

Effect of salt stress on biochemical parameters in rice (*Oryza sativa* L.) genotypes

C. Raja Babu and S. Ramesh*

Department of Crop Physiology, Tamil Nadu Agricultural University, COIMBATORE (T. N.) INDIA

ABSTRACT

The present investigation employing ten rice cultivars was aimed to determine the physiological basis of salt tolerance with particular reference to sodicity. The field experiment was conducted under sodic soil condition prevailing at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli in India. Biochemical parameters like Soluble protein, Proline and Nitrate reductase enzyme recorded higher values in the salt tolerant cultivars. Soluble protein content was estimated in order to find out the photosynthetic capacity of the genotypes under salt stress condition. The genotypic difference in soluble protein content could be related to grain yield. The genotypes like CORH 2, TRY(R) 2, APMS 5B and TRY 1 registered comparatively higher values for the soluble protein implying their salt tolerance behaviour. Proline, an amino acid, has been shown to accumulate in plant tissues in many species when subjected to salt and water stresses. Cultivars such as CORH 2, TRY(R) 2, TRY 1 and CO 43 had recorded higher proline content. The hybrid CORH 2 followed by ARMS 5B, TRY 1 and TRY(R) 2 established their superiority over other cultivars for the enzyme NRase. The decreased NRase activity in salt sensitive rice cultivars is possibly due to the inhibition of enzyme induction under salt stress. Under salt stress condition, uptake of NO_3 by the plants is reduced.

Key words : *Oryza sativa* L., Salt stress, Soluble protein, Proline, Nitrate reductase enzyme.

INTRODUCTION

In India, rice is cultivated in about 44.6 mha under varied eco-systems, which contributes 23 per cent of total world rice production and 45 per cent of total food production in India. In India, an area of nearly 4 mha of rice is affected by soil salinity (Paul and Ghosh, 1986). Therefore, there is a great deal of urgency for developing rice genotypes, which can sustain and set seed under high salt stress condition. Efforts to improve productivity of rice under salt stress condition need understanding of the mechanism to identify traits required for productivity improvement programme.

Understanding of adaptive mechanism for salt stress in rice is complex due to presence of ionic and osmotic compounds. Identification or development of suitable genotypes that can come up well under saline/alkaline soil is one of the immediate requirements. The crop response studies particularly of tolerance mechanism and yielding ability under saline/alkali soil condition are the major efforts for the improvement of rice productivity under salt stress condition. Rice is considered to be a salt sensitive crop (Flowers and Yeo 1981). However, considerable variability for salinity resistance among rice varieties is also apparent (Yeo and Flowers 1982). This paper reports the effect of salt stress on biochemical parameters like soluble protein, Proline and Nitrate reductase enzyme in rice

MATERIALS AND METHODS

Field experiments were conducted under sodic soil condition at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirapalli. Ten rice genotypes were raised in the nursery under moderate level of soil sodicity. Transplanting of seedlings was done 28 days after sowing in the main field under sodic soil condition. Two to three seedlings hill⁻¹ were planted in the main field in the spacing

of 20 x 10 cm. The study was conducted in the wet season (2002-2003) in Randomized Block Design and each treatment was replicated thrice. Plant samples were drawn at transplanting, tillering, panicle initiation and flowering stages for assessing the biochemical characters. Soluble protein content was determined by the procedure described by Lowry *et al.* (1951), and expressed as mg g^{-1} on fresh weight basis. The amino acid proline content was estimated in fully expanded leaf at transplanting, tillering, panicle initiation and flowering stages following the method of Bates *et al.* (1973) and expressed on $\mu\text{g g}^{-1}$ on fresh weight basis. The nitrate reductase activity was estimated as per the method suggested by Nicholas *et al.* (1976), and expressed as $\mu\text{ moles NO}_3\text{ g}^{-1}\text{ h}^{-1}$ fresh weight. The mean values of the above mentioned observations were subjected to the statistical analyses and the genotypes were tested for their significance by adopting the procedure of Panse and Sukhatme (1961).

RESULTS AND DISCUSSION

Soluble Protein Content

Soluble protein content was estimated in order to find out the photosynthetic capacity of the genotypes under salt stress condition. The genotypic difference in soluble protein content could be related to grain yield. The total soluble protein content determines the dry matter accumulation of crops since it represents the efficiency of the RuBPase, the carboxylating enzyme in C₃ plants (Plaut, 1974). A strong positive correlation between soluble protein and grain yield has been established in this study (0.742**). Vijayaraghavan (1994) had reported a reduction in soluble protein content under salt stress situation. However, the genotypes like CORH 2, TRY(R) 2, APMS 5B and TRY 1 registered comparatively higher values for the soluble protein implying their salt tolerance behaviour (Table 1). Protein contents in

* Author for correspondence, Present address : Department of Agronomy, Central Farm Unit TNAU, Coimbatore (T.N.)

Table 1: Effect of salt stress on soluble protein content (mg g⁻¹) in 10 rice genotypes at different phenological stages

S. No.	Genotypes	Transplanting	Tillering	Panicle Initiation	Flowering	Mean
1	IR 62829B	6.20	7.00	7.52	9.35	7.52
2	IR 68885B	5.94	7.84	9.20	11.60	8.65
3	IR 68281 B	5.80	4.40	5.84	7.38	5.86
4	APMS 5B	6.43	9.20	9.70	11.00	9.08
5	TRY1	6.38	9.28	10.00	10.43	9.02
6	TRY (R) 2	6.57	9.30	10.08	11.75	9.43
7	CO 43	6.72	8.38	8.56	11.33	8.75
8	ADT 39	6.10	7.21	7.28	9.44	7.51
9	CORH2	6.82	9.30	10.10	12.10	9.58
10	WHITE PONNI	5.41	5.84	6.12	8.00	6.34
	Mean	6.24	7.78	8.44	10.24	
			Sed		CD (P=0.05)	
S			0.06		0.12	
G			0.14		0.29	
SXG			0.29		0.57	

leaves were found to be correlated with plant growth rate, leaf area index, tillers and panicle number (Jayabalan *et al.*, 1995). Jha and Singh (1997) reported higher protein content of stressed seedlings of tolerant rice cultivars as compared to susceptible cultivars.

Proline

Proline, an amino acid has been shown to accumulate in plant tissues in many species when subjected to salt and water stresses (Mukerjee, 1974). The loss of turgor

due to salt stress triggers proline accumulation in plants contributing to osmotic adjustment and stress tolerance (Aslam *et al.*, 1989; Pessarakkali, 1999). Besides this, proline can serve as a protector of enzyme denaturation, a reservoir of nitrogen and carbon or as a stabiliser of the machinery for protein synthesis (Hamada and Khulaef, 1995). Hence, proline accumulation in plants can be taken as an index to identify the tolerant genotypes. Pessarakkali (1999) reported that accumulation of proline depend on the extent of stress. Proline was considered as salt tolerant

Table 2 : Effect of salt stress on proline ($\mu\text{g g}^{-1}$) in 10 rice genotypes at different phenological stages

S. No.	Genotypes	Transplanting	Tillering	Panicle Initiation	Flowering	Mean
1	IR 62829B	425	635	510	680	563
2	IR 68885B	480	670	590	830	643
3	IR 68281 B	400	590	410	630	508
4	APMS 5B	500	725	520	1050	699
5	TRY1	480	710	700	980	718
6	TRY (R) 2	485	780	680	1000	736
7	CO 43	510	850	810	1150	830
8	ADT39	450	750	630	820	663
9	CORH2	590	830	850	1100	843
10	WHITE PONNI	350	* 670	700	900	655
	Mean	467	721	690	914	
			Sed		CD (P=0.05)	
S			5.10		10.07	
G			11.96		23.61	
SXG			23.93		47.23	

Table 3 : Effect of salt stress on enzyme nitrate reductase activity (p moles NO₂ g⁻¹ hr⁻¹) in 10 rice genotypes at different phenological stages

S. No.	Genotypes	Transplanting	Tillering	Panicle Initiation	Flowering	Mean
1	IR 62829 B	21.5	23.3	22.4	26.0	23.3
2	IR 68885B	25.1	27.2	23.0	28.5	25.9
3	IR 68281 B	21.0	22.5	25.2	26.7	23.9
4	ARMS 5B	32.0	35.4	37.3	39.3	36.0
5	TRY1	29.6	33.4	34.4	35.3	33.2
6	TRY (R) 2	28.4	32.4	32.8	33.5	31.8
7	CO 43	31.2	32.5	36.4	40.3	35.1
8	ADT39	23.8	26.0	27.5	28.5	26.4
9	CORH2	35.0	34.1	36.0	40.1	36.3
10	WHITE PONNI	18.3	20.4	19.5	21.9	20.0
	Mean	26.6	28.7	29.5	32.1	
			Sed		CD(P=0.05)	
S			0.02		0.04	
G			0.05		.0.10	
SXG			0.10		0.19	

mechanism and serve in osmotic regulation (Aslam *et al.*, 1989). Significant variation in proline content was recorded in the 10 rice genotypes. Cultivars such as CORH 2, TRY(R) 2, TRY 1 and CO 43 had recorded higher proline content (Table 2). The positive association of proline with grain yield (0.483*) exhibits the tolerant nature of the above mentioned genotypes under salt stress. The proline accumulation may be due to either non-incorporation of free amino acid proline into protein synthesis due to salt stress or the breakdown of the existing protein molecules into various constituent amino acids with proline being predominant (Somani, 1991 and Mukerjee, 1974).

Nitrate Reductase (NRase)

The pathway of NO₃⁻ assimilation is considered as the major route of conversion of inorganic N into a biologically useful organic compound. The primary step in NO₃⁻ assimilation involves reduction of NO₃⁻ to NO₂⁻ catalysed by the enzyme, NRase. Nitrite is subsequently reduced to NH₄⁺ by the enzyme nitrite reductase. These two enzymes reduce NO₃⁻ to the end product NH₄⁺, which is then incorporated into amino acids. Activity of NRase in plants gives a good estimate of the N status of plants and is very often correlated with growth and yield of crops (Srivastava, 1980). The hybrid CORH 2 followed by APMS 5B, TRY 1 and TRY(R) 2 established their superiority over other cultivars for the enzyme NRase (Table 3). The positive association of the enzyme with grain yield (0.542**) and also soluble protein (0.698**) explained the higher yield of these genotypes under salt stress. The decreased NRase activity in salt sensitive rice cultivars is possibly due to the inhibition of enzyme induction under salt stress (Katiyar and Dubey, 1992). Under salt stress condition, uptake of NO₃⁻ by the plants is reduced. This leads to limited NO₃⁻ availability in the plant tissues and thereby NRase is suppressed, which results in decreased NRase activity (Lacuesta *et al.*, 1990). This decreased NRase activity has been partly attributed to the enhanced degradation of NRase enzyme itself (Plaut, 1974). Several possible explanations have been suggested for the decreased NRase activity in salt sensitive plants under saline stress. The plausible reason appears to be inhibition of enzyme induction under salinization. NRase is a substrate-inducible enzyme. Under saline conditions, NO₃⁻ uptake by the plants is reduced. This causes a limited NO₃⁻ availability in plant tissues and thereby, NRase induction is suppressed, which results in decreased NRase activity (Lacuesta *et al.*, 1990).

REFERENCES

Aslam, M., Qureshi, R.H. and Ahmed, N. (1989). Effect of external NaCl on ionic variations in leaves of rice varieties. *Journal of Agricultural Sciences (Lahore)* **27(4)**: 327-332.

Bates, L.S., Waldren, R.P. and Teere, V. (1973). Rapid determinations of free proline for water stress studies. *Plant and Soil* **39**:205-207.

Flowers, T.J. and Yeo, A.R. (1981). Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *New Phytol.* **88**: 363-373,

Hamada, A.M. and Khulaef, E.M. (1995). Effects of salinity and heat shock on wheat seedling growth and content of carbohydrate, proteins and amino acids. *Biologia Plantarum* **37(3)**: 399-404.

Jayabalan, M., Chandrasekaran, C., Weseley, C.G., Mahalingam P. and Shaji, C. (1995). Metabolic response of rice varieties (*Oryza sativa* L.) under water stress. *Journal of Ecotoxicology and Environmental Monitoring* **5**: 59-66

Jha, B.N. and Singh, R. (1997). Physiological response of rice varieties to different levels of moisture stress. *Indian Journal of Plant Physiology* **2**: 81-84.

Katiyar, S. and Dubey, R.S. (1992). Influence of NaCl salinity on behaviour of nitrate reductase and nitrite reductase in rice seedlings differing in salt tolerance. *Journal of Agronomy and Crop Science* **169**:289-297.

Lacuesra, M., Gonzalez-Moro, B., Ganzale-Murua, C. and Munoz-Rueda, A. (1990). Temporal study of the effect of phosphinothricin on the activity of glutamine synthetase, glutamate dehydrogenase and nitrate reductase in *Medicago sativa*. *Journal of Plant Physiology* **136**: 410-414

Lowry, O.H., Rosebrought, N.J., Fair, L.A. and Randal. R.J. (1951). Protein measurement with Folin Phenol reagent. *Journal of Biological Chemistry* **193**:265-273.

Mukerjee, I. (1974). Effect of potassium on proline accumulation in maize during wilting. *Physiology of Plant* **31**: 288-291.

Nicholas, J.C., Harper, J.E. and Hageman, R.H. (1976). Nitrate reductase activity in soybeans. I. Effect of light and temperature. *Plant Physiology* **58**: 731-735.

Panse, V.G. and Sukhatme, P.V. (1961). Statistical methods for Agricultural workers. ICAR. New Delhi.

Paul, N.K. and Ghosh, P.D. (1986). *In vitro* selection of NaCl tolerant cell cultures in *Oryza sativa* L. *Current Science* **55**: 568-569.

Pessarakkali, K. (1999). Response of green beans (*Phaseolus vulgaris* L.) to salt stress. In: Handbook of plant and crop stress. Marcel Dekker, Inc. New York, pp.827-842.

Plaut Zvi. (1974). Photosynthesis in plant/crops under water and salt stress. In: Handbook of plant and crop physiology. Marcel Dekker, Inc. New York, pp.58-604.

Somani, L.L. (1991). Crop production with saline water. Agro Botanical Publishers (India). Bikaner, p.308.

Srivastava, H.S. (1980). Regulation of nitratereductase activity in higher plants. *Phytochemistry* **19**: 725-733.

Vijayaraghavan, H. (1994). Physiological impact of salt stress on rice (*Oryza sativa* L.). Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore.

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

Received : March, 2006; Accepted : September, 2006