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Effect of salinity on callus induction, growth and proline accumulation in calli of rice genotypes

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ABSTRACT

In vitro studies were conducted to study callus induction, relative callus growth and level of proline accumulation in calli obtained from mature embryos of rice seeds at various salinity levels. Higher salt concentrations delayed callus induction, reduced the relative callus growth and significantly increased proline accumulation in all the genotypes. The study indicated a positive relationship between proline accumulation and salt tolerance of rice genotypes.

Key words: *Salinity, callus, tolerance, rice genotypes*

About 10 million hectares of land in India is affected by salinity and alkalinity (Bhargava,1989). Development of salt tolerant lines has gained interest among breeders only in the recent past. The slow progress of this research compared to other fields is due to complexity of the problem involving salinity in the soil and genetic system of the plants. Tissue culture holds great potential in increasing the stress tolerance of plants (Nabors and Dykes, 1985). The present study was thus conducted to study the *in vivo* behaviour and the cellular responses of the cultivars to salt stress.

MATERIALS AND METHODS

The materials for the study consisted of four rice genotypes of known tolerance level to salinity *viz.,* Jaya (susceptible), Triguna (moderately susceptible), CSR-13 (tolerant) and CSR-26 (moderately tolerant). M.S. medium [supplemented with 2,4D 2 mg 1^{-1}, kinetin 0.5 mg 1^{-1} and sodium chloride at concentrations of 0 (control), 0.25%, 0.5%, 0.75% and 1%] was used for callus induction and growth. Sucrose (30 gm 1^{-1}) and Agar (8 gm 1^{-1}) were added and the pH was adjusted to 5.8 using 0.1 N HCI or 0.1 N NaOH. Seeds of the selected genotypes were surface sterilized with 0.1% $HgCl_2$ and soaked overnight. Embryos were dissected from soaked seeds and inoculated in the test tubes containing MS medium at various concentrations of sodium chloride. The tubes were maintained at day/night temperature of 30/20 ± 1^{o}C, 50/95 ± 5% RH. A photo-period of 14 days/10 night hours with 800 ± 50 m mol photons m^{-2} s^{-1} light was provided by fluorescent tubes. Three replications were tried for each treatment. Test tubes were periodically observed to study the time taken for callus initiation. Fresh weight of calli was recorded at the end of 30 days after inoculation to obtain relative callus growth.

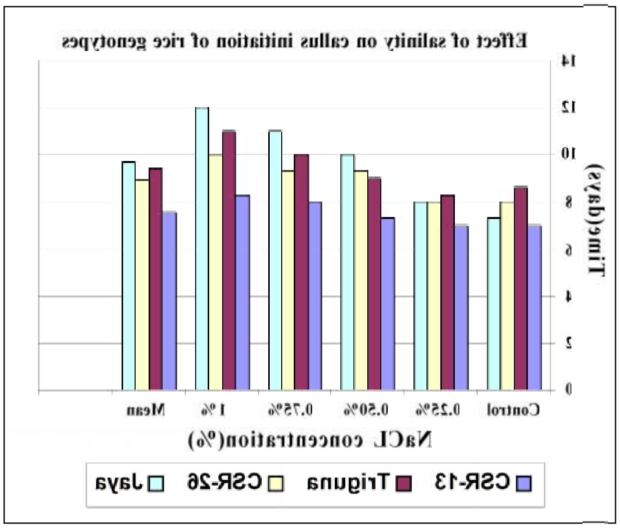
$$\text{Relative callus growth} = \frac{W_2 - W_1}{W_1} \times 100$$

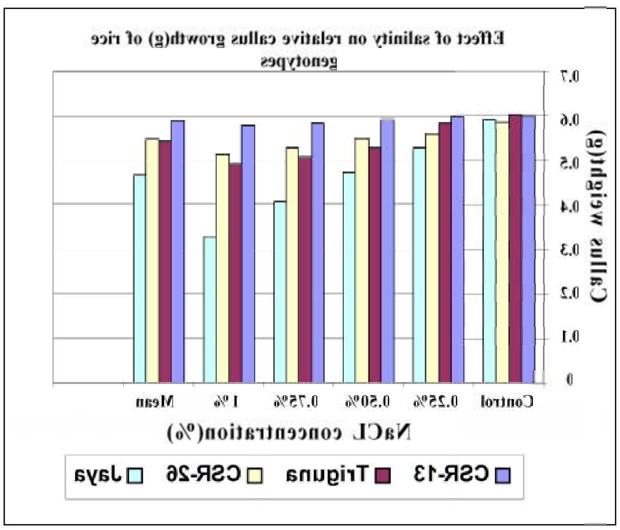
W_1 = Initital weight of explant inoculated
W_2 = Final weight of callus growth on explant

Proline content in calli was estimated by the procedure described by Bates et *al*. (1973). The data were analysed by mixed factorial experiment laid out in completely randomized design.

RESULTS AND DISCUSSION

Salinity treatment has delayed callus induction and reduced the relative callus growth in all the genotypes tested. CSR-13, the salt tolerant cultivar recorded maximum mean relative callus growth (0.59g) (Table 2) (Fig. 2) and minimum time for callus initiation (7.52 days) (Table 1) (Fig. 1) while Jaya, the susceptible cultivar recorded minimum relative callus growth (0.47





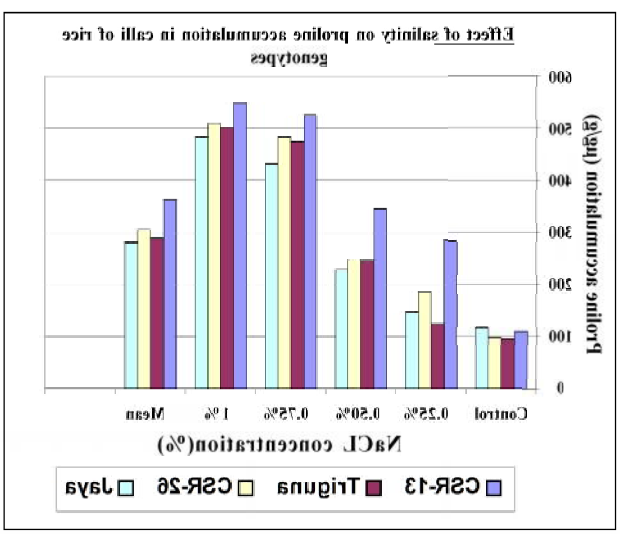


Table 1. Effect of salinity on callus initiation in days in rice genotypes

Genotype\ Treatment (days)	Control	0.25% NaCl	0.5% NaCl	0.75% NaCl	1% NaCl	Mean
CSR-13	7.0	7.0	7.3	8.0	8.3	7.52
Triguna	8.6	8.3	9.0	10.0	11.0	9.38
CSR-26	8.0	8.0	9.3	9.3	10.0	8.92
Jaya	7.3	8.0	10.0	11.0	12.0	966
Mean	7.725	7.825	8.900	9.575	10.325	8.87
	Genotype		Treatment		Interaction	
CD (0.01)	0.528		0.590		1.180	
SEm±	0.261		0.291		0.583	

Table 2. Effect of salinity on relative callus growth (g) in rice genotypes

Genotype\ Treatment	Control	0.25% NaCl	0.5% NaCl	0.75% NaCl	1% NaCl	Mean (g)
CSR-13	0.601	0.598	0.592	0.583	0.579	0.590
Triguna	0.603	0.583	0.529	0.508	0.493	0.543
CSR-26	0.587	0.561	0.550	0.529	0.513	0.548
Jaya	0.591	0.529	0.474	0.407	0.329	0.466
Mean	0.595	0.567	0.536	0.506	0.478	0.536
	Genotype		Treatment		Interaction	
CD (0.01)	0.004		0.005		010	
SEm±	0.002		0.002		0.005	

Table 3. Effect of salinity on proline accumulation (m g^{-1}) in rice calli

Genotype\ Treatment	Control	0.25% NaCl	0.5% NaCl	0.75% NaCl	1% NaCl	Mean
CSR-13	109	285	345	525	549	362.6
Triguna	96	124	245	475	502	288.4
CSR-26	98	187	249	482	509	305.0
Jaya	116	146	228	430	483	280.6
Mean	104.75	185.5	266.75	478	510.75	309.15
	Genotype		Treatment		Interaction	
CD (0.01)	8.228		9.200		18.400	
SEm±	4.067		4.547		9.095	

(Withers and Alderson, 1986).

Delayed callus initiation and reduced callus growth due to salinity was reported Subhashini and Reddy (1989) and Pushpalatha and Padmanabhan (1998) in rice genotypes.

Proline accumulation in calli increased with salt concentration in all the four genotypes. However, maximum proline content (362.6 µg g^{-1}) was recorded in CSR-13, a salt tolerant cultivar and minimum (280.6µg/g) in susceptible cultivar Jaya. CSR-26 and Triguna recorded moderate quantities of proline (305 µg g^{-1} and 288.4 µg g^{-1}, respectively) (Table 3) (Fig. 3).This indicated that greater proline accumulation occured in salt tolerant genotypes when compared to susceptible genotypes. Proline accumulates more under stress for the purpose of osmotic adjustment and aids in combating the adverse effects of stress due to drought, salinity, high and low temperatures (Stewart and Lee, 1974).

Similar increase in proline accumulation due to salinity in salt tolerant genotypes has been reported by Paulas and Sree Rangasamy (1995) and Pushpam and Sree Rangasamy (2000).

The study thus reveals a positive relationship between callus proliferation and proline accumulation with salt tolerance of rice genotypes.

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