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Diallel analysis of callusing and regeneration potential in salt adapted calli of rice (*Oryza sativa* L.)

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ABSTRACT

The genetics of salinity tolerance in rice through in vitro study was investigated using six-parent half-diallel. Test materials were two tolerant (CSR-10 and CSR-5), two moderately tolerant (SAR-41 and NDR501), and two sensitive genotypes (IET12860 and IET11149), and their 15 F_1s. Callus cultures on MS (Murashige and Skoog, 1962) media containing 1.5% salt, were evaluated for callus growth, Na/K ratio, proline content in callus and total regeneration frequency. The tolerant and moderately tolerant genotypes had a higher callus growth and lower Na/K ratio in callus, containing more proline and higher regeneration ability. The sensitive genotypes produced low callus growth and showed no regeneration ability. Na/K ratio and proline content were mainly controlled by dominant genes, while total regeneration and callus growth were controlled by recessive genes. The broad and narrow sense heritabilities were high for all traits. The combining ability analysis demonstrated preponderance of additive gene effect(s) in control of callus growth and regeneration percentage. For the salt tolerance indices –Na/K and proline – both additive and non-additive gene interactions were found important. The tolerant and moderately tolerant parents were good general combiners for all traits, and produced best combinations. A highly significant negative correlation between callus growth and Na/K ratio, and significant positive relationship of callus growth with proline content, and total regeneration potential was observed.

Key words: *Combining ability, regeneration ability, heritability, rice, salt tolerance, callus*

Development of rice varieties with increased salinity tolerance is of prime importance as each year more land is becoming nonproductive because of salt accumulation in soil (Carter, 1975). Salinity is a serious problem affecting one third of all irrigated land in the world (Mass and Haffman 1977). In India 7 million ha are affected by salinity (Subashini and Reddy, 1989). In rice, tissue culture was mostly successful in most of the *japonica* types but served little to *indica* and *African* rices due to low culturability of seeds (Zapata *et al.*, 1987). The physiological status of donor plants and passage induced culture variants generally influence the regeneration potential (Raval and Chatto, 1993). Presence of salt in the regeneration medium also results in lowering regeneration frequencies (Binh *et al*., 1992). Moreover, salt sensitivity of rice varieties/ genotypes consequently depends on the developmental stages of the plants (Yan *et al*., 1992). Reports on diallel analysis have indicated significant additive and dominance genetic effects and high degree of heritability values for most of the traits studied for salt tolerance (Gregorio and Senadhira, 1993).

Study on genetics of salinity tolerance was undertaken using six parent half-diallel analysis in a controlled experiment with a focus on physiological parameters of stressed calli and subsequent regeneration potential, enable to generate new information about the genetics of salinity tolerance in rice. The generated information will elucidate the possible source of desirable genes and type and/or amount of gene action. This will help rice breeders to design an effective and composite breeding programme for developing salt tolerant varieties. In addition to field evaluation, *in vitro* mode of inheritance of traits will provide greater insight into the gene effects and combining ability of tolerant and sensitive rice varieties.

MATERIALS AND METHODS

Genetics of salt tolerance was investigated by using a six parent half-diallel analysis. The selection of parents exhibiting varying degrees of salt tolerance was based on the "screening parameters" for adaptation to saline environment as per IRRI scale. Accordingly, tolerant genotypes (CSR10 and CSR5), moderately tolerant (SAR4 and NDR501) and sensitive genotypes (IET12860 and IET11149) were identified. The parents were involved in diallel crosses to produce 15 F_1s excluding reciprocals.

The dehusked seeds of parents and their F_1s were used for inducing seed calli. Seeds were washed thoroughly in running tap water and surface sterilized with $HgCl_2$ (0.2% w/v) and then followed with EtOH treatment (40% w/v) for 20-30 minutes, to partially remove wax. The seeds were washed 3-4 times with double distilled sterile water, and kept at 24±1°C for 36 hours. Embryos were excised from each seed. For callus induction, three embryos per tube were placed onto solidified MS medium (Murashige and Skoog, 1962) containing 2, 4-D (2 mg l^{-1}). All the tubes were kept under continuous fluorescent light at 28±2 ^{0}C and maintained for 4 weeks. The calli were transferred to fresh medium for proliferation under same conditions. Proliferated calli were divided into small pieces and exposed to solidified agar nutrient MS medium containing 1.5% NaCl, 2,4-D (2 mg l^{-1}) tryptohane (5 mg l^{-1}), and sucrose (30 g l^{-1}). The cultured calli of the parents and their F_1s were subcultured again under same salt medium. After 4 weeks of subculture, the calli were evaluated for their fresh weight (mg), Na/K ratio and proline content. The Na/K ratio and proline content were estimated following Bates *et al.* (1973).

For regeneration, salt-adapted calli were noted prior to transfer to the regeneration medium containing IAA (1mg l^{-1}) and BAP (4 mg l^{-1}). Total regeneration frequency was recorded in terms of development of morphogenic and non-morphogenic calli. Survival ratio at 1.5% NaCl (w/v) was calculated in terms of per cent calli showing organogenesis.

The diallel analysis of Hayman (1954) was used to compute the array variance (Vr) and parents to off spring covariance (Wr) for all the traits from the F_1 data. The general and specific combining ability analyses were carried out according to Griffing (1956) Method II - Model 1, using the computer program DIALL (Ukai, 1997).

RESULTS AND DISSCUSSION

The relative analysis of the salt injury under gradient saline agar at various growth stages of seed such as, seed derived seedlings, seed derived callus system and seed-callus derived regenerants of parental lines and their F_1-hybrid callus of *O. sativa* were studied. These were obtained by 6 parents and reciprocal crosses of genotypes with different degree of salt tolerance at whole plant level. The callus growth was the highest for the salt tolerant genotypes CSR 5 (140.9 mg) and CSR 10 (135.3 mg) followed by the two moderately tolerant genotypes NDR 501 (122.1 mg) and SAR 41 (110.9 mg); the range being 110.9 to 140.9 mg (Table 1). For the two sensitive genotypes, the callus growth was much less 20.7 mg for IET 12860 and 23.6 mg for IET 11149. The hybrid combinations involving the tolerant parents (CSR 5, CSR 10 and NDR 501) showed much higher growth than the parents, while the growth was much less for the cross between two sensitive genotypes. Similar results have been reported by (Yan *et al.*, 1992), where in the selected lines grew markedly better in salt medium than the unselected lines.

The Na/K ratios were lower for the tolerant genotypes compared to those of the sensitive genotypes (Table 1). Also the hybrid combinations among the tolerant genotypes had lower Na/K ratios than the cross between the two sensitive types. Thus, it seems possible to select salt tolerant materials from the crosses among the tolerant genotypes. Proline has been shown to accumulate in a number of plant tissues in response to salt stress (Dix and Pearce, 1981)). It has been suggested that the accumulation of proline mediated tolerance by serving as a source of cytoplasmic osmoticum (Watad *et al.*, 1983). Large differences were observed in the proline contents of the tolerant and sensitive genytypes; the content being 4 to 5 times higher in the tolerant and moderately tolerant genotypes than in those in the sensitive genotypes (Table 1). NDR 501 produced the highest amount of proline content. Also the crosses among the tolerant types and tolerant x moderately tolerant types had higher amount of proline content in their calli than the crosses between sensitive types. However, NDR 501 when crossed with either of the sensitive types produced higher level of proline

Table 1. Average mean data of parents and their F_1's for callus growth, Na/K, proline content and total regeneration in salt medium in 6 x 6 diallel cross

Parents and hybrids	Callus growth (mg)	Na/K ratio	Proline content (μ mole g^{-1})	Total regeneration (%)
CSR 10	135.30	1.59	310.50	51.10
CSR 5	140.97	1.46	394.57	48.27
SAR 41	110.97	2.21	407.23	17.13
NDR 501	122.13	1.82	462.60	20.51
IET12860	20.73	3.50	97.13	0.00
IET11149	23.57	3.27	90.13	0.00
CSR 10 x CSR-5	144.03	2.15	415.50	59.23
CSR 10 x SAR-41	123.70	2.45	211.40	29.00
CSR 10 x NDR501	155.33	1.95	327.67	55.37
CSR 10 x IET12860	119.73	3.01	167.40	21.37
CSR 10 x IET11149	120.03	2.79	197.40	29.63
CSR 5 x SAR-41	130.80	2.31	253.87	28.50
CSR 5 x NDR501	165.50	1.64	480.20	64.53
CSR 5 x IET12860	124.47	2.58	209.73	18.69
CSR 5 x IET11149	112.50	2.70	150.83	16.40
SAR 41 x NDR501	116.00	1.98	359.20	25.83
SAR 41 x IET12860	110.10	2.80	276.33	12.40
SAR 41 x IET11149	98.27	2.96	173.30	10.43
NDR501 x IET12860	131.90	2.03	417.13	23.50
NDR501 x IET11149	121.03	2.33	373.07	23.30
IET12860 x IET11149	45.70	3.27	110.77	0.00

content than the crosses between sensitive genotypes. Yan *et al.* (1992) also reported higher proline content in the selected callus of wheat cultivars than in the unselected callus lines.

The total regeneration (%) was the highest for CSR 10 and CSR 5 (51.01 and 48.27%) followed by NDR 501 and SAR 41, respectively (Table 1). No regeneration was noticed in the sensitive types. The highest regeneration percentage was observed for the cross between CSR 5 x NDR 501 (64.53%) followed by CSR 10 x CSR 5 and CSR 10 x NDR 501, respectively. Although, no regeneration occurred in the cross between two sensitive types, regeneration did occur, when crossed with any of the tolerant/moderately tolerant genotypes, and the percentage ranged from 10.43 to 29.63. The genotypic differences for regeneration ability of subcultured calli were reported earlier also by Yan *et al.* (1992).

Analysis of variance for callus growth, Na/K, Proline content and total regeneration was observed highly significant additive (a) and dominant gene effects (b) (Table 2). Dominance variation (b) was significant, although the mean square and F value of b were not compared with those of a. Further, the significance of b_1 indicated that the dominance was unidirectional while significance of b_2 suggested asymmetrical distribution of genes controlling these traits. The significance of item b_3 indicated presence of residue dominance deviations which were not attributable to b_1 and b_2. Comparison between all four traits showed that the additive genetic variation was high in proline contents, while the dominance variation was also high in proline content. Combining ability indicated for both GCA and SCA variance (Table 2) were highly significant. This suggested importance of both additive and non additive genetics variances for all the four traits. However, the predictability ratio ($2s^2g/2s^2g+s^2s$) showed the preponderance of additive gene effects for callus growth and totoal regeneration. For Na/K, the non-additive gene action was more prominent while both additive and non-additive types of gene actions seemed equally important for proline content.

Table 2. Analysis of variance for callus growth, Na/K ratio, proline content and total regeneration in salt medium of 6 x 6 diallel

Source	df	Callus growth		Na/K ratio		Proline content		Total regeneration	
		MS	F	MS	F	MS	F	MS	F
a	5	13536.13	661.71**	3.60	211.74**	144412.95	2066.91**	3702.90	779.61**
b	15	1522.43	74.43**	0.26	15.09**	14741.32	210.98**	238.10	50.13**
b_1	1	10483.39	472.54**	0.36	21.10**	4553.34	65.17**	208.81	43.96**
b_2	5	864.56	42.27**	0.47	27.50**	16575.40	237.23**	290.52	61.17**
b_3	9	41.92	2.53*	0.13	7.53**	14854.38	212.60**	212.23	44.68**
Error	40	16.55		0.02		69.87		4.75	
GCA	5	6428.94	242.34**	0.77	25.51**	81817.68	1572.09**	2648.69	618.45**
SCA	9	220.030	8.30**	0.52	17.42**	14854.38	285.42**	137.47	32.10**
Error	28	26.53		0.03		52.04		4.28	
$2\sigma^2g/2\sigma^2g+\sigma^2s$		0.88		0.11		0.53		0.82	

** = significant at 1% level, *=significant at 5% level

The additive genetic variance (D) and the three components of dominance (H_1, H_2 and h^2) were found highly significant, indicating the importance of both additive and non-additive types of gene action in the inheritance of callus growth, Na/K ratio, proline content and total regeneration (Table 3). The variances due to additive (D) gene action were higher than due to dominant gene effects (H_1 and H_2). The existence of partial dominance detected in the analysis was confirmed by the fact that $(H_1/D)^{1/2}$ was greater than zero and lower than unity. The significant and positive values of F and the ratio of dominant and recessive alleles $[(4DH_1)^{1/2}+ F]/ [(4DH_1)^{1/2}-F]$ for callus growth, Na/K ratio, proline content, indicated that parents used

Table 3. Estimates of genetic component for callus growth, Na/K ratio, proline content and total regeneration in 6 x 6 diallel crosses under salt medium

GeneticParameters	Estimates ± SE			
	Callus growth	Na/K ratio	Proline content	Total regeneration
D	3052.96 ± 133.85	0.788 ± 0.062	26369.65 ± 666.21	419.62 ± 24.23
F	1253.45 ± 137.85	0.314 ± 0.072	6751.19 ± 651.84	-107.58 ± 20.67
H_1	1619.64 ± 85.15	0.324 ± 0.046	21887.19 ± 593.39	328.96 ± 20.04
H_2	1375.28 ± 70.04	0.212 ± 0.028	16990.83 ± 444.14	238.57 ± 13.75
h_2	2296.66 ± 188.80	0.064 ± 0.030	973.26 ± 200.37	46.80 ± 12.60
E	7.73 ± 1.31	0.007 ± 0.001	20.58 ± 3.29	41.47 ± 0.24
$(H_1/D)^{1/2}$	0.72	0.64	0.91	0.81
$(H_2/4\ H_1)$	0.21	0.16	0.19	0.23
h^2/H_2	2.00	0.36	0.06	0.18
$[(4DH_1)^{1/2}+ F]\ [(4DH_1 - F)^{1/2}]$	1.78	1.90	1.32	-1.33
$h_{(bs)}$	0.99	0.97	0.99	0.98
$h_{(ns)}$	0.76	0.82	0.74	0.99
r(Pr, Wr+Vr)	-0.96	-0.059	-0.48	0.76

D= Additive gene effects, $(H_2/4\ H_1)$ = Ratio of genes with positive and negative effects in the parents
F = Gene distribution, h^2/H_2 = Number of gene groups which control tolerance and exhibit dominance
H_1, H_2, h_2 = dominant effects, $[(4DH_1)^{1/2}+ F]/ [(4DH_1 - F)^{1/2}]$ = Ratio of dominant and recessive genes in the parents
E = Environment effect, $h_{(bs)}$ = Heritability, broad sense
$(H_1/D)^{1/2}$ = Mean degree of dominance , $h_{(ns)}$ = Heritability, narrow sense

in this study had more dominant than recessive genes. On the other hand the negative value of these parameters for total regeneration frequency implied that the parents have more recessive than dominant genes. The values for $(H_1/D)^{½}$ indicated partial dominance for callus growth, Na/K ratio and total regeneration frequency but complete dominance for proline content. The low values of $H_2/4H_1$ (less than 0.25) suggested unequal mean allelic frequencies at the loci influencing these traits, particularly for Na/K rates and proline content. The h^2/H_1 values indicated that the two groups of genes were involved in parental differences for callus growth, but for other traits, only one group of genes was involved. The estimated values of broad and narrow sense heritability were high (0.99, 0.97, 0.99, 0.99) either due to major role of additive gene effects or the low role of non-additive genes and environment effects in the *in vitro* salt stress conditions, a more precisely controlled environment.

The tolerant (CSR10, CSR 5) and the moderately tolerant (NDR501) genotypes were good general combiners for callus growth and total regeneration. CSR 5 and NDR 501 showed good general combining ability for proline content, but none of the parents significant GCA for Na/K ratio (Table 4). The crosses involving CSR 5 and NDR 502 showed maximum SCA effects for all the traits, other combinations showing high SCA were CSR 10 x CSR 5 for proline content and total regeneration, CSR 10 x IET 11149 for proline content, SAR 41 x IET 12860, SAR 41 x IET 11149, NDR 501 x IET 11149 and NDR 501 x IET 12860 for callus growth and proline content (Table 4).

The results suggest that the callus growth and total regeneration had quite similar inheritance pattern that showed high influence of additive gene effects and both characters exhibited partial dominance and high heritability. For both the traits, the tolerant (CSR 10

Table 4. General combining ability (GCA) effects (under line) and specific combing ability (SCA) effect for callus growth, Na/K ratio, proline content and total regeneration in 6 x 6 diallel crosses under salt medium

Parents	CSR10	CSR 5	SAR41	NDR501	IET12860	IET11149
CSR 10	[a]14.39	-9.42	-5.16	-1.23	3.45	12.36
	[b]-0.08	-0.20	0.14	-0.03	0.33	-0.24
	[c]-14.00	120.69	-24.40	-79.01	-46.05	-28.78
	[d]-12.70	6.46	-3.50	2.43*	-3.25	-2.15
CSR 5		18.01	-1.68	5.32	4.57	1.21
		-0.30	0.03	0.19	-0.10	0.08
		33.94	-29.88	35.58	-50.66	-65.73
		13.11	-4.41	11.91	-5.56	-6.59
SAR 41			-6.59	-19.59	14.86	11.57
			0.14	-0.07	-0.09	0.00
			-25.07	-36.41	74.95	15.75
			-7.17	-7.24	-7.52	-7.62
NDR 501				21.12	8.88	6.63
				-0.54	-0.20	0.11
				145.81	45.21	44.64
				13.37	-2.66	-3.72
IET 12860					-19.16	-31.76
					0.30	0.05
					-48.42	-23.44
					-14.94	4.94*
IET 11149						-27.77
						0.47
						-92.25
						-16.99

SE(gi) perents	SE(sij)	F1'S	SE(gi) perents	SE(sij)	F_1'S
[a] Callus growth	16.07	24.01	[c] Proline content	90.64	50.25
[b] Na/K ratio	0.06	0.01	[d] Total regeneration	5.27	4.28

and CSR 5) and the moderately tolerant parents (NDR 501) contained most dominant genes that enhanced callus growth and regeneration ability. The reverse was true for the sensitive (IET 11149, IET 12860) and the moderately tolerant genotypes (SAR 41). For Na/K and proline content, the pattern was different. For Na/K, partial dominance (0.64) was observed, but higher proportion of non-additive gene effect was also observed. The moderately tolerant genotype, NDR 501 (P_4) had low Na/K ratio governed by most dominant genes. On the contrary, CSR 10, a tolerant genotype – contained most of recessive genes for low Na/K ratio, while the sensitive genotype IET 12860, contained most recessive genes for a high Na/K ratio. For proline content, complete dominance was found with both additive and non-additive gene effects having equal effects. In NDR 501, high proline was controlled predominantly by dominant genes and low proline was due to mostly recessive genes. As evident, for all the four traits, NDR 501, the moderately tolerant genotype, possessed dominant genes showing desired increase or decrease in the traits. NDR 501, was also the best combiner for all these traits. The tolerant genotype CSR 10 also had dominant genes controlling increase in callus growth and regeneration frequency, but the two indices of salt tolerance (Na/K and proline content) were controlled mostly by recessive genes.

It is evident from this study that high callus growth, low Na/K rates and high proline content were better for total plant regeneration. The mechanism of gene for control of these traits appears to be different in different parents and a favorable gene (dominant or recessive) seems to be sutured among parents. Selection of appropriate cross combinations for pedigree breeding based on information generated help in further accumulation of favorable genes. The recombinant induced lines (RILs) derived from such crosses, therefore, would be expected to have higher degree of salt tolerance.

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