Genetic analysis for seed germination, callus induction and survival of rice under salt at *in vitro* conditions

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ABSTRACT

The genetics of salt tolerance in rice was investigated both at seed germination and in seed/seedling derived calli using six parents diallel analysis., excluding reciprocals. Test materials involved tolerant (CSR-5 and CSR-10), moderately tolerant (NDR-501 and SAR-41) and susceptible (IET12860 and IET 11149) genotypes. Rate of germination index (RGI), germination stress index (GSI) at seedling phase, fresh and dry weight (FW and DW) of calli, corresponding survival ratio of callus were studied for their combining ability and gene action. Analyses revealed both additive and non-additive gene effects for most of the traits: the former being more pronounced than later, especially under stress medium: for fresh and dry callus weight and callus survival under controled condition, both additive and non-additive gene effects were equally important. The genotypes like CAR-10, CSR=5 (tolerant) and NDR-501 (moderately tolerant) were found to be the best combiners under both Control (CM) and salt medium (SM). The crosses like CSR-10 x CSR-5, CSR-5 x NDR-501, NDR-501 x IET 12860 and NDR-501 x IET 11149 exhibited maximum SCA effects for fresh and dry weight of calli in control medium and salt medium and its terminal survival under CM.

Key words: *Salinity, seed derived callus, rice, combining ability, gene effects*

Soil salinity is a major limiting factor in rice production. It is a serious problem affecting 1/3 of all irrigated land in the world. Strategy to develop varieties with salt tolerance gene is becoming non productive because of salt accumulation (Carter, 1975). Somaclonal variants derived through tissue culture, provide opportunity to select salt tolerant lines (Scoweroft and Larkin, 1982). In rice, tissue culture was successful in most of the japonica type but served little in Indica and African rice (Yoshida *et al.,* 1983). Genetic factors are considered to be involved in tissue culture ability. However, tissue culture is unlikely to succeed because of different gene expression for resistance to salinity at cellular and whole plant levels. Parameters for *in vitro* screening of salt tolerance generally include initial measurement of fresh- and dry-weight, survival ratio of callus etc. (Reddy and Vaidynath, 1986). Reports have indicated significant additive and dominance-gene effects and high degree of heritability values for most traits studied so far in salt tolerant varieties(Gregorio and Senadhira, (1993). Analysis of data on different degree of culturability of genotypes in somatic cell-culture by diallel analysis indicated difference in both general and specific combining ability in rice anther culture (Chu and Croughan, 1988).

In the present case, parents with different levels of salt-adaptability and their crosses were studed in vitro metric traits of seed at germination and seed derived calli in rice using six parents-half-diallel analysis. The idea was to understand the genetics of salt tolerance at plant as well as cellular level which in turn would help design effective breeding for developing salt tolerant varieties.

MATERIALS AND METHODS

The selection of parents was based on their earlier field experiments showing varying degree of salt tolerance. Screening of genotypes for adaptation to saline environment was done as per IRRI scale (Table 1). Based on the above criteria, tolerant genotypes (CSR-5 and CSR-10), moderately tolerant (NDR-501 and SAR-41) and susceptible (IET12860 and IET 11149) genotypes were selected and grown in randomized block

Table 1. Observations on different characters of the parents selected for diallel cross under saline-sodic soil (pH 10.0) .

Cultivars	Plant height (cm)	Seedling* mortality	Na/K Ratio	Ca/Na Ratio	Tiller** mortality (%)	Harvest index DAT	1000 seed weight	Yield t ha^{-1} (g)
CSR-10	90.5	Nil	3.12	2.17	-21.0	62.5	2.3	5.8
CSR-5	91.3	Nil	3.15	2.17	-8.4	62.5	2.3	6.3
SAR-41	85.3	Nil	2.17	2.18	35.9	53.0	2.1	3.6
NDR-501	72.3	Nil	2.72	2.43	14.9	24.0	2.2	3.0
IET-12860	90.9	Nil	0.85	0.75	22.4	58.0	1.0	2.0
IET-11149	91.2	1.5	1.50	1.25	7.8	34.0	0.8	7.6

* = Seedling mortality (12-15 days after planting)

** = (-) negative tiller mortality indicate increase in tiller number after vegetative phase.

design with three replications. At proper stage of flowering, diallel crosses were made to produce 15 F_1's excluding reciprocal.

The dehusked mature seeds of parents and their F_1's were used for inducing seed calli in induction phase. Seeds were washed thoroughly in running tap water and surface sterilization of seeds with $HgCl_2$ (0.2% w/v) was done prior to diping in EtOH (40% w/v) for 20-30 minute, so as to partially remove wax. The seeds were washed in double sterile water, the seed germinated for 36h at 24+1^{0}C. Embryos were excised from seed, three embryos per tube were placed onto solidified agar medium containing Murashige and Skoog medium (1962) with 2,4-D (2 mg/l). All cultures were kept under continuous inflorescent light and maintained for 4 week at 28+^{0}C to induce callus. Callus was transferred to fresh medium and allowed to proliferate for 3 weeks under same conditions. For the screening of salt tolerance, two level of culture media were used, one for raising the seedling to study salt injury under gradients of NaCl levels, while the screening of proliferate divided into small pieces and placed into agar solidified MS media containing 0.0, .0.5, 1.0, 1.5, 2.0 and 2.5% NaCl. Three replications were maintained for 4 weeks. After 3 week, the rate of germination index (RGI) and germination stress Index (GSI) at seedling stage was estimated. Another set was used to investigate the effect of NaCl stress on callus growth. The proliferating callus was divided into small pieces and placed onto solidified MS media (1.5% NaCl) and without NaCl, supplemented with 2,4-D (2 mg/l), typtophan (5 mg/l) and energy source (sucrose 30 g/l). The cultures were maintained at 28+2^{0}C under continuous inflorescent light with three replications. The parents and their crosses were sub-cultured again under same conditions in the presence of NaCl. The parents and their crosses were evaluated for their consistent performance to salt tolerance upon repeated subcultured on saline nutrient medium. Data were recorded for both NaCl and with out NaCl treated callus fresh and dry weight (mg) and callus survival ratio.

Seed germination index was measured after 48 and 168 hr (Krishnasamy and Seshu, 1989).

$$RGI = \frac{\text{No. of seed germinated at 48 hrs}}{\text{No. of seed germinated at 168 hrs)}} \times 100$$

and Germination stress Index (GSI) was analyzed (George, 1967)

$$GSI = \frac{\text{Promptness index of stressed seed (PIS)}}{\text{Promptness index of controlled seeds}} \times 100$$

(PIC) PI = nd2 + nd4 + nd6 + nd8

Where, nd = percent of seeds observed to germinate after 2,4,6 & 8 days

Estimate of general (GCA) and specific (SCA) combining ability were obtain according to Model I and Methods II of Griffing (1956).

RESULTS AND DISCUSSION

Under stress medium, the values for susceptible genotypes were much lower than the tolerant types

(Table 2). The RGI ranged from 89.1 to 79.8% in control media and 45.6 to 13.3% under salt medium. The survival of callus ranged from 89.7% (CSR-10) to 82.5 % (IET12860) in control media (CM) and 45.1 (CSR-10) to 7.5% (IET 11149) in salt media (SM). Similar results were observed for callus fresh and dry weight under both CM and SM (Fig 1-4) and GSI (%) under SM was much higher for the tolerant genotypes than the susceptible. Basu et. al.('1997) also reported higher survival plant generated from NaCl adapted calli than those obtained from non-adapted calli in rice variety Basmati 370. The two sensitive genotypes had also much lower fresh and dry weight than the resistant and moderate resistant genotypes. The GSI (%) was also quite low for the susceptible ones. Decrease in growth rate of callus in the un-selected lines to selected (tolerant) ones has also been reported by Reddy and Vaidyanath (1986). They also reported that calli growth as well as fresh and dry weight of calli reduced with the increase in salt stress.

Combining ability analysis helped to partition the genotypes variance due to general and specific combining effects (Table 3).Both GCA and SCA variances were significant for all the five traits under CM as well as SM suggesting involvement of both additive and non-additive effects in their control. However, high predictability ($2ó^2s/2ó^2g+2ó^2s$) ratio indicated the predominance of additive gene effects, particularly for RGI both under CM and SM. However, for the control medium (CM), both additive and non-additive gene effects were equally important for fresh and dry callus The values of predictability ratio for the survival calli were close to 0.5. The callus induction

Table 2. Mean values of different characters under control (CM) and stressed medium (SM)

Genotypes	RGI (%)		GSI(%)		Callus freshweight (mg)		Callus dryweight (mg)		Survival of callus(%)	
	CM	SM	CM	SM	CM	SM	CM	SM	CM	SM
CSR-10	80.3	35.3	—+	17.4	219.0	220.7	43.6	51.2	89.7	45.1
CSR-5	86.5	45.6	—	20.6	225.1	224.9	51.7	57.3	91.4	47.0
SAR-41	81.6	32.2	—	13.6	201.4	202.7	32.0	40.4	80.5	40.7
NDR-501	87.7	36.6	—	14.3	231.5	215.7	52.5	46.1	88.5	42.8
IET-12860	79.8	20.1	—	7.3	187.2	153.6	25.8	33.7	82.5	22.8
IET-11149	89.1	13.3	—	7.8	208.5	145.8	36.2	29.8	84.0	7.5
Mean	84.1	30.5	—	13.5	212.1	193.9	40.3	43.0	86.1	34.3

+ = No Response

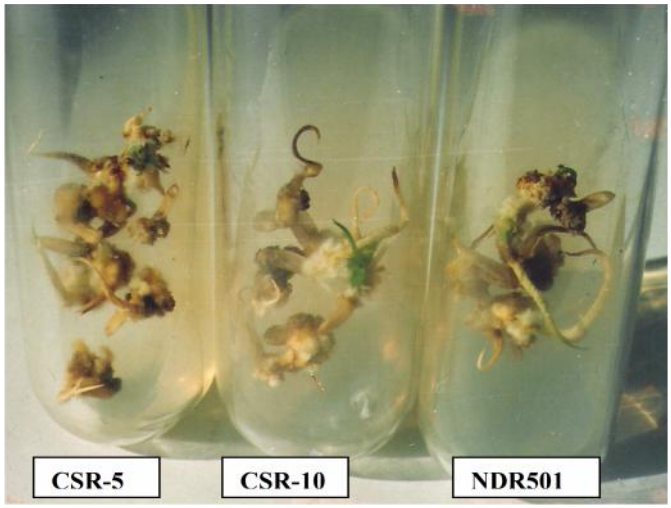

Fig 1. Seed derived callus induction in different parent: CSR-5, CSR-10 and NDR 501 under 2,4-D 2/mg/l; NaCl level : 1.5% w/v.

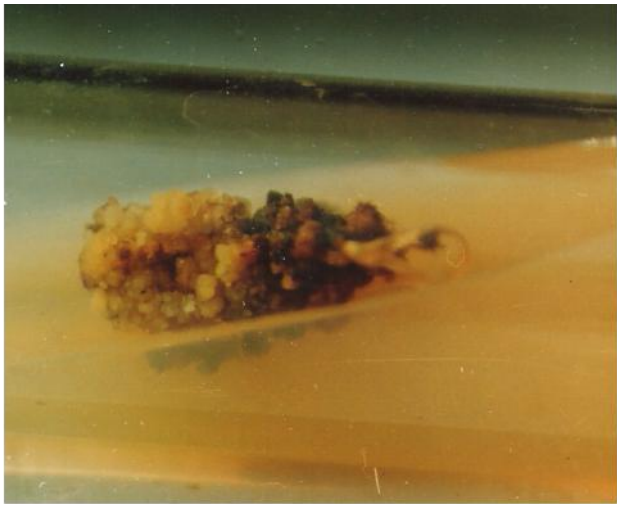

Fig 2. Seed derived callus induction in NDR 501 (moderately tolerant)under 2,4-D 2/mg/l; NaCl level : 1.5 % w/v.

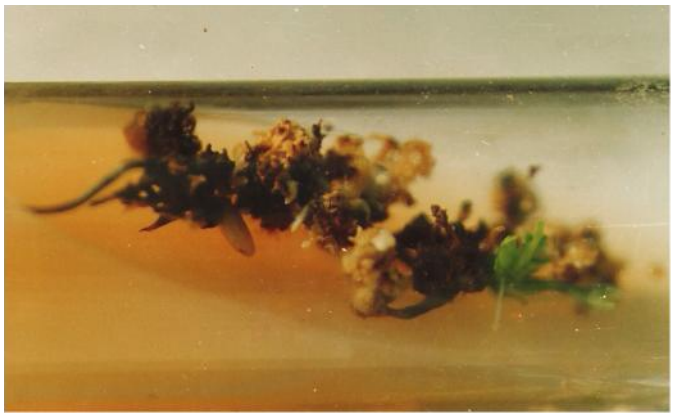

Fig 3. Seed derived callus induction in SAR-41(Moderately tolerant) under 2,4-D 2/mg/l; NaCl level : 1.5% w/v.

Fig 4. Seed derived callus induction in IET 12860 (sensitive genotype) Under 2,4-D 2/mg/l; NaCl level : 1.5% w/v.

Table 3. Mean sums of square due to GCA and SCA and estimates of $2\pm^2g/2\pm^2g+\pm^2s$ in a 6x6 diallel analysis.

Source	D.F.	RGI (%)		GSI (%)		Callus fresh wt. (mg)		Dry wt. (mg)		Survival of callus (%)	
		CM	SM	CM	SM	CM	SM	CM	SM	CM	SM
GCA	5	237.7**	39.5**	—+	151.8**	13630.3**	18896.6**	3498.2**	5153.1**	73.3**	276.6**
SCA	15	32.6**	7.5**	—	20.1**	2330.6**	3000.6**	696.5**	707.4**	26.1**	33.2**
Error	60	23.4	5.3	—	0.9	16.0	26.6	26.6	13.4	10.8	0.6
$2\sigma^2g/2\sigma^2g+\sigma^2s$		0.85	0.78	—	0.63	0.55	0.57	0.51	0.62	0.43	0.65

+ = No. response, Significant at *P(0.05), **P(0.01)

and green plant regeneration involvement of both additive and non-additive gene effects were also reported by Singh et al., 2005, with additive gene effects as a primary contributor.

The salt tolerant genotypes had higher GCA value than sensitive ones for most of the traits (Table 4). NDR 501, a moderately resistant variety, was the best general combiner, showing high and positive GCA value for all the traits except RGI under salt medium. CSR 10 and CSR 5, the well know salt tolerant varieties. Were also good combiners. SAR-41, a moderately resistant variety and the two susceptible genotypes IET12860 and IET11149 showed poor general combing ability, as indicated by their significant negative GCA effects. The trend for the GCA effects of genotypes remained more or less same both under CM and SM, with the exception of NDR501 and IET12860 for RGI. IET 12860 is the only good combiner for RGI under SM.

The three best general combiners, which also were salt tolerant (CSR10, CSR5 and NDR 501) produced the best cross combinations as indicated by high positive and significant SCA value (Table 5). Besides the combination with (CSR 10, CSR 5 and NDR501) when crossed with two sensitive genotypes having negative GCA also produced high positive SCA value for fresh and dry weight, although the value were negative for other traits. As against this CSR-10 and CSR-5 when crossed with IET 12860 and IET 11149, a poor combiner. The SCA was negative in most of the traits, but where crossed with another poor combiner IET 12860, the results were variable. Crosses between the two sensitive lines, which were also the poorest general combiner resulted into negative SCA values. Singh *et al.*, 2005 found that SCA effects were significant and positive between two lines with negative GCA effects, while the crosses between two best combiners gave negative SCA effect for callus induction and positive, not significant SCA for plant regeneration. In the present case, additive x additive types of gene effects contributed more towards the high SCA than the non-additive gene interactions. The mean crosses among the tolerant and moderately tolerant genotypes might throw better segregations for the traits studied here. Further more, NDR 501 is the best combiner

Table 4. Estimates of gca effects of lines for different characters in control (CM) and stressed medium (SM)in 6x6 diallel

Parents	RGI (%)		GSI(%)		Callus fresh wt. (mg)		Callus dry wt. (mg)		Survival of callus(%)	
	CM	SM	CM	SM	CM	SM	CM	SM	CM	SM
CSR-10	-1.94*	-0.95*	—+	1.76**	8.61**	10.11**	3.01**	3.56**	0.60	1.30**
CSR-5	0.39	-0.69	—	2.94**	16.77**	20.96**	9.58**	12.81**	2.01**	3.49**
N\SAR-41	-2.82**	-1.31**	—	-1.55**	-10.24**	-10.24**	-6.56**	-7.08**	-1.26**	-0.80**
NDR-501	2.35*	-1.53**	—	0.97**	24.52**	28.25**	13.17**	15.40**	1.31**	2.58**
IET-12860	3.98**	0.62	—	-1.81**	-7.52**	-7.97**	-11.57**	-4.89**	-1.05	-2.91**
IET-11149	-3.53**	-0.58	—	-2.30**	-31.69**	-36.69**	-14.31**	-16.59**	-1.61**	-3.66**
±S.E.(gi)	0.54	0.14	—	0.14	0.14	0.26	0.83	0.58	0.52	0.10
±S.E.(gi-gj)	0.86	0.14	—	0.14	1.00	0.42	1.28	0.91	0.81	0.14

+ = No. response, Significant at *P(0.05), **P(0.01)

Table 5. Estimates of sca effects of crosses among lines for different characters under control (CM) and stressed medium (SM) in 6 x 6 diallel.

Crossees	RGI (%)		GSI(%)		Callus fresh wt. (mg)		Callus dry wt. (mg)		Survival of callus(%)	
	CM	SM	CM	SM	CM	SM	CM	SM	CM	SM
CSR-10 x CSR-5	2.43	0.45	—+	2.73**	7.80**	7.07**	11.76**	7.83**	2.36	3.89**
CSR-10 x SAAR-41	-0.53	-3.25**	—	-2.21**	14.41**	4.99**	0.45	1.36	1.36	1.29**
CSR-10 xNDR501	1.61	1.46	—	1.84**	0.40	6.32**	8.68**	4.05*	1.93	3.38**
CSR-10 x IET12860	1.39	-0.29	—	-1.89**	2.87*	1.01	0.35	1.40	10.32**	-0.61**
CSR-10 x IET11149	-1.93	0.22	—	-1.16**	8.41**	-6.93**	-11.03**	-6.36**	8.56**	-1.47**
CSR-5 x SAAR-41	-7.56**	-1.30	—	-1.78**	0.32	0.89	-4.38*	-4.51*	-3.35*	-2.79**
CSR-5 x NDR-501	7.89**	1.33	—	0.93**	35.46**	43.60**	23.32**	31.98**	5.60**	6.51**
CSR-5 x IET-12860	-3.87	1.31	—	-0.72**	4.18**	4.08**	-3.88	-0.09	8.34**	-1.67**
CSR-5 x IET-11149	2.65	-0.03	—	-3.40**	-11.37**	-31.83**	-11.51**	-14.53**	10.46**	-2.06**
SAR-41 xNDR-501	1.60	1.08	—	1.21**	-0.15	-3.52**	-2.26	-2.74	-0.32	0.02
SAR-41 x IET12860	2.06	-0.43	—	-0.96**	1.97	2.08*	-3.86	-4.42*	10.75**	-1.53**
SAR-41 x IET11149	-1.50	-0.12	—	-0.78**	-43.88**	-45.88**	-14.45**	-12.98**	9.50**	0.12
NDR501x IET12860	1.59	-0.20	—	-0.68**	23.74**	23.74**	12.19	5.38**	10.70**	-1.53**
NDR501x IET11149	-1.97	-0.49	—	-0.78**	25.43**	25.43**	8.31**	5.80**	8.90**	-1.92**
IET12860xIET11149	6.33	0.25	—	-0.99**	-23.92**	-23.92**	0.93	-3.70*	-12.21**	-12.07**
±SE(sij)	2.41	1.14		0.14	1.20	0.84	2.57	1.83	2.33	0.24

+ = No. response, Significant at *P(0.05), **P(0.01)

followed by CSR-5 and CSR-10, the known salt tolerant varieties which could well be utilized in the breeding programme.

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