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Proline Accumulation and Salt Tolerance in Rice

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ABSTRACT

The morphological and biochemical response of calli and seedlings of different rice cultivars were compared under acid saline conditions. Calli of both tolerant and sensitive varieties showed severe stress symptoms like browning and necrosis, but the onset of stress symptoms was delayed in Pokkali. Seedlings of Pokkali showed minimal stress symptoms in lower salinities, and curling and senescence of older leaves in higher salinities although plants revived on amelioration of stress. Seedlings of the other varieties showed severe stress symptoms even at low salinities and plant death at higher salinities. Salt stress induced accumulation of the putative osmoprotectant proline in calli and seedlings of all varieties. Proline accumulation was higher in sensitive varieties than in Pokkali. These results indicate that proline accumulation is not directly correlated with salt tolerance in rice.

Key words: Salt stress, proline accumulation, Pokkali.

INTRODUCTION

Salinity is known to affect plant growth and metabolism through its osmotic effects, and through specific toxic effects of ions, by disturbing the membrane integrity and function thereby interfering with internal solute balance and uptake of essential nutrients (Poljakoff-Mayber and Gale, 1975). Salinity tolerance is a complex whole plant characteristic with physiological and biochemical functions controlled by numerous genes.

In rice (*Oryza sativa* L.) natural sources of salt tolerance have been identified for incorporation into breeding programmes. They

include several traditional varieties such as Pokkali, Kalarata, and Getu (Ponnamperuma, 1980). However breeding efforts have been hampered by a lack of understanding of the physiological and biochemical basis of salt tolerance in these varieties and also by the non-availability of biochemical genetic markers for selection of salt tolerant segregants in hybridisation programmes.

In the present study an attempt has been made to correlate the salt stress response of different varieties with proline accumulation in seedlings and in dedifferentiated callus cultures to determine whether proline enhancement may be used as a biochemical marker to differentiate between sensitive and tolerant varieties.

MATERIALS AND METHODS

a) Estimation of Proline in Seedlings

Seeds of rice varieties Pokkali, IR-20, Annapoorna, Jyothi and Hrswa obtained from Kerala Agricultural University were germinated in sand. Fourteen day old seedlings were transferred to hydroponic media containing 1/10th strength MS (Murashige and Skoog, 1962) salinised with different proportions of sea water as shown in Table 1. The plants were raised in trays under diffused sunlight conditions. Proline content was estimated (Bates *et al.*, 1973) at 0, 4 and 8 days after transfer to salinised media.

b) Estimation of Proline in Callus

Embryogenic callus cultures were obtained from scutellum of mature embryo explants cultured on MS medium supplemented with 2 mg l^{-1} 2, 4 Dichlorophenoxy acetic acid, 0.5 mg l^{-1} kinetin and 3% sucrose. The cultures were incubated in dark at 25°C. After three months the callus was subcultured on salinised medium as shown in Table 1. Proline content was estimated and the data was statistically analysed by ANOVA.

Table 1: Description of salinised media

Code	Sea water (ml/l)	ECe (micro mhos/cm)
SM0	0	0.73
SM1	125	8.6
SM2	250	15.6
SM3	500	28.0

RESULTS AND DISCUSSION

Rice is sensitive to salt stress during 14 day old seedling stage (Akbar and Ponnamperuma, 1982) and hence this stage was selected for experimentation. The seedlings were salinised with sea water in preference to NaCl and KCl solutions. Many crop plants can stand much higher concentration of salinity if the solution contains physiologically balanced salts as in sea water (Boyko, 1966).

The seedlings showed stress symptoms such as curling of leaves and yellowing of leaf tips. The onset and severity of the symptoms were more in Annapoorna, IR-20, Jyothi and Hrswa as compared to Pokkali which showed stress symptoms only under high salinity. The callus cultures showed stress symptoms such as browning and necrosis even under low salinities, but the onset of symptoms was delayed in Pokkali. Proline content in seedlings increased with salinity and duration of culture in all varieties (Table 2). By comparing the response of the five varieties under study it appears that proline accumulation is lesser in Pokkali than in other varieties and this difference was statistically significant. The critical point i.e., the concentration of salinity above which the proline content rises sharply was found to be higher in Pokkali. The varieties IR-20 and Annapoorna did not differ significantly from one another and had the highest accumulation of proline. Jyothi and Hrswa also did not differ significantly from one another. The 5 varieties may be represented in decreasing order of salt tolerance and in increasing order of proline accumulation as Pokkali > Hrswa > Jyothi > Annapoorna > IR-20. In conclusion, proline accumulation is least and the critical point is highest in the most tolerant variety. Proline content remained high

Table 2: Proline content in 14-day old seedlings after transfer to media salinised with sea water

Media	Day	Variety				
		Annapoorna	Hrswa	IR-20	Jyothi	Pokkali
SM0	0	0.5239[a]	0.6642	0.8248	0.8329	0.6233
	4	0.8651	0.6609	0.7090	0.6797	0.7093
	8	0.3385	0.4487	0.4541	0.5132	0.7526
SM1	4	3.0790	4.1375	6.8995	3.2106	0.7528
	8	6.1123	3.9790	3.5249	3.7292	2.0688
SM2	4	8.3047	5.8490	8.0870	7.5444	3.3556
	8	10.7964	3.2097	7.9393	7.6319	5.8356
SM3	4	8.7937	9.1295	8.9979	7.4852	3.0763
	8	6.0880	10.2037	8.5201	7.3348	3.1005

[a] = proline content in micro moles/gram fresh weight

even on amelioration of stress conditions in sensitive varieties, but Pokkali showed reduction in proline content concomittant with recovery.

In callus cultures, proline accumulation increased in all treatments except in SM3 (Table 3). The varieties differed significantly with respect to accumulation of proline under salt stress conditions. Pokkali and Hrswa produced lesser amount of proline compared to other varieties, Jyothi having the highest accumulation.

Table 3: Proline content in 3-month old callus after transfer to media salinised with sea water

Media	Day	Variety				
		Annapoorna	Hrswa	IR-20	Jyothi	Pokkali
SM0	0	0.7415[a]	1.9721	2.1345	1.8323	1.3702
	4	1.8431	0.9672	0.9403	0.5319	0.7792
	8	2.2076	0.5534	1.4669	2.1762	0.9027
SM1	4	4.8469	0.8221	1.3541	0.3707	0.9834
	8	0.3922	1.4024	1.8108	5.6045	1.3750
SM2	4	8.7695	0.7308	1.3595	3.3584	1.3595
	8	0.8383	1.2198	1.9183	6.4320	0.2042
SM3	4	0.7200	0.9672	0.4137	4.2128	3.7991
	8	0.5105	2.1276	6.0290	1.1177	0.2961

[a] = Proline content in micro moles/gram fresh weight

Over production of proline under stress is reported in several varieties of rice. Pokkali accumulate 13 times more proline in shoot under salt stress than under normal conditions (IRRI Annual report, 1978, 1979). Proline enhancement appears to be a varietal character (Bhattacharya, 1991) and has been correlated to a change in the activity of the proline regulatory enzymes such as pyrroline-5-carboxylate reductase and L-proline dehydrogenase (Roy *et al.*, 1992). The activity of the former enzyme was stimulated in salt resistant varieties under stress conditions. It has therefore been proposed that the content of proline (Prakash & Padayatty, 1988) or the activity of pyrroline-5-carboxylate reductase (Roy *et al.*, 1992) can be used as biological marker for screening sensitive and tolerant varieties during the early germination stage. However, many reports indicate negative correlation between proline accumulation and salt tolerance (Handa *et al.*, 1986). One of the reasons for this could be that in some intact plants, the mechanism of tolerance may be other than or in addition to simple osmotic adjustment e.g., through stress avoidance. In the present study high proline content may possibly be only an indication of injury and may not confer osmotic tolerance.

In the present study callus cultures of sensitive and tolerant varieties did not differ significantly with respect to proline accumulation. It is possible that the behaviour of excised tissue or cell cultures may not always be consistent with the behaviour of whole plants from which they are derived.

In conclusion, although proline over production under salt stress condition is a significant stress response, a lower level of proline accumulation and a higher critical point may be indicative of higher salt tolerance in rice seedlings.

REFERENCES

Akbar, M. and Ponnamperuma, F.N. (1982). Saline Soils of south and south east Asia as potential rice lands. In: Rice research strategies for the future. pp. 265–281 Int. Rice Res. Inst. P.O. Box 933 Manila, Philippines.

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant and soil 39: 205–207.

Bhattacharya, S. (1991). Differential responses of rice (*Oryza sativa* L.). Callus derived from 3 cultivars in saline environment. Phytomorphology. 41 (1 & 2): 133–137.

Boyko, H. (1966). Basic ecological principles of plants growing by irrigation with highly saline or sea water. In Boyko, H. (Ed.), salinity and acidity pp. 131–200 Junk, The Hague.

Handa, S., Handa, A.K., Hasegawa, P.M. and Bressan, R.A. (1986). Plant Physiol. 80: 938.

IRRI. (1978). Annual report for 1977, pp. 548 Los Banos, Philippines.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–493.

Poljakoff-Mayber, A. (1975) Morphological and anatomical changes in plants as a response to salinity stress. In: Poljakoff-Mayber A. and Gale, J. (Eds.), Plants in Saline Environments Springer-Verlag, Berlin.

Ponnamperuma, F.N. and Bandhyopadhya, A.K. (1980). Soil salinity as a constraint on food production in the humid tropics in priorities for alleviating soil-related constraints to food production in the tropics. IRRI, Los Banos, Philippines pp. 203–216.

Prakash, K.S. and Padayatty, J.D. (1989). Transfer of saline tolerance from one strain of rice to another by injection of DNA. Curr. Sci. 58: 991.

Roy, D., Buria, A., Basu, N. and Banerjee, S.K. (1992). Biol. Plant. 34: 159–162.