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BRIEF COMMUNICATION

In vitro selection for salt tolerance in rice

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Abstract

In six cultivars of rice (*Oryza sativa* L.), Pusa Basmati 1, Basmati 370, Type III, Pant Dhan 4, CSR 10 and Pokkali, embryogenic callus growth, plant regeneration, and proline and total protein contents were studied under salt stress (on agar solidified media containing 0, 0.5, 1.0, 1.5 and 2.0 % NaCl). Four weeks after inoculation the callus fresh mass decreased with increasing salt concentration in all the six cultivars. The regeneration frequency in salt stressed callus was also lower as compared to control. 15 d and 30 d after inoculation proline content increased several fold whereas total protein content decreased markedly with increase in salt concentration.

Additional key words: callus growth, *Oryza sativa*, proline, protein, salt stress.

Soil salinization in many parts of the world is continuously reducing the available land for conventional agriculture. The development of salt tolerant crop cultivar is, therefore, considered to be an efficient and economic means of overcoming the salinity problem. The yield of rice is very much affected by soil salinity. *In vitro* culture technique can be used to produce salt tolerant lines of rice. This technique also provides an efficient means of exploiting the genetic variability in this crop (*e.g.* Oono 1981, Yano *et al.* 1982, Kishor and Reddy 1986, Karp 1995, Khanna and Garg 1997). The present study deals with the effect of NaCl salt concentration on proliferation, plant regeneration, and proline and total protein contents in six indica rice cultivars, Pusa Basmati 1, Basmati 370, Type 3, Pant Dhan 4, CSR 10 and Pokkali.

Seeds of cultivars Pusa Basmati 1, Basmati 370, CSR 10 and Pokkali were obtained as a gift from Dr. R.K. Singh, Central Soil Salinity Research Institute, Karnal (Haryana), and Type 3 and Plant Dhan 4 from Prof. S.C. Mani, Department of Genetics and Plant Breeding, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, U.P., India. Dehusked seeds were surface sterilized with 0.1 % $HgCl_2$ for 15 min, washed in sterilized distilled water and cultured onto solidified agar containing Murashige and Skoog (1962) medium supplemented with 2 mg dm^{-3} 2, 4-dichlorophenoxyacetic acid (2, 4-D) and kept in dark at temperature 26 ± 1 °C in a *BOD* incubator (*Techins*, India) for callus initiation. Induced callus was subcultured after three weeks on the same medium for proliferation.

At the end of second subculture, the proliferated calluses were divided into small pieces of about 100 mg fresh mass. These pieces were cultured on MS medium containing 0, 0.5, 1.0, 1.5 or 2.0 % NaCl. After four weeks, fresh mass of the calli was estimated.

15 and 30 d after inoculation three replicates of known mass of callus tissues were homogenized in 3 % sulphosalicylic acid, centrifuged at 5 000 *g*, and the proline content was determined according to Bates *et al.* (1973). Another three replicates of known mass of calli were homogenized in 0.2 M PCA and centrifuged at 5 000 *g*. After centrifugation the pellet was extracted three times with a mixture of ethanol:ether:chloroform (2:2:1; v/v/v). 0.2 M NaOH was added to the residue and left overnight. After centrifugation supernatant was used to estimate the total protein content following the method of Lowry *et al.* (1951).

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Abbreviations: BAP - benzylaminopurine, 2,4-D - 2,4-dichlorophenoxy acetic acid, PCA - perchloric acid.
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At the end of third subculture the salt tolerant calli were subcultured on regeneration medium (MS + 0.5 mg dm^{-3} BAP + NaCl in different concentrations). The number of plants produced per callus were recorded 3 - 4 weeks after inoculation.

Callus initiation in all the cultivars was observed within a week after inoculation of mature embryo explant on MS medium supplemented with 2 mg dm^{-3} 2,4-D and 30 g dm^{-3} sucrose. The fresh mass of proliferated callus dramatically decreased with increasing salt concentration in Pusa Basmati 1, Basmati 370 and Type 3. However, the callus of Pant Dhan 4 and Pokkali gave better proliferation in 0.5 % NaCl, while CSR 10 performed better even at 0.5, 1.0 and 1.5 % NaCl in terms of fresh mass as compared to control (Table 1). The results suggested that CSR 10 was more tolerant to salinity than other cultivars. The salt tolerant callus of CSR 10 may produce organic acids and amino acids or accumulate ions from external medium thereby

Table 1. Fresh mass of callus [mg $callus^{-1}$] in different rice cultivars grown for 4 weeks on medium with different NaCl concentrations (from 0 to 2.0 %). Means ± SE, $n = 15$.

Cultivars	0.0 %	0.5 %	1.0 %	1.5 %	2.0 %
Pusa Basmati 1	832.67 ± 11.15	797.33 ± 5.97	637.33 ± 12.13	319.33 ± 9.97	207.33 ± 10.26
Basmati 370	634.00 ± 18.59	618.00 ± 15.37	588.67 ± 8.56	341.33 ± 6.96	156.67 ± 7.47
Type 3	571.33 ± 4.46	489.00 ± 3.54	404.67 ± 4.12	302.00 ± 3.12	193.33 ± 2.70
Pant Dhan 4	466.67 ± 3.74	524.00 ± 3.05	405.33 ± 3.26	290.00 ± 2.39	200.67 ± 2.06
CSR 10	268.00 ± 2.79	400.00 ± 2.76	366.00 ± 3.05	287.33 ± 2.28	199.33 ± 2.48
Pokkali	478.00 ± 3.17	504.67 ± 2.56	468.67 ± 2.53	281.33 ± 2.36	248.67 ± 1.92

Table 2. Effect of NaCl on proline and total protein content in the callus of different rice cultivars. The experiment was conducted three times, means ± SE, $n = 15$.

Cultivars	NaCl [%]	15 d after inoculation Proline [μmol g^{-1}(FM)]	Protein [mg g^{-1}(FM)]	30 d after inoculation Proline [μmol g^{-1}(FM)]	Protein [mg g^{-1}(FM)]
Pusa Basmati 1	0.0	3.39 ± 0.17	20.47 ± 0.43	10.14 ± 0.13	40.96 ± 0.47
	0.5	6.34 ± 0.27	13.82 ± 0.23	16.56 ± 0.12	33.36 ± 0.76
	1.0	10.13 ± 0.63	12.22 ± 0.18	18.79 ± 0.17	29.47 ± 0.35
	1.5	10.60 ± 0.54	11.27 ± 0.24	19.93 ± 0.19	24.63 ± 0.37
	2.0	10.73 ± 0.46	8.04 ± 0.21	19.52 ± 0.29	18.96 ± 0.28
Basmati 370	0.0	2.94 ± 0.11	19.63 ± 0.17	9.17 ± 0.19	35.57 ± 0.48
	0.5	4.67 ± 0.24	16.14 ± 0.14	11.84 ± 0.33	25.53 ± 0.20
	1.0	6.83 ± 0.28	14.55 ± 0.11	16.73 ± 0.27	21.22 ± 0.31
	1.5	8.48 ± 0.34	10.28 ± 0.32	18.51 ± 0.18	19.29 ± 0.23
	2.0	8.87 ± 0.22	7.53 ± 0.12	8.89 ± 0.16	15.42 ± 0.18
Type3	0.0	5.34 ± 0.12	12.70 ± 0.21	9.92 ± 0.15	22.67 ± 0.16
	0.5	7.70 ± 0.16	10.72 ± 0.15	13.11 ± 0.16	16.52 ± 0.56
	1.0	7.86 ± 0.14	9.86 ± 0.17	17.29 ± 0.24	15.74 ± 0.67
	1.5	14.72 ± 0.20	8.70 ± 0.15	19.95 ± 0.39	14.13 ± 0.56
	2.0	19.59 ± 0.19	6.28 ± 1.00	18.90 ± 0.15	11.53 ± 0.19
Pant Dhan 4	0.0	4.27 ± 0.87	11.36 ± 0.44	5.80 ± 0.11	38.87 ± 0.32
	0.5	5.08 ± 0.95	8.86 ± 0.11	11.45 ± 0.17	36.27 ± 0.52
	1.0	5.71 ± 0.13	7.55 ± 0.33	15.48 ± 0.26	31.22 ± 0.46
	1.5	7.41 ± 0.19	7.25 ± 0.14	20.79 ± 0.21	23.19 ± 0.22
	2.0	9.54 ± 0.20	5.35 ± 0.14	15.42 ± 0.15	20.60 ± 0.59
CSR 10	0.0	2.29 ± 0.48	14.59 ± 0.35	5.78 ± 0.18	23.79 ± 0.32
	0.5	4.53 ± 0.40	9.13 ± 0.15	13.95 ± 0.45	20.36 ± 0.55
	1.0	7.36 ± 0.12	6.85 ± 0.12	19.70 ± 0.66	15.39 ± 0.46
	1.5	8.36 ± 0.12	6.60 ± 0.11	21.23 ± 0.96	12.84 ± 0.23
	2.0	11.39 ± 0.26	4.99 ± 0.20	21.57 ± 0.62	08.49 ± 0.44
Pokkali	0.0	2.18 ± 0.73	13.86 ± 0.24	7.31 ± 0.73	26.83 ± 0.31
	0.5	4.26 ± 0.10	7.58 ± 0.17	14.55 ± 0.14	24.56 ± 0.34
	1.0	7.33 ± 0.85	6.88 ± 0.37	21.37 ± 0.21	18.68 ± 0.58
	1.5	8.77 ± 0.74	6.49 ± 0.98	26.37 ± 0.45	14.24 ± 0.36
	2.0	10.00 ± 0.16	5.29 ± 0.14	26.35 ± 0.54	6.61 ± 0.26

acquiring a high internal concentration of osmotically active solutes to maintain water balance in salt stress condition resulting in a shift towards halophytic nature (Greenway and Munns 1980). Similar results were obtained with the callus of rice cultivar Basmati 27814 grown under various concentrations of NaCl (Reddy and Vaidyanath 1986).

NaCl stressed calli of all the cultivars produced more proline than control, after 15 and 30 d of inoculation. However, the rate of proline accumulation with respect to control was more in Pusa Basmati 1, Basmati 370, Type 3, CSR 10 and Pokkali after 15 d of inoculation. In contrast, Pant Dhan 4 revealed low accumulation rate of proline at 15 d (Table 2). In cv. Basmati 27814 proline content also increased several fold in stressed callus as compared to control (Reddy and Vaidyanath 1986). Similar results have been observed by Kavi Kishor (1988) in cultivar Tellahamsa. In rice cultivars Dee-gee-woo-gen (salt tolerant) and IR-8 (salt sensitive), proline accumulation increased rapidly in seedlings of salt tolerant cultivar whereas slightly in salt sensitive one (Igarashi *et al.* 1997). In the present study, results indicate that proline serves as a compatible solute in all cultivars.

A significant reduction in total protein content of the calli occurred with increasing salt concentration at 15 and 30 d after inoculation in all the cultivars (Table 2). The total protein contents in stressed and controlled calli were higher at 30 d in comparison to 15 d after inoculation. There was a great variation in percent reduction of total protein in stressed calli which may be due to decrease in protein synthesis and or increase in protein degradation. Decreased content of soluble proteins under salinity was also found in callus of rice cv. 27814 (Reddy and Vaidyanath 1986). Similar results were obtained for cv. ECD-1 and IR 58 (Viji *et al.* 1996). Reduced protein content was found also in *Pennisetum typhoides* leaf under saline conditions and the authors explained the accumulation of free amino acids by hydrolysis of proteins (Reddy and Vora 1985). The protein content in the salt tolerant rice cultivar CO-43 was higher as compared to salt sensitive IR8 which was considered as an adaptation for survival in salt stressed condition (Krishnamurthy and Bhagwat 1989). On the contrary, higher protein content was found in NaCl stressed soybean seedlings due to the synthesis of additional stress proteins in the embryogenic axis (Diaz de Leon *et al.* 1990).

The effect of different concentrations of NaCl (0.5 - 2.0 %) for plant regeneration was also tested. Embryogenic calluses of all the six cultivars were cultured on MS medium supplemented with optimum concentrations of BAP (0.5 mg dm^{-3}) and 0.5 - 2 % NaCl. There was no regeneration at 1.5 and 2.0 % NaCl in the medium in any rice cultivar. Pusa Basmati 1 did not give any response even at 0.5 and 1.0 % NaCl. However, Basmati 370 showed 2.5 % regeneration at 0.5 % NaCl concentration. Type 3 and Pant Dhan 4 exhibited 10 % regeneration in 0.5 % NaCl. However, the maximum regeneration of 47.5 %, was found in CSR 10 in 0.5 % NaCl in the medium. Pokkali also showed good regeneration percentage, 32.5 %, at 0.5 % NaCl in the medium. The present results show that CSR 10 is a tolerant cultivar to salinity. Pokkali and Pant Dhan 4 also showed the ability of salt tolerance at low level of salt concentration. Similar results were obtained in other rice cultivars. High proportion of sea water in the medium inhibited plant regeneration in rice cultivar IR 36 (Yano *et al.* 1982) and lower frequency of plant regeneration was observed from NaCl tolerant callus of cv. Tainurg 67 than non-selected callus (Wong *et al.* 1996). Rice hybrids 448, 459, 491, 552 and 641 showed ability to regenerate the plantlets at 0.75 and 1.5 % NaCl (Bihn and Heszky 1990, Bihn *et al.* 1992).

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