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

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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, b; 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

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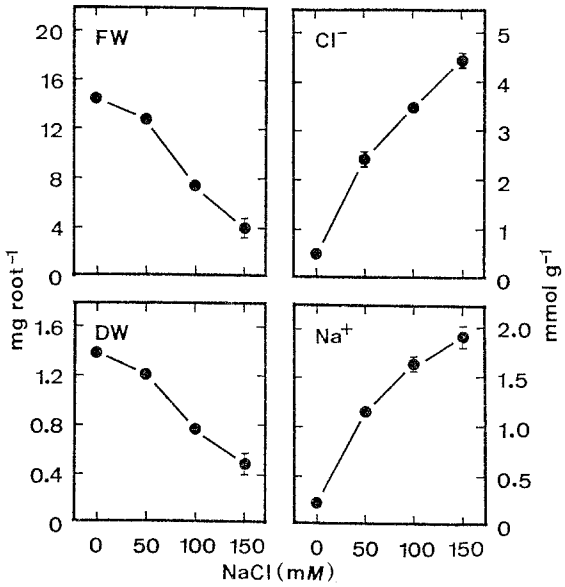

Figure 1. Effects of NaCl on root growth and Na^+ and Cl^- levels in roots of rice seedlings. Root growth and Na^+ and Cl^- levels were measured after 5 days of treatment. Na^+ and Cl^- were expressed on the basis of g DW. Vertical bars represents standard errors.

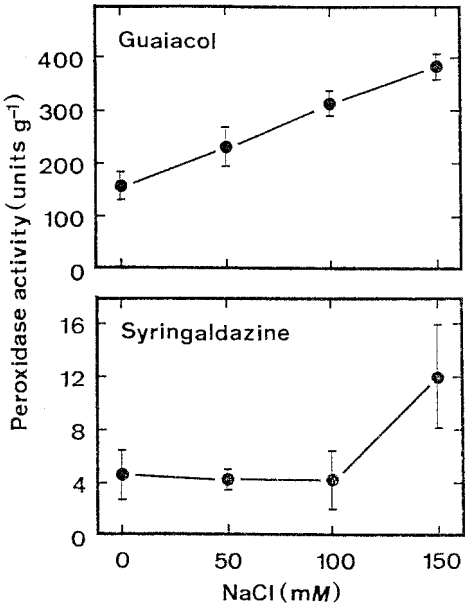

Figure 2. Effects of NaCl on ionically bound peroxidase activities against guaiacol and syringalsazine in roots of rice seedlings. Enzyme activities were determined after 5 days of treatment. Vertical bars represents standard errors.

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°C 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°C 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 m*M* guaiacol, 11.8 m*M* 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 μmol tetraguaiacol per min. When syringaldazine was used as the substrate, the assay medium contained: 10 m*M* potassium phosphate buffer (pH 7.0), 0.018 m*M* syringaldazine, 0.5 m*M* 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, b). For Na^+ determination, harvested roots 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 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 m*M* 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 et al., 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 m*M* 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 m*M*) for 2 days; after this, the seedlings were incubated in distilled water and NaCl (150 m*M*), 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 m*M* 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 m*M*) 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 m*M* 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 m*M* proline inhibited root growth and increased ionically bound peroxidase in roots (Figure 3).

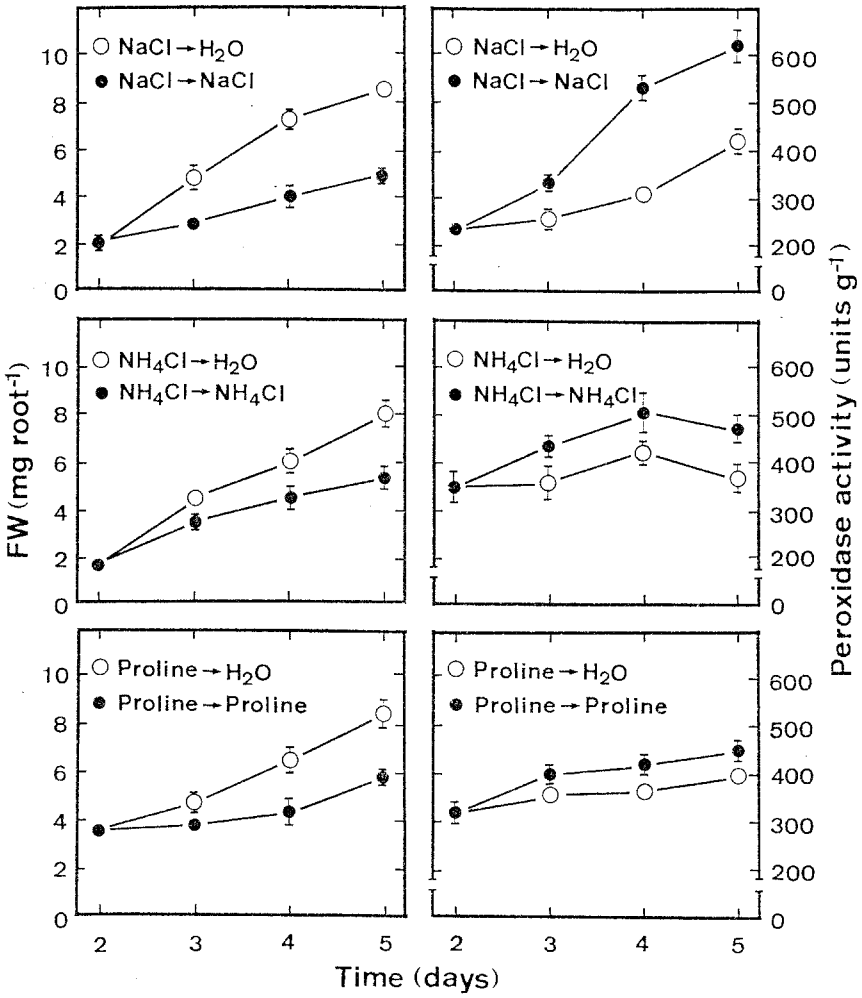

Figure 3. Changes in root growth and ionically bound peroxidase activities in roots of NaCl-, NH_4Cl-, or proline-pretreated rice seedlings grown in the presence or absence of NaCl (150 m*M*), NH_4Cl (4 m*M*), and proline (4 m*M*), respectively. Rice seeds were germinated for 2 days in NaCl (150 m*M*),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

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 growth 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, b). 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

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 m*M*), NH_4Cl (4 m*M*) and proline (4 m*M*), respectively

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

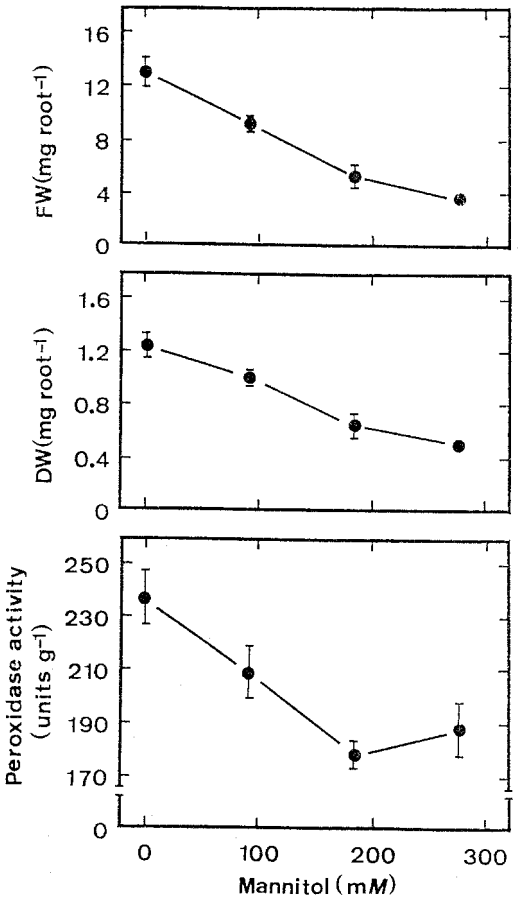

Figure 4. Effects of mannitol on root growth of and ionically bound peroxidase activities in roots of rice seedlings. Mannitol concentrations of 92, 184, 276 m*M* were iso-osmotic with 50, 100, 150 m*M* NaCl, respectively. FW, DW and enzyme activity were measured after 5 days of treatment. Vertical bars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

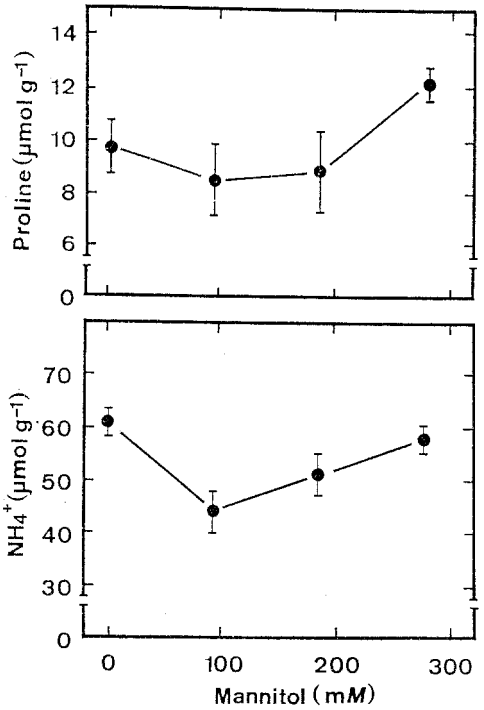

Figure 5. Effects of mannitol on ammonium and proline levels in roots of rice seedlings. Mannitol concentrations of 92, 184, 276 m*M* were iso-osmotic with 50, 100, 150 m*M* NaCl, respectively. Ammonium and proline levels were measured after 5 days of treatment. Vertical bars represents standard errors.

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 m*M*. 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.

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