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Evaluation of $F_{2:3}$ Population for Seedling Stage Salinity Tolerance in Rice (*Oryza Sativa* L.)

K Vijaya Durga, P V Satyanarayana,V Satyanarayana Rao, P Venkata Ramana Rao and K Jayalalitha

Department of Genetics and Plant Breeding, Agricultural College, Bapatla, A.P.

ABSTRACT

Salinity is an important abiotic stress affecting rice production worldwide. Development of salt tolerant varieties is the most feasible approach for improving rice productivity in salt affected soils. In rice, seedling stage salinity tolerance is crucial for better crop establishment. In the present study, 234 $F_{2:3}$ population derived from a cross between a high yielding salt susceptible rice cultivar Sri Druthi (MTU 1121) and salt tolerant variety Indra (MTU 1061) were evaluated for salt tolerance at the seedling stage in a hydroponics experiment at electrical conductivity (6 and 12 dSm^{-1}). Based on modified standard evaluation score for visual salt injury at seedling stage, one line was highly tolerant, forty were tolerant, one hundred fifteen were moderately tolerant, seventy-one were susceptible and the rest seven were highly susceptible.

Keywords: *Rice, Salinity tolerance, seedling stage and $F_{2:3}$ population.*

Rice (*Oryza sativa* L.) is an important staple food crop for one-third of the world's population.Soil salinity is the second major abiotic stress responsible for the reduction in cultivated land area and rice productivity.Salinized soils are known to suppress plant growth in rice and other crops due to high osmotic stress, nutritional disorders and poor soil physical conditions (Venkata Ramana Rao *et al*., 2017). Approximately 20% irrigated and 8% of rain fed agricultural land is affected by salinity and also one-third of the irrigated rice growing areas are affected by salinity (Jahan *et al.*, 2020).

Salinity is one of the major obstacles in increasing rice production worldwide, which is an ever-present threat to crop yield. One of the highly-effective strategy to improve rice production in saline soils is to develop salinity tolerance rice varieties. This is a strategic approach, which is cost effective and practical (Sen *et al*., 2017). The response of rice to salinity varies with growth stage. Several studies indicated that rice plant is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant at maturity (Bhowmik *et al*., 2009). The damaging effect of salt injury on rice plant has been extensively reviewed. It is well established that the excess NaCl alone can cause more toxicity to the rice plant more than mixed salts (Ammar *et al*., 2007).

At seedling stage, salt stress causes significant reduction in germination index and seedling vigor.The seedling stage is the first stage of growth during which salinity has a great influence on rice plant affecting plant height, tillering ability, shoot length and root length.

Recognizing the above challenges, the present study was taken up to identify novel sources of tolerance to salt stress at the seedling stage using $F_{2:3}$ population generated using salt tolerant rice cultivar Indra (MTU 1061). The tolerant $F_{2:3}$ lines identified in this study will facilitate the development of adaptable salt tolerant varieties.

MATERIAL AND METHODS

Choice of Parents and Generation of $F_{2:3}$ Population

Sri Druthi (MTU 1121) a high yielding, early maturing, BPH and leaf blast tolerant variety with low grain shattering but susceptible to salinity was used as recipient parent while Indra (MTU 1061), a high yielding salinity tolerant rice variety was used as donor. Crossing was taken up using Sri Druthi as female and Indra as male parent during Rabi 2017-18. F_1s were evaluated and selfed to generate F_2 population in Kharif 2018. The phenotyping of $F_{2:3}$for seedling salinity tolerance was taken up during Rabi 2018-19 and Kharif 2019.

Seedling Salinity Tolerance Evaluation

A total of 234 $F_{2:3}$ population with both parents (MTU 1061 and MTU 1121) were screened for salinity tolerance at seedling stage in greenhouse following the standard protocol of IRRI with some modifications(Gregorio *et al*., 1997). The screening experiment was conducted in a randomized complete block design with 2 replications. All lines were germinated in the laboratory and were transferred to nutrient solution Yoshida *et al*. (1976) . When the seedlings were at two leaf stage, they were subjected to initial salinity stress of EC=6dsm^{-1} by adding NaCl to nutrient solution.After eight days of initial salinization, the EC was increased to12dsm^{-1}. Initial scoring of the selected individual plants was recorded at 10 days after initial salinization as per SES of IRRI (1997). The description of the standard evaluation score of 1 - 9 is presented in Table 1. The final score was recorded at 16 days after initial salinization (Fig.1). Score 1 indicates that the line is highly tolerant and a score of 9 indicates high susceptibility (Fig. 2). The data on root length and shoot length were recorded. After screening experiment, the shoots of thelines were oven dried at 60 °C for 3 days, and the dry weights were recorded. For measuring the concentrations of Na^+ and K^+ in the shoot, one gram of powdered plant sample was taken in 150 ml Erlenmeyer flask and digestion with diacid mixture (HNO_3 and $HCLO_4$ in 9:4 ratio) at 50–55 °C heating block for 3 h. The total amount of Na^+ and K^+ was measured by a flame photometer. The final concentrations of Na^+ and K^+ ions were computed using the standard curve developed using different dilutions.

Statistical Analyses

The analysis of variance (ANOVA) for each trait was computed by using lines as a fixed effect and replicationas a random effect. Pearson correlation coefficients were computed to determine the relationship among different morphological and physiological traits. Statistical Package for the Social Sciences software version 2007 (www.spss.com) was used for the data analysis. The histograms were constructed in Microsoft Excel 2013 to analyze the distribution of families for each phenotypic trait.

RESULTS AND DISCUSSION

Phenotypic Variation for Seedling Stage Salinity Tolerance Traits

The $F_{2:3}$ population and parents showed a wide range of variation for different morpho-physiological traits in response to salt stress (EC 12 dsm^{-1}) (Table 2). Indra (MTU 1061) recorded lower values consistently for SIS and NaK ratio compared to Sri Druthi (MTU 1121) while the K^+ concentration

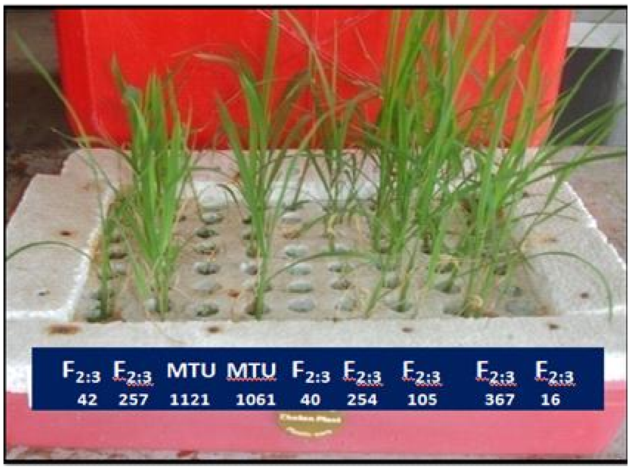

Fig 1. Final Scoring at 16 days after Salinization

Fig 2. Standard Evaluation Score (1-9)

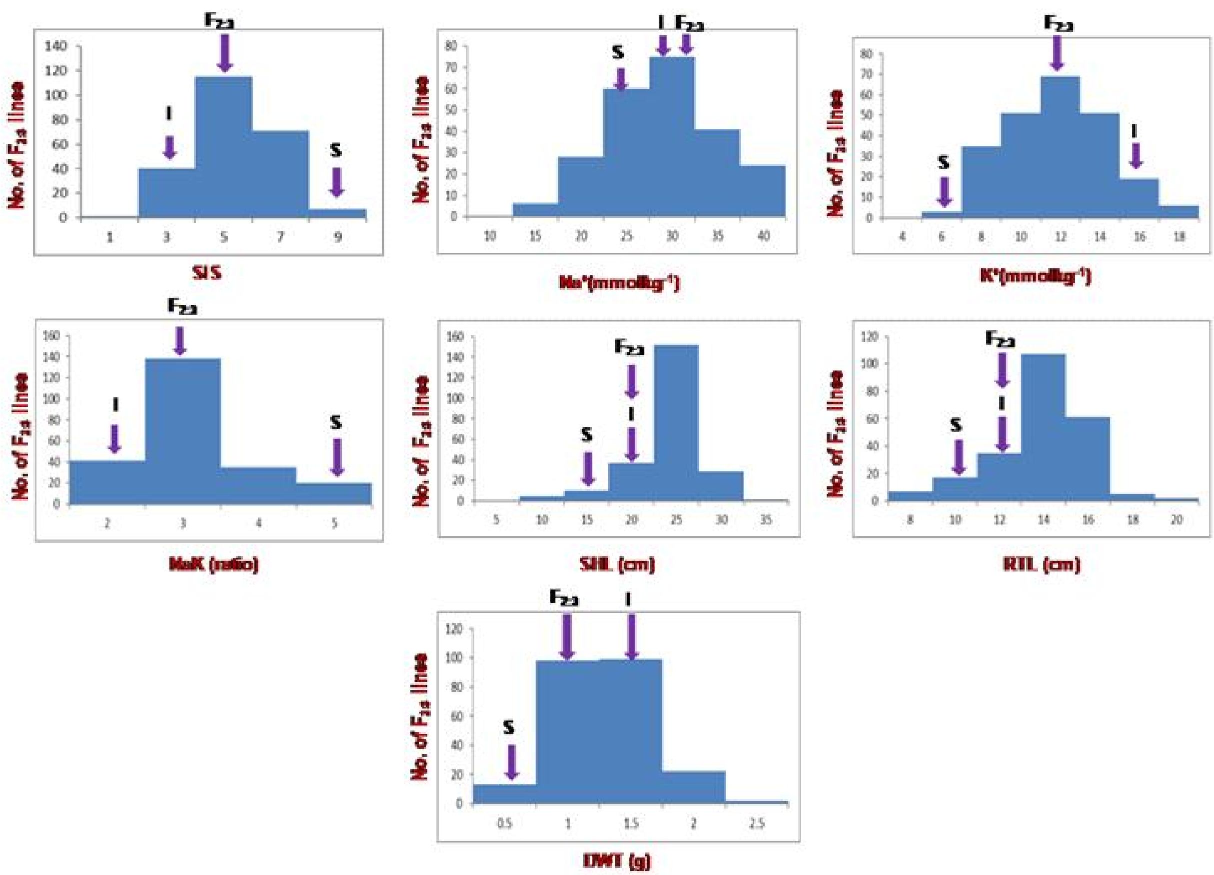

Figure 3. Frequency distribution of $F_{2:3}$ lines for 7 morpho-physiological traits under salt stress (EC = 12 dSm^{-1}). I, S, and F_3 indicate the positions of the mean phenotypic values of Indra, Sri Druthi, and the $F_{2:3}$ population. SIS, salt injury score; Na^+, Na^+ concentration; K^+, K^+ concentration; NaK, Na^+/K^+ratio; SHL, shoot length; RTL, root length; DWT, dry weight.

of Indra was higher than that of Sri Druthi. The mean values of the $F_{2:3}$ lines for all the traits were in between the mean values of parents with the exception for root length.The mean SIS value of Sri Druthi was 9 while it was 3 for Indra and 5.36 for the $F_{2:3}$ lines. The $F_{2:3}$ population had a mean shoot Na^+ concentration of 26 ppmwhich was closer to Indra mean (27 ppm) but higher than that of Sri Druthi (23 ppm). The shoot K^+concentration, the $F_{2:3}$ mean (11 ppm) was closer to Indra mean (15 ppm) and was much higher than that of Sri Druthi (5 ppm). Majority of traits were normally distributed in the $F_{2:3}$ lines(Figure 3).

Correlations among Salinity Tolerance Traits

The Pearson correlation coefficients indicated that there were significant correlations among different morphological and physiological traits for salinity tolerance (Table 3). The SIS score showed positive and significant correlation with Na^+concentration (0.576**), Na/K ratio (0.860**) and significant negative correlation with K^+ concentration (-0.574**), shoot length (-0.670**), root length (-0.584**) and shoot dry weight (-0.598**).These results are in conformity with the findings of Venkata Ramana Rao *et al*. (2017) who evaluated 138 ILs of Jupiter / Nona Bokra at seedling stage.The correlations between Na^+ and K^+(0.195**), $Na^+$and Na/K (0.634**) were positive and significant while it was negative and significant between $Na^+$and shoot length (-0.425**), Na^+ and root length (-0.410**), Na^+ and shoot dry weight (-0.309**).These results were in contrary with the findings of Naveed *et al*. (2018).The K^+ concentration was significant and negatively correlated with Na/K ratio (-0.601**) and significant positive correlation with shoot length (0.437**), root length (0.321**) and shoot dry weight (0.389**). The correlations between Na/K and shoot length (-0.696**), Na/K and root length (-0.586**), Na/K and shoot dry weight (-0.546**) were negative and significant.The findings are in conformity with the reports of Teresa *et al*. (2016) who screened 187 RILs from Bengal and Pokkali at seedling stage.The shoot length had significant and positive correlation with root length (0.704**) and shoot dry weight (0.657**). The root length showed positive and significant correlation with shoot dry weight (0.438**).Similar findings were reported by Lang *et al*. (2017).

Analysis of Tolerant $F_{2:3}$ Population

Forty-one $F_{2:3}$ lines showed high tolerance with a SIS score less than 5.0 and were significantly different from the susceptible parent Sri Druthi (Table 4). The mean SIS score in the highly tolerant $F_{2:3}$ lines ranged from 1 to 3 while the Na^+concentration ranged from 11.39 to 31.54 ppm. The shoot K^+ concentration was lowest in $F_{2:3}$ 33 (7.07 ppm) and highest in $F_{2:3}$ 367(17.94 ppm). For NaKratio, $F_{2:3}$ 367 recorded the lowest ratio of 1.50 which was lower than that of donor Indra (1.81). Most of the tolerant $F_{2:3}$ lines recorded shoot length intermediate between the parents. $F_{2:3}$ 105 recorded highest root length (17.43 cm) while it was lowest in $F_{2:3}$ 286 (11.20 cm). The $F_{2:3}$ 257 recorded highest dry weight of 2.31 g which was more than that of Indra (1.86 g).

$F_{2:3}$ 496 was the highly tolerant line with a SIS score of 1 and NaKratio of 1.54 whereas the corresponding values for Indra were 3 and 1.81, respectively. The $F_{2:3}$ 496 had Na^+concentration between both parents but had K^+ concentration and shoot dry weight more than the donor. The shoot length (24.84 cm) and root length (14.15 cm) was intermediate of both parents. Indra recorded shoot length of 28.86 cm and root length of 16.11 cm while Sri Druthi recorded less shoot length (15.38 cm) and root length (10.21 cm) when compared with Indra.

Table 1. Standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	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; most leaves dry; some plants drying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Table 2. Mean phenotypic performance of the parents and $F_{2:3}$ lines for seedling stage salinity tolerance traits

Trait Name	MTU 1061 mean	$F_{2:3}$ mean	$F_{2:3}$ range	SD
SIS	3	5.36	1.0-9.0	1.52
Na^+(ppm)	27.02	26.35	11.39-40.00	6.06
K^+(ppm)	14.91	10.78	4.52-17.94	2.52
NaK(ratio)	1.81	2.55	1.50-5.00	0.85
SHL (cm)	21.86	21.83	6.58-30.59	3.8
RTL (cm)	12.11	12.88	6.42-19.00	2.09
DWT (g)	1.86	1.03	0.07-2.31	0.37

SIS, salt injury score; Na^+, shoot sodium concentration; K^+, shoot potassium concentration; NaK, ratio of the shoot sodium and shoot potassium concentration; SHL, shoot length; RTL, root length; DWT, shoot dry weight.

Table 3. Pearson correlation matrix of morpho-physiological traits under salt stress in $F_{2:3}$ lines

	SIS	Na^+	K^+	NaK	SHL	RTL	DWT
SIS	1						
Na^+	0.576**	1					
K^+	-0.574**	0.195**	1				
NaK	0.860**	0.634**	-0.601**	1			
SHL	-0.670**	-0.425**	0.437**	-0.696**	1		
RTL	-0.584**	-0.410**	0.321**	-0.586**	0.704**	1	
DWT	-0.598**	-0.309**	0.389**	-0.546**	0.657**	0.438**	1

** Significant at 0.05 (two tail)

Table 4. Mean phenotypic performance of selected salt tolerant $F_{2:3}$ linesunder salt stress EC $12 dSm^{-1}$

S.No	$F_{2:3}$ lines	SIS (Final Score)	Na^+ (ppm)	K^+ (ppm)	NaK (ratio)	SHL (cm)	RTL (cm)	DWT (g)
1	$F_{2:3}$ 496	1	27.00	17.53	1.54	24.84	14.15	1.91
2	$F_{2:3}$ 2	3	19.13	11.56	1.65	24.90	15.75	1.29
3	$F_{2:3}$ 6	3	24.53	14.15	1.73	26.44	14.58	1.35
4	$F_{2:3}$ 7	3	26.88	15.35	1.75	24.48	12.50	1.60
5	$F_{2:3}$ 8	3	15.26	9.40	1.62	25.85	16.05	1.36
6	$F_{2:3}$ 13	3	26.09	14.68	1.77	24.70	14.05	1.49
7	$F_{2:3}$14	3	27.07	15.01	1.80	25.23	15.20	1.39
8	$F_{2:3}$17	3	15.49	9.09	1.70	24.37	15.80	1.28
9	$F_{2:3}$33	3	11.39	7.07	1.61	24.50	13.73	1.17
10	$F_{2:3}$ 36	3	13.74	8.09	1.69	22.03	11.87	1.16
11	$F_{2:3}$72	3	23.21	13.88	1.67	24.92	12.67	1.82
12	$F_{2:3}$ 76	3	20.17	13.20	1.52	23.69	12.37	1.41
13	$F_{2:3}$ 90	3	15.07	7.90	1.90	23.80	15.60	1.14
14	$F_{2:3}$95	3	24.36	15.62	1.55	25.04	14.16	1.35
15	$F_{2:3}$ 96	3	26.07	14.65	1.77	22.72	13.57	1.10
16	$F_{2:3}$ 97	3	24.99	15.36	1.62	29.12	14.56	1.45
17	$F_{2:3}$98	3	28.39	17.35	1.63	22.79	13.03	1.37
18	$F_{2:3}$99	3	21.75	13.26	1.64	24.90	14.90	1.10
19	$F_{2:3}$101	3	17.65	11.09	1.59	22.17	13.88	1.25
20	$F_{2:3}$105	3	12.99	8.28	1.56	27.56	17.43	1.46
21	$F_{2:3}$ 117	3	20.02	12.44	1.60	25.92	12.20	1.30
22	$F_{2:3}$122	3	18.71	10.60	1.76	23.35	14.20	1.40
23	$F_{2:3}$129	3	18.33	12.13	1.51	23.52	12.04	0.77
24	$F_{2:3}$149	3	18.04	11.11	1.62	27.47	13.33	2.07
25	$F_{2:3}$155	3	24.21	15.58	1.55	25.01	12.22	1.35
26	$F_{2:3}$160	3	25.53	14.56	1.75	19.60	15.00	1.29
27	$F_{2:3}$168	3	26.01	14.37	1.81	27.30	15.71	1.44
28	$F_{2:3}$226	3	31.54	17.84	1.76	27.09	15.48	1.29
29	$F_{2:3}$ 255	3	12.58	8.32	1.51	23.95	16.24	1.51
30	$F_{2:3}$ 257	3	14.59	9.61	1.51	30.59	14.39	2.31
31	$F_{2:3}$261	3	20.12	12.56	1.60	24.32	13.84	1.14
32	$F_{2:3}$281	3	24.89	13.35	1.86	24.61	13.15	1.57
33	$F_{2:3}$ 283	3	17.30	11.13	1.55	23.63	14.54	1.60
34	$F_{2:3}$285	3	20.65	12.69	1.62	24.95	14.97	1.84
35	$F_{2:3}$286	3	22.93	12.30	1.86	27.43	11.20	1.22
36	$F_{2:3}$288	3	23.60	13.08	1.80	22.85	15.07	1.53
37	$F_{2:3}$ 292	3	19.42	12.63	1.53	23.71	14.20	0.79
38	$F_{2:3}$298	3	25.69	14.34	1.79	25.24	16.68	1.60
39	$F_{2:3}$ 352	3	15.80	9.72	1.62	24.30	13.27	1.06
40	$F_{2:3}$367	3	27.03	17.94	1.50	26.63	15.40	0.89
41	$F_{2:3}$ 449	3	30.58	17.09	1.78	23.62	13.85	1.05
42	MTU 1061	3	27.02	14.91	1.81	28.86	16.11	1.86
43	MTU 1121	9	23.15	5.28	4.38	15.38	10.21	0.68

CONCLUSION

Salinity tolerance is a complex trait and has strong association with visual symptoms (as indicated by the SIS score) as well as physiological traits viz., shoot Na^+ concentration, shoot K^+ concentration and NaK ratio. Overall phenotypic performance reflected by SIS scores is determined by these key traits. We developed 234 $F_{2:3}$ lines of Indra in the genetic background of Sri Druthi, a high yielding cultivar. Total forty one lines were selected out of the 234 lines based on modified standard evaluation score for visual salt injury and Na/K ratio at seedling stage and selected of $F_{2:3}$ lines will be valuable pre-breeding material for further fine mapping and introgression into elite genotypes to develop salt tolerant varieties.

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