BIOLOGIA PLANTARUM 35(1): 69-72, 1993

Counteraction of exogenous L-proline with NaCl in salt-sensitive cultivar of rice

D. ROY, N. BASU, A. BHUNIA and S.K. BANERJEE

Department of Biochemistry, University College of Science, Calcutta University 35, Ballygunge Circular Road, Calcutta - 700 019, India.

Abstract

The counteraction of exogenous L-proline at different concentrations with salinity (100 mM NaCl at sublethal concentration) were observed on germinating rice. Supplemented 30 mM L-proline was shown to be the most effective; at higher concentration it reduced seedling growth and K^+/Na^+ ratio. Thus 30 mM L-proline can alleviate the salinity stress in rice seedlings.

Introduction

Saline soil inhibits the grown of crop plants and the extent of reduction is dependent on the species of the plant, the salinity level and the ionic composition of the soil.

Rice is a salt-sensitive species and accumulates Na^+ at only moderate external salinities (Yeo and Flowers 1982). Most high yielding modern rice cultivars perform poorly under saline condition (Anwar *et al.* 1987). Among different parameters respond to salt-stress in rice, the most significant one is rapid accumulation of free proline (Krishnamurthy *et al.* 1987, Roy *et al.* 1992). Different suggestions on role of accumulated proline has been registered but it is not yet cleared. The counteraction of proline supplementation against water deficit (Handa *et al.* 1986), and in *Vigna radiata* L. callus cultures (Kumar and Sharma 1989) exposing salinity were tested earlier. The present communication is concerned with the action of proline with salinity (100 mM NaCl at sublethal concentration) during rice germination.

Material and methods

Rice (*Oryza sativa* L.) seeds of 'Ratna' cultivars were collected from Calcutta University seed farm. Seeds were on the surface sterilized with 0.1 % $HgCl_2$, washed repeatedly with distilled water and allowed to germinate after imbibition in dishes.

Received 10 October 1991, *accepted* 23 April 1992.

Arnon and Hoagland (1938) half strength media was served as control while for treatment 100 mM (sublethal concentration) NaCl alone and with L-proline at different concentrations (20 mM, 30 mM, 40 mM, 50 mM) were supplied in half-strength media, 10 cm^3 in each dishes were used. The dishes were kept in a growth chamber under the temperature at 30 ± 2 °C, irradiance of 150 μmol m^{-2} s^{-1} and relative humidity of 85 % to maintain proper growth conditions.

Growth of roots and shoots were measured during germination and analyzed statistically using analysis of variance. Internal free proline level of rice was determined according to Bates *et al.* (1973) during early germination period under saline environment. Internal concentration of Na^+ and K^+ ions were measured in different hours of germination by digesting fixed amount tissues with the help of HNO_3 and $HClO_4$ (3:1) mixture in a digestion chamber. After proper digestion they were estimated by directly coupled (DC) plasma *Scan Analyzer* (*Beckman*) fixing wavelength for Na^+ at 589,59 nm and K^+ at 766.49 nm.

Results and discussion

Application of low concentrations of exogenus proline (20 mM, 30 mM) stimulated the growth of sensitive rice seedlings alleviating the inhibitory effect of 100 mM NaCl. But at high concentrations (40 mM, 50 mM), growth were inhibited more than under the NaCl treated alone (Table 1). From the result it appears that 30 mM concentration of proline was the most efficient in maintaining optimum growth. This confirms the earlier finding regarding stimulatory effect of exogenus proline at low concentraion in NaCl-stressed *Vigna radiata* cells (Kumar and Sharma 1989). Influence of supplemeted L-proline on K^+/Na^+ ratio indicates that higher concentrtions of L-proline (40 mM, 50 mM) decreases the K^+/Na^+ ratio more than 100 mM NaCl alone. But at low concentrations (20 mM, 30 mM), it stimulated K^+/Na^+ ratio in comparison to salt-treated alone (Table 2).

From K^+/Na^+ ratio level it can be predicted that at higher concentration of L-proline, the permeability of cell membrane become non-selective, as at high concentrations of L-proline there was sudden increase in the level of Na^+. On the other hand, the low levels of exogenous L-proline maintained selective permeability for K^+; Na^+ was kept fairly low and non-toxic to germinating rice that was reflected by good growth of rice seedling under such experimental condition. It is supported by the report published earlier (Matoh *et al.* 1988) that high K^+ ion content is regarded as salt tolerance capability and in cereals, salt damage has reported to be due to ion excess rather than water deficit (Lynch and Läuchli 1984). Recently in *Vigna radiata* callus exogenous proline at low concentration has fund effective, however at higher concentrations more influx of Na^+ has also reported (Kumar and Sharma 1989). So, the data presented here may suggest that optimum concentration for exogenous proline is 30 mM which effectively alleviate salt stres and toxicity of sublethal dose of NaCl, thus crop yield can be improved by proper use of supplemented proline at low concentration during germination of rice when exposed to salinity.

Table 1. Effect of exogenous L-proline on length of shoots and roots [cm] of germinating rice seedling under NaCl stress. Results are expressed as means ± S.D. of four sets of experiments.

Age of seedlings [d]	NaCl [mM] / L-proline [mM]	0 / 0	100 / 0	100 / 50	100 / 40	100 / 30	100 / 20
3	Root	3.5 ± 0.10	2.6 ± 0.07	2.4 ± 0.12	2.5 ± 0.10	2.3 ± 0.11	2.4 ± 0.10
	Shoot	1.1 ± 0.03	0.7 ± 0.02	0.6 ± 0.02	0.7 ± 0.02	0.9 ± 0.02	0.7 ± 0.01
5	Root	5.2 ± 0.20	4.4 ± 0.17	4.1 ± 0.12	4.3 ± 0.12	4.9 ± 0.19	4.5 ± 0.14
	Shoot	4.3 ± 0.12	3.6 ± 0.10	3.3 ± 0.13	3.4 ± 0.06	3.8 ± 0.11	3.6 ± 0.08
7	Root	7.2 ± 0.36	5.5 ± 0.22	5.2 ± 0.20	5.4 ± 0.21	6.1 ± 0.18	5.7 ± 0.17
	Shoot	6.5 ± 0.26	5.2 ± 0.15	4.9 ± 0.14	5.0 ± 0.15	5.5 ± 0.20	5.3 ± 0.14

Table 2. Effect of exogenous L-proline on K^+/Na^+ ratio of rice seeds during germination of rice seedling under NaCl stress. Results are expressed as means ± S.D. of four sets of experiments.

Duration of treatment [h]	NaCl [mM] / L-proline [mM]	100 / 0	100 / 50	100 / 40	100 / 30	100 / 20
2.5		14.79	13.53	14.26	14.58	14.35
24		4.35	3.74	4.17	4.41	4.37
48		1.96	1.42	1.73	2.07	1.99
72		1.27	1.03	1.18	1.38	1.30
96		1.09	0.95	0.99	1.17	1.11
120		0.98	0.87	0.91	1.06	1.01

References

Anwar, A.K., Akbar, M., Seshu, V.D.: Ethylene as an indicator of salt tolerance in rice. - Crop Sci. **27**: 1242-1247, 1987.

Arnon, D.I., Hoagland, D.R.: The water culture method for growing plants without soil. - Univ. Calif. Agr. exp. Circ. **347**: 1-39, 1938.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline in water-stressed studies. - Plant Soil **39**: 205-210, 1973.

Handa, S., Handa, A.K., Hasegawa, P.M., Bressan, R.A.: Proline accumulation and the adaptation of cultured plant cells to water stress. - Plant Physiol. **80**: 938-943, 1986.

Kumar, V., Sharma, R.D.: Effect of exogenous proline on growth and ion content in NaCl stressed and non-stressed cells of mung bean, *Vigna radiata* var. radiata. - Indian J. exp. Biol. **27**: 813-815, 1989.

Krishnamurthy, R., Anbazhagan M. Bhagwat, A.K.: Accumulation of free amino acids and distribution of Na^+, Cl^- and K^+ ion rice varieties exposed to NaCl stress. - Indian J. Plant Physiol. **30**: 183-188, 1987.

Lynch, J., Läuchli, A.: Potassium transport in salt-stressed barley roots. - Planta **161**: 295-301, 1984.

Matoh, T., Matsushita, N., Takahashi, E.: Salt tolerance of the reed plant *Phragmites communis*. - Physiol. Plant. **72**: 8-14, 1988.

Roy, D., Bhunia, A., Basu, N., Banerjee, S.K.: Effect of NaCl-salinity on metabolism of proline in salt-sensitive and salt-resistant cultivars of rice. - Biol. Plant. **34**: 159-162, 1992.

Yeo, R.A., Flowers, J.T.: Accumulation and localisation of Na^+ within the shoots of rice (*Oryza sativa* L.) varieties differing in salinity resistance. - Physiol. Plant. **56**: 343-348, 1982.