內含出席國際學術會議心得報告及發表之論文各一份

行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC89-2313-B-002-111

執行期限：88 年 8 月 1 日至 89 年 7 月 31 日

主持人：高景輝　　執行機構及單位名稱：台大農藝系

一、中文摘要

本研究探討過量銅導致水稻切離葉片脯胺酸累積之機制。過量銅所誘導之脯胺酸累積與蛋白質水解增加，P5CR 及 OAT 活性增加有關，但與脯胺酸的利用及 PDH 與 P5CDH 活性無關。過量銅處理會增加 ABA 的含量，但不改變相對水分含量。

銨離子累積與脯胺酸累積間之關係，亦在本研究中一併探討。黑暗下，脯胺酸之累積伴隨著銨離子之累積。能夠使水稻切離葉片內銨離子含量增加之 NH_4Cl 與 MSO 處理，亦可增加脯胺酸含量。雖然鎘或銅處理可增加銨離子含量，但銨離子之增加發生在脯胺酸增加之後。

關鍵詞：銨離子、鎘、銅、水稻、脯胺酸、缺水逆境。

Abstract

Accumulation of proline in response to excess Cu was studied in detached rice leaves. Proline accumulation induced by excess Cu was related to proteolysis and an increase in P5CR or OAT activity and could not be explained by proline utilization or stress-induced modifications in PDA or P5CDH. $CuSO_4$ treatment resulted in an increase in ABA content but had no effect on RWC.

Ammonium accumulation in relation to proline accumulation in detached rice leaves under stress conditions was also investigated. Ammonium accumulation in dark-treated detached rice leaves preceded proline accumulation. Ammonium accumulation caused by water stress coincide closely with proline accumulation in detached rice leaves. Exogenous NH_4Cl and MSO, which caused ammonium accumulation in detached rice leaves, increased proline content.

Although ammonium content increased in Cd- and Cu-treated rice leaves, the increase in ammonium content was only observed after the increase in proline content.

Keywords: Ammonium, Cadmium, Copper, *Oryza sativa*, Proline, Water Stress

二、緣由與目的

植物遇到環境逆境，常會導致脯胺酸累積。高等植物中脯胺酸累積的主要路徑是以 glutamic acid 以及 ornithine 爲前驅物的合成路徑，其主要限制酵素爲 P5C synthetase，P5C reductase (P5CR) 與 ornithine δ-aminotransferase (OAT)。脯胺酸氧化路徑則是經由 proline dehydrogenase (PDH) 與 P5C dehydrogenase (P5CDH)二種酵素控制。蛋白質水解的增加以及脯胺酸利用的減少亦可使得脯胺酸含量增加。本研究即在瞭解過量銅所導致水稻切離葉片脯胺酸累積之控制機制。許多逆境下，植物會累積大量的銨離子。逆境下銨離子的累積是否控制脯胺酸的累積，目前並不清楚。本研究亦一併加以探討。

三、結果與討論

$CuSO_4$ 處理能有效的誘導水稻切離葉片脯胺酸之累積(圖 1)。過量銅處理不會改變切離葉片的相對水分含量，顯示銅所誘導之脯胺酸累積與缺水無關(圖 2)。有趣的是，過量銅處理會增加 ABA 含量(圖 2)。銅所誘導的脯胺酸累積與蛋白質水解(圖 3)，P5CR 與 OAT 活性之增加有關(圖 4)，但與脯胺酸之利用以及 P5CDH 與 PDH 活性無關(圖 5)。

NH_4Cl 處理切離葉片可增加銨離子及脯胺酸之含量(圖 6)。MSO 爲 glutamine synthetase 活性抑制劑，可增加切離葉片內銨離子含量，同時亦可增加脯胺酸含量(圖 7)。水稻切離葉片在黑暗下誘導脯胺酸累積之前，銨離子含量增加(圖 8)。缺水處理脯胺酸累積之同時伴隨著銨離子之累積(圖 9)。然而，鎘與銅所誘導之脯胺酸累積則早於銨離子之累積(圖 10)。

四、計畫成果自評

整個計畫執行順利，達到預期之成果。本計畫成果對水稻葉片在逆境下之脯胺酸累積控制有深入的瞭解。

五、參考文獻

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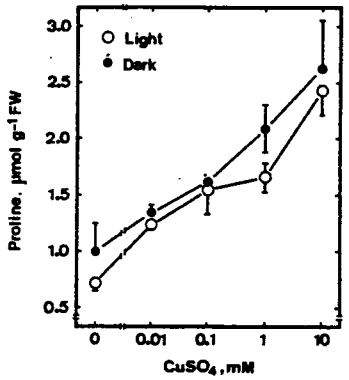

圖 1. $CuSO_4$ 處理對水稻切離葉片脯胺酸含量之影響。

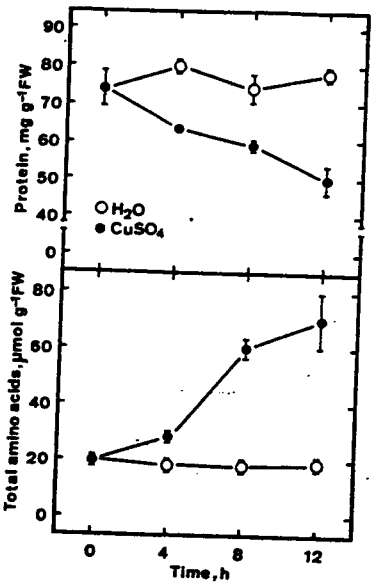

圖 3. $CuSO_4$ (10 mM) 處理對水稻切離葉片蛋白質與總胺基酸含量變化之影響。

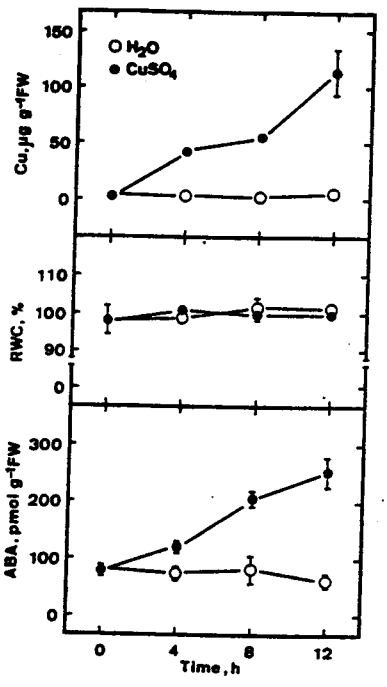

圖 2. $CuSO_4$ (10 mM) 處理對 Cu 含量，相對水分含量與 ABA 含量變化之影響。

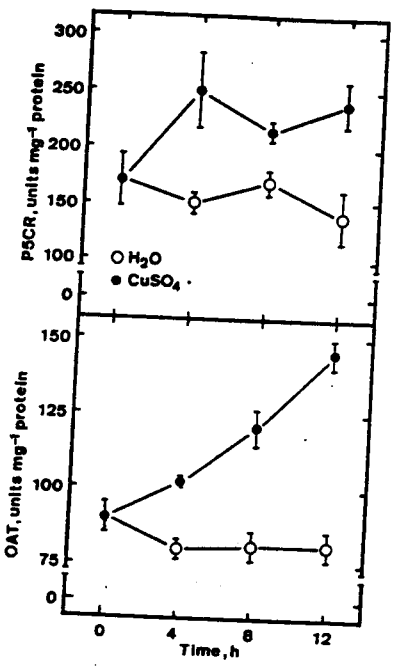

圖 4. $CuSO_4$ (10 mM) 處理對水稻切離葉片 P5CR 與 OAT 活性變化之影響。

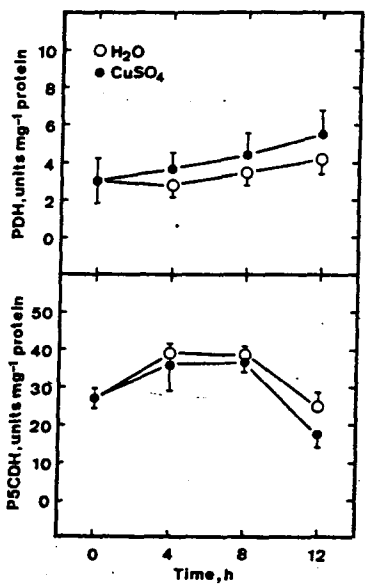

圖 5. $CuSO_4$ (10 mM) 處理對水稻切離葉片PDH與P5CDH活性變化之影響。

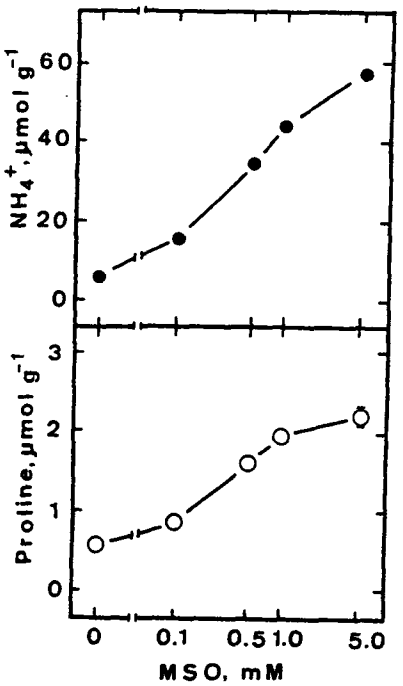

圖 7. MSO 處理對水稻切離葉片銨離子與脯胺酸含量之影響。

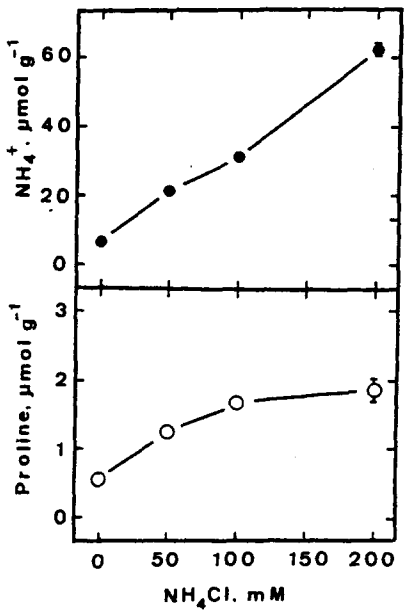

圖 6. NH_4Cl 處理對水稻切離葉片銨離子與脯胺酸含量之影響。

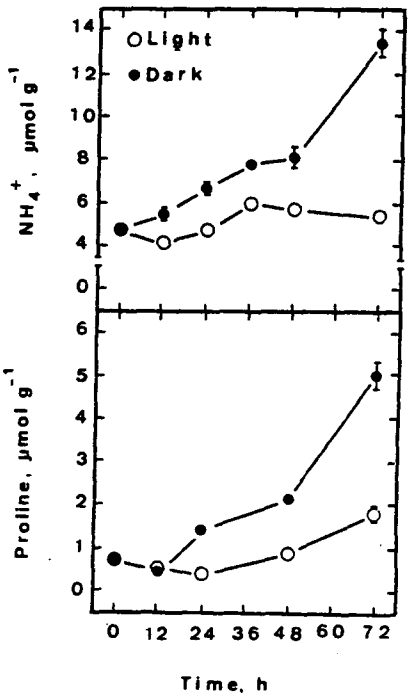

圖 8. 在黑暗與光線下對水稻切離葉片銨離子與脯胺酸含量之影響。

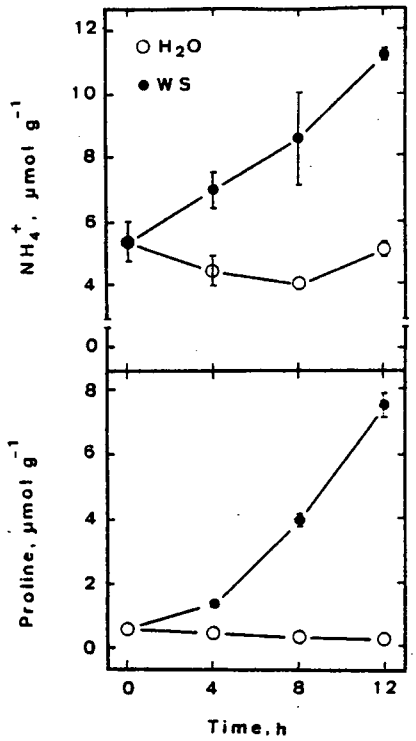

圖 9. 缺水處理對水稻切離葉片銨離子與脯胺酸含量之影響。

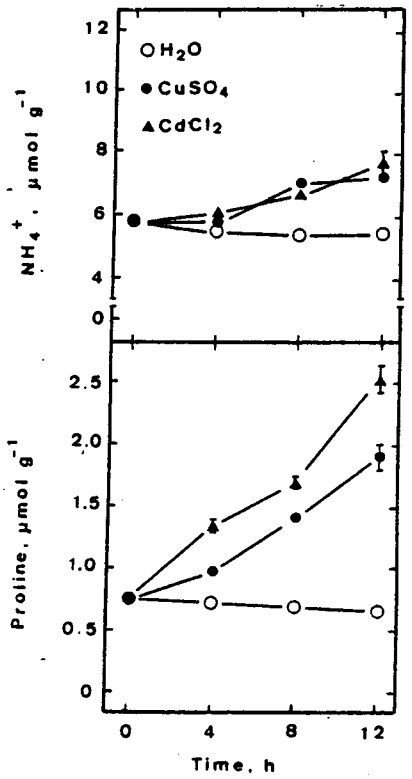

圖 10. $CuSO_4$ 與 $CdCl_2$ 處理對水稻切離葉片銨離子與脯胺酸含量之影響。

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

89年 8 月28 日

附件三

報告人姓名	高景輝	服務機構及職稱	台大生化科系 教授
會議時間地點	89年7月15日至7月19日 美國聖地牙哥	本會核定補助文號	NSC 89-2313-B002-111
會議名稱	（中文）二十一世紀植物生物學會議 （英文）Plant Biology 2000		
發表論文題目	（中文）氯化鈉誘導水稻根部之鍵結型過氧化酵素活性之變化 （英文）NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings		

報告內容應包括下列各項：

一、參加會議經過

二、與會心得

三、考察參觀活動（無是項活動者省略）

四、建議

五、攜回資料名稱及內容

一、前言

二千年植物生物學會議 (Plant Biology 2000) 係由美國植物生理學會 (American Society of Plant Physiologists) 所籌辦之年會。由於美國在植物生理學領域一直是屬領先與領導的地位，且會員涵蓋全世界各國植物生理學家，因此該年會實際上相當於國際性會議。

二千年植物生物學會議會期自 7 月 15 日至 19 日，為期五天。會議地點為美國加州聖地牙哥。參與會議代表約 1000 人，提出論文約有 1300 篇。

二、會議內容

會議期間每天上午 8 時自下午 6 時，安排演講與壁報展示。演講包括五場大型演講 (major symposium) 與 17 場小型演講 (minisymposium)。

五場大型演講主題分別為：

Evolution of photoreception

Leaf development

Redox regulation

Comparative plant genome

Plant through the millennia

十七場小型演講主題則包括：

Plant interaction with pathogens

Genomics: Structure & function

Abiotic stress I

Vegetative plant development

Social issue in GMOs

Algal physiology

Abiotic stress II

Hormone receptor & signaling

Targeting

Membrane transporters I

Genomics: Analysis

Enzymology

Membrane transporters II

Cell walls

Seed biology

Bioenergetics in photosynthesis

Guard cell signal transduction

壁報展示共有 1042 篇。本人研究的興趣爲環境逆境。提出參展的壁報題目爲 " NaCl changes in ionically bound peroxidase activity in roots of rice seedling "，該論文係執行國科會之專題計畫。主要報導氯化鈉抑制水稻根生長係經由過氧化酵素所促進之細胞壁硬化之結果。

三、與會心得

(一) 主辦單位精心籌畫，值得借鏡

每年的美國植物生理學會年會，大約在四、五年前，就已經決定會議的時間與地點。在年會的一年前，即訂下主題與會議初步的議程。在會議前半年開始接受會員們的論文摘要與註冊申請。今年正值 2000 年，主辦單位也應景特別安排了一場大型演講，討論 Plants through millennia。會場內精心安排，井然有序。近年來，國內也常舉辦相當多的研討會，會議的安排，相形之下，仍有相當改善的空間。美國植物生理學會籌畫會議

的流程，是值得我們仿傚的。

(二) 舊的問題，新的方法解決

分子生物技術與基因轉殖，已爲植物生理研究之趨勢。這次會議也是與以前的幾次會議一樣，許多報告是利用這些技術來解決過去無法解決的問題。過量表現 (overexpression) *DREBIA* 基因，可提高阿拉伯芥的耐凍能力。阿拉伯芥的 CBF 基因如在油菜過量的表現亦可增加耐凍性。轉殖棉花過量表現 sucrose phosphate synthase 可產生高品質的棉花纖維。過量表現 S-adenosylmethionine decarboxylase 基因或者過量表現 ACC synthase 與 ACC oxidase 基因，則植物多種環境逆境。

(三) 非生物性逆境 (abiotic stress) 仍爲熱門研究課題

植物之生長分化是遺傳因子與環境交感的整體表現。植物常會遭受生物性與非生物性之逆境。近年來，全球氣候環境變遷，工業發展之結果造成嚴重的環境污染，因此非生物性逆境的問題，漸趨嚴重，也使得植物生理學家對此問題之研究興趣。這次會議亦不例外，非生物性逆境生理與抗該逆境機制研究，仍然是熱門研究課題。涵蓋之範圍有凍害、臭氧、高二氧化碳、重金屬、紫外線、低溫、低氧等逆境。

(四) 基因調控之細胞死亡 (programmed cell death) 問題，從氧化逆境著手

植物發育過程中，細胞常會死亡，此死亡係基因調控。細胞死亡問題在此次會議中有多篇論文提出討論。討論重點都放在細胞死亡與氧化逆境有關，例如，GA 處理可導致糊粉層合成水

解酵素，同時亦可導致 H_2O_2 之形成，catalase 活性降低，而造成細胞死亡。而 ABA 不會誘導水解酵素形成，且不會導致細胞死亡。ABA 與 H_2O_2 同時處理則會導致細胞死亡。細胞死亡與氧化逆境間之關係，似乎是一個值得未來關注的研究方向。

(五) GMO 倫理問題，漸受重視

生物技術之廣泛利用，產生不少的轉殖植物，或者遺傳基因被修飾後之生物，此即所謂的 GMO (genetetically modified organism)。GMO 有其正面的效應，也有許多人士認爲有負面的問題。這之間所衍生的問題，已涉及倫理與管理問題。此問題在會議中也有相當多的論辯與討論。

(六) 與會大陸學者人數顯著增加，意義匪淺

在 1979 年前國際會議中，甚少有大陸學者參與。大陸與美國建交後，參與國際會議的大陸學者，逐年增加。令人意外的是，此次會議參加的大陸學者，已超越台灣學者甚多。值得注意的是，大陸學者所提出的論文品質都有極高的評價。是欣慰還是警惕？值得深思。

四、建議

每次參加國際會議得到的印象是，國外大學或研究機構與會的人員中，除教授級的研究人員外，尚有相當多的博士班學生或博士後研究人員。目前，國內研究環境已有相當改善，國科會似應更積極的鼓勵與補助博士後研究人員與博士班學生參與國際會議，補助條件儘量放寬，使得這些年輕的人員能有機會參與，增廣見聞，增加臨場討論經驗，與直接面對面的參與學術交流。

五、攜回資料名稱與內容

此次會議所攜回之資料為 Programs and Abstracts for Plant Biology 2000。且購買一本由美國植物生理學會首次編輯之參攷書 " Biochemistry & Molecular Biology of Plants "。

NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings

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Key words: NaCl, *Oryza sativa*, peroxidase, root growth

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Abstract

The changes in ionically bound peroxidase activity in roots of NaCl-stressed rice seedlings and their correlation with root growth were investigated. Increasing concentrations of NaCl from 50 to 150 mM progressively decreases root growth. The reduction of root growth by NaCl is closely correlated with the increase in ionically bound peroxidase activity. Since proline and ammonium accumulations are associated with root growth inhibition caused by NaCl, we determined the effects of proline or NH_4Cl on root growth and ionically bound peroxidase activity in roots. External application of proline or NH_4Cl markedly inhibited root growth and increased ionically bound peroxidase activity in roots of rice seedlings in the absence of NaCl. An increase in ionically bound peroxidase activity in roots preceded inhibition of root growth caused by NaCl, NH_4Cl or proline. Mannitol inhibited root growth, but decreased rather than increased ionically bound peroxidase activity at the concentration iso-osmotic with NaCl. The inhibition of root growth and the increase in ionically bound peroxidase activity in roots by NaCl is reversible and is associated with ionic rather than osmotic component.

Abbreviations: DW-dry weight; FW-fresh weight

Introduction

It has been postulated that growth cessation or reduction is likely to result from cell wall stiffening processes related to formation of cross-linkages among cell wall polymers (Fry, 1986). The formation of cross-linkages between cell wall components is mediated by cell wall-associated peroxidase enzymes (Cosgrove, 1997; Fry, 1979; Fry, 1982; Fry, 1986; Hartly et al., 1988; Hartly et al., 1990). Several studies have demonstrated that cell wall peroxidase activity is inversely related to cell growth (Bacon et al., 1997; Cordoba-Pedregrosa et al., 1996; Goldberg et al., 1987; Hohl et al., 1995; Kim et al., 1989; MacAdam et al., 1992a, 1992b; Sanchez et al., 1989, 1995, 1996; Valero et al., 1991; Zheng and van Huystee, 1992). In rice, a role for cell wall peroxidase has been established in controlling anoxia- and ethylene-induced growth of coleoptiles (Lee and Lin, 1995, 1996b) and abscisic acid-, methyl jasmonate- and cadmium-inhibited growth of roots (Chen and Kao, 1995; Lee and Lin, 1996a; Tsai et al., 1997).

The inhibition of plant growth by salinity is a widespread problem in agricultural practice. However, the mechanisms underlying this inhibition are not yet clear (Greenway and Munns, 1980; Munns and Termaat, 1986; Rengel, 1992). Neumann (1993, 1997) suggests that rapid metabolically regulated changes in the physical properties of growing cells, caused by osmotic or other effects, appear to be a factor regulating maize leaf growth responses to root salinization. Neumann et al. (1994) also demonstrated that root growth inhibition caused by salinity was associated with cell wall hardening. From the presently available literature there is no clear evidence for the role of cell wall peroxidase activity in controlling root growth processes during salinity stress, although a potentially caused role for cell wall peroxidase in restricting cell growth during drought has been demonstrated (Bacon et al., 1997). The present investigation was designed to study the changes in ionically bound peroxidase activity in roots of NaCl-stressed rice seedlings and their correlation with root growth.

Materials and Methods

Rice (*Oryza sativa* L., cv. Taichung Native 1) seeds were sterilized with 2.5 % sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dish (20 cm) containing distilled water at 37℃ under dark condition. After 1-day incubation, uniformly germinated seeds were selected and transferred to Petri dishes (9.0 cm) containing two sheets of Whatman No. 1 filter paper moistened with 10 ml of distilled water or test solutions. Each Petri dish contained 20 germinated seeds. Each treatment was replicated 4 times. The germinated seeds were allowed to grow at 27℃ in darkness and 3 ml of distilled water or test solutions was added to each Petri dish on day 3 of the growth. Fresh weight (FW), dry weight (DW), ionically bound peroxidase, ammonium, proline, Na^+ and Cl^- of roots were measured at the times indicated.

Peroxidase was extracted according to the method described by MacAdam et al. (1992a). Briefly, roots were frozen in liquid N and ground with a mortar and pestle and mixed with either 0.05 M potassium phosphate buffer (pH 5.8) to extract soluble peroxidase, or with the same buffer containing 0.8 M KCl to extract both soluble and ionically bound ("total") peroxidase. Ionically bound peroxidase activity is total peroxidase activity minus soluble peroxidase activity. Peroxidase activity was measured using a modification of the procedure described by Curtis (1971). The assay medium contained 0.05 M potassium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H_2O_2 and 0.1 ml enzyme extract in a final assay volume of 3.0 ml. The reaction was initiated by the addition of H_2O_2 and the change in absorbance at 470 nm was measured. Activity was calculated using the extinction coefficient (26.6 mM^{-1} cm^{-1} at 470 nm) for tetraguaiacol. One unit of peroxidase was defined as the amount of enzyme that caused the formation 1 μ mol tetraguaiacol per min. When syringaldazine was used as the substrate, the assay medium contained: 10 mM potassium phosphate buffer (pH 7.0), 0.018 mM syringaldazine, 0.5 mM H_2O_2 (Grison and Pilet, 1985). The reaction was started by introducing 0.5 ml of enzyme preparation. The change in absorbance at 530 nm was monitored. One unit of peroxidase against syringaldazine was expressed as an increase of 1 A_{530} per min. Peroxidase activity was expressed as units per g DW.

Ammonium and proline were extracted and determined according to the methods described previously (Lin and Kao 1996a, 1996b). For Na^+ determination, harvested roots were washed three times (with each one minute) with distilled water,

dried at 65℃ for 2 days, extracted in 1 N HCl at room temperature (Hunt, 1982) and analyzed 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) equipped with chloride ion electrode. The levels of ammonium, proline, Na^+ and Cl^- were expressed on the basis of DW.

Results and discussion

Root growth was followed by measuring FW or DW of roots. Figure 1 shows the effects of NaCl on root growth and Na^+ and Cl^- levels in roots of rice seedlings. Increasing concentrations of NaCl from 50 to 150 mM progressively decreased root growth and increased both Na^+ and Cl^- levels in roots. The reduction of root growth is closely correlated with the increase of Na^+ and Cl^- levels in roots. It is also clear from Figure 1 that Cl^- concentration in roots was approximately twice that of Na^+. Basu and Ghosh (1991) and Basu et al. (1988) also reported that Cl^- concentration in NaCl-treated roots of rice seedlings was much higher than Na^+.

The peroxidase we determined in this study is ionically bound peroxidase (total peroxidase minus soluble peroxidase), removable from homogenized tissues with high ionic strength buffers. The ionically bound fraction of peroxidase has been generally equated with cell wall activity, and several studies have reported an association between increase in ionically bound peroxidase activity and reduction or cessation of cell growth (Gardiner and Cleland, 1974; Goldberg etal., 1986; MacAdam et al., 1992a; Rama Rao et al., 1982; Sanchez et al., 1995).

It has been shown that syringaldazine, as hydrogen donor, has a particularly high affinity for the peroxidase associated with lignification (Grison and Pilet, 1985; Goldberg et al., 1983). Guaiacol and syringaldazine were used as the substrates to establish whether ionically bound peroxidase activity is related to the reduction of root growth caused by NaCl. When guaiacol was used as the substrate, ionically bound peroxidase activity increased with the increasing of NaCl concentration (Figure 2), a pattern similar to that of root growth reduction. However, the increase in the activity of ionically bound peroxidase using syringaldazine as substrate was only observed at a concentration of 150 mM NaCl (Figure 2). Thus, guaiacol was used as the substrate to assay ionically bound peroxidase for all subsequent experiments.

To characterize further the role of ionically bound peroxidase activity on NaCl-induced inhibition of root growth, seeds were grown in NaCl solution (150 mM) for 2 days; after this, the seedlings were incubated in distilled water and NaCl (150 mM), respectively, for another 3 days. When 2-day-old NaCl-treated seedlings were incubated in NaCl, only slight root growth was observed (Figure 3). However, when seedlings were transferred to distilled water, root growth rapidly resumed and increased linearly with increasing duration of incubation (Figure 3). Figure 3 also

shows that ionically bound peroxidase activity in roots of seedlings incubated in NaCl solution is higher than that of seedlings transferred to distilled water.

It is known that ammonium strongly inhibits the growth of many plants (Haynes and Goh, 1978). Exogenous application of NH_4Cl was also found to reduce root growth of rice seedling (Lin and Kao, 1996a). The growth of roots of rice seedlings at 4 mM NH_4Cl was reduced to 50 % of the control value (Lin and Kao, 1996a). It has also been shown that NaCl was effective in stimulating the accumulation of ammonium in roots of rice seedling, and that accumulation of ammonium in roots preceded inhibition of root growth caused by NaCl (Lin and Kao, 1996a). If the increase in ionically bound peroxidase activity is important in regulating growth reduction of roots caused by NaCl, then exogenous application of NH_4Cl would be expected to increase ionically bound peroxidase activity in roots of rice seedlings. Figure 3 shows that FW of and ionically bound peroxidase activity in roots of seedlings in NH_4Cl (4 mM) are lower and higher, respectively, than those of seedlings in distilled water.

In previous studies, we have shown that proline accumulation is correlated with root growth inhibition of rice seedlings induced by NaCl and that exogenous application of proline at 4 mM resulted in a reduction of root growth to 50 % of control value (Lin and Kao, 1996b). In the present study, we demonstrated that addition of 4 mM proline inhibited root growth and increased ionically bound peroxidase in roots (Figure 3).

The observations that rice seedlings fed with NH_4Cl or proline, which resulted in an increase in ionically bound peroxidase activity in roots, reduced root growth in the same way that NaCl did, further support our suggestion that ionically bound peroxidase is likely to participate in the regulation of root growth reduction of rice seedling under NaCl condition.

To test the causal relationship between ionically bound peroxidase activity and root groth reduction caused by NaCl, NH_4Cl or proline, 2-day-old seedlings were transferred to distilled water, NaCl, NH_4Cl and proline, respectively, for 4, 8 and 12 h. Changes in ionically bound peroxidase activity and root growth were then monitored. As indicated in Table 1, an increase in ionically bound peroxidase activity in roots preceded inhibition of root growth caused by NaCl, NH_4Cl or proline. Previously, we observed that proline or ammonium accumulation occurred at 4 h after NaCl treatment (Lin and Kao, 1996a; Lin and Kao, 1996b). Clearly, the links between NaCl

treatment, proline, ammonium, ionically bound peroxidase and root growth are well established.

Munns (1993) hypothesized that plant growth is initially inhibited by cellular response to the osmotic effects of external salt, i.e. by responses to the decreased availability by soil water. Mannitol inhibited root growth, but decreased rather than increased ionically bound peroxidase activity at the concentrations iso-osmotic with NaCl (Figure 4). These results suggest that the reduction of root growth by mannitol (osmotic effects) is not necessarily connected to an increase in ionically bound peroxidase activity. Figure 5 shows the effects of mannitol on ammonium and proline levels in roots of rice seedlings. Mannitol treatment did not increase ammonium level. Slight accumulation of proline was observed only at a concentration of mannitol iso-osmotic with 150 mM. Mannitol effects are unlikely due to a consequence of toxic components caused by its breakdown, because similar results were obtained when mannitol solution was changed daily (data not shown).

The observations that mannitol inhibited root growth but did not increase ionically bound peroxidase activity and proline and ammonium levels in roots of rice seedlings (Figures 4 and 5) and that Na^+ and Cl^- levels in roots increased with the increase of NaCl concentrations (Figure 1) suggest that increase in ionically bound peroxidase activity in NaCl-treated roots is associated with ionic rather than the osmotic component of NaCl stress.

Acknowledgement

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Table 1. Changes in root growth and ionically bound peroxidase (POD) activities in roots of NaCl-, NH_4Cl-, or proline-treated rice seedlings. Rice seeds were germinated in distilled water for 2 days and then were transferred to distilled water, NaCl (150 mM), NH_4Cl (4 mM), and proline (4 mM), respectively

	FW (mg root^{-1})				Peroxidase activity (units g^{-1})			
Time	H_2O	NaCl	NH_4Cl	Proline	H_2O	NaCl	NH_4Cl	Proline
0h	6.30±0.16				225±28			
4h	6.38±0.17	6.52±0.08	6.45±0.12	6.65±0.20	245±30	242±15	240±16	270±22
8h	6.80±0.22	6.66±0.16	6.79±0.17	6.68±0.12	270±20	320±4	356±27	342±20
12h	7.28±0.22	6.80±0.16	6.81±0.17	6.72±0.12	285±27	354±20	380±14	378±32

Figure legends

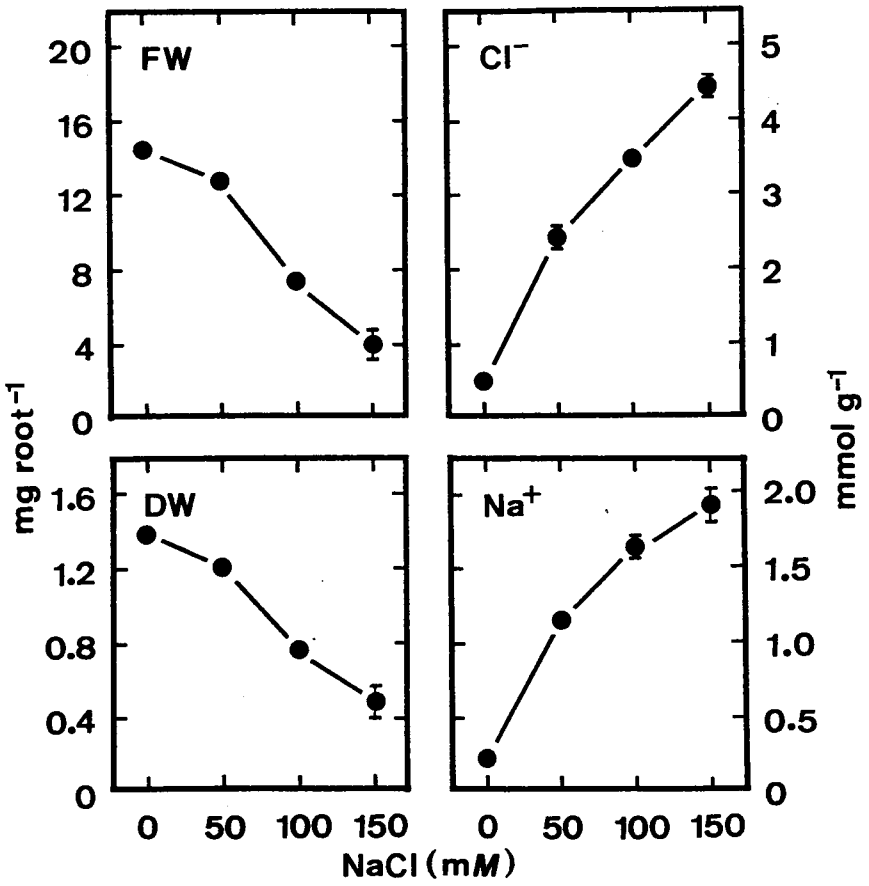
Figure 1. Effects of NaCl on root growth and Na^+ and Cl^- levels in roots of rice seedlings. Root growth and Na^+ and Cl^- levels were measured after 5 days of treatment. Na^+ and Cl^- were expressed on the basis of g DW. Vertical bars represents standard errors.

Figure 2. Effects of NaCl on ionically bound peroxidase activities against guaiacol and syringalsazine in roots of rice seedlings. Enzyme activities were determined after 5 days of treatment. Vertical bars represents standard errors.

Figure 3. Changes in root growth and ionically bound peroxidase activities in roots of NaCl-, NH_4Cl-, or proline-pretreated rice seedlings grown in the presence or absence of NaCl (150 mM), NH_4Cl (4 mM), and proline (4 mM), respectively. Rice seeds were germinated for 2 days in NaCl (150 mM), NH_4Cl (4 mM), and proline (4 mM) and then seedlings were transferred to distilled water or remained in NaCl, NH_4Cl and proline, respectively. Vertical bars represents standard errors.

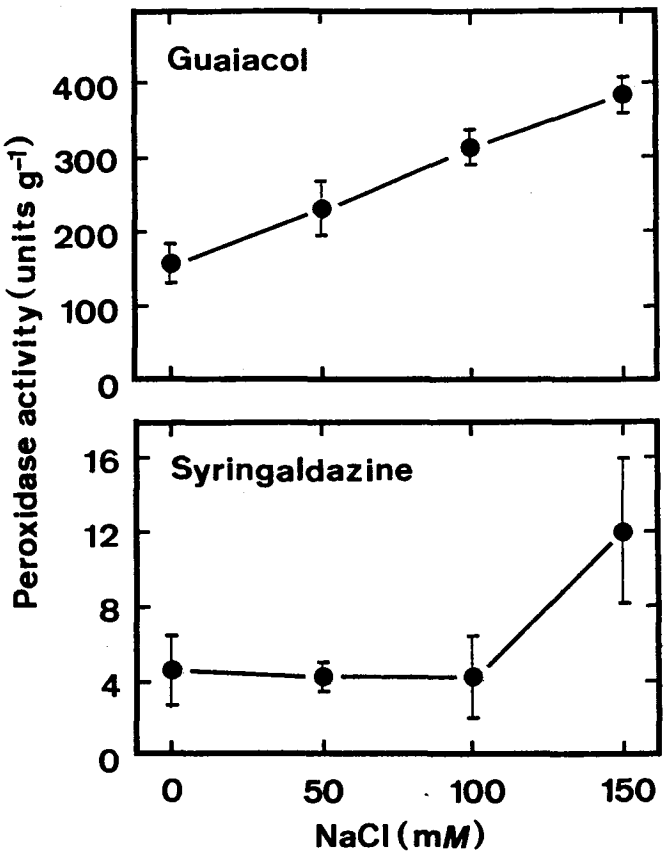
Figure 4. Effects of mannitol on root growth of and ionically bound peroxidase activities in roots of rice seedlings. Mannitol concentrations of 92, 184, 276 mM were iso-osmotic with 50, 100, 150 mM NaCl, respectively. FW, DW and Enzyme activity were measured after 5 days of treatment. Vertical bars represents standard errors.

Figure 5. Effects of mannitol on ammonium and proline levels in roots of rice seedlings. Mannitol concentrations of 92, 184, 276 mM were iso-osmotic with 50, 100, 150 mM NaCl, respectively. Ammonium and proline levels were measured after 5 days of treatment. Vertical bars represents standard errors.



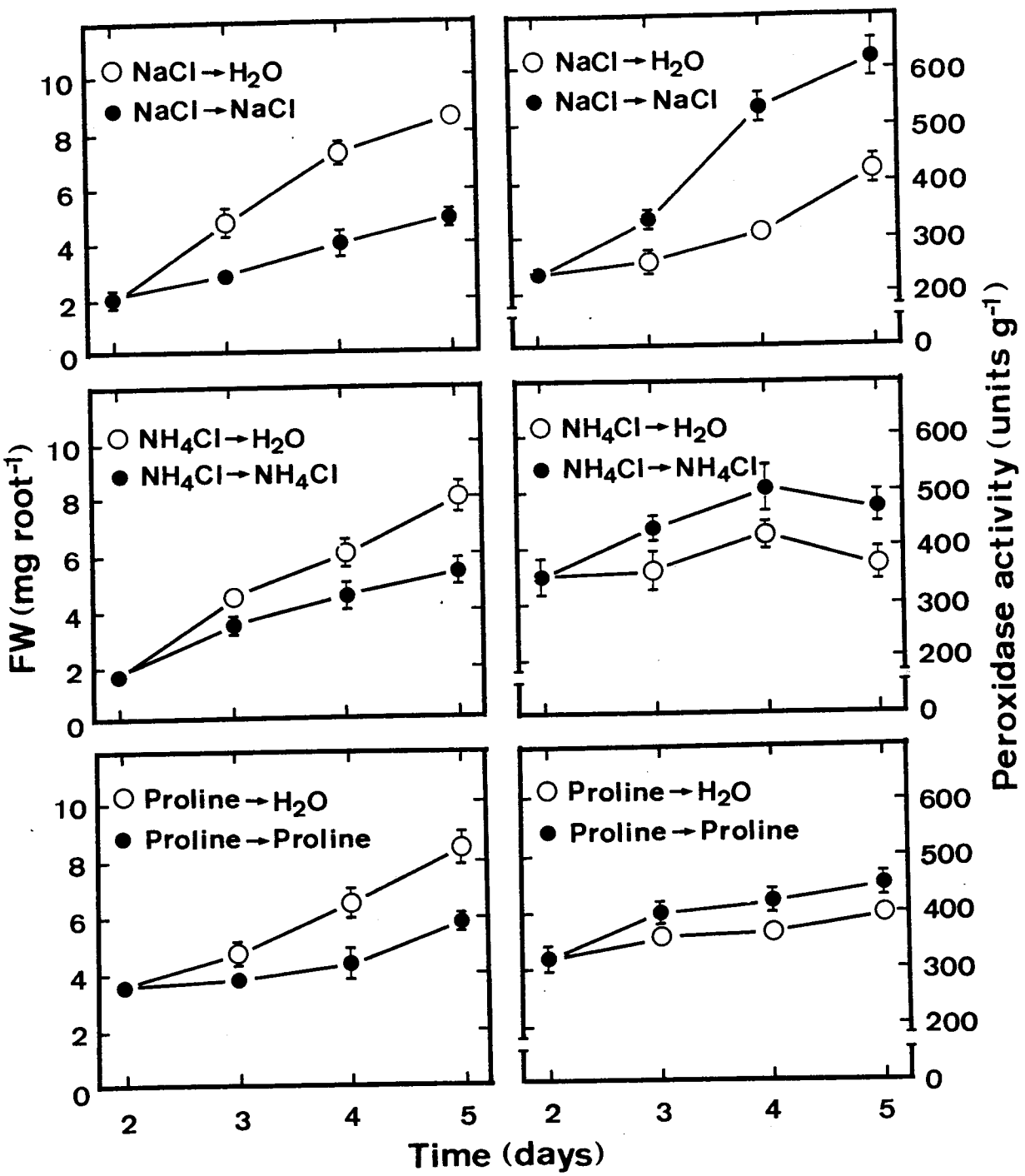
Lind Kao

Fig. 1


Guaiacol
400
300
200
100
0
Syringaldazine
16
12
8
4
0
0
50
100
150
NaCl (mM)
Peroxidase activity (units g⁻¹)

Lin & Kao

Fig. 2


NaCl → H2O
NaCl → NaCl
NH4Cl → H2O
NH4Cl → NH4Cl
Proline → H2O
Proline → Proline
FW (mg root-1)
Peroxidase activity (units g-1)
Time (days)
0
2
4
6
8
10
200
300
400
500
600
2
3
4
5

Lin & Kao

Fig. 3

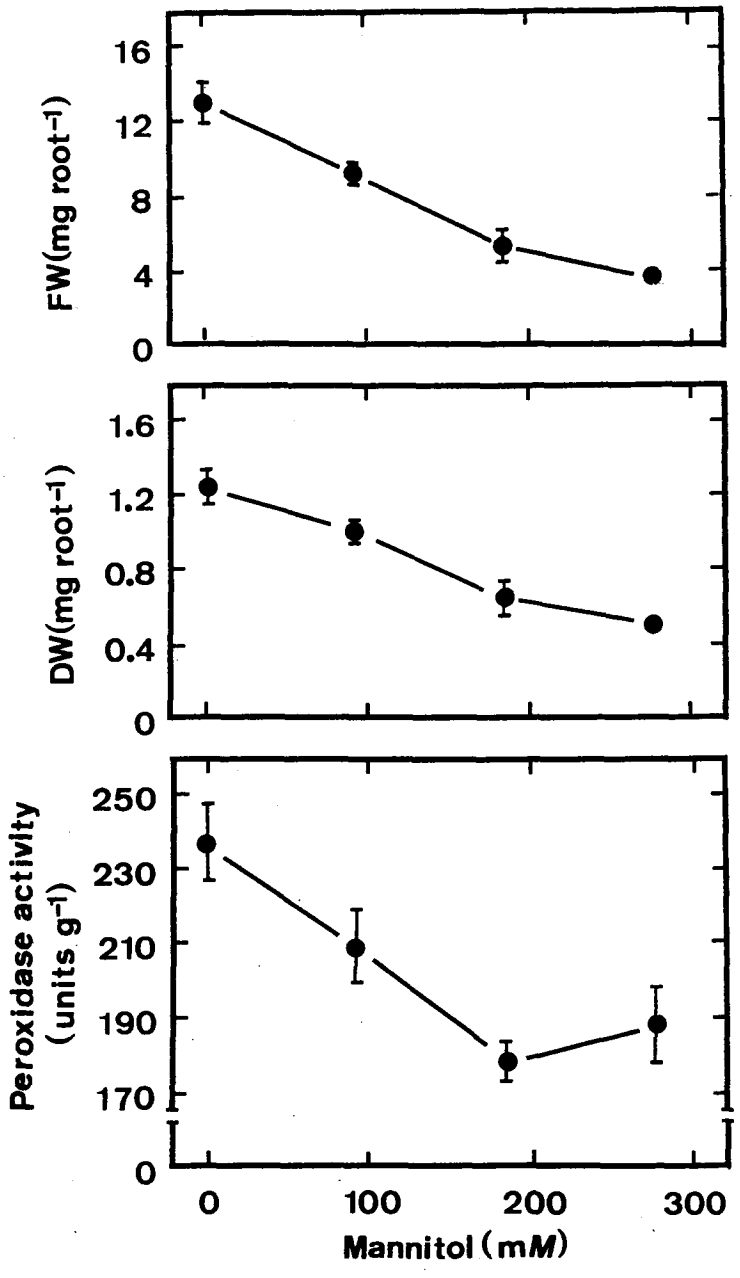
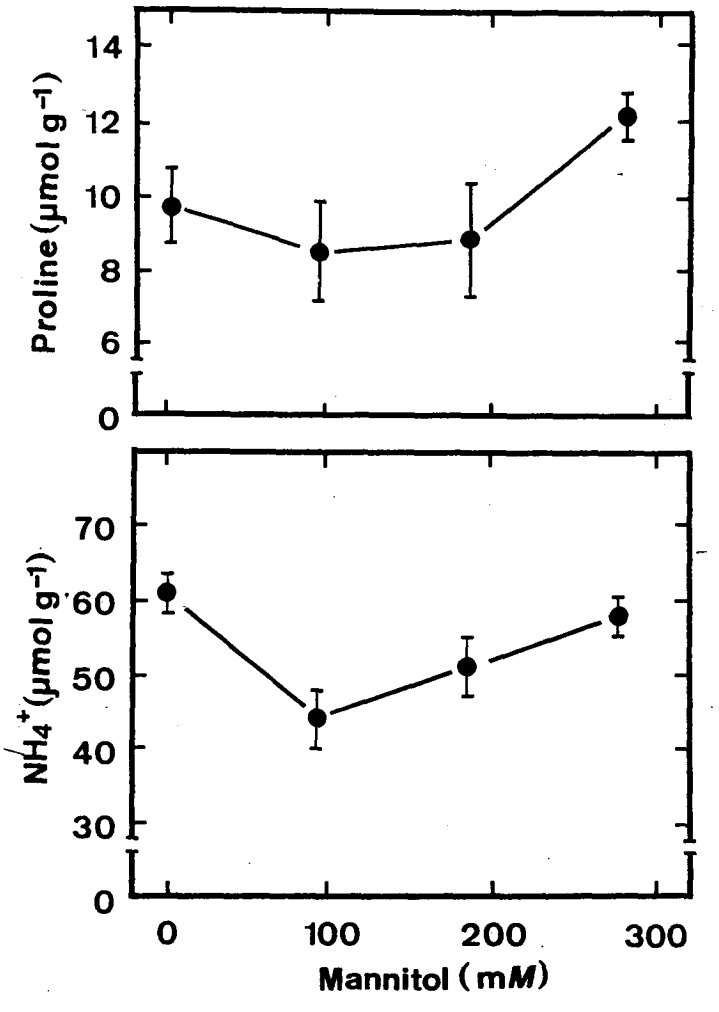
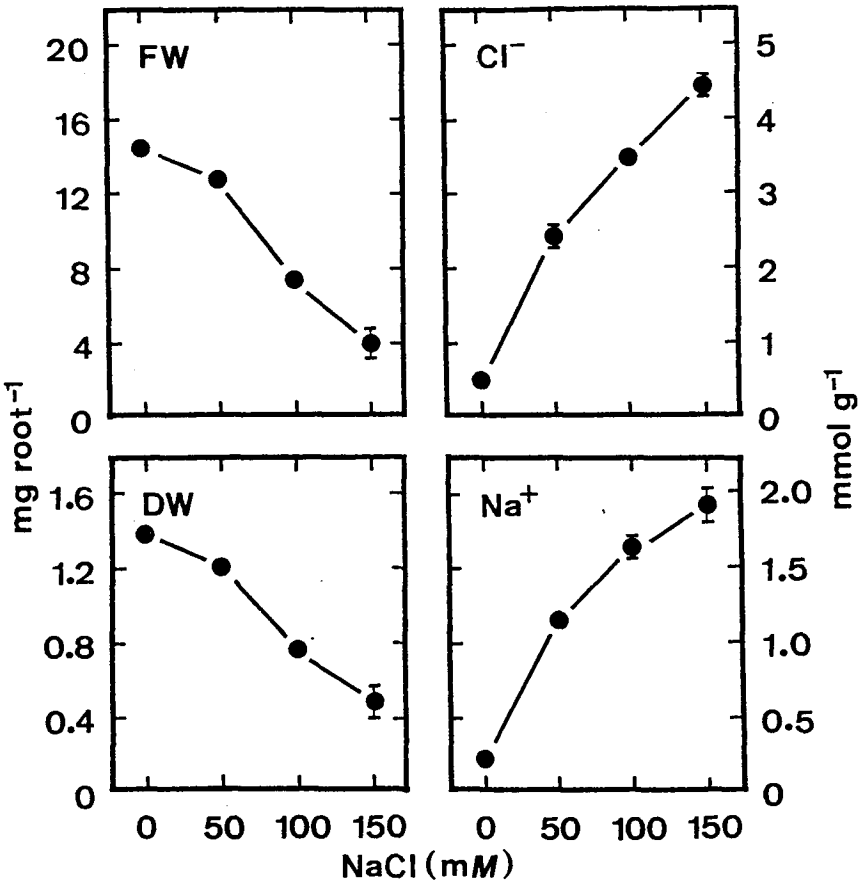

FW(mg root⁻¹)
16
12
8
4
0
DW(mg root⁻¹)
1.6
1.2
0.8
0.4
0
Peroxidase activity (units g⁻¹)
250
230
210
190
170
0
0
100
200
300
Mannitol (mM)

Fig. 4



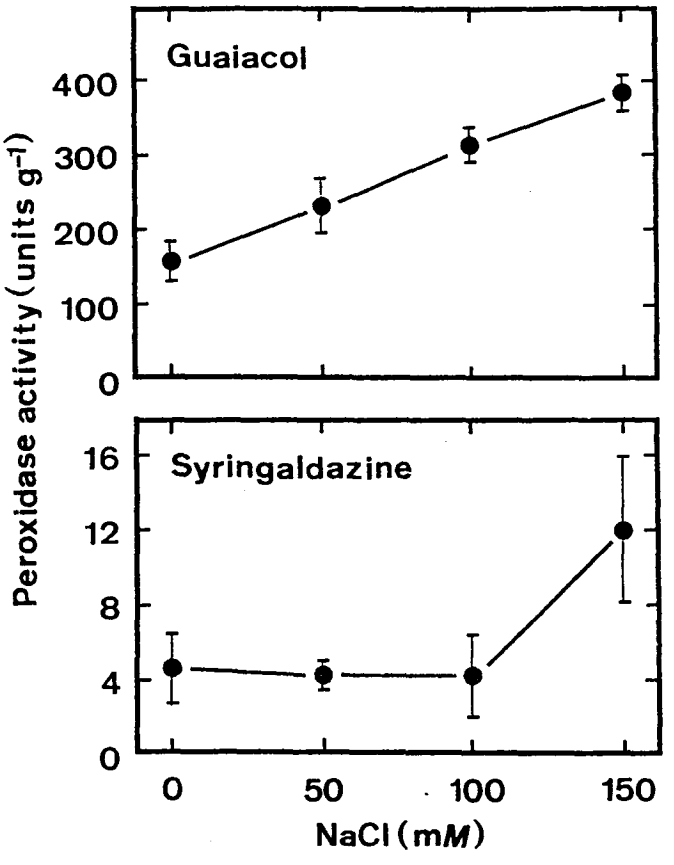
Linda Ka

Fig. 5



Lind Kao

Fig. 1


Guaiacol
400
300
200
100
0
Syringaldazine
16
12
8
4
0
0
50
100
150
NaCl (mM)
Peroxidase activity (units g⁻¹)

Lind ko

Fig. 2

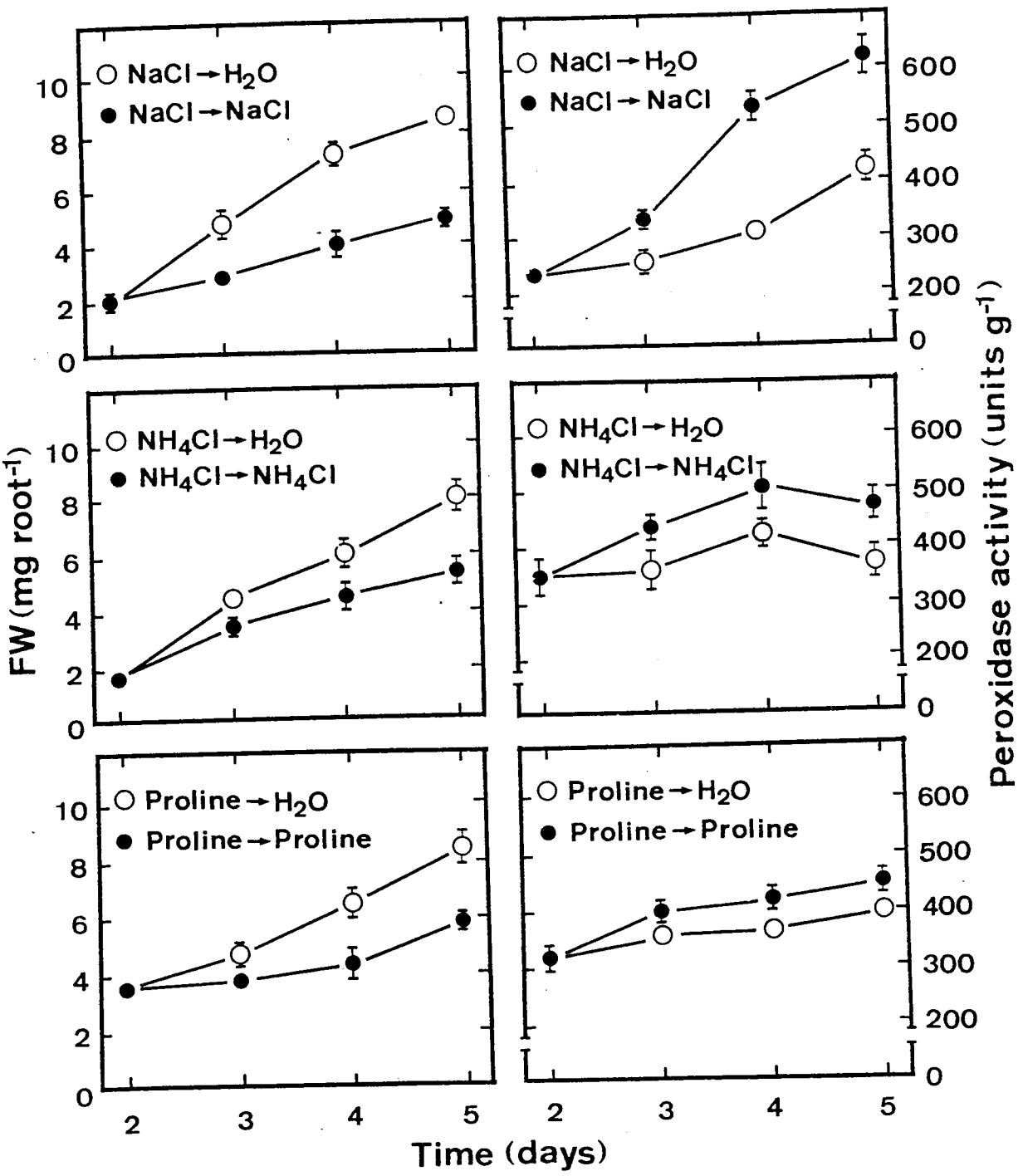

NaCl→H2O
NaCl→NaCl
NH4Cl→H2O
NH4Cl→NH4Cl
Proline→H2O
Proline→Proline
FW (mg root-1)
Peroxidase activity (units g-1)
Time (days)
0
2
4
6
8
10
200
300
400
500
600
2
3
4
5

Fig. 3

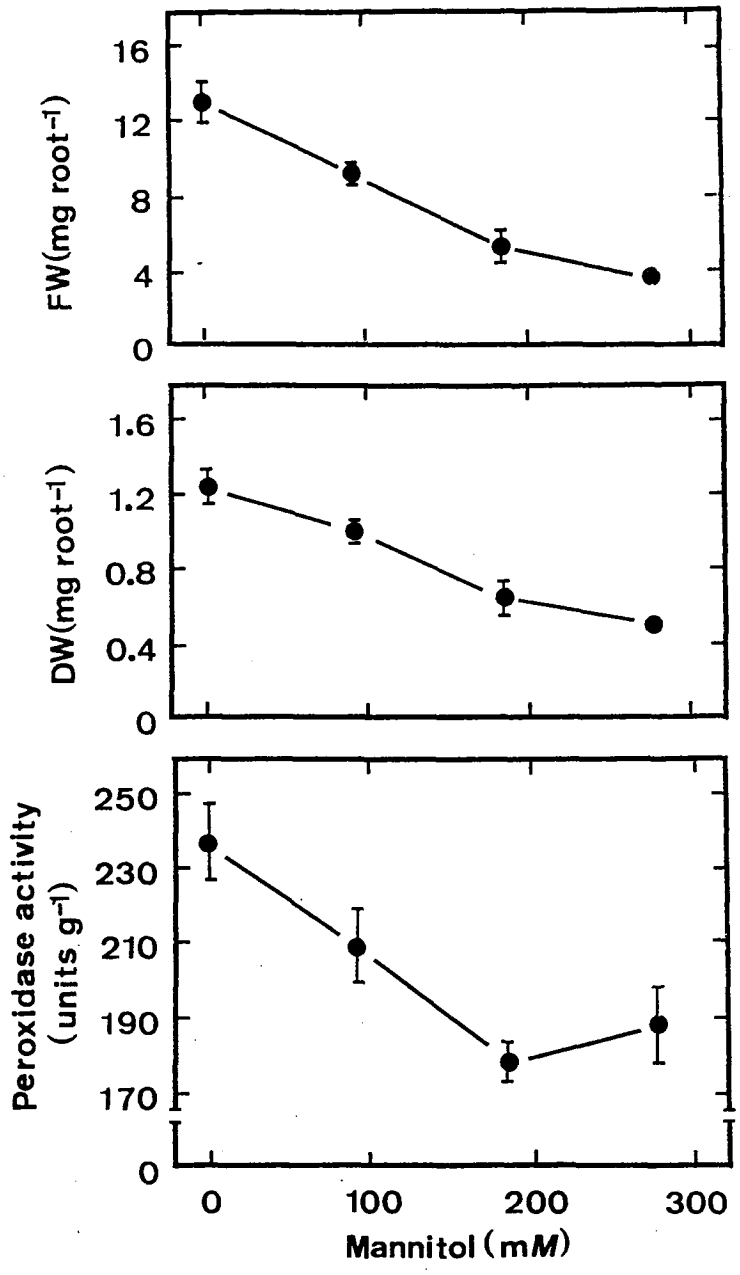
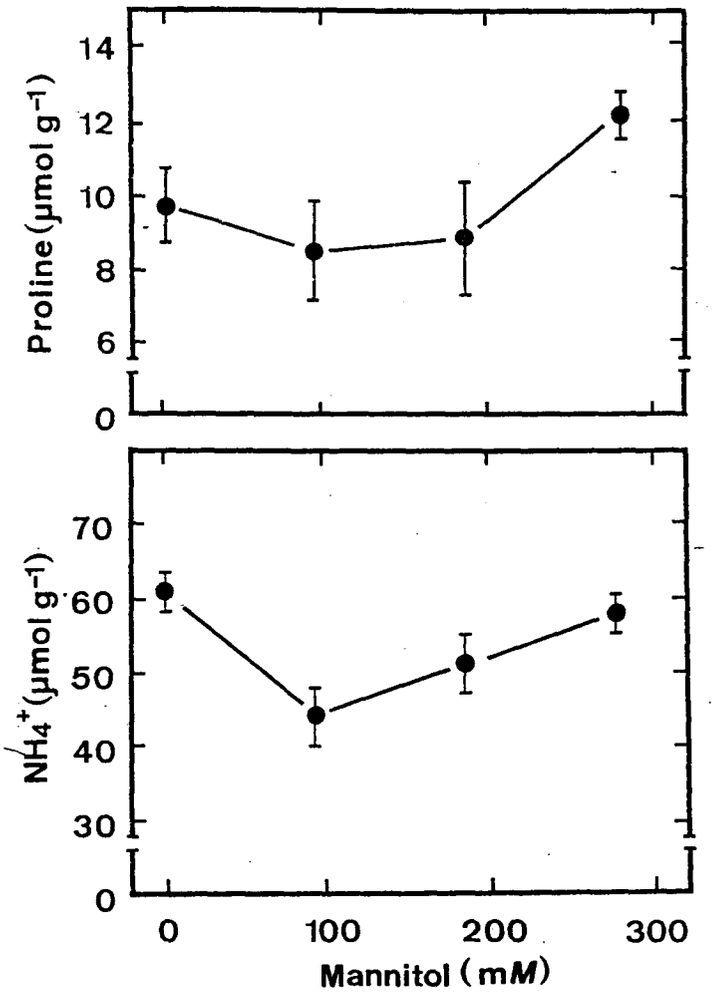

FW(mg root⁻¹)
16
12
8
4
0
DW(mg root⁻¹)
1.6
1.2
0.8
0.4
0
Peroxidase activity (units g⁻¹)
250
230
210
190
170
0
0
100
200
300
Mannitol (mM)

Fig. 4


Proline (µmol g⁻¹)
14
12
10
8
6
0
NH4+ (µmol g⁻¹)
70
60
50
40
30
0
0
100
200
300
Mannitol (mM)

Linda

Fig. 5