BIOLOGIA PLANTARUM 34 (1-2): 159-162, 1992

BRIEF COMMUNICATION

Effect of NaCl-salinity on metabolism of proline in salt-sensitive and salt-resistant cultivars of rice

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

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

Abstract

The effect of NaCl at sublethal concentration was observed on germinating seeds of salt-sensitive and -resistant rice cultivars with respect to the level of proline regulatory enzymes and the growth of seedlings on different days of early germination period. The two enzymes of proline biosynthesis and catabolism, Δ-pyrroline-5-carboxylate reductase and L-proline dehydrogenase, were taken into consideration to observe the effects of 100 mM NaCl on their activities in both rice cultivars. The activity of Δ-pyrroline-5-carboxylate reductase in salt-resistant cultivar was increased twice after 5 d in 100 mM NaCl. Simultaneously, the activity of L-proline dehydrogenase was decreased significantly. High activities of Δ-pyrroline-5-carboxylate reductase may be regarded as a biological marker for screening the sensitive and resistant cultivars of rice seed under NaCl-salinity.

Most high-yielding modern rice cultivars give poor yield under saline conditions (*e.g.* Hoffman 1981). Rapid accumulation of free proline in tissues of many plant species as a response to salt effect, drought or temperature stress, has been atributed to protection of the cellular membrane and enzyme stabilization and/or osmoregulation (Aspinall and Paleg 1981), but no significant enzymatic studies have been done in relation to significant proline accumulation. Δ-Pyrroline-5-carboxylate reductase (L-proline: NAD(P)-5-oxidoreductase, EC 1.5.1.2.) catalyses the final step in the biosynthetic pathway leading from glutamic acid to proline and L-proline dehydrogenase catalyses the reverse step to maintain optimum L-proline concentration for proper growth.

The objective of these investigations were to measure the activities of these proline regulatory enzymes within salt-sensitive and -resistant rice cultivars (namely

---

Received 13 December 1990, *accepted* 13 May 1991.

* To whom correspondence should be sent.

'Ratna' and 'CSR-4') following salinization during early germinating period to ascertain the cause for high proline accumulation under salt stress and to investigate also the potentiality of their activities as a biological marker for screening rice cultivars and breeding lines for salinity tolerance.

Rice (*Oryza sativa* L.) seeds of 'Ratna' (salt-sensitive cultivar) and 'CSR-4' (salt-resistant cultivar) were collected from 'Calcutta University Seed Farm' and 'Central Soil Salinity Research Institute', Canning, India, respectively. Seeds were surface sterilised with 0.1 % $HgCl_2$, washed repeatedly with distilled water and allowed to germinate after imbibition in Petri dishes. Arnon and Hoagland (1938) half-strength medium served as control while for treatment, 100 mM (sublethal concentration) NaCl was supplied in half-strength medium, solutions were replaced everyday by fresh ones. The Petri dishes were kept in a growth chamber under irradiance 150 μmol m^{-2} s^{-1} at 30 ± 2 °C and 85 % air humidity.

Growth of roots and shoots were measured during germination (replicated four times and analyzed statistically using analysis of their variance). Δ-Pyrroline-5-carboxylate reductase and L-proline dehydrogenase from rice seedlings were extracted with 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1 mM cysteine and 0.1 mM EDTA after grinding rice seedlings in a mortar with sea sand. The homogenate was filtered through cheesecloth and centrifuged at 31 000 *g* for 30 min. The above enzymes were determined in the supernatant.

Δ-Pyrroline-5-carboxylate reductase activity was assayed according to Rena and Splittstoesser (1975) after synthesizing DL-1-pyrroline-5-carboxylate from its 2.4-dinitrophenyl hydrazone derivative following the method of Mezl and Knox (1976). The assay mixture contained, in a final volume of 1.0 ml, 0.7 mM DL-1-pyrroline-5-carboxylate, 0.19 mM NADH, 50 mM Tris-base, 25-30 μl of enzyme extract at pH 7.0, the rate of decrease in absorbance at 340 nm (A_{340}), was recorded at 30 °C.

L-proline dehydrogenase activity was also assayed according to Rena and Splittstoesser (1975) using 20 mM L-proline as a substrate, 10 mM NAD, 0.6 ml of extract and 0.1 M Na_2CO_3 - $NaHCO_3$ buffer at pH 10.3 in a final volume of 3.0 ml. The rate of increase in A_{340} was recorded at 30 °C. Protein was estimated, according to Lowry *et al.* (1951) using bovine serum albumin as standard. At different stages of germination a free proline level was measured according to Bates *et al.* (1973).

Growth of roots and shoots was greater in 'CSR-4' than in 'Ratna' under salinity (Table 1).

Δ-Pyrroline-5-carboxylate reductase activity was higher in resistant cultivar than in sensitive cultivar from early period of germination and under salinity, the activity was stimulated by 2.4 times after 5 d germination in 'CSR-4' as compared to 'Ratna' (Table 2). Stimulation of this activity (3 times in *Brevibacterium lactofermentum* under osmotic stress) was reported recently by Yoshio *et al.* (1989). The activity of the enzyme, increased 3.5 times when sedlings of *Pennisetum typhoides* were grown under salt stress (Huber 1974). More recently, activation of Δ-pyrroline-5-carboxylate reductase activity in suspension cultures of *Mesembryanthemum nodiflorum* was reported by Treichel (1986) and in *Chlorella autotrophica* and *C. saccharophila* by Laliberte and Hellebust (1989).

Table 1. Growth of seedlings of salt-sensitive (Ratna) and salt-resistant (CSR-4) rice cultivars under salt-stress (100 mM NaCl). Length of shoots or roots [cm] ± S.D. of four sets of experiments

Time [d]	Ratna control		NaCl		CSR-4 control		NaCl	
	root	shoot	root	shoot	root	shoot	root	shoot
2	2.7±0.05	0.9±0.01	2.1±0.03 **	0.5±0.01 *	3.1±0.05	1.9±0.03	2.4±0.04 *	1.3±0.02 **
4	4.5±0.08	3.2±0.05	3.8±0.07 **	2.8±0.05 **	5.4±0.01	4.4±0.07	4.9±0.09 **	4.1±0.07 *
6	6.6±0.13	5.4±0.10	5.1±0.10 **	4.7±0.08 **	7.1±0.13	6.7±0.12	6.4±0.12 **	6.2±0.11 *

*significant at $P < 0.01$
**significant at $P < 0.001$

On the other hand, L-proline dehydrogenase activity was significantly inhibited in the salt condition of both cultivars. But it was more pronounced in 'CSR-4' compared to 'Ratna' (Table 2). Inhibition of L-proline dehydrogenase activity under a salt-stress condition in horsegram (*Dolichos biflorus* L.) was reported recently by Sudhakar *et al.* (1987).

Table 2. Activities of Δ-pyrroline-5-carboxylate reductase, L-proline dehydrogenase and free proline content in rice seedlings (5 d old) during germination under salt-stress (cultivars see Table 1).

Treatment	Δ-pyrroline-5-carboxylate reductase [ΔA_{340} s^{-1} mg^{-1} (protein)]		L-proline dehydrogenase [ΔA_{340} s^{-1} mg^{-1} (protein)]		Free proline [μg^{-1} (fresh root mass)]	
	Ratna	CSR-4	Ratna	CSR-4	Ratna	CSR-4
control	0.312±0.012	0.657±0.032	0.144±0.005	0.175±0.005	103.27±1.96	153.75±2.92
NaCl	0.384±0.011 *	0.930±0.027 *	0.084±0.004 *	0.087±0.003 *	141.52±2.97 *	250.65±5.26 *

*significant at $P < 0.01$

The results presented here may suggest that significant stimulation of Δ-pyrroline-5-carboxyalate reductase and simultaneous inhibition of L-proline dehydrogenase under a salt-stress condition (100 mM NaCl) causes high production of L-proline that maintains an osmotic balance to alleviate the above stress. The level of Δ-pyrroline-5-carboxylate reductase in resistant cultivar is much higher than in the sensitive cultivar and its higher activity can be used as a biological marker for screening the sensitive and tolerant cultivars of rice during early germination period.

References

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

Aspinall, D., Paleg, L.G.: Proline accumulation - physiological aspects. - In: Paleg, L.G., Aspinal, D. (ed.): The Physiology and Biohemistry of Drought Resistance in Plants. Pp. 206-211. Academic Press, Sydney 1981.

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

Hoffman, G.J.: Alleviating salinity stress. - In: Arhin,C.F., Taylor,H.M. (ed.): Modifying the Rood Environment to Reduce Crop Stress. Pp. 305-343. American Society of Agricultural Engineers, St. Joseph 1981.

Huber, W.: Über den Einfluss von NaCl- oder Abscisinsäurebehandlung auf den Protein-Metabolismus und einige weitere Enzyme des Aminosäurestoffwechsels in Keimlingen von *Pennisetum typhoides*. - Planta **121**: 225-235, 1974.

Laliberte, G., Hellebust, A.J.: Pyrroline-5-carboxylate reductase in *Chlorella autotrophica* and *Chlorella saccharophila* in relation to osmoregulation. - Plant Physiol. **91**: 917-923, 1989.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin-phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Mezl, V.A., Knox, W.E.: Properties and analysis of a stable derivative of pyrroline-5-carboxylic acid for use in metabolic studies. - Anal. Biochem. **74**: 430-440, 1976.

Rena, A., Splittstoesser, W.E.: L-proline dehydrogenase and pyrroline-5-carboxylate reductase from pumpkin cotyledons. - Phytochemistry **14**: 657-661, 1975.

Sudhakar, C., Reddy, P.S., Veeranjaneyulu, K: Biochemical changes during early seedling growth of horsegram (*Dolichos biflorus* L.) subjected to salt-stress. - Indian J. exp. Biol. **25**: 479-482, 1987.

Treichel, S.: The influence of NaCl on proline accumulating cell suspension cultures of *Mesembryanthemum nodiflorum* and other halophytes. - Physiol. Plant. **67**: 173-181, 1986.

Yoshio, K., Tsuyoshi, O., Yasuhiko, Y., Shigeho, I.: Proline in osmoregulation of *Brevibacterium lactofermentum*. - Agr. biol. Chem. **53**: 2475-2479, 1989.