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Original Research Article

Inheritance Pattern of Salinity Tolerance and Combining Ability in Rice (*Oryza sativa* L.)

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A B S T R A C T

The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and narrow sense heritability of salt tolerance. Screening under field conditions is difficult due to stress heterogeneity *i.e.*, presence of other soil related stresses and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. These complexities, cause difficulties in developing and using reliable methods of screening voluminous materials. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone areas. The challenge is to develop high-yielding, salt-tolerant cultivars for the various salt-affected areas of Asia. The tools of biotechnology (specifically, molecular marker-assisted selection [MAS] and genetic engineering) offer a promise to complement existing breeding strategies. Salinity tolerance F_1 were selected for marker assisted selection with the RM 493 and RM 23 primer, the gel analysis resulted that F1's showed band like its tolerant parents were, CSR13, Narendra Usar3, CSR30, NDR359, and Nonabokra.

Keywords

Salinity tolerant, Molecular marker, combining ability

Introduction

Rice is the staple food of more than 50% of the world's population (Aggarwal *et al.*, 2002). By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhuiyan *et al.*, 2002). Salinity is one of the major obstacles in increasing production in rice growing areas worldwide. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice is tolerant during germination, vegetative growth stage and at maturity, and becomes very sensitive during early seedling stage (2-3 leaf stage), pollination and fertilization (Bhowmik, 2009). At present time, to develop salinity tolerance rice varieties along with high-yield is a big challenge to the rice breeders. Breeding for salinity tolerance in rice requires reliable screening techniques. Screening of germplasm at seedling stage is readily acceptable as it is based on a simple criterion of selection; it provides rapid screening difficult at vegetative and reproductive stage (Gregorio *et al.*, 1997)

The biotechnological tools offer an opportunity to the rice breeders along with existing breeding strategies to overcome the salt related tribulations. The ability of the

plant to tolerate the salt stress up to an extent is of paramount importance to manage the resources optimally and this is the reason to develop the tailored crops with higher salt tolerance. Nowadays, major soil reclamation programmes involve both biological and hybrid approaches to combat the salt problem. These techniques must be rapid to keep pace with the large amount of breeding materials generated. Screening under field conditions is difficult due to stress heterogeneity presence of other soil related stresses and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. These complexities, together with the degree of control of salinity and reproducibility, cause difficulties in developing and using reliable methods of screening voluminous materials. The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). This hinders the development of an accurate, rapid and reliable screening technique. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Recent progress and technical advances in DNA marker technology permit reduction of time and accuracy of breeding where pronounced effects of environment lead to poor selection efficiency (Bhowmik, 2009).

Materials and Methods

The present experimental material consisted of 12 F_1's were derived through Line x Tester mating fashion, and 7parents, in which three as a line (female) viz. Sarjoo 52, IR 64 and NDR 359, and four as a testers (male) viz. NDRK5088, CSR 13, Nonabokra and Narendra Usar 3 were diverse to salinity susceptible and resistant rice pollen parents were evaluated during *Kharif* 2008in Randomized Block Design with three replications. Recommended package of practices have been done to raise healthy crop.

Phenotypic study of salinity tolerance at vegetative stage

The genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (*Gregorio*, 1997). Salinized and non-salinized setups with three replications were maintained. The evaluation was done using *Yoshida et al.,* (1976) nutrient solution at the glasshouse. The nutrient solution was salinized by adding crude salt to obtain desired EC (12 dS/m). The modified Standard Evaluation System was used in rating the visual symptoms of salt toxicity (IRRI, 1997). The scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 7 days and 21 days after salinization.

Preparation of stock solutions

Proper preparation of stock solutions is essential to avoid nutrient deficiencies and mineral toxicities. The amounts of solution depend on the number of test entries screened during a two-month period (Davitt *et al.,* 1981). For the macronutrient stock solutions, weight the required amount of reagent was transferred to a 1000 ml beaker and do initial mixing with about 750 ml distilled water. Mix the solution in 2 liter volumetric flask, then add distilled water and make up volume to 2 liter. Mix the solution properly for 15 minute using a glass rod, then transfer to stock solution bottle. Preparation of micronutrient stock solution is critical because most nutrient deficiencies and other toxicities could be traced to

improper preparation. Each reagent of the micronutrient solution (Table 1) was dissolved separately. Only ferric chloride was dissolved in 100 ml distilled water. Mix all solutions together by using 2.0 liter capacity volumetric flask. Add the ferric chloride solution to the mixture just before citric acid and stir the mixture for 15 minute. Finally add 100 ml sulfuric acid to the mixture and make up volume to 2.0 liter. Stir for another 10 minute and store in a dark glass bottle. The final color of the solution was yellowish brown. All stock solutions was properly labeled and kept in separate bottles.

CTAB Mini Preparation DNA Extraction

DNA isolation was done from fresh leaf tissues of 14-day old seedlingsof parents and F_1s were extracted using CTAB method given by *Murray and Thompson* (1980). Fresh leaves of each sample were taken and grinded in liquid nitrogen and transferred into centrifuge tube.

Later, centrifuge tubes were heated in water bath at 65^0C for 1 hour. After that centrifuge tubes were cooled at room temperature and 3 ml of chloroform (Chloroform: Isoamyl:: 24:1) was added and mixed properly by a shaker. Centrifuge tubes were centrifuged at 6,000 rpm for 15 minutes at room temperature. Supernatant was transferred in fresh eppendrof tubes. In supernatant, half volume of 5 M NaCl and equal volume ofice-cold iso-propanol were added andshaken slowly then stored it at 4^0C for overnight. Then, eppendrof tubes were centrifuged at 10,000 rpm for 20 minutes. Pelletes were washed with 70% ethanol (200 µl), spinned for 15 minutes at 5700rpm and then air-dried for 1/2-1hours. Then the ethanol was removed and air-dried. The pelletes were resuspended in 50 µl of TE buffer and stored at 4^0C.

DNA amplification and visualization

A total of 6 SSRs primers were used for the molecular analysis for total rice varieties. Sequence of one primer which has given clear bands. Amplification was carried out in 20µl reaction volume containing 2.0 µl 10 X Taq buffer, 2.0 µl dNTPs, 2.0 µl primer forward, 2.0 µl primer reverse, 0.8 µl Taq polymerase, 9.2 µl distilled water and 2.0 µl of each template DNA samples. The amplification reactions were carried out in a thermo cycler (model PTC-100) at initial denaturing step at 94^0 C for 5 minutes followed by 35 cycles of 94^0C for 1 minute, 55^0C for 1 minute and 72^0C for 2 minute. In the last cycle, primer extension is at 72^0C for 7 minutes.

Salinity screening (SES, 1996)

To confirm the reliability of screening technique, the visual salt-injury symptoms were compared with leaf rolling symptoms of entries obtained in the lab under saline conditions. The Table 2a and 2b shows these symptom score. The score based on visual symptoms relates saline lab condition due to salt stress. The reliability of visual symptoms of salt stress shows after 15 days of salinization. Visual symptom rating is adequate to determine the level of tolerance for breeding purposes. Visual symptom rating correlated well with yield performance in saline condition. The technique can be accelerating rice breeding programs for salt tolerance.

Results and Discussion

The varieties *viz.,* CSR13, Narendra Usar3, Nonabokra, CSR30 and their crosses with Sarjoo52, IR-64, NDR359 *i.e* Sarjoo 52 × CSR13, Sarjoo52 × Narendra Usar3, Sarjoo52x Nonabokra, Sarjoo52 x CSR30, IR 64 × CSR13, IR64 x Nonabokra, IR-64 x

CSR30, NDR 359 × CSR13, NDR359 x Nonabokra, NDR359 x CSR30, NDR359 x Narendra Usar 3 were found tolerant at vegetative stage (Table 2a). Sarjoo 52, IR 64, NDRK-5088, Sarjoo52 x NDRK-5088, IR-64 x NDRK-5088, IR 64 × Narendra Usar 3, NDR 359 × NDRK 5088 were found moderately tolerant at vegetative stage (Table 2a). In reproductive stage CSR13, CSR30, Nonabokra, Narendra Usar3 and the crosses *viz.* Sarjoo 52 × CSR 13, Sarjoo 52 × Narendra Usar3, Sarjoo52x Nonabokra, Sarjoo52 x CSR30, IR 64 × CSR 13, IR 64 × Narendra Usar3, IR64 x Nonabokra, NDR 359 × CSR 13, NDR 359 × Narendra Usar3 and NDR359 x Nonabokra were found tolerant (Table 2b). At reproductive stage Sarjoo52, IR 64, NDR359, Sarjoo 52 × NDRK-5088, IR 64 x NDRK-5088, IR 64 × CSR30, NDR359 x NDRK-5088 were found moderately tolerant (Table 2b) in the salinity screening under sodium (Na) rich solution.

Salt tolerant QTLs

Molecular screening of rice varieties for parents including susceptible and resistance traits are studied with *saltol* marker. Total five primers were used for detecting the *saltol* QTL. Only two primers *viz;* RM23 and RM493 were amplified during the analysis. The parents were analysed first with these two primers. The tolerant parents were, CSR13, Narendra Usar3, CSR30, NDR359, and Nonabokra. The susceptible parents were Sarjoo52, NDRK5088 and IR-64. The line shows polymorphism during their molecular analysis. Selected parents and their F_1s are screened with primer RM493 which showed bands like tolerant parent in F_1,sand RM 23 also showed amplification pattern like their tolerant parents. This result supported that molecular analysis of salinity tolerant varieties were screened on the basis of amplification like tolerant parent (Nguyen *etal*, 2008).

Estimates of general combining ability effects (gca)

The estimates of general combining ability (gca) for the eleven characters were presented in Table 3.Among lines IR64 recorded significantly negative gca effects for days to 50 per cent flowering and days to maturity, while NDR359 and Sarjoo52 recorded significantly negative gca effects (desirable direction) for plant height. Sarjoo52 also exhibited positive significant gca effects for flag leaf area, harvest index, panicle bearing tillers per plant, grain yield per plant, test weight, spikelets per panicle and biological yield.

Thus Sarjoo52 was found a good general combiner for all the traits except spikelets fertility. Among testers Narendra Usar 3 showed good general combining ability for flag leaf area, harvest index, biological yield and grain yield per plant.

Estimates of specific combining ability effects

Specific combining ability effects (sca) for eleven traits are presented in Table 4.The general and specific combining ability is associated with interaction effects, which may be due to dominance and epistatic components of genetic variation that are non-fixable in nature. Out of 12 cross combinations, only four crosses exhibited significant positive sca effects were Sarjoo52 x Narendra Usar 3 (3.351), NDR 359 x IR 29 (1.561), IR 64 x CSR13 (1.326) and NDR359 x NDRK5088 (0.894) for grain yield per plant. The cross Sarjoo 52 x Narendra Usar 3 also showed significant positive sca effects for some other yield contributing traits like biological yield (3.317), harvest-index (3.074), spikelets per panicle (5.167) and days to 50% flowering (4.222).

Fig.1 Salinity scoring at vegetative stage (7, 14 and 21 days)

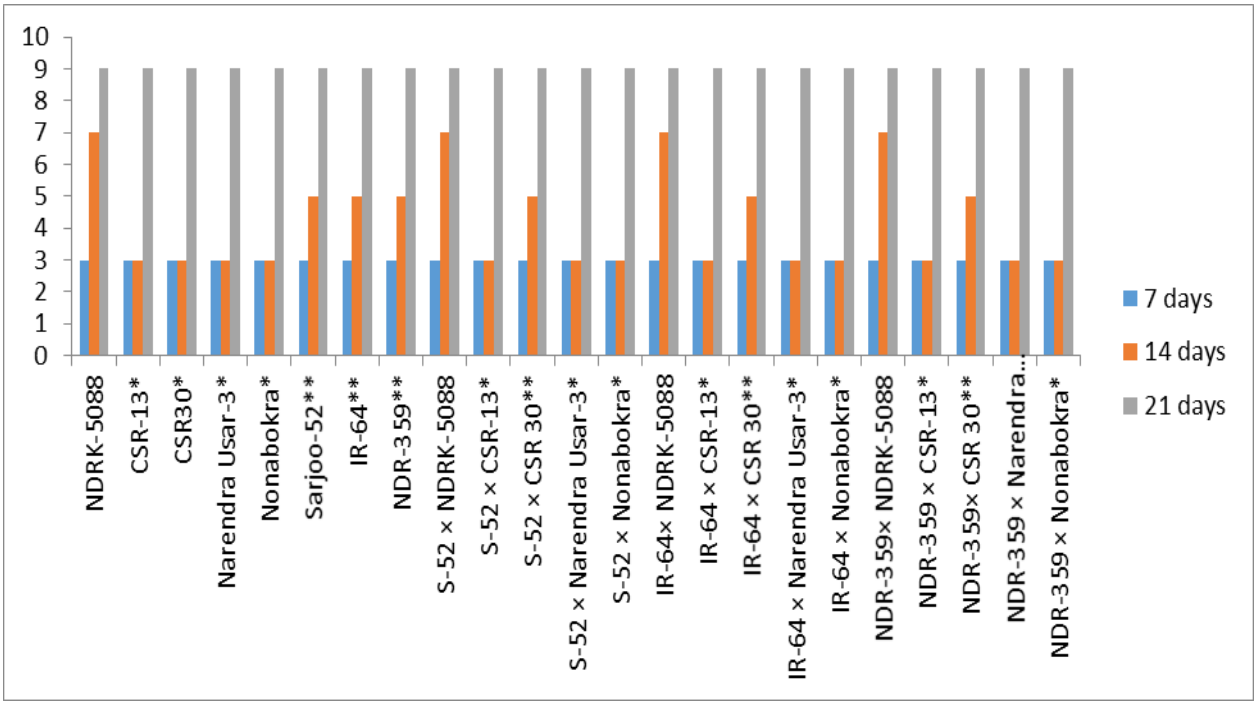


Fig.2 Salinity scoring at reproductive stage (7, 14 and 21 days)

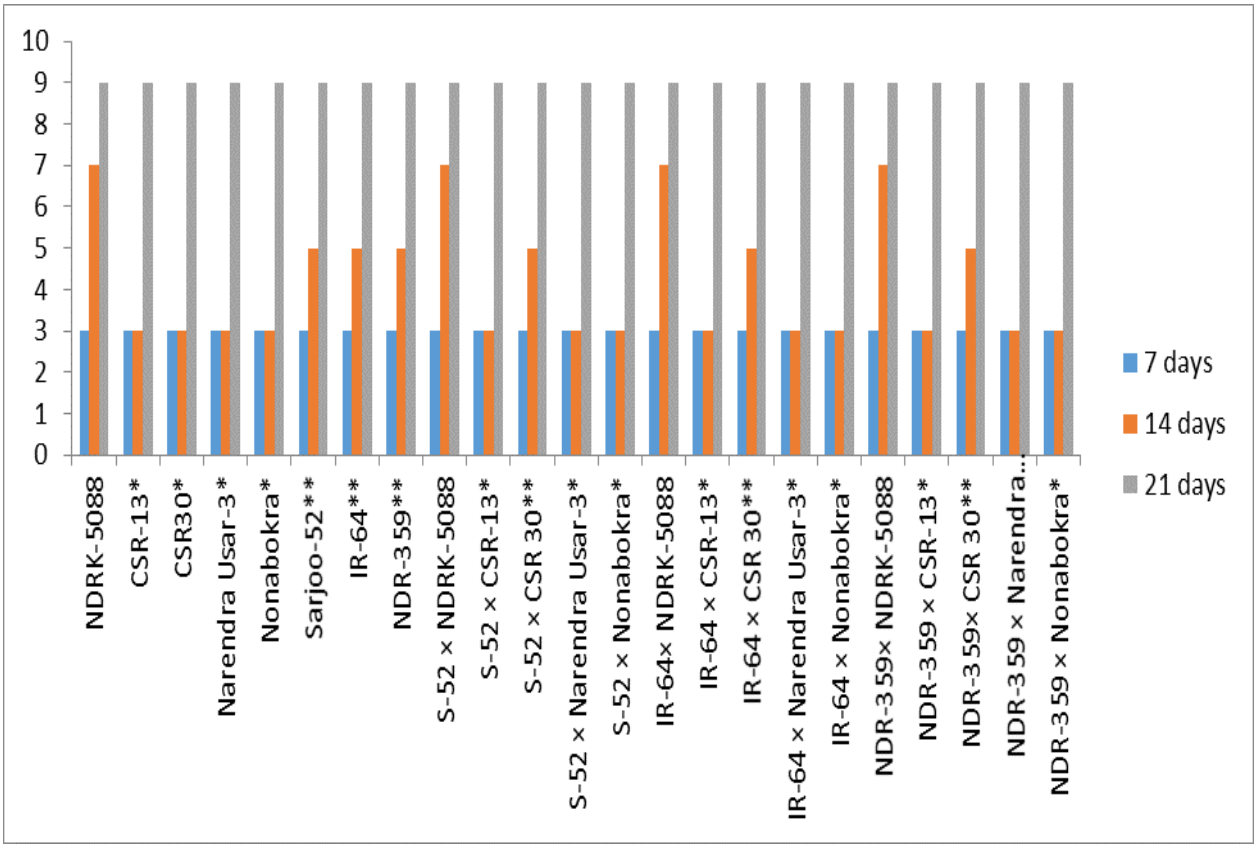
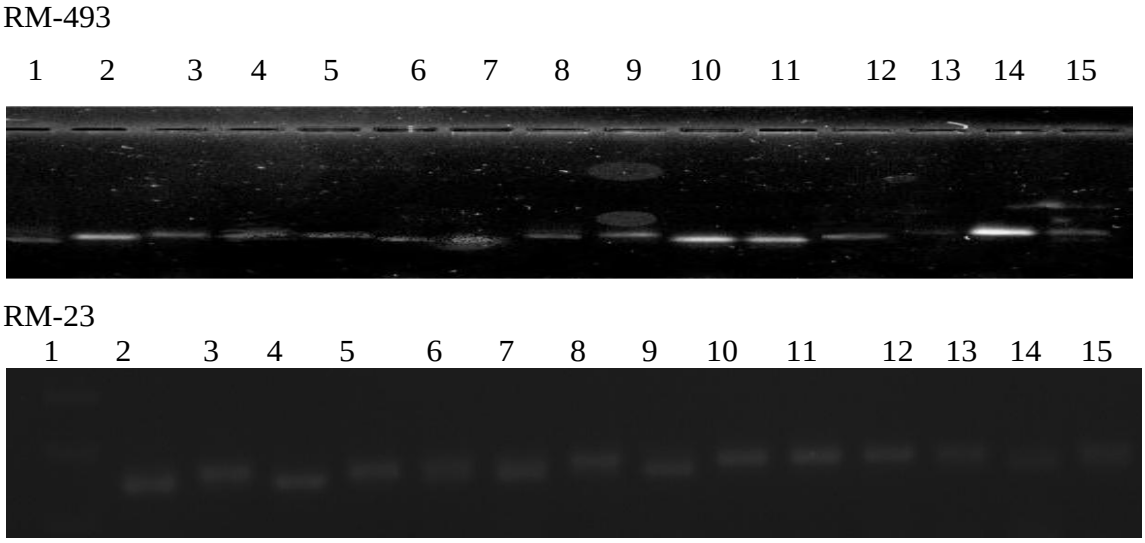


Fig.3 2% Agrose gel electrophoresis of Rice samples



Sequence of SSRs primer-pairs provided clear amplification in Rice genotypes

S. No.	Primer code	Sequence (5'→3')	Forward/ Reverse
1	RM493	TGCGTTTGTAAGCATTCTTCA	F
		AGGTATCCAATATCCAACTTG	R
2	RM23	GGAGCTATGTTGGAGGATGA	F
		CCTTTTTGCATGGGTTGTAT T	R

Table.1 Preparation of stock solution

Element	**Reagent (AR grade)**
Macronutrient	
N	Ammonium nitrate (NH_4NO_2)
P	Sodium phosphate monobasic monohydrate ($NaH_2PO_4H_2O$)
K	Potassium sulfate (K_2SO_4)
Ca	Calcium chloride dehydrate ($CaCl_2$ $2H_2O$)
Mg	Magnesium sulfate 7-hydrate ($MgSO_4$ $7H_2O$)
Micronutrient(dissolve each reagent separately and mix in 2litter distilled water then add 200ml H_2SO_4)	
Mn	Manganese chloride 4-hydrate (MnC 13-4 H_2O)
Mo	Ammonium molybdate 4-hydrate [$(NH_4)_3$Mo/O24 $4H_2O$]
Zn	Zinc sulfate 7 hydrate ($ZnSO_4$ $7H_2O$)
B	Boric acid (H_2SO_4)
Cu	Cupric sulfate 5 hydrate ($CuSO_4$ $5H_2O$)
Fe	Ferric chloride 6 hydrate ($FeCl_2$ $6H_2O$)
	Citric acid monohydrate

Source: Adapted from *Yoshida et al.,* (1976); Note: For easy handling and storage, hydrate reagents are preferred

Table.2a Salinity score at vegetative stage in lab condition

S. No.	Name of variety/line	Salinity score		
		7 days	14 days	21 days
1	NDRK-5088	3	7	9
2	CSR-13*	3	3	9
3	CSR-30*	3	3	9
4	Narendra Usar-3*	3	3	9
5	Nonabokra*	3	3	9
6	Sarjoo-52**	3	5	9
7	IR-64**	3	5	9
8	NDR-359**	3	5	9
9	S-52 × NDRK-5088	3	7	9
10	S-52 × CSR-13*	3	3	9
11	S-52 × CSR 30**	3	5	9
12	S-52 × Narendra Usar-3*	3	3	9
13	S-52 x Nonabokra	3	3	9
14	IR-64× NDRK-5088	3	7	9
15	IR-64 × CSR-13*	3	3	9
16	IR-64 × CSR 30**	3	5	9
17	IR-64 × Narendra Usar-3**	3	3	9
18	IR 64 x Nonabokra	3	3	9
19	NDR-359× NDRK-5088	3	7	9
20	NDR-359 × CSR-13*	3	3	9
21	NDR-359× CSR 30**	3	5	9
22	NDR-359 × Narendra Usar-3*	3	3	9
23	NDR 359 x Nonabokra	3	3	9

*Tolerate till to 15 days, ** moderately tolerant till to 15 days

Table.2b Salinity score at reproductive stage in lab condition

S. No.	Name of variety /line	Salinity score		
		7	14	21
1	NDRK-5088	3	7	9
2	CSR-13*	3	3	9
3	CSR30*	3	3	9
4	Narendra Usar-3*	3	3	9
5	Nonabokra*	3	3	9
6	Sarjoo-52**	3	5	9
7	IR-64**	3	5	9
8	NDR-359**	3	5	9
9	S-52 × NDRK-5088	3	7	9
10	S-52 × CSR-13*	3	3	9
11	S-52 × CSR 30**	3	5	9
12	S-52 × Narendra Usar-3*	3	3	9
13	S-52 × Nonabokra*	3	3	9
14	IR-64× NDRK-5088	3	7	9
15	IR-64 × CSR-13*	3	3	9
16	IR-64 × CSR 30**	3	5	9
17	IR-64 × Narendra Usar-3*	3	3	9
18	IR-64 × Nonabokra*	3	3	9
19	NDR-359× NDRK-5088	3	7	9
20	NDR-359 × CSR-13*	3	3	9
21	NDR-359× CSR 30**	3	5	9
22	NDR-359 × Narendra Usar-3*	3	3	9
23	NDR-359 × Nonabokra*	3	3	9

*Tolerate till to 15 days, ** Moderately tolerant till to 15 days

Table.3 Estimate of GCA effect of parent (males and females) for 11 characters in rice

Parents	Days to 50% flowering	Days to maturity	Plant height	Panicle bearing tillers/ plant	Flag leaf area	Spikelets/ panicle	Spikelets fertility	Test weight	Harvest Index	Biological Yield	Grain yield/ plant
Lines											
Sarjoo52	0.44	-0.11	-0.90**	1.14**	0.49*	3.61**	-5.64**	2.38**	1.06*	3.89**	2.10**
IR64	-1.89*	-1.44**	4.82**	0.06	-1.82**	-6.56**	0.99**	-0.33**	-1.60**	-3.74**	-2.52**
NDR359	1.4*	1.56**	-3.92**	-1.19**	1.33**	2.94**	4.64**	-2.05**	0.54	-0.16	0.42**
SE(gi) lines	0.664	0.502	0.273	0.343	0.201	0.991	0.367	0.098	0.38	0.31	0.185
SE(gi-gj) lines	1.327	1.005	0.545	0.686	0.402	1.982	0.734	0.198	0.761	0.691	0.369
TESTERS											
NDRK5088	-2.97**	-0.31	0.18	1.17*	0.40	6.08**	-1.46**	0.66**	0.00	-1.05*	-0.30
CSR13	2.81**	1.92**	0.96**	-1.28**	-2.63**	-6.14**	1.90**	2.75**	-2.93**	1.72**	-0.98**
IR29	-0.19	-0.86	-0.99**	0.36	-0.82**	1.64	-0.49	-1.01**	1.58**	-3.15**	-0.47*
Narendra Usar 3	0.36	-0.75	-0.15	-0.28	3.05**	-1.58	0.06	-2.40**	1.35**	2.48**	1.74**
SE(gi) tester	0.813	0.615	0.334	0.42	0.246	1.214	0.449	0.121	0.466	0.379	0.226
SE(gi-gj) tester	1.15	0.87	0.472	0.594	0.348	1.717	0.635	0.17	0.659	0.536	0.32

*, ** Significant at 5% and 1% probability levels

Table.4 Estimate of SCA effect of hybrids for 11 characters in rice

Hybrid	Days to 50% flowering	Days to maturity	Plant height	Panicle bearing tillers/ plant	Flag leaf area	Spikelets/ panicle	Spikelets fertility	Test weight	Harvest Index	Biological Yield	Grain yield/ plant
S-52 × NDRK5088	0.556	1.889*	-1.456**	1.083	-0.297	-1.833	-0.748	1.596**	1.648*	-2.761**	-0.543
S-52 × CSR-13	-5.222**	-4.000**	-0.067	0.194	1.100**	-7.611**	1.241	1.175**	-4.655**	1.106	-1.898**
S-52 × IR-29	0.444	1.111	1.844**	0.861	0.766*	4.278*	-1.219	0.022	0.066	-1.661**	-0.910*
S-52 × Usar-3	4.222**	1.000	-0.322	-2.139**	-1.569**	5.167**	0.726	-2.793**	3.074**	3.317**	3.351**
IR-64× NDRK-5088	-0.111	1.889*	-0.139	-0.500	0.078	-1.667	-0.097	-1.653**	0.911	-1.828**	-0.351
IR-64 × CSR-13	2.444*	-1.000	-0.017	0.278	-1.854**	6.556**	0.002	-1.024**	1.227	1.372	1.326*
IR-64 × IR-29	-0.556	-0.889	-0.072	-0.056	-0.755*	-8.889**	1.405*	0.681**	1.193	-2.528**	-0.651
IR-64 × Usar-3	-1.778	0.000	0.228	0.278	2.531**	4.000*	-1.310	1.995**	-3.331**	2.983**	-0.324
NDR-359× NDRK-5088	-0.444	-3.778**	1.594**	-0.583	0.219	3.500	0.845	0.057	-2.559**	4.589**	0.894*
NDR-359 × CSR-13	2.778*	5.000**	0.083	-0.472	0.754*	1.056	-1.243	-0.151	3.428**	-2.478**	0.572
NDR-359× IR-29	0.111	-0.222	-1.772**	-0.806	-0.011	4.611*	-0.186	-0.703**	-1.126	4.189**	1.561*
NDR-359 × Usar-3	02.444	-1.000	0.094	1.861**	-0.962**	-9.167**	0.585	0.798**	0.257	-6.300**	-3.028**
SE (Sij)	1.15	0.87	0.472	0.594	0.348	1.717	0.635	0.17	0.659	0.536	0.32
SE (Sij-skl)	2.655	2.01	1.091	1.372	0.804	3.964	1.467	0.394	1.522	1.239	0.739

*, ** Significant at 5% and 1% probability levels

Table.5 Ranking of five desirable hybrids on the basis of *per se* performance and sca effect for 11 characters in rice

Character	Desirable crosses based on *per se* performance	Best specific combiner	Best crosses based on *per se* performance and sca effects
Days to 50% flowering	IR64XNDRK5088	Sarjoo52XNarendra Usar3	NDR359XNarendra Usar3
	IR64XNarendraUsar3	NDR359XCSR13	
	IR64XIR29	IR64XCSR13	
	NDR359XNDRK5088	NDR359XNarendra Usae3	
Days to maturity	IR64XIR29	NDR359X NDRK5088	NDR359X NDRK5088
	NDR359XNDRK5088	IR64XNDRK5088	
	IR64XNarendra Usar3	Sarjoo52XNDRK5088	
	Sarjoo52xCSR13	Sarjoo52xIR29	
Plant height	NDR359XIR29	Sarjoo52XIR29	NDR359XNarendra Usar3
	NDR359XNarendra Usar3	NDR359XNDRK5088	
	NDR359XCSR13	IR64XNarendra Usar3	
	Sarjoo52xNDRK5088	NDR359XNarendra Usar3	
Panicle bearing tillers/ plant	Sarjoo52XNDRK5088	NDR359XNarendra Usar3	Sarjoo52XNDRK5088
	Sarjoo52XIR29	Sarjoo52XNDRK5088	Sarjoo52XIR29
	IR64XCSR13	Sarjoo52XIR29	IR64XCSR13
	IR64XIR29	IR64XCSR13	
Flag leaf area	NDR359XNarendra Usar3	IR64XNarendra Usar3	Sarjoo52xNarendra Usar3
	Sarjoo52xNarendra Usar3	Sarjoo52XCSR13	
	NDR359XNDRK5088	Sarjoo52xNarendra Usar3	
	Sarjoo52XNDRK5088	NDR359XCSR13	
Spikelets/ panicle	NDR359XNDRK5088	IR64XCSR13	NDR359XIR29
	Sarjoo52XIR29	Sarjoo52xNarendra Usar3	Sarjoo52XIR29
	NDR359XIR29	NDR359XIR29	
	Sarjoo52XNDRK5088	Sarjoo52XIR29	
Spikelets fertility	NDR359XCSR13	IR64XIR29	NDR359XNDRK5088
	Sarjoo52xNarendra Usar3	Sarjoo52XCSR13	Sarjoo52xNarendra Usar3
	NDR359XNDRK5088	NDR359XNDRK5088	
	NDR359XIR29	Sarjoo52xNarendra Usar3	
Test Weight	Sarjoo52XCSR13	IR64XNarendra Usar3	Sarjoo52XNDRK5088
	Sarjoo52XNDRK5088	Sarjoo52XNDRK5088	Sarjoo52XCSR13
	Sarjoo52XIR29	Sarjoo52XCSR13	
	IR64XCSR13	NDR359XNarendra Usar3	
Harvest Index	Sarjoo52xNarendra Usar3	NDR359XCSR13	Sarjoo52xNarendra Usar3
	Sarjoo52XNDRK5088	Sarjoo52xNarendra Usar3	Sarjoo52XNDRK5088
	Sarjoo52XIR29	Sarjoo52XNDRK5088	
	NDR359XNarendra Usar3	IR64XCSR13	
Biological Yield	Sarjoo52xNarendra Usar3	NDR359XNDRK5088	Sarjoo52xNarendra Usar3
	Sarjoo52XCSR13	NDR359XIR29	IR64XNarendra Usar3
	NDR359XCSR13	Sarjoo52xNarendra Usar3	
	IR64XNarendra Usar3	IR64XNarendra Usar3	
Grain yield/ plant	Sarjoo52xNarendra Usar3	Sarjoo52xNarendra Usar3	Sarjoo52xNarendra Usar3
	NDR359XIR29	NDR359XIR29	NDR359XIR29
	Sarjoo52XNDRK5088	IR64XCSR13	
	NDR359XNDRK5088	NDR359XNDRK5088	NDR359XNDRK5088

The cross NDR359 x CSR 13 showed significant and positive sca effects in desirable direction for plant height (5.000), days to 50 per cent flowering (2.778) and flag leaf area (0.754). The sca effect of the crosses is an estimate for making selection of best cross combinations. High specific combining ability denotes, undoubtedly a high heterotic response, however this, does not mean high performance of the hybrids as well. In general, maximum number of crosses which showed significant sca effects was invariably associated with better *pre se* performance for respective traits (table 5). In majority of cases, the crosses exhibiting high sca effects were found to have both or one of the parents as good general combiner for the characters under study. The results are in agreement with the findings of Chitra *et al.*, (2007).

The present investigation including 5 lines *i.e.*, CSR-13, Narendra Usar-13, CSR-30, NDRK-5088, Nonabokra and 3 testers (Sarjoo-52, NDR-359, IR-64) and their crosses were screened by two means for salinity tolerance.

Firstly salinity screening by morphological scoring (SES, 1996) and secondly screening of salinity tolerance with molecular marker (RM 493& RM 23). Salinity tolerance F_1 are selected for marker assisted selection with the RM 493 and RM 23 the gel analysis was shown that F_1's showed the amplification like their parents. During molecular analysis ofparentsCSR13, Narendra Usar3, CSR30, NDR359, and Nonabokra found tolerance against salinity stress. The susceptible parents were Sarjoo52, NDRK5088 and IR 64.Combining ability analysis elucidated that among parents Sarjoo 52 and Narendra Usar3 identified as most desirable donor parents for yield and yield contributing characters; and among the crosses namely, Sarjoo52 x Narendra Usar3 and NDR359 x NDRK5088 may be considered for exploitation of heterosis for grain yield.

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