相對重要性，(三) 銨離子累積與氯化鈉加速水稻葉片老化間之關係，(四) 氯化鈉誘導水稻葉片脯胺酸累積之機制。研究結果顯示氯化鈉抑制根之生長侷限於根尖端部位與氯化鈉抑制根之生長與 $Na^+$ 而非 $Cl^-$ 有關。氯化鈉可促進葉片之老化與銨離子之累積。氯化鈉所誘導之銨離子累積是由於硝酸鹽離子還原作用之加速以及 glutamine synthetase (GS) 活性之降低所造成。氯化鈉導致之銨離子累積早於葉片老化之促進。外加氯化銨處理亦可加速葉片老化。這些結果說明銨離子累積可能與氯化鈉造成葉片老化加速有關。氯化鈉所造成之脯胺酸累積與蛋白質水解，ornithine-δ-aminotransferase 活性增加與 proline dehydrogenase 活性降低，以及脯胺酸利用之降低有關。結果也顯示氯化鈉增加脯胺酸含量之同時，亦可造成銨離子之累積。

關鍵詞：氯化鈉、生長、老化、脯胺酸、水稻

**Abstract：**

The present work studied (1) the localization of NaCl-inhibited root growth of rice seedlings, (2) the relative importance of $Na^+$ and $Cl^-$ in NaCl-inhibited root growth of rice seedlings, (3) the relationship between $NH_4^+$ accumulation and NaCl-induced proline accumulation in rice leaves. Results demonstrated that NaCl-inhibited root growth was mainly localized in the apical part of roots and NaCl-inhibited root growth was attributable to $Na^+$ rather than $Cl^-$. NaCl was effective in promoting senescence and in increasing $NH_4^+$ content of detached rice leaves. NaCl-induced $NH_4^+$ accumulation was due to enhanced nitrate reduction and decreased GS activity. Exogenous $NH_4Cl$, which caused an accumulation of $NH_4^+$ in detached rice leaves, also promoted senescence. It was found that increase in $NH_4^+$ content preceded the occurrence of senescence caused by NaCl. The current results suggest that $NH_4^+$ accumulation is linked to NaCl-induced leaf senescence. Proline accumulation caused by NaCl was related to proteolysis, an increase in ornithine-δ-aminotransferase activity, a decrease in proline dehydrogenase activity, and a decrease in proline utilization. Results also show that proline accumulation caused by NaCl was associated with $NH_4^+$ accumulation.

**Key words:** NaCl, growth, senescence, proline, *Oryza sativa*

二、緣由與目的：

土壤中鹽分濃度過高，抑制根的生長，是廣泛存在的事實。然而，生長抑制之機制目前還不清楚。本研究室的研究結果，顯示氯化鈉抑制水稻根之生長與過氧化氫含量增加及細胞壁過氧化

酵素活性之增加有關 (Lin and Kao 1999，Lin and Kao 2001a，2001b)，顯示細胞壁硬化是一可能的機制。氯化鈉抑制水稻根生長的部分是否侷限在某一部位，抑或是影響整個根，目前尚不清楚，此為可以探討的問題。氯化鈉對根生長之影響可能是 $Na^+$，$Cl^-$ 或是二者。本計畫亦對 $Na^+$ 及 $Cl^-$ 對根生長的重要性加以探討。

鹽分會加速葉片老化，但其影響之機制並不清楚。近年來本研究室之研究，顯示銨離子之累積與水稻葉片老化有關 (Chen and Kao 1997，Chen et al. 1997)。氯化鈉加速水稻葉片老化之機制是否是經由銨離子累積所造成，應是一有趣的研究問題。

鹽分逆境造成脯胺酸累積是一極普遍的現象。脯胺酸累積可經由蛋白質水解之增加，脯胺酸合成之增加，脯胺酸分解之降低，脯胺酸利用之降低，以及合成脯胺酸之前身物之增加而造成。鹽分造成水稻葉片脯胺酸累積之機制瞭解的不多，因此極具探討之價值。

本研究計畫基本上是延續過去三年之研究，探討水稻氯化鈉逆境相關之問題，將於三年完成。第一年著重於瞭解氯化鈉對水稻幼苗根不同部位之生長之影響。同時也探討 $Na^+$ 與 $Cl^-$ 對氯化鈉所抑制根生長之相對重要性。第二年則進行瞭解銨離子累積與氯化鈉加速水稻葉片老化間之關係。第三年則希望瞭解氯化鈉誘導水稻葉片脯胺酸累積之機制。

三、結果與討論

本研究部份成果已發表在 SCI 期刊 (Lin and Kao 2001c，Lin et al. 2002)，有興趣之學者可逕行參考。

圖 1 的結果明顯指出氯化鈉對根之生長抑制是侷限在尖端部位。雖然根尖端與基部之部位含有等量的 $Na^+$ 與 $Cl^-$，但氯化鈉只能顯著的增加根尖端部位之

過氧化氫、脯胺酸及銨離子含量與細胞壁過氧化酵素活性 (圖 2)。這些結果說明氯化鈉抑制生長的部位侷限在尖端。

以 sodium gluconate 處理，水稻根僅吸收鈉離子，但其抑制根生長的程度與氯化鈉處理相同 (圖 3)。氯化鈉處理會使水稻根之脯胺酸累積、銨離子累積、離子鍵結型式的細胞壁過氧化酵素活性增加以及過氧化氫含量增加，sodium gluconate 亦會誘致這些生理變化 (圖 4)。這些結果說明，$Na^+$ 而非 $Cl^-$ 是與根生長抑制有關。

葉片老化係以葉綠素與蛋白質含量之降低為指標。研究結果顯示，氯化鈉所誘導的水稻切離葉片銨離子累積與葉片老化之加速有關。主要的證據為 (一) 氯化鈉所誘導之銨離子含量增加，早於葉片老化 (圖 5) 與 (二) 外加氯化銨處理可加速葉片老化 (圖 6)。氯化鈉所誘導銨離子累積主要是由於硝酸鹽離子還原之加速及 glutamine synthetase (GS) 之活性下降所造成 (圖 7)。

氯化鈉處理所造成之脯胺酸含量之增加，伴隨著蛋白質含量之減少與總胺基酸含量之增加 (圖 8)，顯示蛋白質之水解可能會造成脯胺酸含量之增加。氯化鈉處理亦可造成水稻切離葉片銨離子含量 (圖 8) 以及脯胺酸合成前身物 glutamate (Glu)，ornithine (Orn) 與 arginine (Arg) 之增加 (圖 9)。脯胺酸合成酵素 ornithine-δ-aminotransferase (OAT) 與 Δ′-pyrroline-5-carboxylate reductase (P5CR) 活性，亦可因氯化鈉處理而增加，但是氯化鈉處理會降低脯胺酸分解酵素 proline dehydrogenase (PDH) 之活性 (圖 10)。脯胺酸之利用亦可影響脯胺酸之含量。表 1 顯示氯化鈉可抑制脯胺酸之利用。

## 四、計畫成果與自評

整個計畫執行順利，達到預期效果。本計畫成果可使本研究室對水稻氯化鈉生理有更深一層

之瞭解。

## 五、參考文獻


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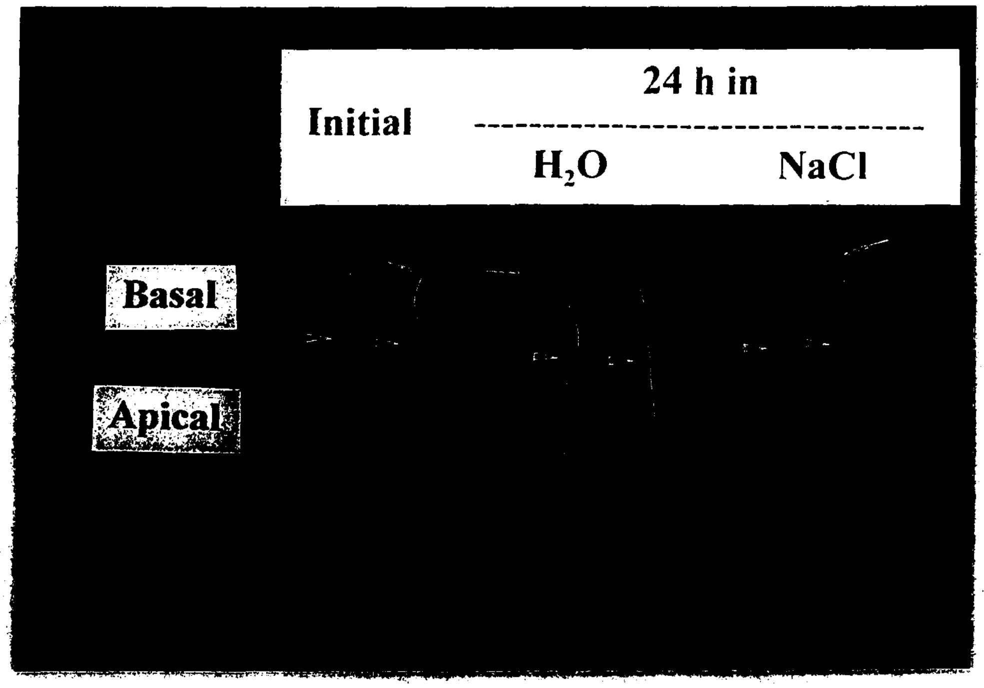


Figure 1. Effect of NaCl on the growth of apical and basel parts of rice roots.

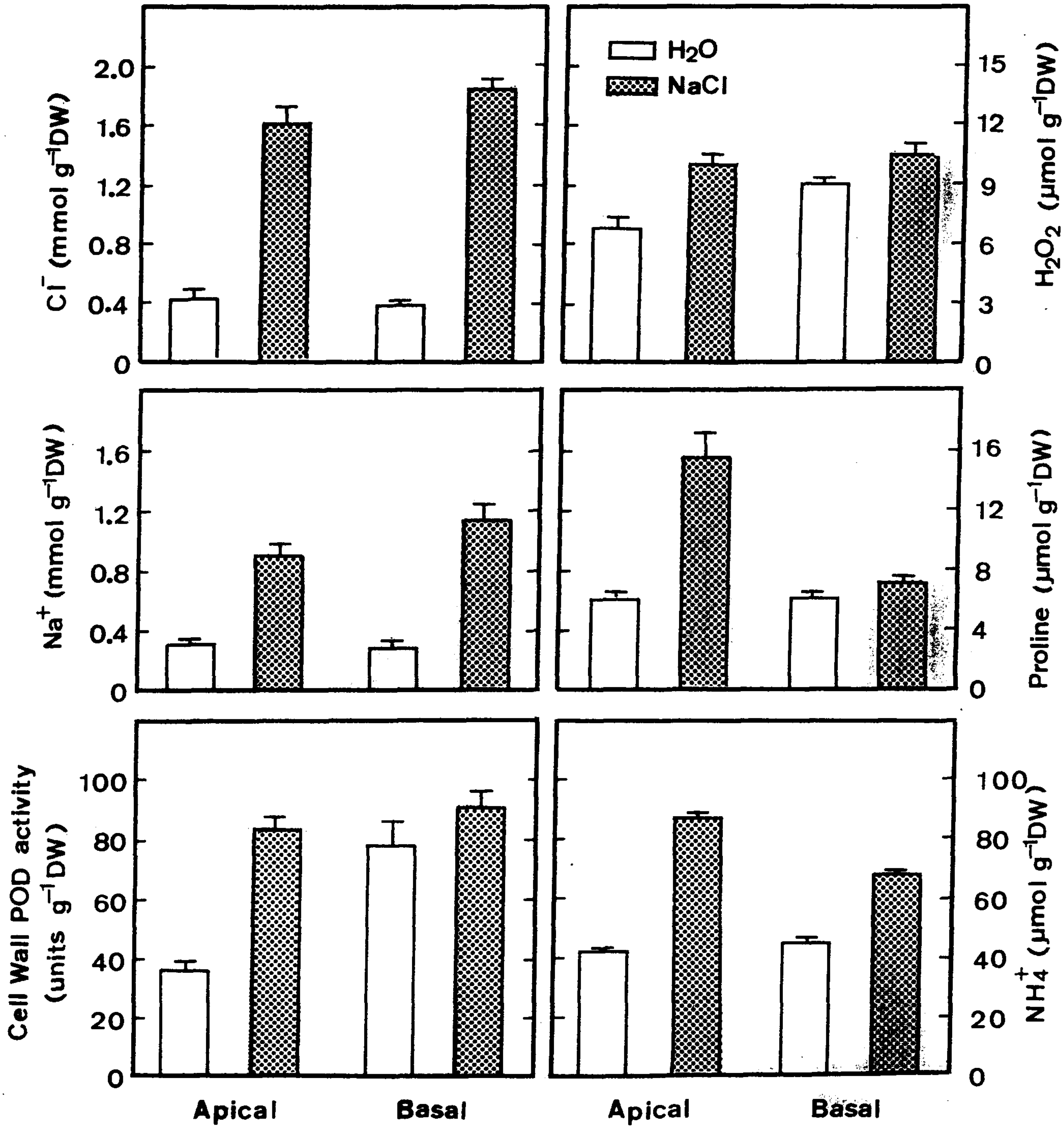


Figure 2. Effect of NaCl on $Cl^-$ and $Na^+$ contents, cell wall peroxidase (POD) activity, and $H_2O_2$, proline and $NH_4^+$ contents in the apical and basel parts of rice seedlings. All measurements were made 24 h after treatment.

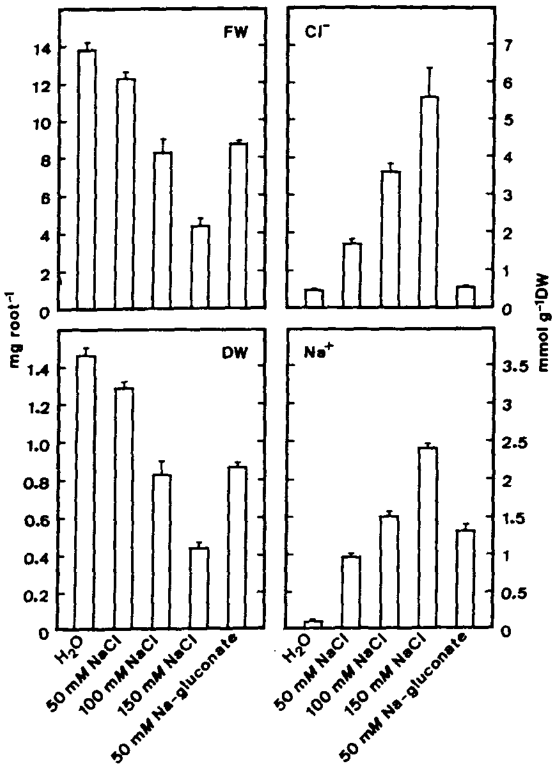


*Figure 3.* Effects of NaCl and Na-gluconate on root growth and $Na^+$ and $Cl^-$ levels in roots of rice seedlings. Root growth and $Na^+$ and $Cl^-$ levels were measured after 5 days of treatment. Bars represent standard errors (*n*=4).

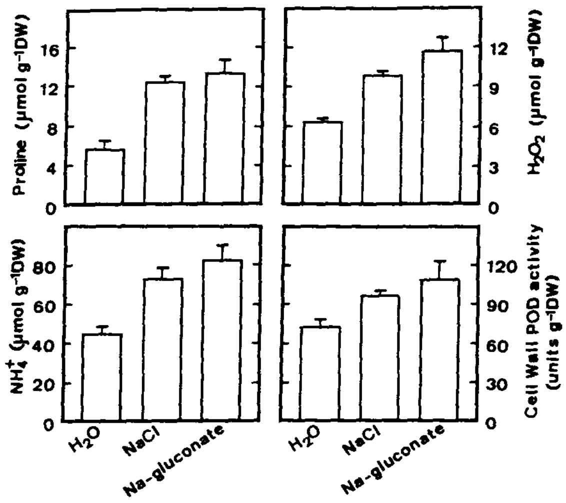


*Figure 4.* Effects of NaCl (100 m*M*) and Na-gluconate (50 m*M*) on ammonium, proline and $H_2O_2$ levels, and cell wall POD activity in roots of rice seedlings. All measurements were made after 5 days of treatment. Bars represent standard errors (*n*=4).

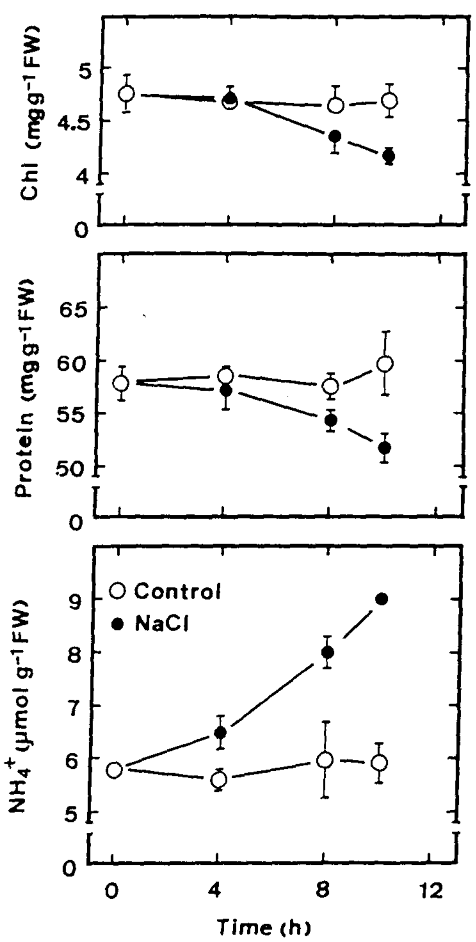


*Figure 5.* Time courses of NaCl effect on the contents of Chl, protein, and $NH_4^+$ in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) with or without NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

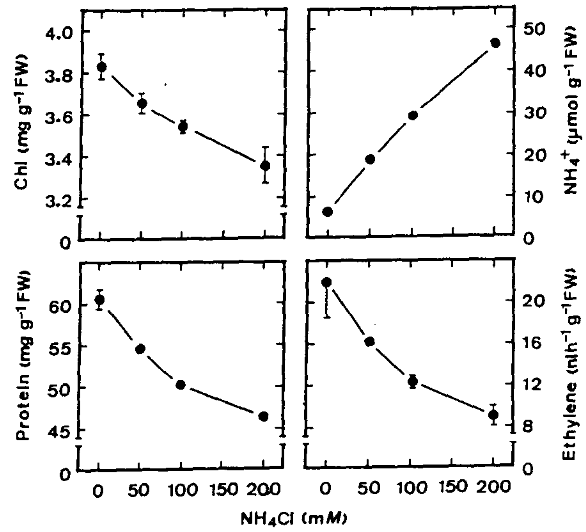


*Figure 6.* Effect of $NH_4Cl$ on Chl, protein and $NH_4^+$ contents, and ethylene production in the detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence of NaCl (0–200 mM). Measurements were made 3 days after treatment in the light. Vertical bars represent standard errors (n = 4).

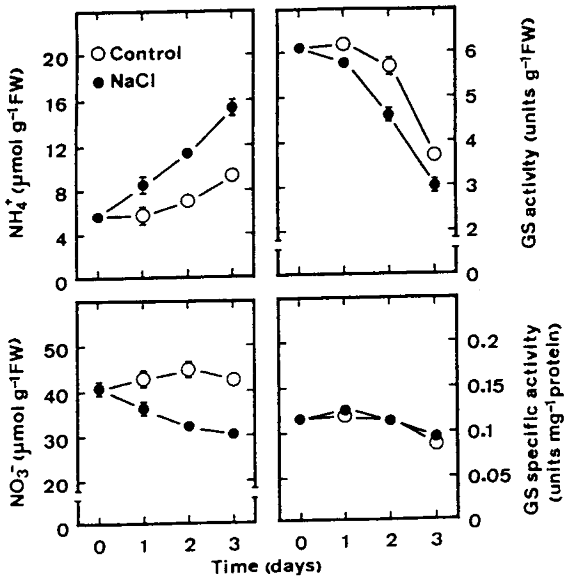


*Figure 7* Time courses of NaCl effect on the contents of $NH_4^+$ and nitrate, and activity and specific activity of GS in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) with or without NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

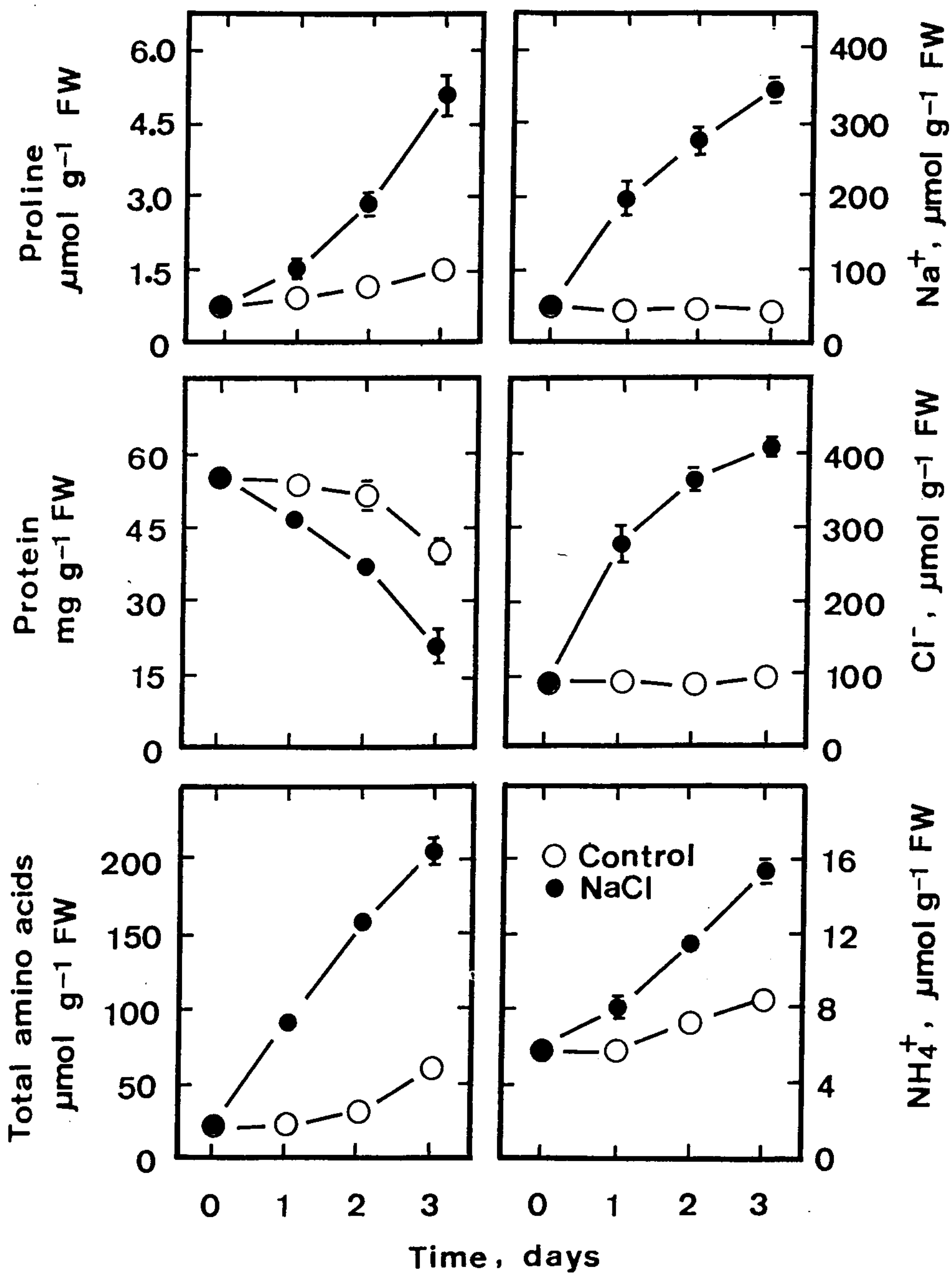


*Figure 8* Time courses of the NaCl effect on $Na^+$, $Cl^-$, protein, proline, total amino acid, and $NH_4^+$ contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

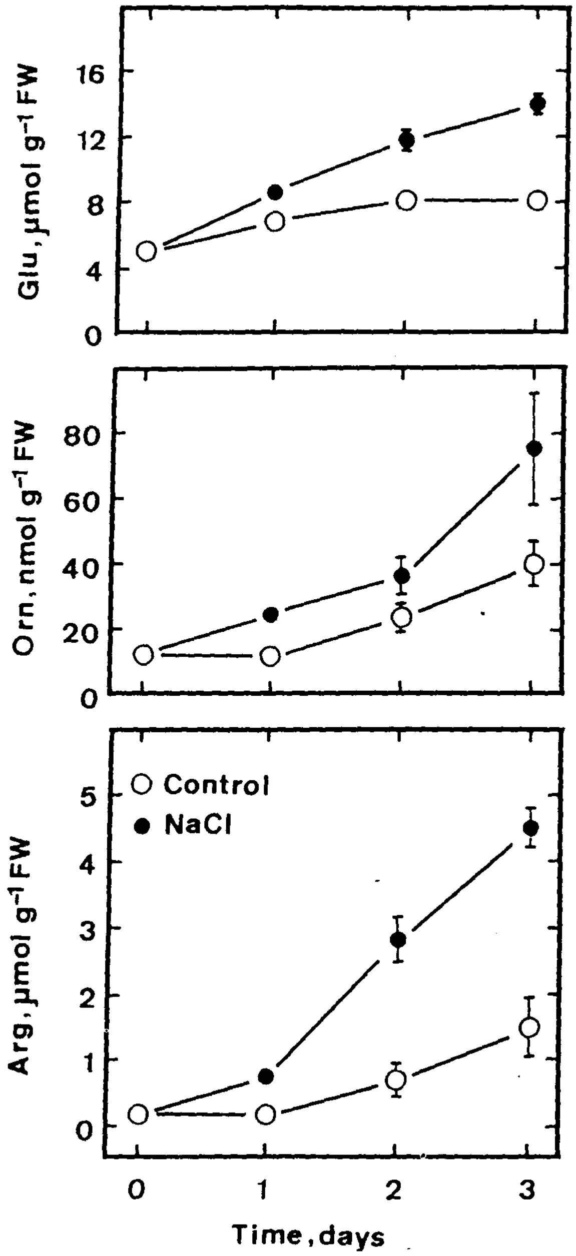


*Figure* 9 Time courses of the NaCl effect on glutamic acid (Glu), ornithine (Orn), and arginine (Arg) contents in detached rice leaves. Detached rice leaves were incubated in 5 mM sodium phosphate buffer (pH 7.0) in the presence or absence of NaCl (200 mM) in the light. Vertical bars represent standard errors (n = 4).

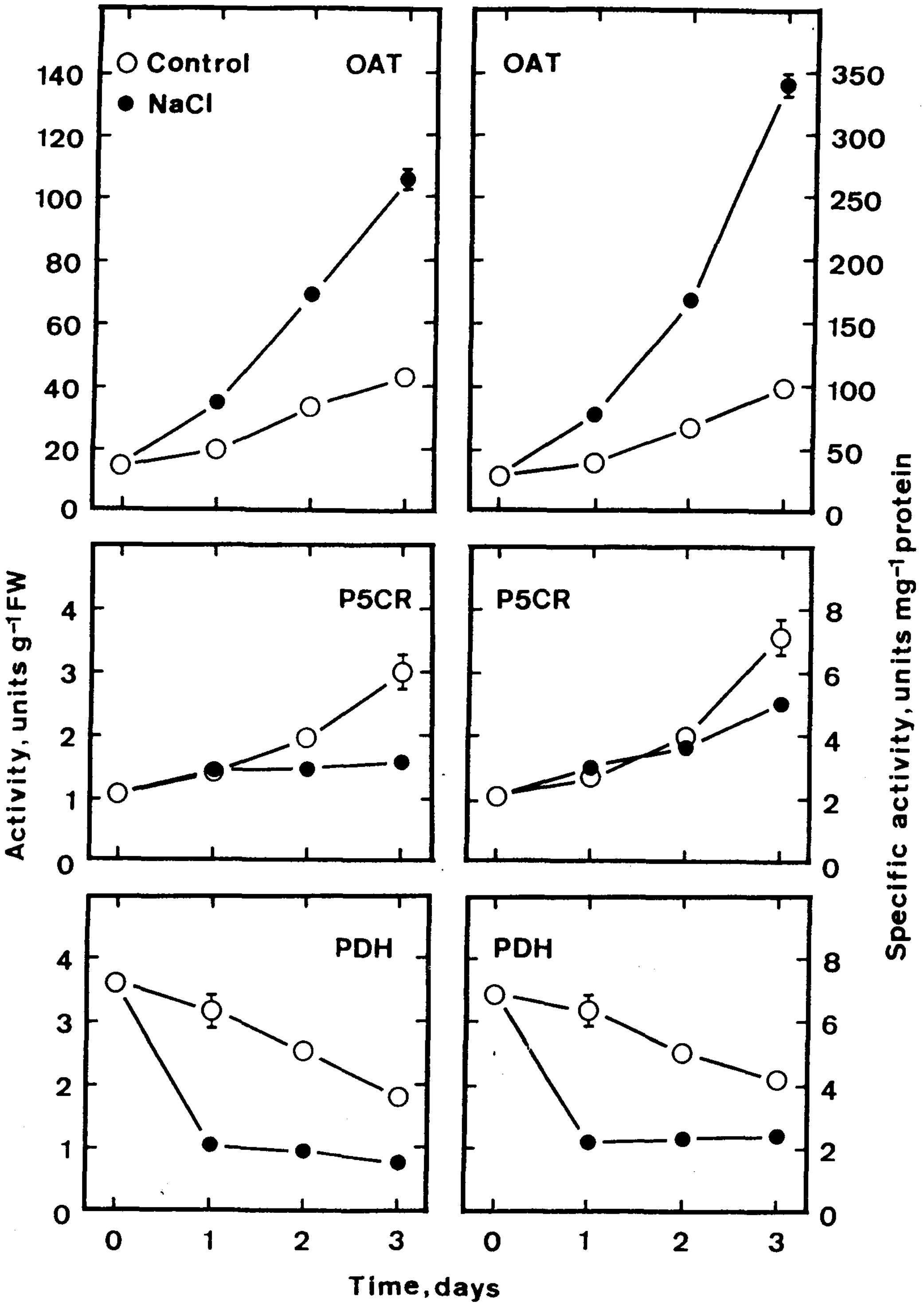


*Figure* [illegible] Time courses of the NaCl effect on the activities or specific activities of $\Delta^1$-pyrroline-5-carboxylate reductase (P5CR), ornithine-$\delta$-aminotransferase (OAT), and proline dehydrogenase (PDH) in detached rice leaves. Detached rice leaves were incubated in 50 mM sodium 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* [illegible]. Proline content in ornithine-pretreated detached rice leaves incubated in sodium phosphate buffer in the presence or absence of NaCl

| Treatment | Proline, $\mu$mol 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 omnithine 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).