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Research Article

Correlation, path analysis and stress indices studies of *Saltol* introgressed lines of rice for salinity tolerance

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Abstract
The correlation, path analysis and stress indices for yield and its component traits were studied in *Saltol* introgressed backcross inbred lines (BILs) developed in the background of ADT 37 and CR 1009 *Sub 1* under normal and saline conditions. The score of seedling stage salinity tolerance indicated that the line BIL 1102 was tolerant to salinity. Correlation studies indicated positive association of number of total grains per panicle and number of filled grains per panicle with grain yield in both saline and normal conditions. The direct positive effects of traits like number of productive tillers and panicle length would be effective for selection on grain yield improvement under both environments.The characters *viz.,* days to fifty percent flowering, number of filled grains per panicle and thousand grain weightalso had direct effects on grain yield under salinity condition. The lines *viz.,* BIL 1102, BIL 752 and BIL 1094 were selected as saline tolerant lines based on stress susceptibility index (SSI) and stress tolerance index (STI).

Key words
Salt stress, Seedling stage tolerance, Correlation, Path analysis, STI

INTRODUCTION

Rice is the most promising human food since nearly half of the world's population relied on it. As the basic food crop, rice is cultivated over one-fourth of the gross cropped area of India. Since it is a staple food crop, it accounts for about 48% of total food grain production in India. Therefore, improving the productivity of rice is an essential one to sustain the food availability and economic development of the country. Due to increasing population and industrialization, the area under cultivation has been reduced which force the rice cultivation to less productive area such as saline, drought and flood-prone areas(Krishnamurthy *et al.,* 2014).Salinity is one of the cruel environmental factors limiting the productivity of crop plants. The state level estimates showed 6.73 million hectares salt affected soils in India(Krishnamurthy *et al.,* 2014). The salinized areas are increasing at the rate of 10% annually for various reasons including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water and poor cultural practices (Shrivastava and Kumar, 2015). It has been estimated that more than 50% of the arable land will be salinized by the year 2050(Jamil *et al.,* 2011). Salinity not only decreases the agriculture production of most crops, but also affects soil phytochemical properties and ecological balance of the area (Shrivastava and Kumar, 2015).

Rice crop exhibit a spectrum of responses under salt stress. The detrimental symptoms include the reduction in plant metabolism, reduced water potential, ion imbalance, toxicity and reduction in the crop field and in the extreme condition it may lead to total failure of crop(Krishnamurthy *et al.,* 2014). Generally, the effects of salinity related to the stage of plant development at which salinity occurs, concentration of the salt and the duration of salinization (Zeng *et al.,* 2001). The biochemical characters of rice are such as plant height, number of productive tillers, panicle length, panicle weight, single plant yield, quality and quantity of grains were decreased as the level of salinity increases. Breeding of saline tolerant lines is emerged as an important breeding aspect to overcome the problem of salt stress. So far, breeders make use of several landraces as the salt-tolerant genotypes like Nona bokra & Pokkali. However, the indirect detrimental characters present in these genotypes leads to the difficulty in cultivation of these landraces. Introgressing QTL for salinity tolerance from these landraces to our elite varieties can be a solution to this problem. But, the conventional breeding approaches have a lot of challenges as it takes more time to select the desirable lines.With the advancement in the field of Marker Assisted Selection (MAS), it is possible to introgress the QTL in a lesser period of time using the strategy, several improved versions of rice varieties have been developed (Banumathy *et al.,* 2018).

The genotype FL 478, a derivative of saline tolerant land race Pokkali was utilized as a donor due to its high level of tolerance to salinity. The back cross inbred lines were developed using FL478 as donor parent of *Saltol* QTL introgressed in the background of popular rice varieties ADT 37, CR 1009 *Sub1* were used in the study. The practical understanding of the association of several characters with the yield has extended the value of any breeder and makes it easier in selection with more accuracy (Ratna *et al.,* 2015). The selection based on correlation without considering the interaction between the characters may lead to the wrong conclusion. Thus, the technique of path coefficient analysis should be used to have an idea of direct and indirect effects of a trait towards the yield.With these points in view, the present research was carried out to understand the effects of salinity on genetic variability, correlation, direct and indirect effects, stress Indices of grain yield with important yield components.

MATERIALS AND METHODS

The experimental material comprised of backcross inbred lines (BILs) along with donor parent (FL 478) and two recurrent parents (ADT 37 and CR 1009 *Sub 1*).The lines BIL 33, BIL 44, BIL 66, BIL 752 and BIL 772 were derived by crossing of ADT 37 & FL 478. Similarly, the lines BIL 1094, BIL 1095, BIL 1096 & BIL 1102 were derived by crossing of CR 1009 *Sub 1*and FL 478. These BIL lines (BC_3F_4generation)were selected through Marker Assisted Selection (MAS) to confirm the presence of *Saltol* QTL on chromosome 1 and attained homogeneity. The materials were screened under normal condition (EC_{sw} - 0.3 ds/m) at Agricultural College and Research Institute, Madurai as well as under saline stress condition (EC_{sw} - 3.30 ds/m) at Anbil Dharmalingam Agricultural College & Research Institute, Trichy.

The seeds of each BIL lines along with their parents were raised on the nursery beds. The seedling stage salt stress was imposed during June – July before the onset of monsoon rain at ADAC & RI, Trichy. During this period, the salinity level in soil as well as in the irrigation water was observed as a natural salt stress environment. The difference between EC and pH values recorded before experimentationin both locations is given in **Table 1**.The genotypes were screened for their seedling stage salinity tolerance using IRRI standard protocol (Gregorio *et al.,* 1997) which is given in **Table 2**.The scoring was done for seedling stage injuryon 10th, 16th, 21th, 26th daysat salinity location.

The size of field plot was 4m x 2m with the spacing of 20cm between rows, 10cm between plants. The RBD (Randomized block design) was followed as the experimental design. The crop production packages were followed as recommended. The 25 days seedlings of each BIL lines which showed tolerance to salt were selected and transplanted to main field condition at Trichy, whereas in Madurai all the seedlings exhibited good vigour and transplanted to main field. The characters *viz.,*days to fifty percent flowering, plant height (cm), number of total tillers, number of productive tillers, panicle length (cm), panicle weight (g), number of total grains per panicle, number of filled grains per panicle, thousand grain weight (g) and single plant yield (g) were observed on five randomly selected plants in each replications from both the environments. The SSI & STI for each genotype were calculated as per Fischer and Maurer, (1978) andFernandez, (1992) respectively.

The genotypic and phenotypic correlations between yield and its component traits and among themselves were analysed as per the method suggested by Johnson *et al.* (1955). The direct and indirect effects of each characters were interpreted from path analysis given by Dewey and Lu, (1959).

Table 1. The EC & pH values at Madurai and Trichy locations

Components	pH		EC	
	Madurai	Trichy	Madurai	Trichy
Standing water (SW)	7.2	8.75	0.3	3.30
Irrigation water (IW)	7.0	7.36	0.28	2.85
Soil	7.1	8.21	0.31	1.31

Table 2.Modified Standard Evaluation Score (SES) of visual salt injury at seedling stage

Score	Observations	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; leaves dry; some plants dying	Most Susceptible
9	Almost all plants dead or dying	Highly susceptible

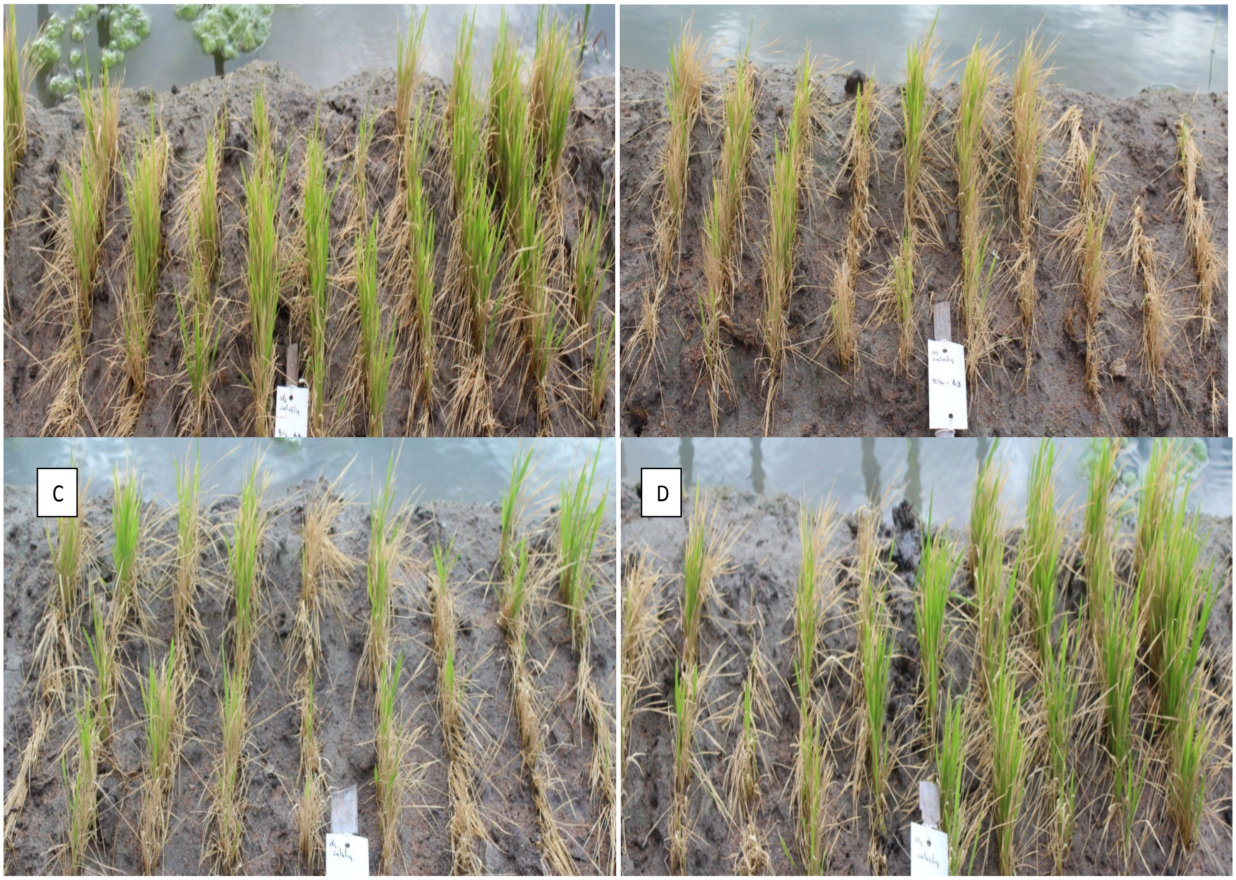
RESULTS AND DISCUSSION

All nine genotypes showed variations in their expression of tolerance to salinity. The genotypes grown in the normal condition did not show any symptom and well established in their development. The seedlings emerged were showing the starting symptoms of leaf tips whitish and rolling and the extreme symptoms of complete cessation of growth and dying of most plants with dried leaves. From the standard evaluation system of salt injury score, the BIL lines were categorized as tolerant (3), moderately tolerant(5), susceptible(7) and highly susceptible(9) (**Table 3**; **Fig. 1**).The line BIL 1102 showed tolerance to salt, while some of the other lines *viz.,* BIL 33, BIL 752 and BIL 1094 showed moderate tolerance. The tolerant check, FL 478 exhibited moderate toleranceto salinity stress while the susceptible checks, ADT 37 and CR 1009 *Sub 1* completely died at 10 DAS. The significant differences among the BIL lines for all ten characters were determined by analysis of variance. Thus, indicating the presence of sufficient genetic variation among the lines which leads to improvement in salt stress tolerance. The characters *viz.,* thousand grain weight (8.3%), panicle length (16%), panicle weight (18%) and plant height (23%) were less affected by salinity, whereas the characters number of total tillers (43%), number of total grains (40%), number of filled grains (36.2%) were highly affected by salt stress.

Table 3.Salt injury scores of BIL lines along with parents

BIL lines	Score			
	10th day	16th day	21st day	26th day
BIL 33	3	5	5	5
BIL 44	3	5	5	7
BIL 63	3	5	5	7
BIL 752	3	5	5	5
BIL 772	3	5	7	7
BIL 1094	3	5	5	5
BIL 1095	3	5	5	7
BIL 1096	3	5	5	7
BIL 1102	3	3	3	3
ADT 37	3	9	9	9
CR 1009 *Sub 1*	3	9	9	9
FL 478	5	5	5	5

The relations among the ten characters were estimated using genetic and phenotypic correlation coefficient (**Table 4**). Previous studies revealed that if genotypic correlation coefficients were higher than their phenotypic correlations, the interconnections were influenced as a minimum level. This is in conformity of the findings of Jayasudha and Sharma, (2010)and Anbanandan *et al.* (2009). Grain yield per plant showed significant and positive association with plant height (0.938 and 0.918), panicle length (0.914 and 0.854), panicle weight (0.739 and 0.695), number of total grains per panicle (0.777 and 0.735), and number of filled grains per panicle (0.891 and 0.832) at genotypic and phenotypic levels respectively under normal environment. A positive significant estimate of character association reveals that the strong association was present among these traits with yield (Banumathy *et al.,* 2018).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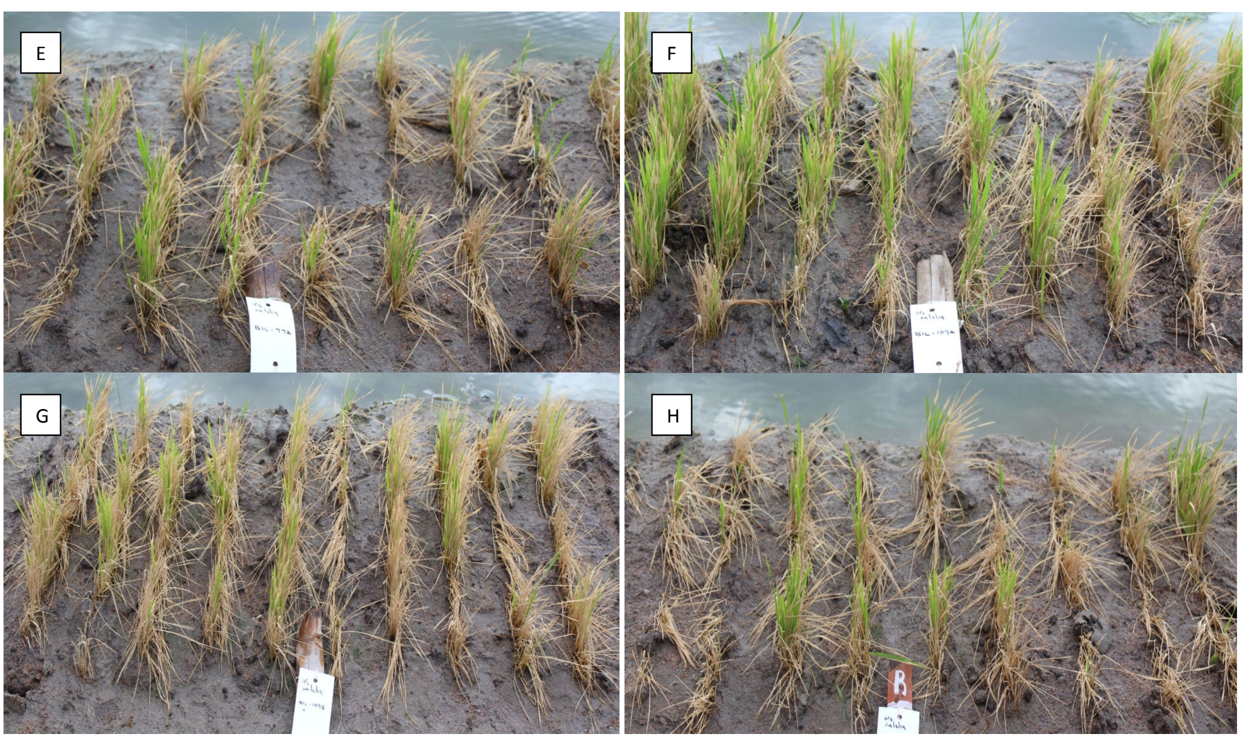
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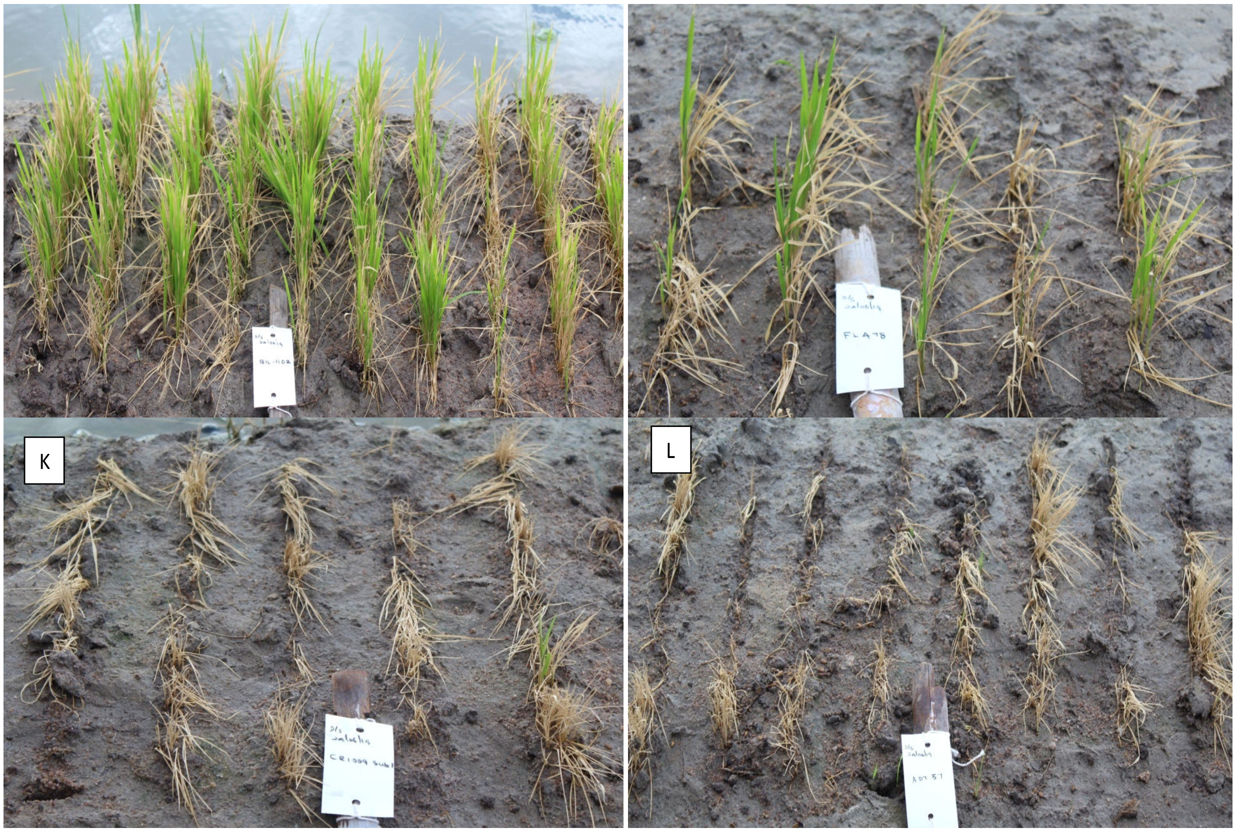

Fig. 1. Seedling stage screening of BIL lines under natural salt stress condtion at ADAC & RI, Trichy. (A) BIL 33; (B) BIL 44; (C) BIL 63; (D) BIL 752; (E) BIL 772; (F) BIL 1094; (G) BIL 1095; (H)BIL 1096; (I) BIL 1102; (J) Tolerent check FL 478; (K) and (L) were susceptible checks CR 1009 *Sub 1* and ADT 37 respectively

Under saline stress condition, grain yield per plant had significant and positive association with number of total tillers (0.730 and 0.617), number of productive tillers (0.938 and 0.795), number of total grains per panicle (0.759 and 0.613), number of filled grains (0.743 and0.590).This results coincide with the findings of Shanthi *et al.,* (2011), as the positive association of grain yield with different traits *viz.,* number of productive tillers, number of filled grains and spikelet fertility.Thus, these characters could be evaluated as the important yield contributing traits in rice. These findings indicated that selection of genotypes under normal and saline stress conditions were entirely different. The dissimilar reaction exhibited by the traits under salt stress might be due to the complication of the traits under stress. As said, grain yield itself is a complex trait governed by multiple genes that makes the improvement of yield under stress conditions a real challenge for breeders. Since, the characters thousand grain weight, panicle length, panicle weight and plant height were less affected by salt stress, selection based on these traits would be effective.

Path analysis provides actual contribution of traits on the yield in the form of direct and indirect effects (Table 5). For grain yield, the direct and positive effect were recorded by number of productive tillers, panicle length, plant height, panicle weight, number of total grains per panicle under normal environment.It reveals their importance while selecting for grain yield. Under salinity stress, the direct and positive effect on grain yield were showed for number of productive tillers, days to fifty percent flowering, panicle length, number of filled grains per panicle, thousand grain weight. Direct selection based on these traits would be effective for improving the grain yield. Positive direct effects of various traits on grain yield observed in this study are in accordance with the findings of Shanthi priya *et al.* (2017). Number of total tillers showed direct but negative effect under both the environments. But this trait contributed indirectly through number of productive tillers. Under salt stress condition, plant height, panicle weight and number of filled grains per panicle showed negative direct effect. Butthese characters showed positive indirect effect through number of productive tillers, number of filled grains per panicle, number of filled grains per panicle, respectively, on grain yield improvement. Hence, selection should also be practiced for the traits which are having positive indirect effects.

Table 4. Genotypic and phenotypic correlations for yield and yield contributing traits in rice under normal and saline conditions

Characters	Correlation	Environments	PH	NTT	NPT	DFF	PL	PW	NTG	NFG	TGW	SPY
PH	G	NORMAL	1.000	0.609*	0.582*	0.647*	0.891**	0.616*	0.741**	0.846**	-0.500	0.938**
		SALINE	1.000	0.640*	0.517	0.176	0.731**	0.444	0.465	0.427	0.354	0.379
	P	NORMAL	1.000	0.572	0.564	0.634*	0.821**	0.574	0.68*	0.751**	-0.475	0.918**
		SALINE	1.000	0.574	0.461	0.178	0.673*	0.417	0.401	0.378	0.302	0.370
NTT	G	NORMAL		1.000	0.998**	0.675*	0.402	0.132	0.322	0.312	-0.466	0.505
		SALINE		1.000	0.798**	0.503	0.847**	0.613*	0.563	0.686*	-0.166	0.730**
	P	NORMAL		1.000	0.960**	0.618*	0.314	0.120	0.318	0.266	-0.412	0.465
		SALINE		1.000	0.749**	0.453	0.718**	0.511	0.443	0.504	-0.080	0.617*
NPT	G	NORMAL			1.000	0.695	0.374	0.176	0.350	0.310	-0.398	0.473
		SALINE			1.000	0.148	0.578*	0.442	0.545	0.557	-0.289	0.938**
	P	NORMAL			1.000	0.654	0.310	0.158	0.348	0.289	-0.354	0.458
		SALINE			1.000	0.133	0.501	0.363	0.439	0.471	-0.181	0.795**
DFF	G	NORMAL				1.000	0.514	0.240	0.405	0.372	-0.139	0.496
		SALINE				1.000	0.487	0.591*	0.562	0.585*	-0.339	0.272
	P	NORMAL				1.000	0.463	0.230	0.378	0.338	-0.132	0.485
		SALINE				1.000	0.460	0.576*	0.445	0.486	-0.293	0.261
PL	G	NORMAL					1.000	0.639*	0.574	0.697*	-0.209	0.914**
		SALINE					1.000	0.406	0.462	0.443	-0.032	0.440
	P	NORMAL					1.000	0.557	0.475	0.574	-0.128	0.854**
		SALINE					1.000	0.379	0.354	0.297	0.002	0.418
PW	G	NORMAL						1.000	0.564	0.713**	-0.003	0.739**
		SALINE						1.000	0.939**	0.998**	-0.439	0.566
	P	NORMAL						1.000	0.511	0.642*	0.007	0.695*
		SALINE						1.000	0.704*	0.789**	-0.332	0.541
NTG	G	NORMAL							1.000	0.964**	-0.575	0.777**
		SALINE							1.000	0.940**	-0.392	0.759**
	P	NORMAL							1.000	0.928**	-0.523	0.735**
		SALINE							1.000	0.859**	-0.277	0.613*
NFG	G	NORMAL								1.000	-0.585	0.891**
		SALINE								1.000	-0.361	0.743**
	P	NORMAL								1.000	-0.522	0.832**
		SALINE								1.000	-0.256	0.590*
TGW	G	NORMAL									1.000	-0.466
		SALINE									1.000	-0.252
	P	NORMAL									1.000	-0.419
		SALINE									1.000	-0.215
SPY	G	NORMAL										1.000
		SALINE										1.000
	P	NORMAL										1.000
		SALINE										1.000

Table r value 5%=0.576 & 1% = 0.708.* Significance @ 5% level **Significance @ 1% level

DFF=Days to 50 % flowering, PH=Plant height, NTT=Number of total tillers per plant, NPT=Number of productive tillers per plant, PL=Panicle length, PW- Panicle weight, NFG=Number of filled grains per panicle, NTG=Number of total grains per panicle, TGW=Thousand grain weight, SPY=Single plant yield.

Table 5. Direct and indirect effects of different traits in rice on grain yield under normal and saline conditions

Characters	Environments	PH	NTT	NPL	DFF	PL	PW	NTG	NFG	TGW	Genotypic Correlation
PH	NORMAL	**0.280**	-0.919	0.779	-0.004	0.664	0.314	0.344	-0.854	0.332	0.938**
	SALINE	**-0.702**	-0.968	0.878	0.039	0.582	-0.079	-0.246	0.659	0.215	0.379
NTT	NORMAL	0.171	**-1.509**	1.335	-0.004	0.300	0.067	0.149	-0.314	0.310	0.505
	SALINE	-0.449	**-1.512**	1.354	0.112	0.674	-0.109	-0.297	1.059	-0.101	0.730**
NPT	NORMAL	0.163	-1.506	**1.337**	-0.004	0.279	0.090	0.162	-0.313	0.264	0.473
	SALINE	-0.363	-1.207	**1.696**	0.033	0.460	-0.079	-0.288	0.861	-0.176	0.938**
DFF	NORMAL	0.181	-1.018	0.929	**-0.006**	0.383	0.122	0.188	-0.375	0.092	0.496
	SALINE	-0.124	-0.760	0.250	**0.223**	0.387	-0.105	-0.297	0.903	-0.206	0.272
PL	NORMAL	0.250	-0.607	0.500	-0.003	**0.746**	0.326	0.266	-0.703	0.139	0.914**
	SALINE	-0.513	-1.281	0.981	0.108	**0.796**	-0.072	-0.244	0.685	-0.020	0.440
PW	NORMAL	0.173	-0.199	0.236	-0.002	0.477	**0.510**	0.262	-0.719	0.002	0.739**
	SALINE	-0.312	-0.927	0.750	0.132	0.323	**-0.178**	-0.496	1.542	-0.267	0.566
NTG	NORMAL	0.208	-0.486	0.468	-0.003	0.428	0.288	**0.464**	-0.972	0.382	0.777**
	SALINE	-0.326	-0.851	0.925	0.125	0.368	-0.167	**-0.528**	1.452	-0.238	0.759**
NFG	NORMAL	0.237	-0.470	0.415	-0.002	0.520	0.364	0.447	**-1.008**	0.389	0.891**
	SALINE	-0.300	-1.037	0.946	0.130	0.353	-0.177	-0.497	**1.545**	-0.220	0.743**
TGW	NORMAL	-0.140	0.703	-0.532	0.001	-0.156	-0.001	-0.266	0.590	**-0.665**	-0.466
	SALINE	-0.248	0.252	-0.490	-0.075	-0.026	0.078	0.207	-0.558	**0.608**	-0.252

Residual effect – Normal- 0.196; saline- 0.352; * Significance @ 5% level **Significance @ 1% level

DFF=Days to 50 % flowering, PH=Plant height, NTT=Number of total tillers per plant, NPT=Number of productive tillers per plant, PL=Panicle length, PW- Panicle weight, NFG=Number of filled grains per panicle, NTG=Number of total grains per panicle, TGW=Thousand grain weight, SPY=Single plant yield.

Table 6. Salinity stress susceptibility and salinity stress tolerance indices for grain yield and its component traits in rice genotypes

Introgressed Lines	Mean performance under salinity				Mean performance under normal				SSI				STI			
	PH	NPT	NFG	SPY	PH	npt	NFG	SPY	PH	NPT	NFG	SPY	PH	NPT	NFG	SPY
BIL 33	84.53	11.67	128.33	36.33	132.33	16.33	257.33	57.93	1.78	0.83	1.39	0.96	0.79	0.70	0.85	1.06
BIL 44	86.60	11.00	138.00	29.21	131.00	16.00	228.33	55.12	1.65	0.91	1.10	1.21	0.80	0.65	0.81	0.81
BIL 63	90.43	12.00	139.33	33.01	125.33	17.67	237.00	51.35	1.35	0.93	1.14	0.92	0.80	0.78	0.85	0.85
BIL 752	83.63	10.67	122.33	30.45	101.33	12.33	134.33	35.52	0.85	0.39	0.25	0.37	0.60	0.48	0.42	0.55
BIL 772	80.50	8.33	87.67	14.20	95.00	12.33	108.33	19.83	0.74	0.94	0.53	0.73	0.54	0.38	0.24	0.14
BIL 1094	93.57	10.20	123.00	30.49	115.50	15.33	154.67	34.42	0.92	0.97	0.57	0.29	0.76	0.58	0.49	0.53
BIL 1095	91.03	9.33	132.00	27.88	116.33	19.67	179.33	45.77	1.06	1.52	0.73	1.01	0.74	0.68	0.61	0.64
BIL 1096	102.13	8.77	118.33	26.23	122.67	14.33	208.67	42.11	0.81	1.13	1.20	0.97	0.88	0.46	0.63	0.56
BIL 1102	100.37	12.33	137.33	36.51	121.00	13.33	258.33	50.32	0.83	0.22	1.30	0.71	0.85	0.61	0.91	0.93
Mean of BIL lines	90.31	10.48	125.15	29.37	117.83	15.26	196.26	43.60	1.11	0.87	0.91	0.80	0.75	0.59	0.64	0.67
ADT 37	91.77	7.27	124.00	16.54	103.67	17.67	184.00	30.13	0.56	1.71	0.91	1.16	0.67	0.47	0.58	0.25
CR 1009 *sub* 1	108.20	9.00	133.67	23.50	133.33	23.33	217.67	50.47	0.92	1.78	1.07	1.37	1.01	0.77	0.74	0.60
FL 478	124.67	12.33	135.33	39.22	134.33	19.33	204.00	56.34	0.35	1.05	0.94	0.78	1.18	0.88	0.71	1.11
Overall mean	**94.44**	**10.26**	**126.50**	**28.69**	**119.21**	**16.38**	**197.56**	**44.07**	**0.99**	**1.02**	**0.93**	**0.87**	**0.80**	**0.62**	**0.65**	**0.67**

PH=Plant height, NPT=Number of productive tillers per plant, NFG=Number of filled grains per panicle, SPY=Single plant yield

The stress indices were used to select the best salt tolerant lines (**Table 6**). The stress susceptibility index (SSI) values for grain yield ranged from 0.29 (BIL 1094) to 1.10 (BIL 44), while the stress tolerance index (STI) values for grain yield ranged from BIL 33 (1.06) to BIL 772 (0.14). According to Fischer and Maurer (1978), genotypes experiencing low yield reduction under stress compared to normal conditions are differentiated by SSI. Lower values of SSI indicated lower differences in yield across stress and normal conditions. The higher values of salt tolerant index indicate superiority of genotypes having both higher yield potential and stress tolerance. Thus, the better tolerance, adaptability and suitability of saline tolerant lines can be evolved by stress susceptible and stress tolerance index.

In this study, it was observed that the traits *viz.,* thousand grain weight, panicle length, panicle weight and plant height were less affected under salt stress condition. Hence, selection based on these traits might be a desirable one. The genotypes *viz.,* BIL 752, BIL 1094 and BIL 1102 showed better performance under both normal and stress environments. They could be useful in increasing productivity under salt stress environment.

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