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Amelioration of NaCl stress by arginine in rice seedlings: changes in endogenous polyamines

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Abstract

Effects of NaCl (0.1 - 0.2 M) alone or in combination with 1 mM arginine on growth and endogenous polyamine (PA) content have been observed in two cultivars of rice differing in NaCl stress tolerance. The germination, seedlings fresh mass and water content decreased with increase in salinity in both the cultivars. This inhibition was partially alleviated by application of arginine. Cv. CSR-27 exhibited relatively better germination than cv. Bas-370 at different salinities. Total PA content increased in both the cultivars under NaCl stress alone and in combination with arginine. Putrescine to spermidine and spermine ratio was higher in NaCl-treated seedlings being more in cv. Bas-370 as compared to cv. CSR-27 and the ratio reversed to almost control level when arginine was applied along with NaCl.

Additional key words: *Oryza sativa*, putrescine, spermidine, spermine, salt stress.

Introduction

Salt stress results in depletion of water content (osmotic effect) and also causes specific ionic effects. Salinity tolerance is multigenic trait controlled by several gene products, which regulate hormonal balance, nutrient status, redox potential as well as inter- and intracellular signalling and transport capacity (Yeo 1998, Hare *et al.* 1998). Plants subjected to salt stress accumulate an array of osmolytes (sugars, glycine betaine, proline and polyamines) in their cytosol. In addition, plants under salinity survive by restricting the absorption of Na^+ and Cl ions and/or their compartmentation.

Polyamines (PAs) are ubiquitous polycationic nitrogenous bases reported to be involved in a variety of biological processes such as cell division, growth and differentiation (for reviews see, Walden *et al.* 1997, Malmberg *et al.* 1998, Kakkar *et al.* 2000). Being positively charged, PAs can interact with anionic components of plasma membrane such as phospholipids and with phosphate groups of DNA and RNA (Roberts *et al.* 1986, Kaur-Sawhney and Applewhite 1993) and retard membrane deterioration. They have also been shown to prevent oxidative damageby scavenging free radicals (Drolet *et al.* 1986). Alteration in PA content and enzymes of their biosynthesis have been reported to occur in response to various kinds of stresses, such as osmotic shock, mineral deficiency, salt stress, chilling stress and atmospheric pollutants. Accumulation of PAs especially putrescine (Put) have been reported in rice (Basu *et al.* 1988, Katiyar and Dubey 1990) and maize (Willadino *et al.* 1996) under salt stress. On the other hand, in salt tolerant species content of PAs decreased (Priebe and Jager 1978, Zacchini *et al.* 1997). In rice, foliar application of Put improved growth and yield under salinity (Prakash and Prathapsanen 1988, Krishnamurthy 1991). However, Put excess had some negative effects (Strogonov *et al.* 1972, Di-Tomaso *et al.* 1989). NaCl stress has also induced accumulation of spermidine and spermine (Krishnamurthy and Bhagwat 1989, Friedman *et al.* 1989, Shevyakova *et al.* 1985, Santa-Cruz *et al.* 1997, Tattini *et al.* 1993).

Since, high concentrations of NaCl damaged membrane integrity resulting in severe injury to the cells,

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Abbreviations: PA(s) - polyamine(s); Put - putrescine; Spd - spermidine; Spm - spermine.
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whereas PAs are membrane stabilizers and oxidative protectants, we designed an experiment to determine the effects of NaCl alone and in combination with arginine (a precursor of putrescine synthesis) on growth performance and endogenous PA content in two cultivars of rice exhibiting different salinity tolerance. We have chosen seedling stage, which is suggested to be more prone to stress conditions.

Materials and methods

Plants: Seeds of *Oryza sativa* L. cultivars Bas-370 (salt-sensitive) and CSR-27 (salt-tolerant) procured from CSSRI, Karnal (Haryana), India, were surface sterilized with 0.1 % $HgCl_2$ for 2 min followed by thorough washing with distilled water. The seeds were germinated in Petri dishes lined with filter paper containing 10 cm^3 of 0.1 to 0.2 M NaCl. Seedlings were raised in an incubator under continuous illumination (PAR 40 μmol m^{-2} s^{-1}) and temperature of 25 ± 2 °C. Three replicates each of 25 seeds were used. Germination counts were taken after 5 d. Fresh mass of ten seedlings in triplicate was recorded on the fifth day. Later, the seedlings were oven dried at 80 °C for 48 h and their dry mass was determined.

Polyamine extraction and determination: Extraction and benzoylation of PAs from tissues were performed as described previously (Kakkar and Nagar 1997). The tissue (5.0 g fresh mass) was homogenized in 10 % (v/v) perchloric acid. The homogenates were kept at 4 °C for 1 h and then centrifuged at 15 000 g for 30 min at 5 °C. The supernatant was treated with insoluble polyvinyl pyrollidone (5.0 mg cm^{-3} extract) and stirred for 1 h to remove phenolics and other interferring compounds that do not allow accurate estimation of PAs. These were then filtered and approx. 0.5 cm^3 of 0.01 M HCl was used to clear out the vials. 1 cm^3 of 2 M NaOH and 0.5 cm^3 of PA aliquot was mixed followed by 0.025 cm^3 benzoyl chloride. After vortexing for 30 s and incubating for 25 min at 28 °C, 2 cm^3 of saturated NaCl solution was added to stop the reaction. Benzoylated PAs were extracted with 2 cm^3 of cold diethyl ether. The samples were centrifuged at 1 500 *g* at 4 °C for 10 min and 1 cm^3 of the ether phase was evaporated to dryness under a stream of air and redissolved in 0.5 cm^3 of methanol (HPLC grade) and 0.02 cm^3 of the extract was injected into the HPLC (*Kontron system 400*, Switzerland). The elution was carried out at a rate of 1.2 cm^3 min^{-1} in 64 % methanol for 15 min at 30 °C with UV detector at 254 nm. Peak areas were integrated by a digital integrator and concentrations were calculated from standard curve responses of the known PAs (*Sigma Chemical Co.*, St. Louis, USA).

Results and discussion

In the present study, effects of NaCl (0.1 - 0.2 M) alone and along with arginine (1 mM) on growth performance (Tables 1, 2) and endogenous PA content (Table 3) of tolerant rice cutivar CSR-27 and sensitive cv. Bas-370 were observed. The germination percentage and seedling fresh mass and water content decreased with increased salinity in both the cultivars. This inhibition was partially alleviated by 1 mM arginine. Although there were no significant differences in dry mass between control and NaCl-treated rice seedlings, the differences did exist among cultivars: CSR-27 showed more dry mass and higher water content than Bas-370. Moreover, CSR-27 exhibited relatively better germination capacity than Bas-370 at different salinity levels. The total PA content increased in both the cultivars under NaCl stress alone and in combination with arginine. However, Bas-370

Table 1. Effect of NaCl (0.1 - 0.2 M) on the growth performance of two rice cultivars after five day treatment. Means ± SD of three replicates of 25 seedlings.

NaCl [M]	Germination [%]		Fresh mass [mg seedling^{-1}]		Dry mass [mg seedling^{-1}]		Water content [%(d.m.)]	
	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27
Control	98.0 ± 0.6	96.0 ± 0.8	33.2 ± 2.3	47.5 ± 5.8	16.5 ± 2.3	20.9 ± 4.9	101.21	127.81
0.10	80.0 ± 2.4	82.0 ± 3.2	29.5 ± 4.3	34.6 ± 4.9	16.7 ± 1.3	21.2 ± 4.5	76.43	62.90
0.15	62.0 ± 4.8	69.0 ± 5.9	27.4 ± 3.8	32.9 ± 4.7	17.3 ± 3.6	21.7 ± 4.8	58.24	47.06
0.20	34.0 ± 4.2	42.0 ± 5.6	21.5 ± 3.2	31.8 ± 5.7	17.5 ± 3.4	22.5 ± 4.8	22.85	41.60

Table 2. Effects of arginine (1 mM) alone and along with NaCl (0.15 M) on the growth performance of two rice cultivars after five day treatment. Means ± SD of three replicates of 25 seedlings.

Treatment	Germination [%]		Fresh mass [mg seedling^{-1}]		Dry mass [mg seedling^{-1}]		Water content [%(d.m.)]	
	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27
Control	98.0 ± 0.6	96.0 ± 0.8	33.2 ± 2.3	47.5 ± 5.8	16.5 ± 2.3	20.9 ± 4.9	101.21	127.81
Arginine	97.0 ± 0.8	98.0 ± 0.4	37.3 ± 3.2	49.2 ± 6.2	17.2 ± 2.6	21.2 ± 4.7	116.8	132.07
Arg. + NaCl	72.0 ± 2.9	76.0 ± 3.6	29.4 ± 3.6	34.9 ± 4.2	17.8 ± 2.5	22.4 ± 4.9	65.16	56.07

Table 3. Efiects of NaCl (0.15 M) and arginine (1 mM) alone and in combination on the content of different endogenous polyamines [nmol g^{-1}(f.m.)] in two rice cultivars after five day treatment. Means ± SD of two replicates from a single experiments.

Treatment	Put		Spd		Spm		Put/Spd+Spm ratio	
	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27	Bas-370	CSR-27
Control	680 ± 24	640 ± 18	590 ± 32	570 ± 28	115 ± 14	105 ± 12	0.97	0.95
NaCl	1360 ± 52	935 ± 34	795 ± 48	690 ± 44	95 ± 12	80 ± 8	1.53	1.21
Arginine	735 ± 38	525 ± 22	585 ± 34	465 ± 28	85 ± 4	95 ± 6	1.09	0.93
Arg. + NaCl	825 ± 54	780 ± 42	735 ± 26	680 ± 42	110 ± 8	125 ± 12	0.97	0.96

showed more accumulation as compared to CSR-27. Arginine treatment did not affect Put content in CSR-27. However, Put to Spd and Spm ratio was higher in NaCl-treated seedlings being more in Bas-370 as compared to CSR-27 and the ratio reversed to almost control level when arginine was applied along with NaCl.

NaCl stress can result in anion/cation imbalance, reduction in water absorption, cell division, cell enlargement, and production of free radical. Whereas, diamine Put and polyamine Spd and Spm are suggested to be involved in stabilization of nucleic acid, proteins (Bestford *et al.* 1991) and ionic balance (Lucarini and Sangwan 1987). Differences in PA response under salt stress have been reported among and within plant species (Friedman *et al.* 1989). The Put inerease under salt stress in our studies is consistent with the results obtained in maize callus (Willadino *et al.* 1996) and rice plantlets (Krishnamurthy and Bhagwat 1989, Krishnamurthy 1991) and embryo axes (Katiyar and Dubey 1990). However, decrease in free Put content after arginine treatment in both the cultivars might be due to the conjugation of the former with small molecules forming soluble conjugates and/or their binding with proteins, nucleic acids to form insoluble conjugates. Improvement in germination percentage under salt stress after pretreatment with Put in *Brassica*, *Vigna* and *Triticum* has also been reported (Kakkar and Rai 1992, Mishra and Sharma 1994). In another study, putrescine increased the growth and leaf viability in five rice cultivars differing in salinity tolerance under NaCl stress (Lutts *et al.* 1996).

Putrescine content and Put/Spd ratio has also been shown to increase under salt stress (Flores and Galston 1984) and Put/Spd ratio can be better indicator of stress than Put content alone (Minocha *et al.* 1996). Our results are in close agreement with Krishnamurthy and Bhagwat (1989) and Zacchnini *et al.* (1997) who observed higher increase in Put/PA ratio under NaCl stress in salt-sensitive cultivar than in salt-tolerant one. Recently, Santa-Cruz *et al.* (1998) reported higher Put content in tolerant tomato cultivar compared to sensitive one. Thus, present findings suggest that screening the plants for altered PA content and metabolism could be very useful to investigate their role in the mechanisms of salt tolerance. Further investigations are needed on the conjugated and bound PA content after arginine treatment.

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