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PLANT SCIENCE

Proline accumulation is associated with inhibition of rice seedling root growth caused by NaCl

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Abstract

The effects of NaCl on changes in the proline level in roots and root growth of rice seedlings were investigated. NaCl was effective in inhibiting root growth and in increasing proline accumulation in roots. Mannitol was also effective in inhibiting root growth, but proline accumulation was observed only at the concentration iso-osmotic with 150 mM NaCl. Accumulation of proline in roots preceded inhibition of root growth caused by NaCl. The inhibition of root growth and accumulation of proline in roots by NaCl are reversible. Exogenous L-proline and gabaculine, which were found to enhance in an accumulation of proline in roots, inhibited root growth of rice seedlings. The relationship between growth inhibition of roots caused by NaCl and changes in the proline level in roots is discussed.

1. Introduction

Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon [1,2]. Proline is believed to protect plant tissues against stress by acting as an N-storage compound, osmo-solute and hydrophobic protectant for enzymes and cellular structures [3]. However, its role in imparting resistance to salt stress continues to be controversial. Some workers consider enhanced proline levels simply as a stress effect, rather than a cause of stress tolerance [4–6]. There are reports that proline accumulation is correlated with growth inhibition induced by NaCl [7,8]. The

role of proline accumulation in salinity stress therefore needs to be critically examined. In this paper, we investigate the role of proline in the control of NaCl-inhibited root growth of rice seedlings.

2. 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 dishes (20-cm) containing distilled water at 37°C under dark conditions. After 1-day incubation, uniformly germinated seeds were selected and transferred to a Petri dish (9.0-cm) containing 2

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sheets of Whatman No. 1 filter paper moistened with 10 ml distilled water or test solution. All test compounds were dissolved in distilled water. Their pHs are similar to that of distilled water, which is 5.5. 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 an additional 3 ml distilled water or test solutions was added to each Petri dish on day 3 of the growth.

Proline was extracted and its concentration determined by the method of Bates et al. [9]. Roots were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined. Levels of proline are expressed as μmol/g dry weight.

For all measurements, each treatment was repeated 4 times. All experiments described here were repeated 3 times. Similar results and identical trends obtained each time. The data reported here are from a single experiment.

3. Results

Fig. 1 shows the effect of NaCl on the growth of rice seedlings. NaCl significantly reduced growth of shoots and roots, as judged by fresh weight and dry weight, of rice seedlings. Increasing concentrations of NaCl from 50 to 150 mM progressively decreased root and shoot growth. Similar findings were recently reported when seedling growth was measured in terms of root and shoot length [10]. These results are in general agreement with those reported by Flowers and Yeo [11].

The effects of NaCl on proline levels in shoots and roots of rice seedlings are shown in Fig. 2. Increasing concentrations of NaCl from 50 to 150 mM progressively increased proline levels in roots. However, proline accumulation in shoots was observed only when seedlings were treated at high concentration (150 mM) of NaCl. Clearly, the reduction of root growth, but not shoot growth, is closely associated with the accumulation of proline in roots.

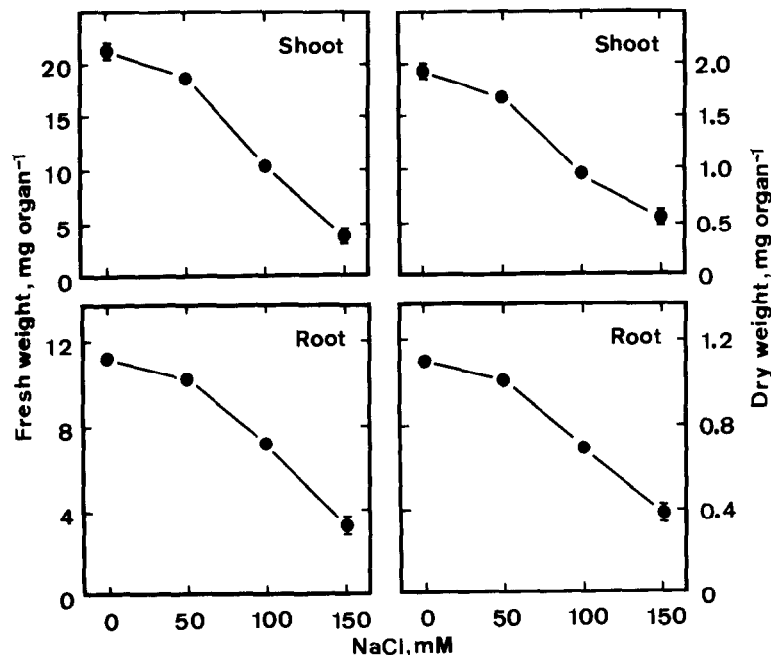Mannitol inhibited root growth, but proline ac-

Fig. 1. Effects of NaCl on seedling growth of rice. Seedling growth was measured after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.
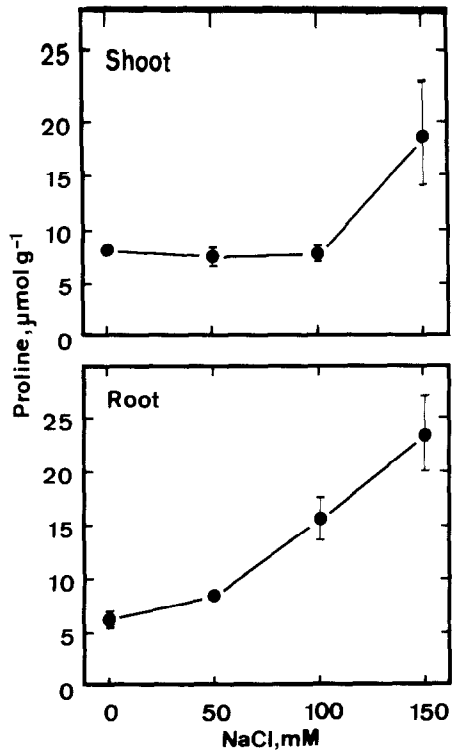
Fig. 2. Effects of NaCl on proline levels in shoots and roots of rice seedlings. Proline level was determined after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.
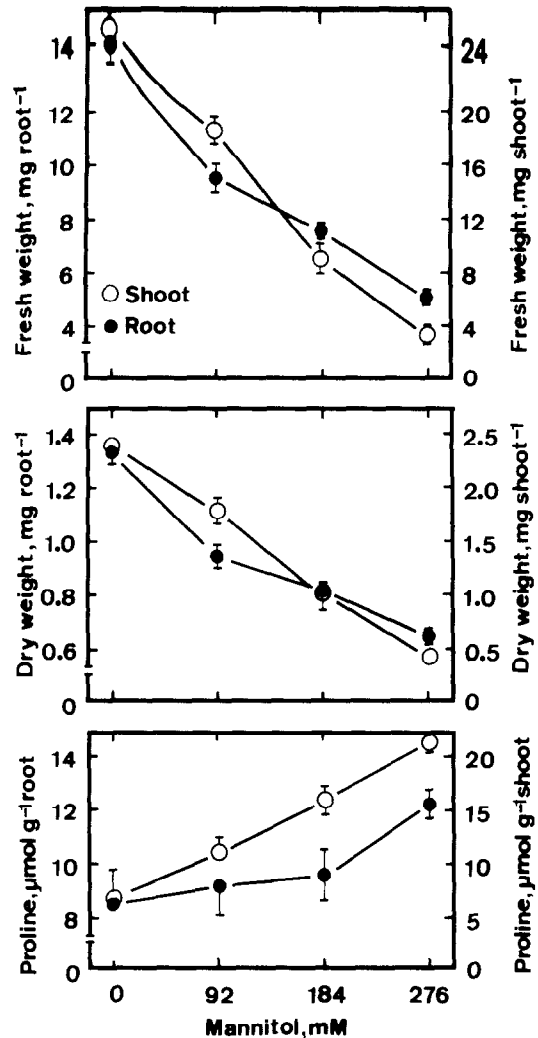

Fig. 3. Effects of mannitol on seedling growth and proline level in rice seedlings. Mannitol concentrations of 92, 184, 276 mM were iso-osmotic with 50, 100 and 150 mM, respectively. Seedling growth and proline level were measured after 5 days of treatment. Vertical bars represent standard errors.

cumulation was observed only at a concentration iso-osmotic with 150 mM NaCl (Fig. 3). Mannitol treatment also inhibited shoot growth and resulted in an accumulation of proline. All these results suggest that proline accumulation in NaCl-treated roots and shoots is associated with the ionic rather than the osmotic component of NaCl stress.

If proline accumulation is important in regulating growth inhibition of roots but not shoots of rice seedlings caused by NaCl, then exogenous application of proline is expected to inhibit root growth but not shoot growth of rice seedlings. As indicated in Fig. 4, exogenous L-proline treatment resulted in an inhibition of root growth and an increase in proline level in roots. However, addition of L-proline did not affect shoot growth (Fig. 4). The effect of L-proline on root growth is unlikely to be due to microorganisms, since inhibition of root growth was also obtained when L-proline was added together with chloramphenicol

(0.25 mg/l) to prevent bacterial growth. Chloramphenicol itself had no effect on root growth. In a recent work, we demonstrated that L-proline enhanced NaCl-induced inhibition of root growth, but reversed the inhibitory effect of NaCl on shoot growth of rice seedlings [12]. It seems that proline may play different roles in regulating shoot and root growth of rice seedlings under NaCl conditions.
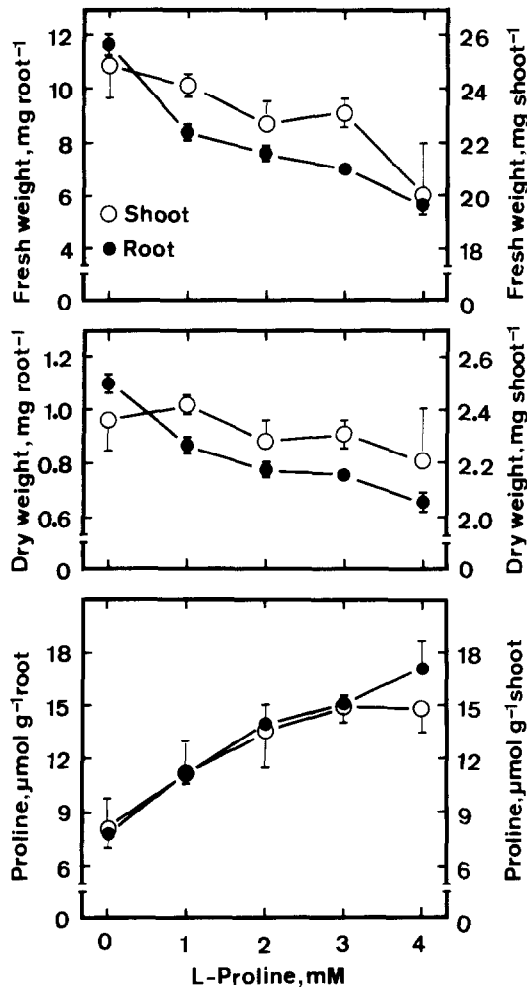
Fig. 4. Effects of L-proline on seedling growth and proline level in rice seedlings. Seedling growth and proline level were measured after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.
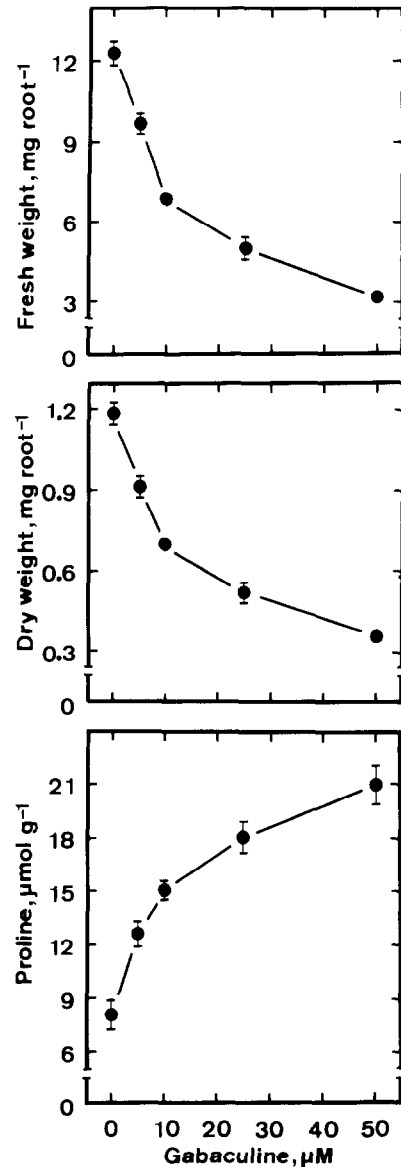

Fig. 5. Effects of gabaculine on root growth and proline level in roots of rice seedlings. Root growth and proline level were measured after 5 days of treatment. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.

Kandpal and Rao [13] have proposed that ornithine aminotransferase may be a potential point of control of proline synthesis in plants subjected to stress. Gabaculine is known to inhibit ornithine aminotransferase [14] and has been shown to increase proline accumulation in radish cotyledons [15]. To characterize further the role of proline accumulation in regulating root growth reduction of rice seedlings under NaCl stress, rice seedlings were grown in the presence of various concentrations of gabaculine. As indicated in Fig. 5, gabaculine increased the proline level in roots and

inhibited root growth. From the shape of the curves, it is apparent that the proline level is closely linked with root growth reduction.

If rice seedlings maintained for 2 days on NaCl

or gabaculine are transferred to distilled water, they then resume root growth (Figs. 6 and 7). Root growth was only slightly restored if seedlings remained in NaCl or gabaculine (Figs. 6 and 7). Figs. 6 and 7 also show that the proline level in roots of seedlings remaining in NaCl or gabaculine
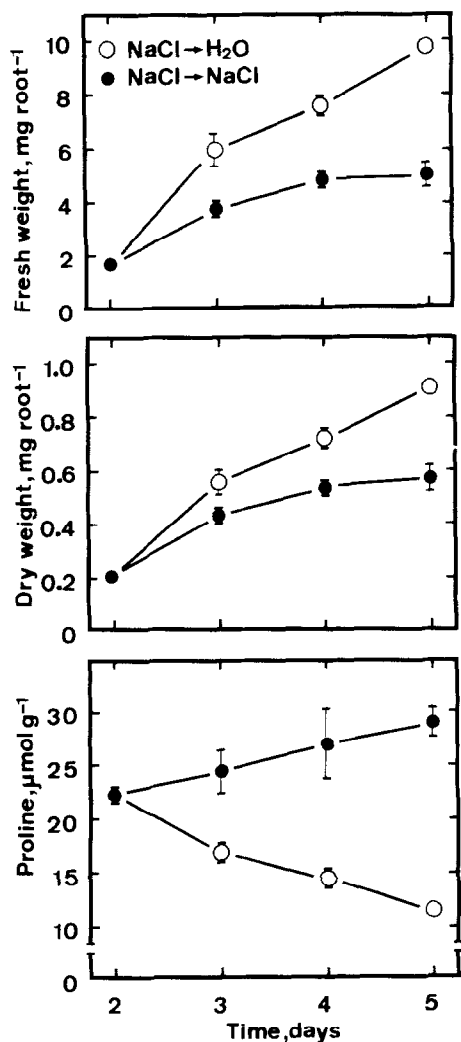

Fig. 6. Changes in growth of and proline level in roots of NaCl-treated rice seedlings grown in the presence or absence of NaCl (150 mM). Rice seeds were germinated for 2 days in NaCl (150 mM) and then seedlings were transferred to distilled water (O) and NaCl (●), respectively. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.
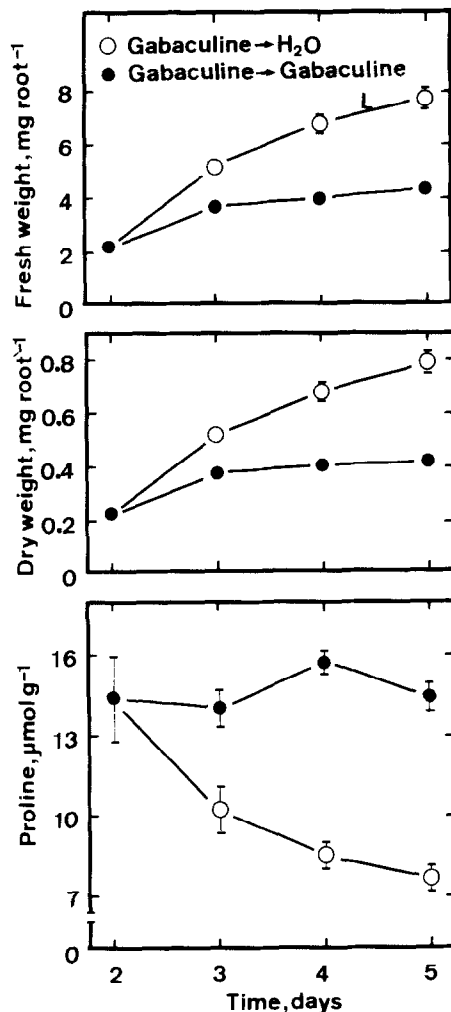

Fig. 7. Changes in growth of and proline level in roots of gabaculine-treated rice seedlings grown in the presence or absence of gabaculine (25 μM). Rice seeds were germinated for 2 days in gabaculine (25 μM) and then seedlings were transferred to distilled water (O) and gabaculine (●), respectively. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.

medium is higher than that of seedlings transferred to distilled water.

To test the causal relationship between proline accumulation and root growth reduction caused by NaCl, 2-day old seedlings were transferred to distilled water and NaCl medium for 4, 8, 12 and 16 h, respectively. Changes in proline levels in roots and root growth were then monitored. As in-
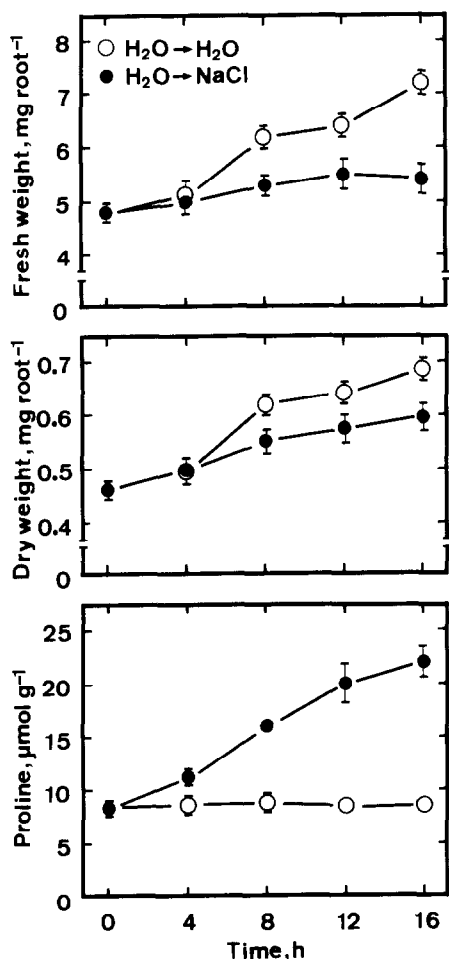
Fig. 8. Changes in growth of and proline level in roots of NaCl-treated rice seedlings grown in the presence or absence of NaCl (150 mM). Rice seeds were germinated for 2 days in distilled water and then seedlings were transferred to distilled water (O) and NaCl (●), respectively. Vertical bars represent standard errors. Only those standard errors larger than symbol size are shown.

dicated in Fig. 8, proline accumulation was observed at 4 h after NaCl treatment, whereas root growth was observed to be inhibited at 8 h.

4. Discussion

Rice is a salt-sensitive species and accumulates sodium ion at only moderate external salinities [16]. Among different parameters responding to

NaCl-stress in rice, the most significant one is rapid accumulation of proline [17,18]. The present investigation shows that the proline level in roots is closely associated with the NaCl concentration applied, whereas proline accumulation in shoots was observed only at high concentration of NaCl (150 mM). We also indicate that proline accumulation in NaCl-treated roots is associated with the ionic rather than the osmotic component of NaCl stress.

The present investigation indicates that proline accumulation participates in the regulation of root growth of rice seedlings under NaCl conditions. This conclusion was based on the observations that (a) proline accumulation in roots preceded root growth reduction caused by NaCl, (b) proline level in roots is inversely associated with root growth and (c) NaCl-induced root growth reduction is produced in rice seedlings fed with L-proline or gabaculine which was found to result in a proline accumulation. When rice seedlings pretreated with NaCl or gabaculine were transferred to distilled water, root growth resumed and proline level in roots declined. These results suggest that growth inhibition of roots induced by NaCl is not irreversible and further support the close link between proline accumulation and root growth reduction induced by NaCl. Proline accumulation induced by NaCl or Na_2SO_4 has also been demonstrated to be correlated with growth inhibition of callus [7,8].

Le Dily et al. [19] reported that the polyamine level of a habituated callus of Beta vulgaris was strongly diminished after treatment with gabaculine. It has been shown that exogenous application of polyamine can overcome the harmful effects of NaCl [20–22] and endogenous levels of polyamines decreased under NaCl stress [23]. However, our recent work provided evidence to show that endogenous polyamines do not play a significant role in the control of NaCl-inhibited growth of rice seedlings [24]. Thus, the inhibition effect of gabaculine on root growth of rice seedlings is unlikely to be due to the decrease of polyamine levels.

Two pathways of proline biosynthesis, glutamate and ornithine pathways, have been reported [25]. In higher plants, the proline biosynthesis

route is generally thought to involve the glutamate pathway which implies pyrroline 5-carboxylate synthase and pyrroline 5-carboxylate reductase [25]. In a few studies with germinated seedlings, a pathway using ornithine as the proline precursor has been put forward, in which ornithine aminotransferase is the key enzyme [25]. We do not know whether these two pathways contribute to the accumulation of proline in NaCl-treated roots. Further studies should provide valuable information on the mechanism of proline accumulation in rice roots in response to NaCl. In animal systems, ornithine aminotransferase is a reversible enzyme. It has been proposed that ornithine aminotransferase, which transforms glutamate 5-semialdehyde and glutamate into ornithine and ketoglutarate, could link proline catabolism to the synthesis of ornithine [26]. Gabaculine has been shown to be a potent inhibitor of ornithine aminotransferase [27]. It appears that proline accumulation in gabaculine-treated rice roots is most likely due to the inhibition of proline catabolism.

Possible functional roles of proline under stress conditions, as noted by Aspinall and Paleg [1], may include (a) cytoplasmic osmoticum, (b) the hydration of polymers, and (c) serving as a nitrogen source compound during periods of inhibited growth. Since growth is generally considered to be an energy-requiring process, elevated proline levels caused by NaCl are most likely acting as a way to save energy by inhibiting root growth and as a readily utilizable source of energy and amino groups once NaCl is relieved.

Acknowledgements

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