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Research Article

Evaluation of saltol introgressed back cross inbred lines for salinity tolerance in rice (*Oryza sativa* L.)

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Abstract
Fifty one backcross inbred lines (BC_3F_3 population) of ADT 37 introgressed with s*altol* loci from FL478 controlling salinity tolerance in rice and 17 lines of CR 1009 Sub1 introgressed with saltol loci from FL478 were evaluated for their salinity tolerance along with respective recurrent parents. All 68 backcross inbred lines (BIL) were subjected to genotyping using RM3412 which led to the identification of 20 lines in the genetic background of ADT 37 and 12 lines in the genetic background of CR 1009 harboring *saltol* loci from FL478. Seedlings of all 32 positive progenies were subjected to phenotypic evaluation under hydroponic conditions (EC levels of 6 dSm^{-1} and EC 12 dSm^{-1}) by following IRRI's standard protocol (1 - 9 scale scoring). Out of 32 selected BILs, 14 lines were found to be tolerant and remaining 18 lines were identified to be moderately tolerant. Tolerant lines exhibited very less reduction in their root growth with increased salinity level. Among the tolerant lines, minimum root and shoot length reduction was observed in BIL 1102, BIL 1079 and BIL 1091 at EC 6 dSm^{-1} level. At EC 12 dSm^{-1}, minimum root and shoot length reduction was observed in BIL 33, BIL 44, BIL 772, BIL 1079, BIL 1091, BIL 1096, BIL 1101 and BIL 1102. Saline tolerant lines *viz.,* BIL 33, BIL 1094, BIL 1096 and BIL 1101 have recorded maximum uptake of K^+ in both root and shoot which resulted in low Na^+/ K^+ ratio. The salt tolerant lines identified in this study will be further evaluated in saline soil under different locations to develop salt tolerant varieties.

Keywords
Genotypic screening, phenotypic screening, rice, *Saltol* introgressed lines, salt ion

Introduction

Rice (*Oryza sativa* L.) is the staple food for more than three billion people in the Wor (Ma *et al.,* 2007). Asian farmers constitute about 92 % of the world's total rice producing group (Mitin, 2009). Asia has produced over 95% of global rice with China (208.00 million tonnes) and India (104.80 million tonnes), ranking first and second, respectively contributing half of the world's rice production. It is imperative to increase rice production in different rice growing ecosystems to feed the increasing world population (Khush, 2005). By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhuiyan *et al.,* 2002).The conversion of some highly productive rice lands for industrial and residential purposes has pushed rice cultivation to less productive areas such as saline, drought and flood prone areas.

Salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increase rice production (Gregorio *et al.,* 1997). In India, salt affected area accounts for 6.73 million hectares of land (Krishnamurthy *et al.,* 2014). Salinity is one of the most important abiotic stresses directly affect the plant growth and development (Arshad *et al*., 2012). The cheapest and easiest way to address the problem of salinity is through the development of a salt tolerant variety. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice 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 (Lutts *et al.,* 1995).

The conventional method of plant selection for salt tolerance is 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. Hence, screening under laboratory conditions is considered to be advantageous over field screening. Screening under controlled condition has the benefit of reduced environment effects and the hydroponic system is free of difficulties associated with soil related stress factors. In rice, the screening can be done independently at its two salt sensitive stages but screening at seedling stage is comparatively easier than reproductive stage and also rapid. Also screening of genotypes for salt tolerance at early stages may be important since there is considerable saving in time.

However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Recent progress in DNA marker technology permits reduction of time and accuracy of breeding. With the advancements in the field of Marker Assisted Selection (MAS), it is possible to introgress QTLs in the desired genetic background. Using this strategy, several improved versions of rice varieties have been developed. This demonstrated the feasibility of developing improved versions of rice varieties exhibiting salinity tolerance. SSR markers are playing important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. The MAS derived back cross lines (BC_3F_3) of rice for salinity tolerance developed under DBT-Bio-CARe scheme at Rice Research Station, Tirur were used for the present study. These lines developed in the background of popular rice varieties ADT 37 and CR I009 *Sub 1* using FL 478 as the donor for *Saltol*, due to its high level of tolerance, without the tallness, photoperiod sensitivity and late flowering of the original Pokkali. Hence, the aim of the present study was to screen MAS derived Backcross Inbred lines (BIL) with the *Saltol* allele under saline conditions and to evaluate SSR markers for the identification of salt tolerant lines at the seedling stage.

Materials and Methods

Fifty one *Saltol* introgressed backcross inbred lines (BC_3F_3 population) of ADT 37 × FL 478 and 17 introgressed lines of CR 1009 *Sub1*× FL 478 were used to identify the lines with salt tolerance through genotypic and phenotypic evaluation. The sixty eight lines were evaluated along with two recurrent parents ADT 37 and CR 1009 *Sub* 1, a tolerant parent FL 478 and a susceptible check IR 29.

Young fresh leaves of 20-25 days old seedlings were collected and were used for DNA extraction. From the collected samples, 0.5 g was extracted using 450 µl of preheated CTAB buffer. Extracted samples were incubated in the water bath for 30 minutes at 65º C with occasional shaking. After incubation, around 450µl of Chloroform: Isoamyl alcohol (24:1) was added into the tubes and inverted twice to mix. Then the tube was kept in centrifuge for 15 minutes at 10000 rpm. After that, the aqueous layer was taken and transferred to the new eppendorf tubes. Ice cold isopropanol was added (twice the volume of supernatant) to Eppendorf tubes containing the supernatant and it was allowed to stay overnight at -20º C. It was again centrifuged at 10000 rpm for 15 minutes on the next day morning. The supernatant was discarded and the pellet settled in the bottom was allowed to air dry for 30 minutes. An amount of 200 µl TE buffer and 50 µl of 3M sodium acetate was added into the tubes and kept at -20 ºC for 5 minutes. The samples were centrifuged at 10000 rpm for 10 minutes. After centrifugation supernatant was carefully decanted from each tube having ensured that the pellets remained inside the tubes. To the tubes containing pellets, 200 µl of 70 per cent ethanol was added to the tubes followed by centrifugation at 10000 rpm for 10 minutes. Pellets were obtained by carefully decanting the supernatant from each tube and then allowed to air dry for one hour. Completely dried pellets were re-suspended in 100µl of TE buffer and incubated overnight at 4 °C to allow them to dissolve completely. Dissolved DNA samples were stored in -20 °C for long term use.

The SSR marker RM 3412 linked to *Saltol* QTL was used for screening of *Saltol* introgressed backcross inbred lines along with salt tolerant parent and salt susceptible check. PCR reaction was carried out in a total volume of 10 µl. The reaction mixture containing 1.0 µl 10 X buffer, 1.0 µl dNTPs, 1.0 µl primer forward, 1.0 µl primer reverse, 0.2 µl taq polymerase, 3.8 µl double distilled H_2O and 2.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94^{o}C for 5 minutes, followed by 34 cycles of denaturation at 94^{o}C for 1 minute, annealing at 55^{o}C for 1 minute and polymerization at 72^{o}C for 2 minutes and final extension by 7 minutes at 72^{o}C. Banding pattern of the genotypes was scored comparing the banding pattern of FL 478. The lines which showed similar banding pattern like FL 478, were considered as tolerant and had different banding pattern were considered as susceptible.

The genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (Gregorio *et al.,* 1997) at the green house facility available at Agricultural College and Research Institute, Madurai. Salinized and non-salinized setups with 3 replications were maintained. The evaluation was done using Yoshida (Yoshida *et al.,* 1976) nutrient solution. The nutrient solution was salinized by adding crude salt to obtain EC levels of 6 dSm^{-1} and 12 dSm^{-1}. The trays were filled with this solution high enough to touch the nylon net bottom of the Styrofoam. The effective culture solution needed per tray was about 11 litres. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity. Initial and final scoring was done at 14 days and 22 days after salinization. For phenotypic observation, root length and shoot length were recorded at salinized and non-salinized conditions.

Modified standard evaluation score (SES) of visual salt injury at seedling stage (Gregorio *et al.*, 1997)

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

The total sodium and potassium in the leaf sample was estimated using the Triple acid extract - Flame photometer method (Piper, 1966). It is based on the principle that atoms of some specific element take energy from flame and get excited to the higher orbit. Such atoms release energy of wavelength which is specific to that element and is proportional to the concentration of the atoms of that element.

Rice leaves were shade dried and oven dried for eight hours and ground into powder form and the powdered leaf sample (1g) was put into conical flask. Triple acid 15 ml was added to the conical flask and allowed for digestion overnight. After digestion, the conical flask was kept in sand bath for eight hours until the colour turned from light yellow to white. Samples were filtered and the volume was made to 100 ml in volumetric flask. 5 ml of triacid extract was added into 25 ml volumetric flask and neutralized with ammonium hydroxide (the piece of red litmus is placed in the flask ammonium hydroxide is added till the paper colour turns blue) and make up the volume to 25 ml with distilled water. Concentration of sodium and potassium in the solution were estimated using flame photometer.

Results and Discussion

Microsatellite marker analysis is promising to identify major gene locus for plant breeders to develop new cultivars. Sixty eight BC_3F_3 lines of ADT 37 × FL 478 and CR 1009 *Sub1* × FL 478 crosses were genotypically screened along with two recurrent parents ADT 37 and CR 1009 *Sub 1*, tolerant parent FL 478 and a susceptible check IR 29 using foreground marker RM 3412. The bands obtained from the lines were compared to the banding pattern of FL 478. The lines having similar banding pattern to FL 478 were considered as tolerant and having different banding pattern to FL 478 were considered as susceptible. Among the 68 lines tested, 32 BC_3F_3lines showed the presence of RM 3412 marker (Table 1). The selected marker RM 3412 was able to discriminate tolerant lines from susceptible. So this marker has relationship with salt tolerance alleles studied in introgressed lines. The identified linked marker RM 3412 can be used in marker-assisted selection programme in identifying tolerant lines and also gene pyramiding of rice salinity breeding.

Siddika *et al*. (2007) observed that genotypes *viz.*, BRRI Dhan 40, PNR-519, Y-1281, TNDB-100 and RD-586 were found as salt tolerant when samples were amplified with RM 9. Mohammadi-Nejad *et al.* (2008) has found that rice genotypes possessing the Pokkali band type for locus RM 8094 marker were either highly tolerant or tolerant to salinity stress at the seedling stage. Therefore, this marker appears to have a strong and positively associated with seedling salt tolerance in rice. Some of salt-tolerant genotypes had the Pokkali marker allele for RM 10745. Whereas Moniruzzaman *et al.* (2012) has identified salt tolerant lines using three markers RM 510, RM 585 and RM 336.

Nguyen *et al.* (2001) found that the marker RM 315 had association with NaCl tolerant alleles at seedling population IR 64/OM 1706 and IR 64/FR 13A under EC 18 dSm^{-1} and salt stress genes were located at loci in chromosomes 1 and 8. Similar result was

reported by Lang *et al. (*2000). They found that RM 223 was closely linked to salt tolerance gene in chromosome 8. Since the markers were used in this study showed polymorphism with the genotypes, these markers could be used in tagging salt tolerant genes, in marker-assisted selection and quantitative trait loci (QTL) mapping.

All genotypes were grown robustly and showed uniform green colour and height in the non-salinized condition. In salinized condition, seedling growth was suppressed under salinity stress. The lines showed variation in phenotypic observation ranging from score 1 (highly tolerant), 3 (tolerant) and 5 (moderately tolerant). The most salinity tolerant lines were BIL 33, BIL 44, BIL 63, BIL 108, BIL 752, BIL 772, BIL 1047, BIL 1079, BIL 1091, BIL 1094, BIL 1095, BIL 1096, BIL 1101and BIL 1102 and remaining lines were identified as moderately tolerant (Table 2).

Islam *et al*. (2007) also observed wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) lines using modified SES of IRRI standard protocol. According to Titov *et al.* (2009), all the genotypes showed a wide variation in phenotypes. Salt tolerant seedlings were distinct from the sensitive seedlings grown in salinized condition. Seedlings grown in salinized condition showed different visual symptoms of salt injury. The symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish of leaf tip, drying of leaves and also reduction in root growth, stunted shoot growth with thickened stem leading to a complete cessation of growth and dying of seedlings (Gregorio *et al.*, 1997).

Seedling height was shorter in saline condition when compared to seedling growth in non-salinized condition. The tolerant lines were less affected by salt stress compared to moderately tolerant lines for shoot length and root length (Table 3).

Among the tolerant lines, minimum root length reduction was observed in BIL 1079 (13.18 %) followed by BIL 1091 (13.59 %), BIL 1102 (14.02 %) and FL 478 (14.29 %) and minimum shoot length reduction was observed in FL 478 (9.62 %), BIL 1079 (10.75 %), BIL 1094 (11.80 %) and BIL 1091 (11.96%) at EC 6 dSm^{-1} level. At EC 12 dSm^{-1}, minimum root length reduction was observed in FL 408 (23.08 %) followed by BIL 1102 (26.19 %), BIL 1096 (27.04 %) and BIL 1094 (27.71 %) and minimum shoot length reduction was observed in FL 478 (21.54 %), BIL 33 (24.60 %), BIL 44 (25.00 %) and BIL 772 (25.20 %). Akbar and Yabuno (1974) also found that root length and emergence of new roots decreases significantly at salinized condition (EC 5-6 dSm^{-1}). Munns & Tester, (2008) also reported that salinity might directly or indirectly inhibit cell division and enlargement during plant growing period. As a result, leaves and stems of the affected plants appeared stunted. According to Titov *et al.* (2009), tolerant cultivars had shown less growth reduction than sensitive types. At EC 12 dSm^{-1}, minimum root and shoot length reduction was observed in BIL 33, BIL 44, BIL 772, BIL 1079, BIL 1091, BIL 1096, BIL 1101 and BIL 1102.

The lines which were identified as saline tolerant under hydroponic system with minimum root length and shoot length reduction at EC 6 dSm^{-1} and EC 12 dSm^{-1} were chosen for salt ion concentration analysis. Totally eight BC_3F_3 lines along with salt tolerant donor FL 478 were used for this analysis and the results are presented in the Table 4.

In root, high Na^+ content was observed in FL 478 (1.03 ppm) followed by BIL 1101 (0.96 ppm), ADT 37 (0.91 ppm) and low Na^+ uptake was observed in BIL 1079 (0.55 ppm) followed by BIL 44 and BIL 1047 (0.68 ppm) and BIL 1091 (0.69 ppm). In root, high K^+ content was observed in FL 478 (1.90 ppm) followed by BIL 1102 (1.42 ppm) and BIL 1096 (1.46 ppm). In root, FL 478 had recorded low Na^+/K^+ ratio (0.54) followed by BIL 1094 (0.56) and BIL 1095 and BIL 1096 (0.57).

Maximum Na^+ content was observed in FL 478 (1.05 ppm) followed by CR 1009 *Sub 1* and IR 29 (1.00 ppm), BIL 1095 (0.97 ppm) and minimum Na^+ content was observed in BIL 772 (0.75 ppm) followed by BIL 33 (0.80 ppm) and BIL 1096 (0.81 ppm). In shoot, maximum K^+ content was recorded in FL 478 (2.11 ppm) followed by BIL 1101 (1.58 ppm) and BIL 1102 (1.49 ppm). In shoot, minimum Na^+/K^+ ratio was observed in FL 478 (0.50), BIL 33 and BIL 1096 (0.56) and BIL 1101 (0.59).

Salinity in rice was associated with Na+ exclusion and increased absorption of K+ to maintain a good Na+/K+ balance in the shoot under saline condition. It is considered that damage of leaves was attributed to accumulation of Na+ from the root to the shoot in external high concentration (Lin *et al.,* 2004). In several species including rice, salt stress might

increase or even include the expression of specific genes and repress or completely suppress the expression of others (Hasegawa *et al.,* 2000).

Out of 68 BILs, 32 lines were found as saline tolerant using foreground marker RM 3412. Out of thirty two selected BILs, 14 lines were found as tolerant and remaining 18 lines were identified as moderately tolerant. Among the 14 lines, six lines have performed better with high root and shoot length while comparing with other lines. The tolerant lines had exhibited very less root reduction with increased salinity level. Among the tolerant lines, minimum root and shoot length reduction was observed in BIL 1102, BIL 1079 and BIL 1091 at EC 6 dSm^{-1} level. At EC 12 dSm^{-1}, minimum root and shoot length reduction was observed in BIL 33, BIL 44, BIL 772, BIL 1079, BIL 1091, BIL 1096, BIL 1101 and BIL 1102. Saline tolerant lines *viz.*, BIL 33, BIL 1094, BIL 1096 and BIL 1101 have recorded maximum uptake of K^+ in both root and shoot. K^+ uptake was higher than Na^+ uptake which resulted in low Na^+/K^+ ratio. The selected salt tolerant lines will be further evaluated in saline areas to observe yield potentiality for developing high yielding saline tolerant varieties.

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Table 1. Genotypic performances of Backcross inbred lines (BILs) and parents against SSR marker RM 3412 for foreground selection

S.No	BILs/parents	Parentage	RM 3412 score	Remarks
1.	BIL 33	ADT 37/FL 478	1	Tolerant
2.	BIL 44	ADT 37/FL 478	1	Tolerant
3.	BIL 63	ADT 37/FL 478	1	Tolerant
4.	BIL 108	ADT 37/FL 478	1	Tolerant
5.	BIL 110	ADT 37/FL 478	1	Tolerant
6.	BIL 111	ADT 37/FL 478	1	Tolerant
7.	BIL 114	ADT 37/FL 478	1	Tolerant
8.	BIL 115	ADT 37/FL 478	1	Tolerant
9.	BIL 116	ADT 37/FL 478	1	Tolerant
10.	BIL 117	ADT 37/FL 478	1	Tolerant
11.	BIL 119	ADT 37/FL 478	1	Tolerant
12.	BIL 137	ADT 37/FL 478	1	Tolerant
13.	BIL 578	ADT 37/FL 478	1	Tolerant
14.	BIL 608	ADT 37/FL 478	1	Tolerant
15.	BIL 752	ADT 37/FL 478	1	Tolerant
16.	BIL 757	ADT 37/FL 478	0	Susceptible
17.	BIL 768	ADT 37/FL 478	0	Susceptible
18.	BIL 770	ADT 37/FL 478	0	Susceptible
19.	BIL 772	ADT 37/FL 478	1	Tolerant
20.	BIL 773	ADT 37/FL 478	0	Susceptible
21.	BIL 774	ADT 37/FL 478	0	Susceptible
22.	BIL 775	ADT 37/FL 478	0	Susceptible
23.	BIL 776	ADT 37/FL 478	0	Susceptible
24.	BIL 777	ADT 37/FL 478	0	Susceptible
25.	BIL 800	ADT 37/FL 478	0	Susceptible
26.	BIL 801	ADT 37/FL 478	0	Susceptible
27.	BIL 802	ADT 37/FL 478	0	Susceptible
28.	BIL 808	ADT 37/FL 478	0	Susceptible
29.	BIL 809	ADT 37/FL 478	0	Susceptible
30.	BIL 810	ADT 37/FL 478	0	Susceptible
31.	BIL 811	ADT 37/FL 478	0	Susceptible
32.	BIL 812	ADT 37/FL 478	0	Susceptible
33.	BIL 814	ADT 37/FL 478	0	Susceptible
34.	BIL 815	ADT 37/FL 478	0	Susceptible
35.	BIL 820	ADT 37/FL 478	0	Susceptible
36.	BIL1000	ADT 37/FL 478	0	Susceptible

S.No	BILs/parents	Parentage	RM 3412 score	Remarks
37.	BIL 1002	ADT 37/FL 478	0	Susceptible
38.	BIL 1004	ADT 37/FL 478	0	Susceptible
39.	BIL 1005	ADT 37/FL 478	0	Susceptible
40.	BIL 1006	ADT 37/FL 478	0	Susceptible
41.	BIL 1024	ADT 37/FL 478	0	Susceptible
42.	BIL 1025	ADT 37/FL 478	0	Susceptible
43.	BIL 1026	ADT 37/FL 478	0	Susceptible
44.	BIL 1036	ADT 37/FL 478	0	Susceptible
45.	BIL 1037	ADT 37/FL 478	0	Susceptible
46.	BIL 1038	ADT 37/FL 478	0	Susceptible
47.	BIL 1039	ADT 37/FL 478	0	Susceptible
48.	BIL 1049	ADT 37/FL 478	1	Tolerant
49.	BIL 1050	ADT 37/FL 478	1	Tolerant
50.	BIL 1047	ADT 37/FL 478	1	Tolerant
51.	BIL 1056	ADT 37/FL 478	1	Tolerant
52.	BIL 1068	CR 1009 *Sub*1/ FL 478	1	Tolerant
53.	BIL 1069	CR 1009 *Sub*1/ FL 478	1	Tolerant
54.	BIL 1070	CR 1009 *Sub*1/ FL 478	1	Tolerant
55.	BIL 1072	CR 1009 *Sub*1/ FL 478	1	Tolerant
56.	BIL 1073	CR 1009 *Sub*1/ FL 478	0	Susceptible
57.	BIL 1074	CR 1009 *Sub*1/ FL 478	0	Susceptible
58.	BIL 1075	CR 1009 *Sub*1/ FL 478	0	Susceptible
59.	BIL 1076	CR 1009 *Sub*1/ FL 478	1	Tolerant
60.	BIL 1078	CR 1009 *Sub*1/ FL 478	0	Susceptible
61.	BIL 1079	CR 1009 *Sub*1/ FL 478	1	Tolerant
62.	BIL 1081	CR 1009 *Sub*1/ FL 478	0	Susceptible
63.	BIL 1091	CR 1009 *Sub*1/ FL 478	1	Tolerant
64.	BIL 1094	CR 1009 *Sub*1/ FL 478	1	Tolerant
65.	BIL 1095	CR 1009 *Sub*1/ FL 478	1	Tolerant
66.	BIL 1096	CR 1009 *Sub*1/ FL 478	1	Tolerant
67.	BIL 1101	CR 1009 *Sub*1/ FL 478	1	Tolerant
68.	BIL 1102	CR 1009 *Sub*1/ FL 478	1	Tolerant
69.	ADT 37		0	Susceptible
70.	CR 1009 sub1		0	Susceptible
71.	FL 478 (Tolerant check)		1	Tolerant

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Table 2. Salinity scoring of Backcross Inbred lines at EC 6dSm^{-1} and EC 12 dSm^{-1}

S.No.	BILs/parents	Parentage	SES Scoring		Salinity reaction
			6 EC dSm^{-1}	12 EC dSm^{-1}	
1.	BIL 33	ADT 37/FL 478	1	3	Tolerant
2.	BIL44	ADT 37/FL 478	3	3	Tolerant
3.	BIL 63	ADT 37/FL 478	3	3	Tolerant
4.	BIL 108	ADT 37/FL 478	3	3	Tolerant
5.	BIL 110	ADT 37/FL 478	5	5	Moderately Tolerant
6.	BIL 111	ADT 37/FL 478	5	5	Moderately Tolerant
7.	BIL 114	ADT 37/FL 478	5	5	Moderately Tolerant
8.	BIL 115	ADT 37/FL 478	5	5	Moderately Tolerant
9.	BIL 116	ADT 37/FL 478	3	5	Moderately Tolerant
10.	BIL 117	ADT 37/FL 478	5	5	Moderately Tolerant
11.	BIL 119	ADT 37/FL 478	5	5	Moderately Tolerant
12.	BIL 137	ADT 37/FL 478	5	5	Moderately Tolerant
13.	BIL 578	ADT 37/FL 478	5	5	Moderately Tolerant
14.	BIL 608	ADT 37/FL 478	5	5	Moderately Tolerant
15.	BIL 752	ADT 37/FL 478	3	3	Tolerant
16.	BIL 772	ADT 37/FL 478	3	3	Tolerant
17.	BIL 1047	ADT 37/FL 478	3	3	Tolerant
18.	BIL 1049	ADT 37/FL 478	3	5	Moderately Tolerant
19.	BIL 1050	ADT 37/FL 478	5	5	Moderately Tolerant
20.	BIL 1056	ADT 37/FL 478	3	5	Moderately Tolerant
21.	BIL 1068	ADT 37/FL 478	5	5	Moderately Tolerant
22.	BIL 1069	ADT 37/FL 478	5	5	Moderately Tolerant
23.	BIL 1070	CR 1009 *Sub1*/ FL 478	5	5	Moderately Tolerant
24.	BIL 1072	CR 1009 *Sub1*/ FL 478	5	5	Moderately Tolerant
25.	BIL 1076	CR 1009 *Sub1*/ FL 478	5	5	Moderately Tolerant
26.	BIL 1079	CR 1009 *Sub1*/ FL 478	3	3	Tolerant
27.	BIL 1091	CR 1009 *Sub1*/ FL 478	3	3	Tolerant
28.	BIL 1094	CR 1009 *Sub1*/ FL 478	1	3	Tolerant
29.	BIL 1095	CR 1009 *Sub1*/ FL 478	3	3	Tolerant
30.	BIL 1096	CR 1009 *Sub1*/ FL 478	1	3	Tolerant
31.	BIL 1101	CR 1009 *Sub1*/ FL 478	1	3	Tolerant
32.	BIL 1102	CR 1009 *Sub1*/ FL 478	3	3	Tolerant
33.	ADT 37		7	7	Susceptible
34.	CR 1009 *Sub* 1		7	7	Susceptible
35.	FL 478		3	3	Tolerant
36.	IR 29 (Susceptible check)		9	9	Highly susceptible

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Table 3 Mean value of root length, shoot length of backcross inbred lines under salinized condition

Backcross inbred lines/parents	Tolerant reaction	Non-salinized condition		EC6 dSm-1		EC 12 dSm-1		Percentage reduction under salinity			
		Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length % reduction		Shoot length % reduction	
								EC 6 dSm-1	EC 12 dSm-1	EC6 dSm-1	EC 12 dSm-1
BIL 33	T	16.0	25.2	13.0	21.0	11.5	19.0	18.75	28.13	16.67	24.60
BIL 44	T	14.5	28.0	12.0	24.0	10.2	21.0	17.24	29.66	14.29	25.00
BIL 63	T	18.2	24.6	15.5	20.0	13.2	18.0	14.84	27.47	18.70	26.83
BIL 108	T	17.7	22.4	14.5	19.0	12.7	16.5	18.08	28.25	15.18	26.34
BIL 110	MT	15.6	21.6	11.3	16.5	9.6	14.5	27.56	38.46	23.61	32.87
BIL 111	MT	17.6	22.5	12.9	17.6	11.0	13.9	26.70	37.50	21.78	38.22
BIL 114	MT	18.5	22.1	12.5	18.6	11.6	14.8	32.43	37.30	15.84	33.03
BIL 115	MT	16.5	23.6	12.0	18.3	10.3	15.5	27.27	37.58	22.46	34.32
BIL 116	MT	18.6	24.5	12.7	19.4	11.8	16.0	31.72	36.56	20.82	34.69
BIL 117	MT	18.5	22.3	13.5	16.5	12.4	14.9	27.03	32.97	26.01	33.18
BIL 119	MT	16.6	24.6	12.0	18.6	10.5	15.2	27.71	36.75	24.39	38.21
BIL 137	MT	15.9	23.7	13.6	16.8	10.6	15.1	14.47	33.33	29.11	36.29
BIL 578	MT	15.6	22.8	11.6	18.3	9.8	13.8	25.64	37.18	19.74	39.47
BIL 608	MT	19.5	21.7	13.0	15.2	12.0	14.9	33.33	38.46	29.95	31.34
BIL 752	T	21.7	25.4	17.8	21.5	15.7	18.0	17.97	27.65	15.35	29.13
BIL 772	T	16.2	25.4	13.0	22.0	11.5	19.0	19.75	29.01	13.39	25.20
BIL 1047	T	19.3	24.0	14.0	19.5	13.6	17.6	27.46	29.53	18.75	26.67
BIL 1049	MT	20.3	26.5	14.5	18.4	12.2	17.0	28.57	39.90	30.57	35.85
BIL 1050	MT	21.3	27.8	15.0	18.9	13.1	16.8	29.58	38.50	32.01	39.57
BIL 1056	MT	18.6	25.6	12.9	18.3	12.2	15.8	30.65	34.41	28.52	38.28
BIL 1068	MT	19.5	22.5	13.3	16.5	12.2	15.1	31.79	37.44	26.67	32.89
BIL 1069	MT	17.6	22.7	13.5	17.5	10.6	14.5	23.30	39.77	22.91	36.12

BIL 1070	MT	19.7	23.8	14.4	16.2	12.0	15.5	26.90	39.09	31.93	34.87
BIL 1072	MT	17.8	22.4	13.6	18.4	10.9	13.6	23.60	38.76	17.86	39.29
BIL 1076	MT	19.6	23.5	13.9	18.0	11.8	14.4	29.08	39.80	23.40	38.72
BIL 1079	T	20.6	27.5	17.9	24.5	15.0	20.0	13.11	27.18	10.91	27.27
BIL 1091	T	19.5	25.5	16.9	22.5	14.0	19.0	13.33	28.21	11.76	25.49
BIL 1094	T	19.9	25.9	16.0	22.8	14.4	18.2	19.60	27.64	11.97	29.73
BIL 1095	T	18.8	26.3	15.8	22.9	13.2	18.5	15.96	29.79	12.93	29.66
BIL 1096	T	20.6	26.5	17.5	23.1	15.0	19.0	15.05	27.18	12.83	28.30
BIL 1101	T	18.6	24.4	15.9	21.3	13.3	18.0	14.52	28.49	12.70	26.23
BIL 1102	T	18.9	27.4	16.3	23.9	14.0	20.0	13.76	25.93	12.77	27.01
ADT 37	S	16.5	24.1	12.5	15.5	8.4	12.8	24.24	49.09	35.68	46.89
CR 1009 *Sub* 1	S	15.5	25.6	11.0	16.3	8.1	13.8	29.03	47.74	36.33	46.09
FL 478	T	18.2	26.0	15.6	23.5	14.0	20.4	14.29	23.08	9.62	21.54
IR 29	HS	17.9	24.7	10.5	14.5	7.1	11.0	41.34	60.34	41.30	55.47
Mean		**18.22**	**24.53**	**13.94**	**19.33**	**11.93**	**16.42**	**23.49**	**34.67**	**21.35**	**33.19**
SE		**0.30**	**0.30**	**0.32**	**0.47**	**0.33**	**0.40**	**1.20**	**1.26**	**1.38**	**1.20**
CV		**9.77**	**7.38**	**13.92**	**14.49**	**16.51**	**14.62**	**30.69**	**21.75**	**38.64**	**21.76**

Table 4. Estimation of salt ion concentration in selected tolerant lines

S.No	Backcross inbred lines/parents	Root			Shoot		
		Na^+(ppm)	K^+(ppm)	Na^+/K^+ (Ratio)	Na^+(ppm)	K^+(ppm)	Na^+/K^+ (Ratio)
1	BIL 33	0.83	1.14	0.73	0.80	1.42	0.56
2	BIL 44	0.68	0.90	0.76	0.83	1.01	0.82
3	BIL 63	0.75	1.10	0.68	0.92	1.15	0.80
4	BIL 108	0.77	1.12	0.69	0.85	1.05	0.81
5	BIL 772	0.80	1.15	0.70	0.87	1.24	0.70
6	BIL 772	0.73	1.15	0.63	0.75	1.16	0.65
7	BIL 1047	0.68	1.12	0.61	0.92	1.45	0.63
8	BIL 1079	0.55	0.95	0.58	0.99	1.20	0.83
9	BIL 1091	0.69	1.05	0.66	0.95	1.30	0.73
10	BIL 1094	0.70	1.24	0.56	0.92	1.20	0.77
11	BIL 1095	0.71	1.25	0.57	0.97	1.32	0.73
12	BIL 1096	0.80	1.40	0.57	0.81	1.45	0.56
13	BIL 1101	0.96	1.35	0.71	0.94	1.58	0.59
14	BIL 1102	0.84	1.42	0.59	0.95	1.49	0.64
15	ADT 37	0.91	0.75	1.21	0.95	0.80	1.19
16	CR 1009 *Sub 1*	0.95	0.80	1.19	1.00	0.88	1.14
17	FL 478	1.03	1.90	0.54	1.05	2.11	0.50
18	IR 29	0.97	0.60	1.62	1.00	0.65	1.54
Mean		**0.80**	**1.13**	**0.76**	**0.92**	**1.25**	**0.79**
SE		**0.03**	**0.07**	**0.07**	**0.02**	**0.08**	**0.06**
CV		**15.93**	**25.80**	**38.20**	**8.82**	**26.38**	**33.15**