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SALT TOLERANCE IN PARENTAL LINES OF RICE HYBRIDS THROUGH PHYSIOLOGICAL ATTRIBUTES AND MOLECULAR MARKERS

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ABSTRACT

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An experiment with 26 parental lines of hybrids was conducted in saline microplots at the Central Soil Salinity Research Institute, India during T. aman season of 2008 to identify salt tolerant ones for future development of salt tolerant hybrids. Salinity treatments (Control, 6 dS/m, 10 dS/m) were imposed from tillering stage to maturity. Results revealed that salinity increased Na^+, decreased K^+ and K^+/Na^+ ratio in flag leaves. Plant height, panicle length, 100-grain weight and biological yield decreased with increasing salinity. Number of tillers and panicles/plant and grain yield decreased similarly by the salinity of 6 and 10 dS/m. Number of dry leaves increased with the increase in salinity although total number of leaves were not affected by the salinity levels. IR60997-16-2-3-2-2R, IR29723, BR827-35, BCW-56, KMR-3, IR80155B and CSR23 performed better under salinity compared to other genotypes. The parental line BCW showed the lowest Na^+ and the highest K^+ and K^+/Na^+ ratio in flag leaves. The view of the DNA structures did not show significant variation in the genotypes.

Key words: *salt tolerance, parental lines of rice hybrids, ions, molecular markers, yield and yield attributes*

INTRODUCTION

Salinity is an environmental condition which affects the physiological processes of plants and it is the most important factor which severely affects crop production. These adverse effects may be attributed to non-availability of water, disturbance in nutrient uptake causing deficiency and ion-toxicity to plants. Among abiotic stress, salinity is foremost and second most widespread problem causing reduction in growth and productivity (Gregario *et al*. 1997; Ashraf and O'Leary, 1996; Munns *et al*. 2006). Plants growing under saline condition invariably face increased concentrations of toxic ions in their tissues resulting from increased uptake of ions mainly Na and Cl under salinity. Three major hazards associated with salinity are; osmotic (water) stress arising from more negative osmotic potential (higher osmotic pressure) of the rooting medium, specific ion toxicity– excess of Na^+, Cl^-, SO_4^{-2} or other ions, and nutritional imbalance. Rice is an important stable food crop and two-third of world population depends on it, however due to adverse biotic, abiotic and soil factors, the productivity is declining and unable to meet out for growing population. So, the possible ways are reclamation of soil and breeding new varieties suitable for saline soils, however reclamation needs more financial needs, laborious man power and not always practically feasible. The other possible strategy is breeding to enhance salinity tolerance, but it has been slow due to limited knowledge about the genetics of salt tolerance, inadequate screening techniques, low selection efficiency and poor GxE interactions.

Generally, salinity tolerance is a polygenic trait. Screening rice germplasms to locate salt-tolerant genes for use in improving the currently grown varieties is of continuous importance to plant biotechnologists (Flowers 2004). Integration of breeding with recent marker assisted selection technology; it is now feasible to analyze simply inherited and quantitative traits at early seedling stage, which fasten the breeding program. Molecular markers can now be used to tag quantitative trait loci and to evaluate their contribution to the phenotype by selecting favorable alleles at these loci using Marker-Assisted Selection (MAS) to accelerate genetic improvement. DNA based molecular markers have been used extensively to assess the genetic diversity in crops. Among the marker technology, microsatellite markers have been effectively used to identify genetic variation among rice cultivars (Garland *et al*. 1999). They can be easily amplified by PCR reaction using DNA nucleotide primers. This study focused on the rice genotypes to analyze the morpho-physiological attributes with Na^+, K^+ and Na^+/K^+ ratio utilizing microsatellite markers. Salinity tolerance levels of parental lines were assessed to identify salt tolerant ones for future development of salt tolerant hybrids.

MATERIALS AND METHODS

Seeds of the 26 parental lines of hybrids (collected from IRRI) were sown on raised beds for raising nursery for their transplantation in microplots at the Central Soil Salinity Research Institute, India to study whole plant salinity tolerance. The nursery seedlings were transplanted in three replications in microplots on 1.9.2008. The experiment was assigned as RCBD with three replications. Recommended doses of fertilizers were applied and other cultural practices were followed as and when necessary. The saline irrigation treatments of EC 6 and 10 dS/m besides control were imposed on 14.10.2008. Na^+ and K^+ contents were estimated from flag leaves of selected 8 parental lines (4 from apparently tolerant and 4 from susceptible ones on the basis of vigor score) of

hybrids under different treatments. Sodium and potassium content was assessed in triple acid (9:2:1 parts of HNO_3:H_2SO_4:$HCLO_4$) extract using Flame Photometer and expressed as mg g^{-1} on weight basis (Jackson 1964). Data on morphological attributes and yield of rice genotypes were also collected. Statistical analysis was done as per design used and DMRT was used to compare means.

DNA Extraction

Leaf samples were collected from 30 days old plants of rice genotypes, grown at the Central Soil Salinity Research Institute, India in 2008. Around 5-8 young tender leaves from each genotype were collected in an ice box and the collected leaf samples were used to extract DNA following the CTAB method (Dellaporta *et al*. 1983). Prior to extraction, the pestle and mortar, spatula and scissors were sterilized by autoclaving. Two grams of leaves were cut into bits with the help of sterile scissors and transferred to mortar. The leaf tissues were frozen in liquid nitrogen and ground into fine powder. The fine powder was allowed to thaw in the presence of 15 mL of pre-heated CTAB extraction buffer containing β mercaptoethanol, in polypropylene centrifuge tubes and incubated for 45 min. at 65°C with occasional mixing. The tubes were removed from the water bath and allowed to cool at room temperature. Equal volume of chloroform and isoamyl alcohol mixture (24:1) was added and mixed by inversion for 15 min. It was centrifuged at 4000 rpm for 20 min in room temperature. The clear aqueous phase was transferred to a new sterile tube. One third volume of ice-cold isopropanol was added and mixed gently by inversion until DNA was precipitated out and incubate it at 20°C for 6 h. Then, it was centrifuged at 4000 rpm for 20 min in room temperature to pellet the DNA and the supernatant was discarded. The DNA pellet was washed with 70% alcohol. The DNA was air dried after washing with 70% alcohol. Depending upon the pellet size the DNA was dissolved in 200-500 µL of TE buffer (pH 8.0). To eliminate the contaminated RNA from DNA one-tenth volume of RNase (10 mg mL^{-1}) was added to DNA sample and incubated at 37°C for 30 min. Equal volume of chloroform: isoamyl alcohol mixture (500 µL) was added and mixed thoroughly by repeated inversions. The mixture was centrifuged at 12000 rpm for 10 min at 4°C and the aqueous phase was transferred to another micro centrifuge tube without disturbing the inner phase. Two volume of absolute alcohol and 1/10 volume of 3 M sodium acetate were added and incubated at -20°C overnight. Then, it was centrifuged to pellet the DNA and the supernatant was discarded. The pellet was washed twice with 70% ethanol. The alcohol was discarded and DNA was air dried completely. Depending upon the size of the pellet, DNA was dissolved in 250-500 µL of TE (pH 8.0) and stored at 4°C. Primers ISSR HB-10 and ISSR 844 A were used in this study.

RESULTS

Results revealed that salinity increased Na^+, decreased K^+ and K^+/Na^+ ratio in flag leaves (Table 1). The parental line BCW showed the lowest Na and the highest K and K^+/Na^+ ratio in flag leaves. Plant height, panicle length, 100-grain weight and biological yield decreased with increasing salinity (Table 2). Number of tillers and panicles/plant and grain yield decreased similarly by the salinity of 6 and 10 dS/m. Number of dry leaves increased with the increase in salinity although total number of leaves were not affected by the salinity levels. The pattern of DNA (Fig.1, 2, 3 and 4) of the rice genotypes did not show much difference. IR60997-16-2-3-2-2R, IR29723, BR827-35, BCW-56, KMR-3, IR80155B and CSR23 performed better under salinity compared to other genotypes.

DISCUSSION

Salinity increased Na^+, decreased K^+ and K^+/Na^+ ratio in rice leaves are in conformity with those of many authors (Hakim *et al*. 2005; Islam and Salam, 1997; Islam *et al*. 1995; Islam *et al*. 1998 and Sen *et al*. 2004). Rice plants tolerate salinity stress by 3 mechanisms acting upon singly or jointly (Munns *et al*. 2008; Islam 2009). These mechanisms are Exclusion, Dilusion, Compartmentalization or maintenance of high K^+/Na^+ ratio. (i) Exclusion: This refers to the restricted uptake of Na ions by tolerant rice varieties. (ii) Dilution effect: Usually the tolerant varieties grow faster than non tolerant varieties under saline condition. It was experimentally found that lower shoot Na content in Pokkali variety is not due to any better control of Na^+ transport by its roots but is directly attribute to the dilution effect of its rapid vegetative growth. (iii) Compartmentalization: When a rice plant is exposed to saline condition the older leaves die due to high amount of Na^+ accumulation while younger ones remain green and growing. This physiological behavior of rice plant is called compartmentalization which is a useful feature of gramineae. High K^+/ Na^+ ratio: It is now established that salt tolerant varieties maintain a higher K^+/Na^+ ratio compared to that in non-tolerant variety (Senguttuvel *et al*. 2010). Relatively higher amount of K^+ than Na^+ ions is probably required in panicles for the protection of growing panicles from the toxic effect of Na ion. The decrease in plant height, number of tillers and panicles/plant, panicle length, 100-grain weight, biological yield and grain yield of rice under salinity are in agreement with those of Islam *et al*. 1996, 2005. The decrease in these parameters depends mainly on salinity levels, varieties and stages of salinity imposed. The parental lines of hybrids showed similar patter of DNA, however, showed significant differences in morpho-physiological attributes and Na^+, K^+ content and K^+/Na^+

ratio. These results indicate that QTLs (Quantitative Trait Locci) for salt tolerance of these parental lines of hybrids may not tightly link and their tolerance to salinity expressed in respect to morpho-physiological attributes and K^+/Na^+ ratio.

Table 1. Na^+, K^+ and K^+/Na^+ ratio in flag leaves of rice genotypes under different salinity levels

Treatment	Na^+ (mg/gfwt)	K^+ (mg/gfwt)	K^+/Na^+ ratio
Control	2.34 c	3.11 a	3.91 a
6 dS/m	15.20 b	1.67 c	0.29 b
10 dS/m	19.30 a	1.87 b	0.25 b
Genotypes			
IR60997-16-2-3-2-2R	7.71 e	1.95 e	1.44 bc
BCW-56	1.53 g	3.99 a	6.49 a
IR80151B	5.00 f	2.00 e	1.33 c
IR80155B	7.74 e	1.17 g	0.45 d
IR62037-12-1-2-2R	18.90 c	2.12 d	0.17 e
DRR-6B	22.98 a	1.82 f	0.14 e
DRR-4B	19.33 b	2.21 c	0.27 e
VSR156	15.04 d	2.51 b	0.56 b

Figures having common letters in a column do not differ significantly at 5% level by DMRT

Table 2. Morphological and yield attributes of rice genotypes under different soil salinity levels in micro plots of Central Soil Salinity Research Institute, India during 2008

Salinity levels	Plant height (cm)	Leaves /plant (No.)	Dry leaves /plant (No.)	Tillers /plant (No.)	Panicles /plant (No.)	Panicle length (cm)	100-grain weight (g)	Biologi-cal yield (g) /2 m line	Grain yield/ (g) /2m line	Vigor score
Control	77.8 a	20.1 a	6.8 b	5.8 a	4.5 a	20.8 a	1.58 a	76 a	11.98 a	1
6 dS/m	68.9 b	20.1 a	11.5 a	5.1 b	3.2 b	17.1 b	1.14 b	62 b	3.95 b	4
10 dS/m	66.1 c	20.0 a	12.6 a	5.4 b	3.5 b	3.3 c	1.01 c	46 c	2.42 b	4
Genotypes										
IR56381-139-2-2R	64.7 i-l	17.2 d-g	13.6 a	5.2 c-f	4.7 a	13.1 c-f	1.39 a-e	57 d-h	8.24 b-e	3
IR60997-16-2-3-2-2R	68.0 g-j	20.2 b-f	10.4 a-f	6.2 a-d	4.4 a-d	14.3 a-d	1.42 a-d	85 ab	14.86 a	2
IR62037-12-1-2-2R	63.4 j-m	17.6 d-g	10.7 a-e	6.0 a-d	3.3 e-h	14.0 b-e	1.18 c-g	48 e-i	5.45 def	4
IR29723	73.5 efg	26.0 abc	10.9 a-e	7.2 a	2.6 h	14.0 b-e	1.14 d-h	93 a	1.47 ef	2
IR62653-8-3-3R	75.1 c-f	16.2 fg	11.3 a-e	4.2 fgh	3.6 b-h	13.6 b-e	1.22 c-g	47 e-i	6.37 c-f	3
IR63879-195-2-2-3-2R	70.1 f-i	18.7 c-f	11.7 a-d	5.0 d-h	4.2 a-e	13.7 b-e	1.11 d-h	60 d-g	5.53 def	3
BR827-35	77.5 b-e	27.8 a	10.0 a-f	6.5 abc	4.0 a-f	13.4 b-e	1.47 abc	100 a	12.29 abc	1
IR65622-151-1-2-2-2R	66.6 h-k	19.0 c-f	12.3 a-d	5.0 d-h	3.6 b-h	13.5 b-e	1.35 a-e	40 f-i	3.69 def	3
IR46	76.3 b-f	22.5 a-f	9.7 a-f	5.9 a-d	4.0 a-e	14.0 b-e	1.22 c-g	78 a-d	4.66 def	3
IR63875-196-2-2-1-3R	68.5 g-j	19.5 b-f	11.3 a-e	5.1 d-g	4.2 a-e	14.8 a-d	0.99 fgh	48 e-i	4.57 def	4
IR69702-52-3-3R	74.0 d-g	26.6 ab	9.6 b-f	6.7 ab	4.2 a-e	13.6 b-e	1.08 e-h	84 abc	6.20 c-f	2
1096	80.2 bcd	19.3 c-f	9.0 def	5.1 d-g	3.5 b-h	14.1 a-e	1.37 a-e	67 b-e	5.54 def	3
BCW-56	91.3 a	23.9 a-e	7.4 ef	5.0 d-h	3.5 c-h	13.7 b-e	1.63 a	91 a	12.20 abc	2
KMR-3	82.1 b	24.4 a-d	8.9 def	5.6 b-e	3.7 a-g	15.7 ab	1.28 b-f	83 abc	7.03 c-f	1
IR80151B	76.7 b-e	20.8 a-f	10.8 a-e	5.8 b-e	4.5 ab	14.4 a-d	1.33 a-e	60 d-g	6.92 c-f	2
IR80154B	70.1 f-i	21.7 a-f	8.7 def	6.0 a-d	4.7 a	15.2 abc	1.40 a-d	53 e-i	4.74 def	3
IR80155B	72.3 e-h	15.8 fg	8.4 def	4.6 e-h	4.2 a-e	14.8 a-d	1.34 a-e	65 b-e	7.67 c-f	1
IR80555B	6.4 klm	20.2 b-f	12.3 a-d	5.6 b-e	3.9 a-f	12.6 def	0.96 gh	40 f-i	3.05 def	3
IR80559B	73.9 d-g	18.6 def	10.1 a-f	6.1 a-d	4.4 abc	14.2 a-d	1.30 b-e	63 c-f	8.83 a-d	3
DRR-4B	54.4 n	19.9 b-f	13.0 abc	5.2 c- f	2.7 h	10.9 f	0.86 h	38 ghi	2.33 def	5
DRR-6B	58.0 mn	18.6 def	13.6 ab	5.0 d-h	3.0 fgh	12.4 def	0.92 gh	32 i	0.81 f	5
DRR-9B	67.4 g-k	18.3 def	8.5 def	5.0 d-h	3.5 b-h	12.6 def	1.19 c-g	62 c-f	3.92 def	3
DRR-10B	63.8 i-m	19.8 b-f	9.3 c-f	5.6 b-e	3.4 d-h	12.7 c-f	0.94 gh	50 e-i	4.14 def	3
DRR-13B	59.2 lmn	16.8 efg	9.6 b-f	3.9 gh	2.7 gh	11.6 ef	1.12 d-h	34 hi	2.26 def	4
CSR23	75.0 c-f	22.0 a-f	10.8 a-e	6.0 a-d	4.1 a-e	16.5 a	1.49 abc	87 ab	14.08 ab	2
VSR156	81.4 bc	10.9 g	6.6 f	3.8 h	2.7 h	13.2 b-f	1.57 ab	38 ghi	2.08 def	6

Figures having common letters in a column do not differ significantly at 5% level by DMRT

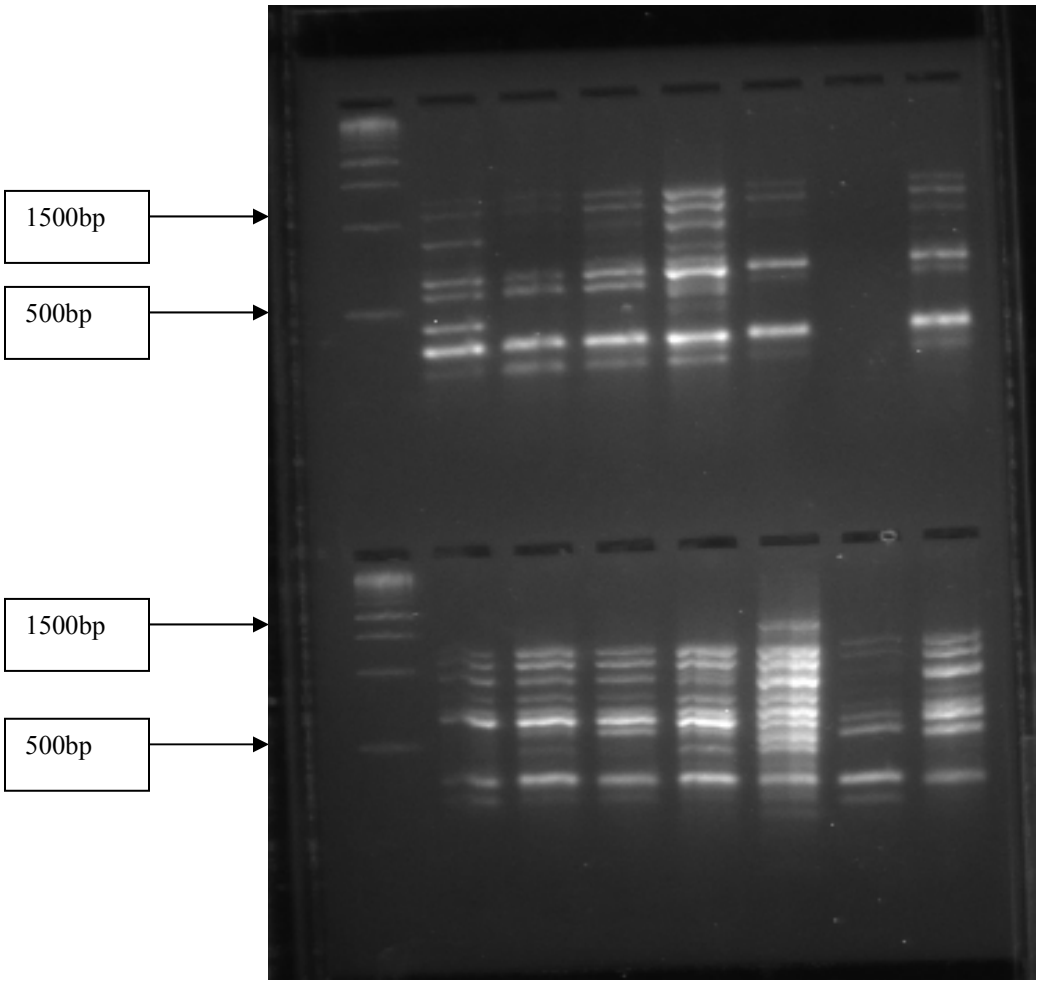

Fig. 1. ISSR profile for rice genus generated by Primer ISSR HB-10:-Lane 1: 500BP DNA Ladder, Lane 2: IR 56381-139-2-2R, Lane 3: IR 60997-16-2-3-2-2R, Lane 4: IR 62037-12-1-2-2R, Lane 5: IR 29723, Lane 6: IR 62653-8-3-3R, Lane 7: IR 66, Lane 8: IR 63879-195-2-2-3-2R, Lane 9: BR 827-35, Lane 10: IR 65622-151-1-2-2-2R, Lane 11: IR 46, Lane 12: IR 63875-196-2-2-1-3R, Lane 13: IR 69702-52-3-3R, Lane 14: 1005

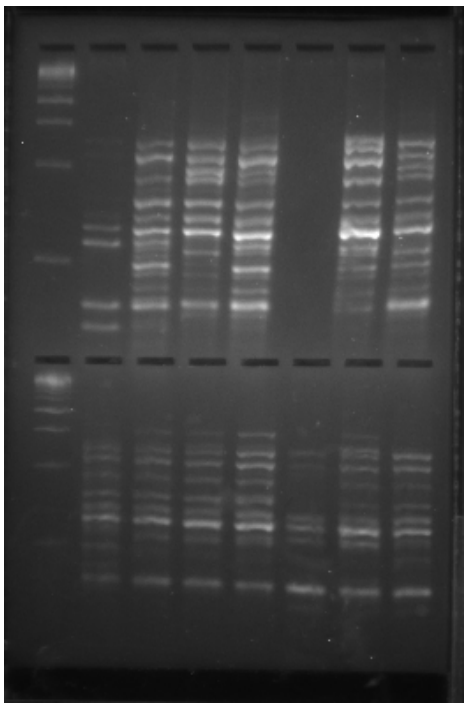

Fig. 2. ISSR profile for rice genus generated by Primer ISSR HB-10:-Lane 1: 500BP DNA Ladder, Lane 2: BCW-56, Lane 3: KMR-3, Lane 4: IR 80151B, Lane 5: IR 80154B, Lane 6: IR 80155B, Lane7: IR 80555B, Lane 8: IR 80559B, Lane 9: IR 80561B, Lane 10: DRR-4B, Lane 11: DRR-6B, Lane 12: DRR-9B, Lane 13: : DRR-10B, Lane14: DRR -13B

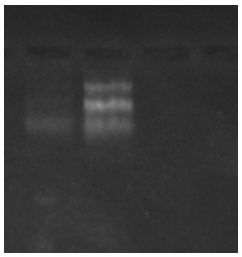

Fig. 3. ISSR profile for rice genus generated by Primer ISSR HB-10:- Lane 1: VSR156, Lane 2: CSR 36

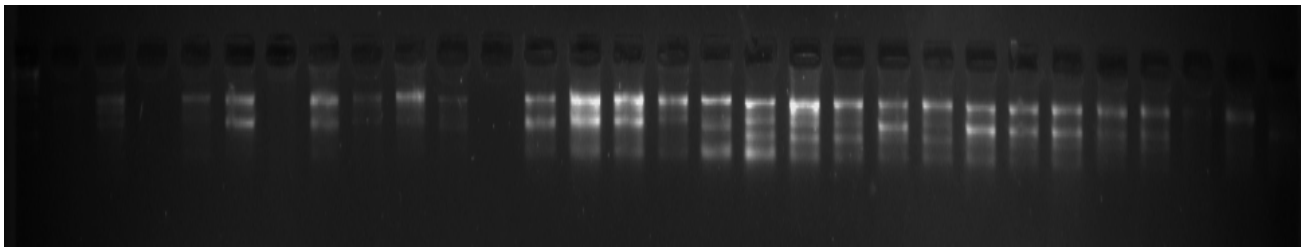

Fig. 4. ISSR profile for rice genus generated by Primer ISSR 844 A:-Lane 1: IR 56381-139-2-2R, Lane 2: IR 60997-16-2-3-2-2R, Lane 3: IR 62037-12-1-2-2R, Lane 4: IR 29723, Lane 5: IR 62653-8-3-3R, Lane 6: IR 66, Lane7: IR 63879-195-2-2-3-2R, Lane 8: BR 827-35, Lane 9: IR 65622-151-1-2-2-2R, Lane 10: IR 46, Lane 11: IR 63875-196-2-2-1-3R, Lane 12: IR 69702-52-3-3R, Lane 13: 1005, Lane14: 1096, Lane 15: BCW-56, Lane 16: KMR-3, Lane 17: IR 80151B, Lane 18: IR 80154B, Lane 19: IR 80155B, Lane 20: IR 80555B, Lane 21: IR 80559B, Lane 22: IR 80561B, Lane 23: DRR-4B, Lane 24: DRR-6B, Lane 25: DRR-9B, Lane 26: DRR-10B, Lane 27: DRR -13B, Lane 28: CSR 23, Lane 29: VSR156, Lane 30: CSR 36

CONCLUSION

The parental lines viz. IR60997-16-2-3-2-2R, IR29723, BR827-35, BCW-56, KMR-3, IR80155B and CSR23 may be preliminary selected for the development of salt tolerant rice hybrids. Quantification of DNA, SSR and SSR-PCR analysis, Marker and Morphological Trait Association of these lines may be studied in future.

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REFERENCES

Ashraf M, O'Leary JW (1996) Responses of newly developed salt tolerant genotype of spring wheat to salt stress: Yield components and ion distribution. *Agron. Crop Sci*., 176, 91-101.

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. *Plant Mol. Biol*. Rep., 1, 19-21.

Flowers TJ (2004) Improving crop salt tolerance. *J. Exp. Bot*., 55, 307-319.

Gregario GB, Senadhira D, Mendoza RD (1997) Screening rice for salinity tolerance. IRRI, Manila, Philippines.

Hakim MA, Islam MT, Fakir MSA, Mia MJ, Rahman MM (2005) Effect of salinity on salt ion concentration in plant tissue, yield and yield attributes of boro rice. *J. Bangladesh Soc. Agril. Sci. and Technol*., 2(1 & 2), 85-88.

Islam MT (2009) Physiological basis of salt tolerance in crop plants and salinity management strategies. *Bangladesh Soc. Agric. Sci. Technol*., 6 (1 & 2), 185-190.

Islam MT, Salam MA (1996) Growth and yield performances of some selected rice lines under different salinity regimes. *Bangladesh. J. Train. and Devt*., 9(2), 53-56.

Islam MT, Salam MA (1997) Salinity tolerance studies in some rice lines/varieties. *Bangladesh J. Sci. Res.*, 15(1), 163-166.

Islam MT, Rashid MA, Razzaque AHM (2005) Effect of salinity at reproductive stages of transplanted aman rice mutants. *Progress. Agric*., 16(2), 7-9.

Islam MT, Salam MA, Dutta RK (1998) Salinity tolerance in rice lines and their production potential. *Bangladesh J. Nuclear Agric*., 14, 63-69.

Islam MT, Salam MA, Badruddin M, Dutta RK (1995) Effect of salinity stress on the ionic balance and tolerance of rice genotypes. *Bangladesh J. Nuclear Agric*., 11, 59-66.

Jackson ML (1964) Soil chemical analysis. Prentice Hall, India.

Jena KK, Mackill DJ (2008) Molecular markers and their use in Marker-Assisted Selection in rice. *Crop Sci*., 48, 1266-1276.

Munns R, James RA, Lauchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot*., 57, 1025-1043.

Sen AK, Islam MT, Islam MO (2004) Effect of salinity stress on ion uptake and yield attributes in some rice cultivars. *Bangladesh J. Nuclear Agric*., 19 & 20, 57-63.

Senguttuvel P, Raveendran M, Vijayalakshmi C, Thiyagarajan K, Bapu JRK, Viraktamath BC (2010) Molecular mechanism of salt tolerance for genetic diversity analysed in association with Na^+/K^+ ratio through SSR markers in rice (*Oryza sativa* L.). *Int. J. Agric. Res*., 5, 708-719.