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BRIEF COMMUNICATION

NaCl-induced changes in putrescine content and diamine oxidase activity in roots of rice seedlings

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Abstract

The effects of NaCl on putrescine (Put) content and diamine oxidase (DAO) activity in roots of rice seedlings were examined. NaCl treatment lowered the content of Put and increased the activity of DAO in roots. Our current results indicate that Cl^- is not required for NaCl-induced decline in Put content and increase in DAO activity in roots. Put content in roots of rice seedlings exposed to NaCl is possibly regulated by DAO activity.

Additional key words: *Oryza sativa*, salinity.

Polyamines are ubiquitous nitrogen compounds classified as plant growth substances (Evans and Malmberg 1989). The most common polyamines in higher plants include putrescine (Put), spermidine, and spermine. Accumulation of Put in plant tissues has been demonstrated in potassium deficiency, drought, osmotic stress, chilling and pollution (*e.g.* Kao 1997, Bouchereau *et al.* 1999). NaCl stress, which is closely related to osmotic stress, also causes marked changes in Put content (Basu *et al.* 1988, Prakash *et al.* 1988, Friedmann *et al.* 1989, Krishamurthy and Bhagwat 1989, Lin and Kao 1995, Benavides *et al.* 1997, Santa-Cruz *et al.* 1997, Mansour 2000). However, the effects of NaCl stress on the changes in Put contents are not always clear-cut and the mechanism involved is generally less understood than the changes caused by osmotic stress. It has been shown that the content of Put decreased in rice seedlings under NaCl stress (Prakash *et al.* 1988). However, Put accumulation in rice seedlings in response to NaCl has also been reported (Basu *et al.* 1988). Furthermore, Krishnamurthy and Bhagwat (1989) reported that Put was accumulated in salt-sensitive rice cultivars and was significantly reduced in concentration in salt-tolerant ones under saline condition. However, we demonstrated that NaCl drastically lowered Put content in shoot and roots of rice seedlings of a salt-sensitive cultivar Taichung Native 1 (Lin and Kao 1995).

In most of the studies concerning salt stress, the induced changes in Put has been assumed to rely mainly on Put biosynthesis (Flores 1991). In recent studies, the effect of salinity on the activity of arginine decarboxylase, an enzyme responsible for Put biosynthesis, as well as its transcript level, was compared in salt-sensitive and salt-tolerant rice cultivars (Chattopathay *et al.* 1997). Put content can also be regulated by the catabolism of Put. Diamine oxidases (DAO) catalyse the catabolism of diamines, especially Put, to their corresponding aldehydes, hydrogen peroxide and ammonia (Bouchereau *et al.* 1999). The enzyme has been demonstrated to be substrate inducible and elevated contents of Put in the plant parts cause an increased enzyme activity (Srivastava *et al.* 1977). However, it is not known whether the changes in Put contents caused by salt stress are attributed to the changes in DAO activity. Therefore, the aim of this paper was to find relationships between the DAO activity and endogenous Put content in rice seedling roots subjected to NaCl stress.

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Abbreviations: DAO - diamine oxidase; DIDS - 4, 4'-diisothiocyano-2,2'-disulfonic acid; d.m. - dry mass; Put - putrescine.

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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. Then, the seeds germinated in Petri dishes (20 cm) containing distilled water at temperature of 37 °C under dark. After 1-d 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 cm^3 of distilled water or test solutions. Each Petri dish contained 20 germinated seeds, and each treatment was replicated 4 times. The germinated seeds were allowed to grow at 27 °C in darkness and additional 3 cm^3 of distilled water or test solutions was added to each Petri dish on day 3 of the growth.

For Put extraction, root samples were homogenised in a mortar and pestle in 5 % perchloric acid. Put content was determined using high performance liquid chromatography (*Waters 484*, Milford, USA) after benzoylation as described previously (Chen and Kao 1991). For extraction of DAO, roots were homogenised with ice-cold phosphate buffer (50 mM, pH 7.8) using a pestle and mortar. The homogenate was centrifuged at 10 000 *g* for 20 min at 4 °C. DAO activity was measured by the method of Naik *et al.* (1981). The incubation mixture contained 50 mM phosphate buffer (pH 7.8), 10 mM Put, 0.1 mM pyridoxal phosphate and enzyme extract in a total volume of 4 cm^3. After incubation at 30 °C for 1 h, the reaction was terminated using 1 cm^3 20 % (m/v) trichloroacetic acid. After 30 min, the incubation mixture was centrifuged at 5 000 *g* for 15 min. One cm^3 of ninhydrin mixture (250 mg ninhydrin in 6 cm^3 acetic acid and 4 cm^3 phosphoric acid) was added to the supernatant. Colour was developed at 100 °C for 30 min. After adding 1 cm^3 of acetic acid, absorbance was measured at 510 nm. In controls, trichloroacetic acid was added prior to the enzyme solution. One unit (U) of DAO was defined as an increase of A_{510} per h.

For Na^+ determination, harvested roots were washed three times (each one minute) with distilled water, dried at 65 °C for 2 d, extracted in 1 M HCl at room temperature (Hunt 1982) and analyzed with a flame photometer (*Evans Electroselenium LTD.*, London, England). Chloride was estimated in a separate extract made according to the method described by Hodson *et al.* (1985) and estimated using an ionmeter (*Mittler Delta 350*, London, UK) equipped with chloride ion electrode.

All experiments described here were repeated at least three times; within each experiment, treatments were replicated 4 times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Put, spermidine and spermine were present in roots of rice seedlings. However, no detectable contents of cadaverine, a diamine usually found in the family *Leguminosae*, and diaminopropane, an oxidation product of spermidine and spermine, were observed in roots of rice seedlings. NaCl treatment resulted in a decline of the content of Put in roots (Table 1). 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 (Lin and Kao 1995). However, our results are inconsistent with those reported by Krishamurthy and Bhagwat (1989) who showed that Put accumulated in NaCl sensitive rice cultivars under salinity. The decline of Put content in response to NaCl stress is not restricted to the roots of rice seedlings. We also observed that NaCl treatment resulted in a decline of Put content in the endosperm of rice seeds during rice germination and in detached rice leaves of 12-d-old rice seedlings grown in greenhouse, under natural light and the day/light temperature of 30/25 °C (data not shown).

DAO was present in roots of rice seedlings (Table 1). The reduction of Put content with increasing NaCl concentration is correlated with an increase in DAO activity (Table 1). To be sure that the described decline of Put and increase in DAO activity was related to an increase in root Na^+ and/or Cl^- content, Na^+ and Cl^- contents were determined in roots treated with either water or NaCl (Table 1). The decrease in Put content and increase in DAO activity was closely correlated with the increase of Na^+ and Cl^- content in roots. However, the Cl^- content in roots was approximately twice than Na^+ content (Table 1). Basu *et al.* (1988) and Basu and Ghosh (1991) also reported that Cl^- content in NaCl-treated roots of rice seedlings was much higher than Na^+ content.

Table 1. Effects of NaCl on the contents of Cl^-, Na^+ and Put, and the activity of DAO in roots of rice seedlings. All measurements were made 5 d after treatment. Means ± SE, *n* = 4.

NaCl [mM]	Cl^- [mmol g^{-1}(d.m.)]	Na^+ [mmol g^{-1}(d.m.)]	Put [μmol g^{-1}(d.m.)]	DAO [U g^{-1}(d.m.)]
0	0.54 ± 0.01	0.14 ± 0.01	15.40 ± 1.52	6.34 ± 0.33
50	1.70 ± 0.10	0.97 ± 0.03	5.48 ± 0.28	7.65 ± 0.38
100	3.62 ± 0.27	1.54 ± 0.03	4.13 ± 0.32	10.93 ± 0.18
150	5.61 ± 0.74	2.43 ± 0.04	2.87 ± 0.18	14.89 ± 1.56

Table 2. Effects of NaCl (150 mM) on the contents of Cl^-, Na^+ and Put, and the activity of DAO in roots of rice seedlings in the presence or absence of DIDS (0.05 mM). All measurements were made 5 d after treatment. Means ± SE, $n = 4$.

Treatment	Cl^- [mmol g^{-1}(d.m.)]	Na^+ [mmol g^{-1}(d.m.)]	Put [μmol g^{-1}(d.m.)]	DAO [U g^{-1}(d.m.)]
H_2O	0.52 ± 0.01	0.12 ± 0.01	15.80 ± 1.03	5.78 ± 0.29
DIDS	0.50 ± 0.01	0.13 ± 0.02	14.70 ± 0.96	6.22 ± 0.33
NaCl	5.61 ± 0.24	2.43 ± 0.04	2.83 ± 0.21	12.91 ± 0.17
NaCl + DIDS	3.13 ± 0.31	2.48 ± 0.05	2.74 ± 0.06	13.32 ± 0.29

Table 3. Effects of NaCl (100 mM) and Na-gluconate (50 mM) on the contents of Cl^-, Na^+ and Put, and the activity of DAO in roots of rice seedlings. All measurements were made 5 d after treatment. Means ± SE, $n = 4$.

Treatment	Cl^- [mmol g^{-1}(d.m.)]	Na^+ [mmol g^{-1}(d.m.)]	Put [μmol g^{-1}(d.m.)]	DAO [U g^{-1}(d.m.)]
H_2O	0.52 ± 0.01	0.12 ± 0.01	15.83 ± 1.03	5.78 ± 0.29
NaCl	3.62 ± 0.17	1.51 ± 0.03	3.41 ± 0.33	9.59 ± 0.27
Na-gluconate	0.58 ± 0.03	1.33 ± 0.04	3.24 ± 0.17	10.38 ± 0.74

The effect of NaCl on the decline in Put content and increase in DAO activity in roots of rice seedlings could be attributed to Na^+, Cl^- or both. Previously, we have reported that NaCl treatment resulted in an accumulation of proline in roots of rice seedlings (Lin and Kao 1996). We also observed that NaCl-induced proline accumulation in roots of rice seedlings was mainly due to Na^+, rather than Cl^- (unpublished data). Thus, it is of great interest to know whether the decline in Put and increase in DAO activity caused by NaCl in roots of rice seedlings are also due to Na^+ rather than Cl^-. To test this possibility, we determined the effect of 4,4'-diisothio-cyano-2,2'-disulfonic acid (DIDS), a nonpermeating amino-reactive disulfonic acid known to inhibit the uptake of Cl^- (Lin 1981), on NaCl-induced decline in Put content and increase in DAO activity of roots of rice seedlings. Treatment with NaCl in the presence of DIDS caused less increase in Cl^- concntration in roots than that in absence of DIDS but did not affect Na^+ and Put contents and DAO activity when compared with NaCl treatment (Table 2). This suggests that Cl^- is not involved in NaCl-induced decline in Put content and increase in DAO activity in roots of rice seedlings. DIDS treatment resulted in about 40 % reduction of Cl^- in NaCl-treated roots (Table 2). Thus, no effect of DIDS on Put content and DAO activity in roots of rice seedlings treated with NaCl can be explained by the possibility that the content of Cl^- is still high enough to exert its effect. In order to test further the involvement of Cl^-, experiments were performed to compare the effect of NaCl with that of Na-gluconate, the anion of which is not able to permeate the membrane (Blumwald and Poole 1985). Treatment with 50 mM Na-gluconate caused similar Na^+ content but had much less Cl^- content in roots in comparison with treatment with 100 mM NaCl. The reduction of Put content and increase in DAO activity in roots treated by 50 mM Na-gluconate was similar to that treated by 100 mM NaCl. These results strongly suggest that Cl^- is not required for NaCl-induced reduction in Put content and increase in DAO activity in roots of rice seedlings.

In conclusion, our results indicate that DAO activity in roots of rice seedlings exposed to NaCl is negatively correlated with Put content. We also observed that Cl^- is not involved in NaCl-induced changes in Put content and DAO activity in roots of rice seedlings.

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