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Levels of endogenous polyamines and NaCl-inhibited growth of rice seedlings

Chuan Chi Lin & Ching Huei Kao*
*Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China (*author for correspondence)*

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Abstract

The role of endogenous polyamines in the control of NaCl-inhibited growth of rice seedlings was investigated. Putrescine, spermidine and spermine were all present in shoots and roots of rice seedlings. NaCl treatment did not affect spermine levels in shoots and roots. Spermidine levels in shoots and roots were increased with increasing concentrations of applied NaCl. NaCl at a concentration of 50 m*M*, which caused only slight growth inhibition, drastically lowered the level of putrescine in shoots and roots. Addition of precursors of putrescine biosynthesis (L-arginine and L-ornithine) resulted in an increase in putrescine levels in NaCl-treated shoots and roots, but did not allow recovery of the growth inhibition of rice seedlings induced by NaCl. Pretreatment of rice seeds with putrescine caused an increase in putrescine level in shoots, but could not alleviate the inhibition effect of NaCl on seedling growth. The current results suggest that endogenous polyamines may not play a significant role in the control of NaCl-inhibited growth of rice seedlings.

Abbreviations: PUT – putrescine; SPD – spermidine; SPM – spermine.

1. Introduction

Aliphatic polyamines (PUT, SPD, and SPM), recently recognized as a new class of plant growth substances [4], are present in all plants examined to date. Polyamine levels and plant growth rates have been positively correlated in a wide variety of conditions; high levels of polyamines are associated with rapidly growing tissues [1, 4].

Several reports have shown that exogenous applications of PUT can overcome the harmful effects of NaCl stress [6, 9, 10]. In addition, carrot cells treated for 2 years in culture with 5 m*M* PUT showed greater plating efficiency on a saline medium than cultures raised in the absence of PUT [8]. NaCl is known to inhibit the growth of rice seedlings [5, 9, 10, 11]. It has been shown that PUT can reduce NaCl-induced inhibition of early seedling growth of rice [10] and that endogenous levels of polyamines decreased in rice seedlings under NaCl stress [11]. However, polyamine accumulation in rice seedlings in response to NaCl has also been reported [2]. Furthermore, Krishnamurthy and Bhagwat [7] reported that PUT was accumulated in salt-sensitive rice cultivars and was significantly reduced in concentration in salt-tolerant ones under saline condition. In view of the contradictory results, the present investigation was conducted to determine the role of endogenous polyamines in the control of NaCl-inhibited 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 thoroughly with distilled water. The seeds were then germinated in Petri dishes (20 cm) containing distilled water at 37 °C under dark condition. After incubation of 1-day, uniformly germinated seeds were selected and transferred to Petri dishes (9.0

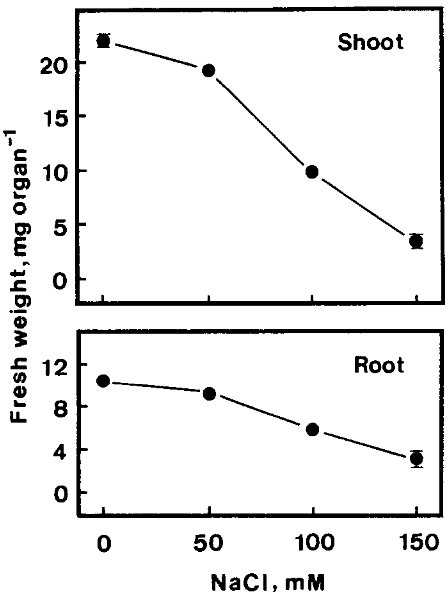

Fig. 1. Effect of NaCl on seedling growth of rice. Seedling growth was measured after 5 days of treatment. Vertical bars represent standard errors (n = 4). Only those standard errors larger than the symbols are shown.

cm) containing two sheets of Whatman No. 1 filter paper moistened with 10 mL of distilled water or test solutions. 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. Each Petri dish contained 20 germinated seeds. Each treatment was replicated 4 times. Fresh weight of shoot and root was measured after 5 days in darkness.

For polyamine extraction, shoot and root samples were homogenized in a motar and pestle in 5% perchloric acid. Polyamine levels were determined using high performance liquid chromatography after benzoylation as described previously [3]. Polyamine levels were expressed as nmol per g fresh weight.

For all measurements, each treatment was repeated four times and all experiments were repeated three times. Similar results were obtained each time. The data reported here are from a single experiment.

Results and discussion

The growth of shoots and roots was followed by measuring the fresh weight of shoot and root. Figure 1 shows that the growth of seedlings decreased with increase in concentration of NaCl. The growth of shoots and roots at 150 m*M* NaCl was reduced to 15 and 30%, respectively, of the control values. These results are, in general, consistent with those reported by Flowers and Yeo [5].

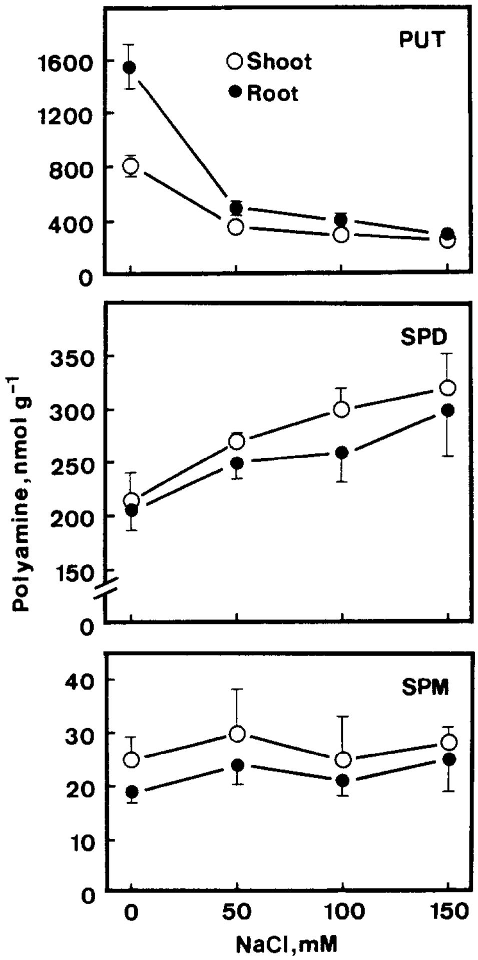

Fig. 2. Effect of NaCl on levels of polyamines in rice seedlings. Polyamines were determined after 5 days of treatment. Vertical bars represent standard errors (n = 4). Only those standard errors larger than the symbols are shown.

PUT, SPD, and SPM were present in both shoots and roots of rice seedlings. However, no detectable levels of cadaverine, a diamine usually found in the family Leguminosae, and diaminopropane, an oxidation product of naturally occurring polyamines, were observed in shoots and roots of rice seedlings. Figure 2 shows that NaCl treatment had no effect on the levels of SPM in shoots and roots of rice seedlings. The effect of NaCl on the levels of SPD in shoots and roots showed a distinct pattern. The levels of SPD in shoots

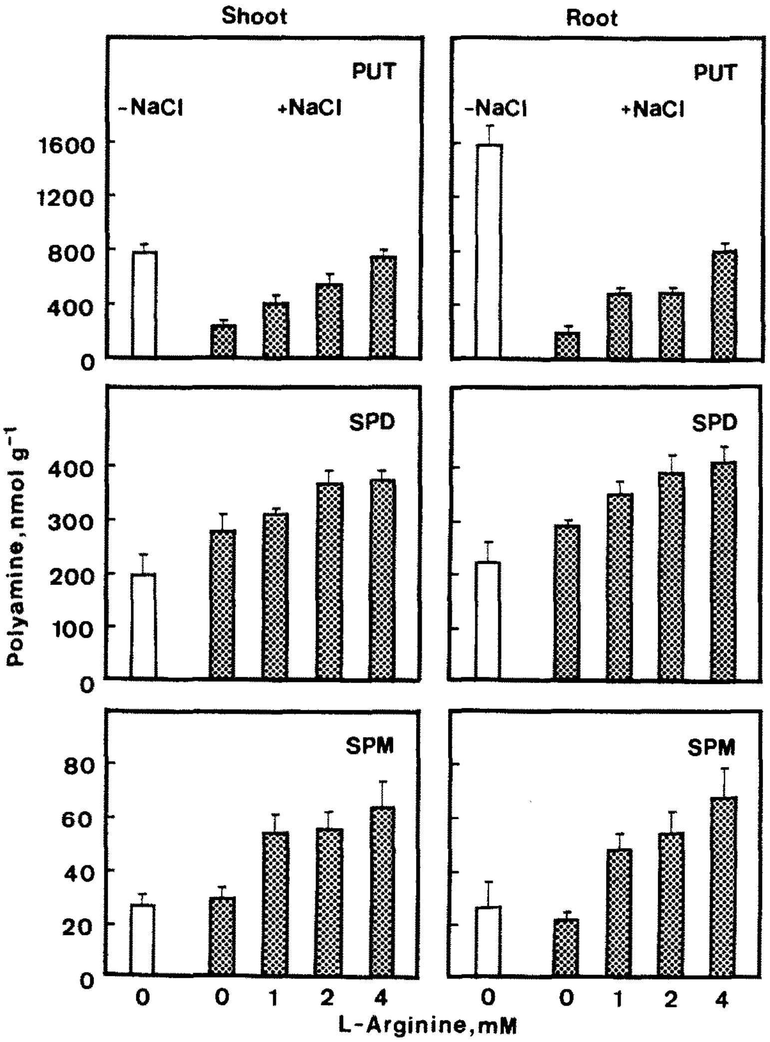

Fig. 3. Effect of L-arginine on polyamine levels in rice seedlings treated with NaCl. The concentration of NaCl was 150 m*M*. Polyamines were measured after 5 days of treatment. Vertical bars represent standard errors (n = 4).

and roots were found to increase with increasing NaCl concentrations (Fig. 2). All these results suggest that endogenous levels of SPD and SPM are not associated with growth inhibition of shoot and root in response to NaCl.

NaCl treatment resulted in the decline of the levels of PUT in shoots and roots (Fig. 2). NaCl at the concentration of 50 m*M*, which caused only slight growth inhibition, drastically lowered the level of PUT in shoots and roots. As judged by the growth of rice seedlings in response to NaCl stress, the rice cultivar Taichung Native 1 used in this study is considered to be NaCl sensitive. However, our results are inconsistent with those reported by Krishnamurthy and Bhagwat [7] who showed that PUT level accumulated in NaCl sensitive rice cultivars under saline condition.

To characterize the role of endogenous polyamines in NaCl-inhibited growth of seedlings, precursors of the biosynthesis of PUT, such as L-arginine and L-ornithine, were used to increase the level of intracellular PUT in NaCl-treated shoots and roots. L-Arginine treatment resulted in the increase of PUT, SPD and SPM levels in both shoots and roots of NaCl-treated seedlings (Fig. 3). The increase of PUT by L-arginine in NaCl-treated shoots was found to be in a concentration dependent manner (Fig. 3). L-Ornithine at a concentration of 4 m*M* also increased PUT, SPD and SPM levels in NaCl-treated shoots and roots (Fig. 4).

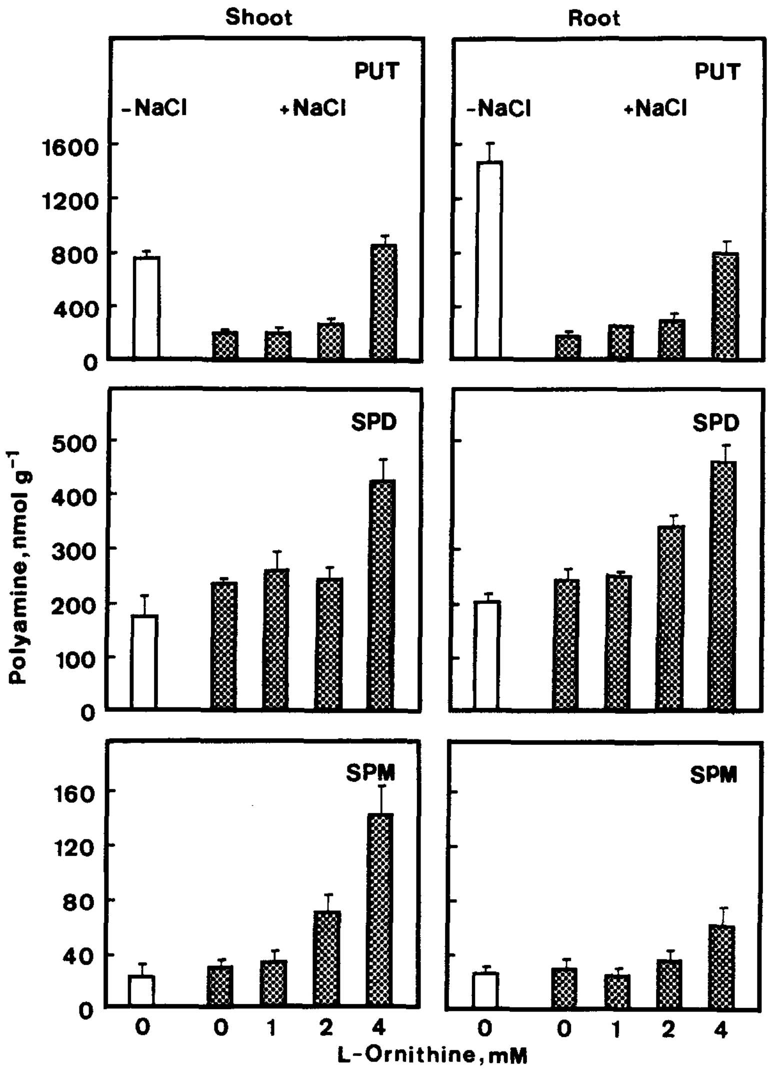

Fig. 4. Effect of L-ornithine on polyamine levels in rice seedlings treated with NaCl. The concentration of NaCl was 150 m*M*. Polyamines were measured after 5 days of treatment. Vertical bars represent standard errors (n = 4).

However, L-arginine and L-ornithine did not reduce growth inhibition of roots under NaCl condition (Fig. 5) and L-ornithine did not counteract the growth inhibition of shoots induced by NaCl (Fig. 5). L-Arginine, which increased PUT level in NaCl-treated shoots in a concentration dependent manner, alleviated growth inhibition of shoots induced by NaCl, but not in a concentration dependent manner (Fig. 5).

To further characterize the role of PUT on the growth of shoot and root of rice seedlings in response to NaCl, we raised rice seedlings in the presence of PUT and tested its effect on growth inhibition by NaCl. Rice seeds were soaked either in distilled water or PUT (1 m*M*) for 24 h in darkness and germinated seeds were transferred to distilled water or NaCl (150 m*M*) for 5 days. Seedlings raised in the presence of additional PUT contained high internal PUT in endosperm and shoots when compared with those raised in distilled water (Table 1). However, the growth of both shoots and roots in NaCl medium was not enhanced by pretreating seeds with PUT (Fig. 6). Those results are inconsistent with those of Prakash and Prathapasenan [10], who demonstrated that pretreatment of rice seeds with PUT could alleviate the inhibitory effect of NaCl on seedling growth. The large reduction in PUT in the PUT-treated seedlings placed in NaCl, compared with

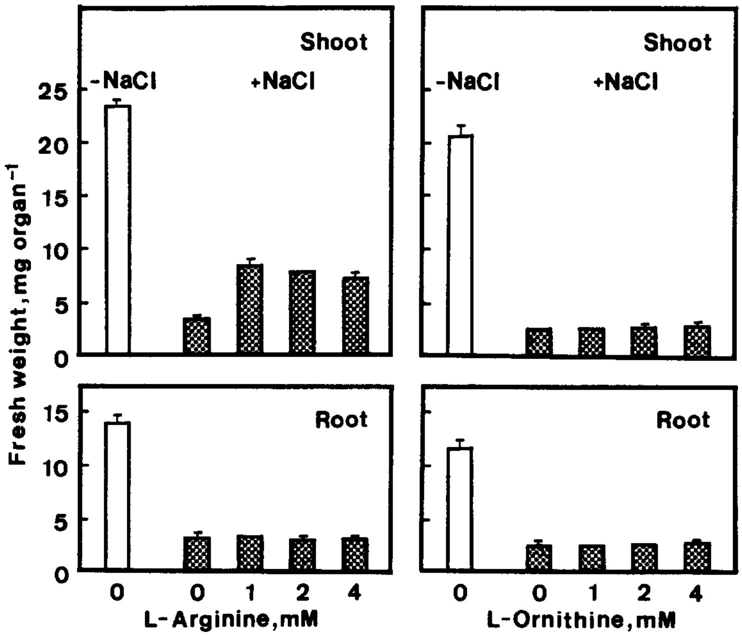

Fig. 5. Effect of L-arginine and L-ornithine on NaCl-inhibited growth of rice seedlings. The concentration of NaCl was 150 m*M*. The growth of rice seedlings was measured after 5 days of treatment. Vertical bars represent standard errors (n = 4).

Table 1. Effect of PUT pretreatment on the level of PUT in endosperm, shoots and roots of rice seedlings. Seeds were pretreated with water or PUT (1 m*M*) for 24 h and then transferred to water or NaCl (150 m*M*). The levels of PUT in endosperm was determined after 24 h of pretreatment and those in shoots and roots were determined after 5 days of treatment

Treatment	PUT (nmol g^{-1})		
	Endosperm	Shoot	Root
Water, 24 h	25.4 ± 2.6	–	–
PUT, 24 h	73.9 ± 1.4	–	–
Water → Water	–	755 ± 68	1079 ± 85
PUT → Water	–	1043 ± 45	1104 ± 44
Water → NaCl	–	238 ± 24	223 ± 15
PUT → NaCl	–	319 ± 7	245 ± 31

PUT-treated, water controls is most probably due to the effect of NaCl on metabolism of PUT. Further work is required to clarify this issue.

Taking all data into account, we conclude that the changes in polyamine levels are not associated with the growth inhibition induced by NaCl. The decline in PUT level seems to be part of the overall expression of NaCl effect on rice seedlings.

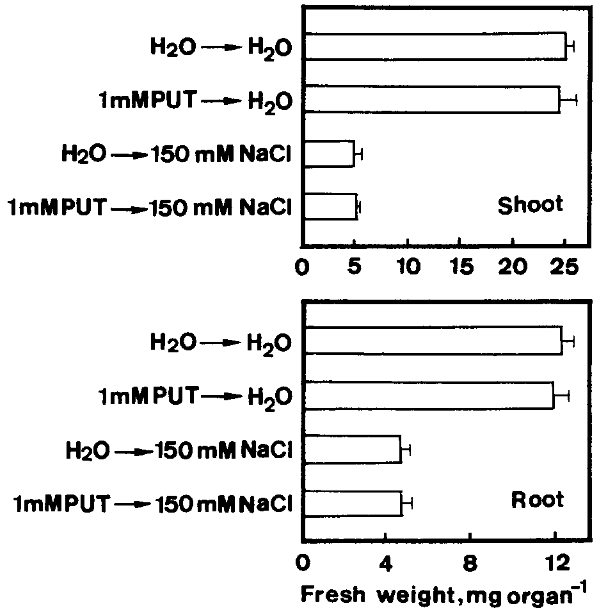

Fig. 6. Effect of PUT pretreatment on NaCl-inhibited growth of rice seedlings. Seeds were pretreated with water or PUT (1 m*M*) for 24 h and then transferred to water or NaCl (150 m*M*) for 5 days. Mean ± SE, 4 replicates.

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