SABRAO Journal of Breeding and Genetics 48 (2) 172-179, 2016



## IDENTIFICATION AND QUANTIFICATION OF SALINITY TOLERANCE THROUGH SALT STRESS INDICES AND VARIABILITY STUDIES IN RICE (Oryza sativa L.)

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#### SUMMARY

Rice is generally shows variable level of sensitivity to salinity during its developmental stages and remarkably sensitive during young seedling stage and early reproductive phase. Quantifying the level of salt tolerance based on salt stress indices is an effective method in identification of tolerant genotypes. In this study, the salt stress indices were used to identify best performing genotypes based on salt susceptibility indices (SSI) and salt response indices (SRI) as relevant parameters. Among the 39 genotypes studied, 29 showed better endurance to salt susceptibility indices, among them Pokkali, IR72132-AC-6-1 and IR70869-B-P-13-2 performed well with least SSI, which refers more tolerance with lesser yield reduction, whereas IR29 was vice-versa to the above. Genotypes IR72593-B-19-2-3-1, IR73104-B-1-1-3-2-1 and IR74802-3R-7-1-2 had a better performance with early flowering and highly responsive to salt stress with lower percent yield reduction. The phenotypic variation is higher than genotypic variation for all traits except for flowering duration and it indicates presence of variability for different traits. High heritability with variability, genetic advance and better salt stress indices involvement of additive gene action and can be manipulated and utilized in further breeding program.

Key words: Rice, salt tolerance indices, salt susceptibility index, salt response index

**Key findings:** The salt stress indices are effective selection parameter in identification of tolerant genotypes with maximum yield under saline conditions and in comparison with normal conditions.

Manuscript received: September 18, 2015; Decision on manuscript: March 28, 2016; Manuscript accepted: May 8, 2016. © Society for the Advancement of Breeding Research in Asia and Oceania (SABRAO) 2016

Communicating Editor: Bertrand Collard

#### **INTRODUCTION**

Salinity is gaining primary importance in recent decades and increasing tremendously along coastal and inland areas due to decrease in level of ground water level and improper management cum cultivation practices. Salinity is the most common and most extensive problem (Senadhira and Akbar, 1991). Breeding rice varieties with inbuilt salt tolerance is realized as the most promising, less resource consuming, economically viable and socially acceptable approach. Salt tolerance is a multigenic trait that allows plants to grow and maintain economic yield in the presence of non-physiologically high and relatively constant levels of salt, in particular NaCl (Hurkman, 1992).

Rice is considered as highly sensitive to salinity at seedling and reproductive stages

and moderately tolerant to rest of the crop stages. However, genotypes respond differently under salinity. Therefore, for achieving desirable extent of genetic adaptation of rice to salinity, it is essential that sufficient heritable variability exists among genotypes. Genetic improvement in the crops depends on the magnitude of genetic variation and heritability of characters of economic importance. Hence, knowledge about the variability using parameters i.e. genetic coefficient of variation, heritability and genetic advance is of paramount importance for initiating an efficient breeding programme in crop like rice. Burton (1952) suggested that GCV together with heritability estimates would give the best picture of the extent of advance to be expected by selection. The parameters such as phenotypic variance, genotypic variance and genetic advance for various characters are expressed in their respective units and standard unit less measures like phenotypic coefficient of variation, genotypic coefficient of variation and environmental coefficient of variation have been calculated. IRRI has screened more than 101,261 rice varieties/breeding lines for salinity, 19.6% of which was rated to be moderately tolerant (Neue, 1991). Such genetic variability could be used for developing rice with higher salinity tolerance.

Indexing yield to some quantifiable measure of stress severity is therefore the only mean of quantitatively evaluating relative stress resistance in a large collection of cultivars (Robin, 1997). This approach sounds pragmatic since cultivars cannot be bred for salt tolerance alone but aiming to breed a cultivar that is capable of performing acceptably well in response to all the local factors affecting yield. For improving salt tolerance, genetic constitution of a genotype for specific adaptation needs variability within and across the cultivars to be incorporated through breeding program. In this study, the stress indices established to quantify drought stress had been utilized for measuring the relative tolerance among the cultivars to salt stress, as salt stress chiefly imposes osmotic stress, which is characteristic feature of drought. Nevertheless the formulae adopted for drought and stress quantification involves only the yield and yield components and not the putative traits which are specific to drought. Hence, such indices can be used to

quantify salt stress also in a justifiable manner. The present study focused on identification of best genotypes among the germplasm based on the salt stress indices and variability studies.

## MATERIALS AND METHODS

The study materials consisted of 39 germplasm lines includes IRRI-INGER entries, 6 maintainer lines and salt tolerant and sensitive lines from India. The field experiment was carried out in 2 environments (control and salinity condition) during Kharif 2014, one being naturally occurring salt stress at Machilipatnam and the other location was Indian Institute of Rice Research farm, Rajendranagar where the experiment was conducted under normal condition (Table 1). The experimental design followed was Randomized complete block design with 3 replications. The seedlings were germinated in the nursery and transplanted to the main field at 28 days after sowing (DAS). Two seedlings hill<sup>-1</sup> were planted following a spacing of 20 x 20 cm. In both the environments various biometrical measurements were recorded and at the seedling to maturity stage.

## Salt stress indices

The salt stress susceptibility index (S) was calculated for each genotype under salt stress condition using the formula suggested by Fischer and Maurer (1978).

Salt stress susceptibility = 
$$(1-Yd/Yp)$$
  
index (S) SD

Where, Yp is yield under normal soil (potential yield), Yd is yield under saline soil (stress yield) and SD is salt stress intensity.

Salt response index (SRI) was calculated for all the genotypes using the expressions made regression model by Bidinger *et al.* (1987). The model assumes yield of stress environment (Ysi) with several variables.

Ysi = a + bYpi + cFLi + SRi + SE

Where, Ysi - Stress yield estimated, Ypi is yield potential in the non-stress environment. FLi is time to flowering, SRi is salt response, SE is random error and parameters a, b and c were estimated by minimizing residuals.

Where, Yp – Yield under normal soil (potential yield) and Yd-Yield under saline soil (stress yield).

#### FL = Time of flowering

### **Coefficient of variations**

For each character PCV and GCV were computed based on the methods given by Burton (1952). The coefficient of variation was categorized as proposed by Sivasubramanian and Menon (1973). Heritability  $(h^2)$  in the broad sense was calculated according to Lush (1940). Genetic advance was expressed as percentage of mean by using the formula suggested by Johnson *et al.* (1955).

#### Statistical analysis

The mean data of selected plants for each genotype per replication were subjected to analysis of variance for all the characters appropriate for randomized complete block design (Panse and Sukhatme, 1954). Heritability was calculated in SAS (1999) using the program Proc VARCOMP. The analysis was done using software INDOSTAT ver. 8.1.

Table 1. Estimates of variability an	d genetic parameters for fou	urteen characters of rice under salt
affected field condition.		

No.	Characters	Mean	Range	Variance		PCV	GCV			GA
				PV	GV	%	%	Heritability	GA	as % of
1	SES for visual salt	5.51	3-8.3	0.684	0.67	14.98	14.89	0.987	1.68	30.48
2	Day to flowering	86.62	70-112	52.92	52.43	8.38	8.34	0.990	14.84	17.12
3	Plant height (cm)	85.56	51-145	259.33	258.02	18.81	18.77	0.994	33.00	38.57
4	Number of tillers	10.00	4-21	4.078	3.76	19.99	19.22	0.926	3.843	38.05
5	Number of productive tillers	9.45	4-17	3.54	3.32	19.67	19.06	0.941	3.637	38.02
6	Panicle length (cm)	23.13	16.3-29	6.03	5.75	10.62	10.37	0.982	4.825	20.86
7	Number of grains per panicle	84.82	30-169	672.11	665.58	30.52	30.37	0.990	52.88	62.27
8	Spikelet fertility	72.50	33.5-87.8	69.62	67.74	11.50	11.35	0.972	16.72	23.06
9	Hundred grain weight	2.260	1.36-3.2	0.087	0.082	13.05	12.72	0.950	0.578	25.55
10	Single plant yield	15.21	1.6-26.9	16.90	16.60	27.01	26.77	0.982	8.321	54.67
11	Straw yield	19.10	9.5-34.5	27.97	27.67	27.69	27.53	0.989	10.77	56.42
12	Harvest index	44.14	11.39- 56.39	31.41	29.59	12.69	12.32	0.942	10.87	24.63
13	SPAD	42.98	29.7-53.3	11.58	10.36	7.91	7.490	0.895	6.27	14.59
14	Na <sup>+</sup> :K <sup>+</sup> ratio	0.188	0.099- 0.412	0.0045	0.0044	35.47	35.29	0.990	0.136	72.35

#### RESULTS

#### Salt stress indices

The salt susceptibility index, salt tolerance index and percent reduction in yield under salt stress condition are presented in Table 2. The values for potential yield were taken from the normal environment and stress yield was obtained from salt affected field condition. The susceptibility index ranged from 0.07 (IR72132-AC-6-1) to 14.9 (IR29) and 24 genotypes showed salt susceptibility index with lesser values than the mean susceptibility index of 4.0. Salt tolerance index ranged from 0.5 (IR29) to 1.0 (IR72132-AC-6-1) with mean index of 0.7.

The percent reduction in yield ranged from 0.2 to 52.6. Maximum reduction was observed in IR29 (52.6%). This was followed

by IR68888B (44.33%) and IR68886B (40.7%). The genotypes IR72132-AC-6-1 (0.2%) and IR70869-B-P-13-2 (0.9%) had recorded the lowest reduction in grain yield.

## **Stress Response Index (SRI)**

The SRI values ranged from -2.9 to 1.7 (Table 2). The genotypes which showed nonsignificant SRI values (> 1.2) were IR66401-2B-6-1-3, IR77799-11-3-1-1-1-3, Pokkali, IR74096-AC-30, TRY2 and IR74802-3R-7-1-1. The genotypes IR74802-3R-7-1-1 and TRY2 recorded maximum SRI value of 1.7 and 1.7 respectively. The days to flowering and percent yield reduction indicated that IR74802-3R-7-1-1, IR72593-B-19-2-3-1 and IR73104-B-1-1-3-2-1 had better performance with least days to flowering and percent yield reduction.

## Variability studies

To understand the extent to which the observed variation was due to genetic factors, the value of genotypic and phenotypic variance, phenotypic and genotypic coefficients of variability, heritability (broad sense) and genetic advance for different characters under saline condition were estimated and furnished in Table 1.

The number of grains per panicle had the highest phenotypic and genotypic variances with the value of 672.1 and 665.6, respectively. The magnitude of phenotypic and genotypic variances was low for  $Na^+/K^+$  ratio (0.005 and 0.004 for phenotypic variance and genotypic variance).

# Phenotypic and genotypic coefficient of variation

The phenotypic and genotypic coefficient of variation was maximum for Na<sup>+</sup>/K<sup>+</sup> ratio (35.5 and 35.3) followed by number of grains per panicle (30.5 and 3.8). The lowest PCV and GCV estimate was observed for the traits SPAD (7.9 and 7.5), days to flowering (8.4 and 8.3) and panicle length (10.6 and 10.4).

## Heritability and genetic advance

Estimates of heritability (broad sense), genetic advance and genetic advance as percentage of mean are furnished in Table 1. The genotypes

showed high heritability values for all the characters. The maximum heritability was expressed by the trait plant height (99.5), days to flowering and Na<sup>+</sup>: K<sup>+</sup> ratio (99.1) followed by number of filled grains per panicle (99.0) and the traits with the lowest heritability value was recorded by SPAD (89.5). The character that recorded high (> 20%) GA as percent of mean were  $Na^+$ :  $K^+$  ratio (72.3), number of grains per panicle (62.3), straw yield (56.7), single plant yield (54.7), plant height (38.6), number of tillers (38.0), number of productive tillers(38.0), SES for visual salt injury (30.5), grain weight (25.5), hundred harvest index(24.6) and spikelet fertility(23.1). The characters that recorded moderate (10 to 20%) level of genetic advance as percent of mean were days to flowering (17.1) and SPAD reading (14.6). No trait had low (< 10%) level of GA as percent of mean. High heritability with high genetic advance was observed for Na<sup>+</sup>: K<sup>+</sup> ratio (99.1% and 72.4%) followed by number of filled grains per panicle (99.0% and 62.3% respectively) and single plant yield (98.3% and 54.7% respectively). High heritability with low genetic advance was recorded for days to flowering (99.1% and 17.1%) and panicle length (98.3% and 54.7%).

## DISCUSSION

Analysis of variance for the 14 characters revealed the presence of significant genotypic difference justifying further analysis. This significant difference could also be attributed to the compositions of the population, which is made of diverse genotypes.

## Salt Susceptibility index (S)

Susceptibility index along with tolerant index was used to determine the degree of tolerance of different genotypes towards salinity. The results of this study connoted 78 genotypes as endurant and among them IR72132-AC-6-1, Pokkali and IR70869-B-P-13-2 had the least values. These genotypes manifested lower values of salt susceptibility index than their mean values. Lower susceptibility index value refers to higher salt tolerance and this could be substantiated with percent reduction in yield under stress condition realized from this study.

No.	Genotypes	Potential yield	Stress yield	Days to	Susceptibility	Tolerance	% reduction	Estimated	SRI
1		(g)	(g)	flowering	index	index	in yield	yield (Ysi)	0.00
1	IR 65775-4B-23-1-2	17.92	15.48	89.5	3.85	0.86*	13.59	15.87	-0.22
2	IR 66401-2B-6-1-3	23.27	22.12	80.0	1.40	0.95*	4.93	19.83	1.30
3	IR 70869-B-P-13-2	11.03	10.93	80.0	0.26	0.99*	0.91	9.21	0.97
4	IR 70870-B-P-6-3	10.08	9.48	81.0	1.69	0.94*	5.96	8.98	0.28
5	IR 71895-3R-81-1	23.81	20.45	81.0	3.99	0.86*	14.11	20.38	0.04
6	IR 72593-B-19-2-3-1	10.92	10.52	78.0	1.04	0.96*	3.66	9.35	0.66
7	IR 72046-B-R-15-3-2-1	12.80	11.75	80.5	2.33	0.92*	8.22	11.09	0.37
8	IR 70023-4B-R-12-3-1	15.71	14.44	99.5	2.28	0.92*	8.05	14.08	0.20
9	IR 71829-3R-89-1-1	11.70	10.36	81.0	3.24	0.89*	11.46	10.05	0.18
10	IR 71895-3R-60-3-1	28.10	24.00	81.0	4.13	0.85*	14.6	23.33	0.38
11	IR 71830-3R-2-2-3	18.79	17.38	91.5	2.12	0.93*	7.48	16.23	0.65
12	IR 71831-3R-1-3-3	11.22	10.35	98.0	2.19	0.92*	7.72	10.28	0.04
13	IR 74095-AC-45	23.92	21.53	81.0	2.84	0.90*	10.03	20.25	0.73
14	IR 74096-AC-30	19.23	18.98	90.5	0.37	0.99*	1.30	16.55	1.38
15	IR 74096-AC-32	20.91	15.44	81.5	7.41	0.74	26.18	17.49	-1.17
16	IR 71865-3R-3-1-1-1	12.53	10.51	82.0	4.57	0.84	16.13	10.74	-0.13
17	IR 73103-B-6-1-2-1	15.22	13.06	84.0	4.03	0.86*	14.22	13.23	-0.10
18	IR 73104-B-1-1-3-2-1	14.36	14.00	79.5	0.71	0.98*	2.49	11.95	1.16
19	IR 72476-B-P-9-3-1-1	12.99	12.36	79.5	1.38	0.95*	4.89	10.97	0.79
20	IR 74802-3R-7-1-2	20.79	20.17	76.5	0.84	0.97*	2.98	17.14	1.72
21	IR 72132-AC-6-1	14.25	14.22	102.0	0.07	1.00*	0.23	12.70	0.86
22	IR 77799-11-3-1-1-3	17.21	16.77	91.5	0.73	0.97*	2.56	14.42	1.33
23	IR 29	13.19	6.25	85.5	14.89	0.47	52.60	11.28	-2.86
24	IR 64	11.96	10.00	104.5	4.64	0.84	16.39	10.32	-0.18
25	VSR 156	14.33	13.75	86.5	1.15	0.96*	4.06	12.28	0.83
26	CSR 27	17.66	16.60	92.0	1.70	0.94*	6.00	15.33	0.72
27	MI 48	19.61	13.63	84.5	8.63	0.69	30.50	16.59	-1.68
28	TRY 2	27.50	26.25	87.5	1.29	0.95*	4.55	23.29	1.68
29	CSR 10	11.63	10.63	70.5	2.45	0.91*	8.64	9.23	0.79
30	CSR 23	21.68	20.65	100.5	1.34	0.95*	4.75	18.84	1.03
31	Pokkali	21.00	21.20	111.0	4.35	0.96*	2.28	21.30	1.20
32	JGL 384	21.65	20.12	100.0	2.01	0.93*	7.09	18.89	0.69
33	JGL 384 JGL 1789	21.05	13.29	85.0	10.77	0.62	38.07	17.99	-2.67
33 34	IR 69619 B	14.65	9.95	84.5	9.07	0.68	32.06		-2.07
34 35	IR 68897 B	14.65	9.95 13.95	84.5 83.5	8.55	0.88	30.20	12.34 16.62	-1.50
35 36		19.99	13.95	83.5 77.0		0.70		16.62	-1.52 -2.20
	IR 68886 B				11.51		40.66		
37	IR 58025 B	22.73	20.19	93.5	3.17	0.89*	11.20	19.15	0.59
38	IR 79156 B	21.80	20.14	89.5	2.15	0.92*	7.59	18.50	0.93
39	IR 68888 B	16.15	8.99	94.0	12.55	0.56	44.33	13.72	-2.69
	Mean	17.502	15.145	87.141	3.889	0.673	13.402	14.985	0.121

Table 2. Estimation of salt susceptibility index (S) and salt response index (SRI) under salt affected field conditions.

Ysi = -3.996 + 0.05073Yp + 0.83438FL + SRi + SE (SE = 1.7607)

A parallelism between cultivars selected as endurant based on susceptibility index and salt tolerant index was also evidenced in this study (Table 1). Similarly Birari et al. (1995) reported the existence of positive association between grain yield under stress and susceptibility index. On perusal of percent reduction in grain yield under stress than potential yield, the genotypes IR29, IR72046-B-R-15-3-2-1 and IR68888B had shown the maximum reduction among the genotypes studied, whereas genotypes IR72132-AC-6-1 and IR70869-B-P-13-2 had lowest reduction in percent grain yield under salt stress condition. As per Blum (1973) resistance was considered to be indicated by a minimal decrease in yield under stress as compared with non-stress condition. Accordingly the aforementioned genotypes are identified as tolerant for salt stress. The reduction in grain yield may be through salt effect on individual components. Since plant development sequences are correlated, component interactions may compensate by increase in some components for reduction in others under the effect of stress (Blum, 1973; Peipho, 1995). But no such perceptible compensation effect on any trait could be observed from this study.

## Salt response index (SRI)

The results of this study revealed that the SRI for genotypes was found with differential magnitude. Salt stress response is said to be zero if the calculated index is less than 1.3 (Bidinger et al., 1987). (The threshold value of 'Z' of 1.3 was chosen as SRI selected those genotypes in the upper and lower 10% of the normal distribution of predicted yield under stress). From the results of this study, IR66401-2B-6-1.3, IR77799-11-3-1-1-3, IR 74096-AC-30, IR72593-B-19-2-3-1 and IR73104-B-1-1-3-2-1, have the SRI value zero indicating that within the limits of experimental error, they had no specific response to stress and were found not affected by salt stress. The genotypes observed to respond to the salt stress with a varying degree was IR74802-3R-7-1-2 and TRY2 which had relatively high responsiveness to the salt stress environments. Based on the early flowering and lower percent yield reduction, the genotypes IR72593-B-19-2-3-1, IR73104-B-1-1-3-2-1 and IR74802-3R-7-1-2 had a better performance under salt affected field condition.

High phenotypic variability, which encompasses genotypic, environmental and genotype x environmental interaction components was evident from the range of values for different characters. In this study, values of phenotypic variance were greater than the genotypic variance for most of the traits except for the days to flowering which showed a phenotypic variance lesser than the genotypic variance. This indicates that except days to flowering all other characters are influenced by environment.

As per the classification given by Sivasubramanian and Menon (1973), the values were grouped into high, medium and low. High GCV and PCV estimates were recorded for Na<sup>+</sup>/ K<sup>+</sup> ratio, number of filled grains per panicle, single plant yield and straw yield. Low GCV estimates were recorded for SPAD value, days to flowering, panicle length, spikelet fertility, hundred grain weight, SES for visual salt injury, plant height, number of tillers and number of productive tillers. Similar finding have been reported in the earlier studies for the above characters (Dey Choudhury and Das, 1998 and Maiti et al.. 2006). Aslam (1989) confirmed Intervarietal variability in rice with respect to its tolerance to salt stress. His findings showed that a tolerant variety of rice always maintained a lower concentration of Na<sup>+</sup> and Cl<sup>-</sup>, higher concentration of  $K^+$  and  $Zn^{2+}$  and higher  $K^+/Na^+$  and  $Zn^{2+}$ : P ratios in the shoot compared with a salt sensitive variety.

genotypic The and phenotypic coefficient of variation indicated the extent of variability for different traits. For assessing the the magnitude heritable variation, of heritability is the most important aspect in the breeding material, which has close bearing on the response to selection with fixable additive gene action. The high heritability in broad sense recorded for all the characters studied, indicates that genotype plays the most important role than the environment in determining these phenotypes. Similar results were reported by Suresh and Anbu Selvam (2005) and Maiti et al. (2006).

The advance in the mean value of population as a result of selection depends on (1) heritability of the characters (2) phenotypic variation and (3) selection pressure. Even if the heritability is 100%, there will be little genetic advance when there is little genotypic variation. The information on heritability alone may not help in pin pointing characters for enforcing selection. Nevertheless. the heritability estimates in conjunction with predicted genetic advance will be more reliable (Johnson et al., 1955). Heritability gives the information on the magnitude of inheritance of traits, while genetic advance in formulating suitable selection helps procedures. These 2 criteria are the foremost importance for successful breeding programme.

High heritability along with high genetic advance was observed for  $Na^+$ :  $K^+$  ratio, filled grains per panicle, straw yield and single plant yield indicating that these traits can be manipulated by selection for appropriate traits since these traits are predominantly governed by additive gene action and therefore selection would be effective for improving these traits. Suresh and Anbu Selvam (2005) made variability studies for yield and its component traits in rice observing considerable amount of genotypic coefficient of variation, heritability and genetic advance for several yield components.

Foolad, (1996) had shown that selection can be made under salt stress conditions, provided the heritability estimates are high. Similar results were obtained by Maiti et al. (2006). High heritability with medium genetic advance was observed for plant height, number of tillers, Number of productive tillers and SES for visual salt injury. This is due to the fact that these characters may be partially governed by the additive gene effect. The effect of genotype x environment interactions is minimal in tolerant genotype as it scores lowest stress indices. The best performing genotypes also have high heritability and genetic advance which has buffering capacity to the stress environment. However, the performance of entries in multiseason depends on soil stress and rainfall. Similar findings were reported in rice by Agarwal (2003). Ali et al. (2006) reported that narrow sense heritability decreased as salt concentration in increased progressively, i.e. heritability decreased as stress increased. Mishra et al. (1990) have reported a greater importance of additive gene action based on a salinity tolerance score at reproductive stage. The heritability values for these characters are also high, ranging from 63% to 74%, showing

the greater importance of additive gene action. High heritability and high genetic advance for salinity tolerance traits offers good scope for selection and genetic improvement.

## CONCLUSION

Salt susceptibility and resistance index are reliable parameter in estimation of stress level and identification of potential salt tolerance donors under problem soils. The present study with 39 genotypes under normal and saline environment was conducted and 29 showed better endurance with least salt susceptibility index with lesser yield reduction. The best of them are Pokkali, IR72132-AC-6-1 and IR70869-B-P-13-2, whereas IR 29 being the most susceptible. Similarly, the genotypes IR 72593-B-19-2-3-1, IR73104-B-1-1-3-2-1 and IR74802-3R-7-1-2 had early flowering with lower yield reduction under stress condition. The variability studies indicated the presence of significant variability for most of the yield contributing traits and these parameters can be used as selection indices in future breeding programme for salt tolerance.

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