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Short Communication

Phenotyping and QTL-linked marker-based genotyping of rice lines with varying level of salt tolerance at flowering stage

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Abstract

Rice is sensitive to salinity stress at early seedling and reproductive stages. Surprisingly, major QTL/gene responsible for salinity tolerance at flowering stage is yet to be identified, mainly due to unavailability of suitable donors. In this investigation, a set of eight genotypes were phenotyped for grain yield and various yield attributing traits as influenced by salt-stress (EC= 8 dS m^{-1}) at flowering stage and subsequently genotyped by using microsatellite markers associated with known QTLs for salt tolerance in rice. One Pokkali and two Chettivirippu accessions were found tolerant to moderately tolerant. These genotypes were genetically distant from the susceptible variety, Swarna indicating the scope of deducing novel QTLs for salt-tolerance at flowering stage.

Key words: *Oryza sativa*, salinity tolerance, reproductive stage, QTL, microsatellite markers

Various yield components such as panicle weight, panicle length, spikelets per panicle, primary branches per panicle, number of tillers per plant and harvest index are sensitive to salinity stress induced at flowering stage in rice [1, 2]. However, it is difficult to screen genotypes at reproductive stage for highly quantitative nature of component traits collectively responsible for salinity tolerance. Therefore, phenotypic assessment should necessary be complemented by DNA markers in the identification and characterization of genetic variation [1]. Apart from QTLs detected for salt-tolerance at late vegetative stage [3, 4], a few reports are also available about QTLs for salt-tolerance at reproductive stage [5, 6]. But SSR markers associated with these QTLs are yet to be validated across the population. Hence, an investigation was undertaken to assess genetic diversity of rice genotypes at reproductive stage based on agro-morphological traits and polymorphism of microsatellite markers associated with QTLs to advocate appropriate parental combination in breeding program for incorporating salt tolerance at flowering stage in elite rice cultivars.

One month old seedlings from four accessions of Chettivirippu [(AC39388 (1), AC39389 (2), AC39394 (3)] and Pokkali (AC41585) from Kerala, two landraces Talmugur and Kamini from Sundarban area of West Bengal, an improved breeding line IRRI147 from IRRI, Philippines and high yielding Indian cultivar, Swarna (MTU 7029) were planted in perforated pots filled with fertilized soil and salinity stress was imposed before booting stage (EC = 8 dSm^{-1}). Analysis of variances (ANOVA) for important agro-morphological traits were done and graphical presentations were made for each trait to compare significant changes in phenotypes under salt stress with the help of SPSS 11.0.

DNA was extracted from seven days old young leaves following manufacturer guidelines using QIAGEN extraction kit (QUAGEN, USA). Based on the earlier reports [3-6] twenty two SSR primers (RM562, RM8231, RM543, RM212, RM8094, RM5699, RM262, RM145, RM7000, RM416, RM1022, RM3827, RM340, RM162, RM11, RM5481, RM125, RM209, RM287, RM206, RM224, RM229) associated with QTLs

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for salt tolerance at reproductive and late vegetative stages located on chromosomes 1, 3, 6, 7 and 11 were chosen for the study. PCR reaction was carried out, DNA bands were visualized in UV and polymorphic Information Content (PIC) values for each primer were calculated. Standardized phenotypic data were used to construct Dist distance coefficients matrix whereas, binary data derived from PCR-assay were employed to construct Jaccard's similarity coefficients matrix. Matrices were compared using Mantel matrix-correspondence test and dendrograms from each data set were generated by Unweighted Pair-Group Method of Arithmetic mean (UPGMA) algorithm using SHAN sub-programme of NTSYS-pc Version 2.1 software.

At the end of the experiment it was found that 'Kamini' didn't flower. Under saline condition, except pollen sterility all parameters were significantly differed. Similarly, under non-saline condition except spikelet sterility, all traits were found significant. Salinity stress at flowering stage, therefore, had significant impact on seed yield and important component traits. Plant height decreased significantly in all the varieties irrespective of their tolerant reaction under stress. This trait was found negatively (r = –0.44) contributed with seed yield in non-salinized condition, while its contribution was significantly positive (r = 0.52) under salinized condition. Significant effect of salinity on plant growth at all the stages were experimentally proof by the previous workers [7]. In the present experiment the comparatively less effect of salt stress on plant growth in Pokkali (Fig. 1) certainly helped in attaining better tolerance as revealed by less yield reduction. On the other hand, non-significant effect of salinity stress on pollen sterility was in disagreement with earlier report [8]. Panicle emergence was generally delayed by salinity. In the present experiment, lowest increment in days to flowering was noted in Pokkali, indicating least effect of salinity stress on this entry in respect of panicle initiation. In contrary to non-salinized condition, in salinized setup it was observed that panicle length (r = 0.98) and number of spikelets/panicle (r = 0.78) were main significant contributing factors to grain yield, while spikelet sterility (r = –0.98) had major significant negative effect on grain yield. Previous investigation [9] revealed that spikelet number/panicle and tiller number/plant were significantly reduced by salinity. In the present investigation, all entries except Pokkali had reduced panicle length and spikelet numbers/panicle under stress condition. No significant change in number of spikelets/panicle was observed in Chettivirippu-2. Though mean spikelet sterility was increased significantly under stress, Pokkali, IRRI147 and Chettivirippu-2 did not show any significant changes. Highest grain yield-decline was observed in Swarna

Table 1. Polymorphic and informative microsatellite primers associated with QTLs for salt tolerance employed in assessing rice germplasm

S.No.	Primer name	Chromosome number	Associated traits to salt-tolerance*	No. of alleles	Range of band position (bp)	PIC value
1	RM8231	1	Number of unfilled grain Na+ uptake [4, 5]	2	190-211	0.65
2	RM212	1	Shoot Na-K ratio Na-K ratio in shoot at vegetative stage [6]	3	125-150	0.76
3	RM7000	3	Shoot length/plant height [4, 5]	5	135-250	0.84
4	RM1022	3	Green leaf area, K+ uptake, panicle length [4, 5]	2	135-150	0.74
5	RM340	6	Na-K uptake ratio, number of branches [4, 5]	3	110-170	0.88
6	RM11	7	Number of filled grain [5]	2	125-140	0.62
7	RM5481	7	Number of filled grain [5]	2	163-170	0.73
8	RM125	7	Dry weight of shoot [4]	2	120-127	0.34
9	RM209	11	Fresh weight of shoot, relative growth rate [3, 6]	2	134-144	0.13
10	RM206	11	Fresh weight of shoot, Na-K ratio in shoot at vegetative stage [3, 6]	2	147-156	0.65
11	RM224	11	Fresh weight of shoot [3]	2	140-155	0.70
12	RM229	11	Fresh weight of shoot [3]	2	116-120	0.70

*No. in parentheses indicate Reference cited

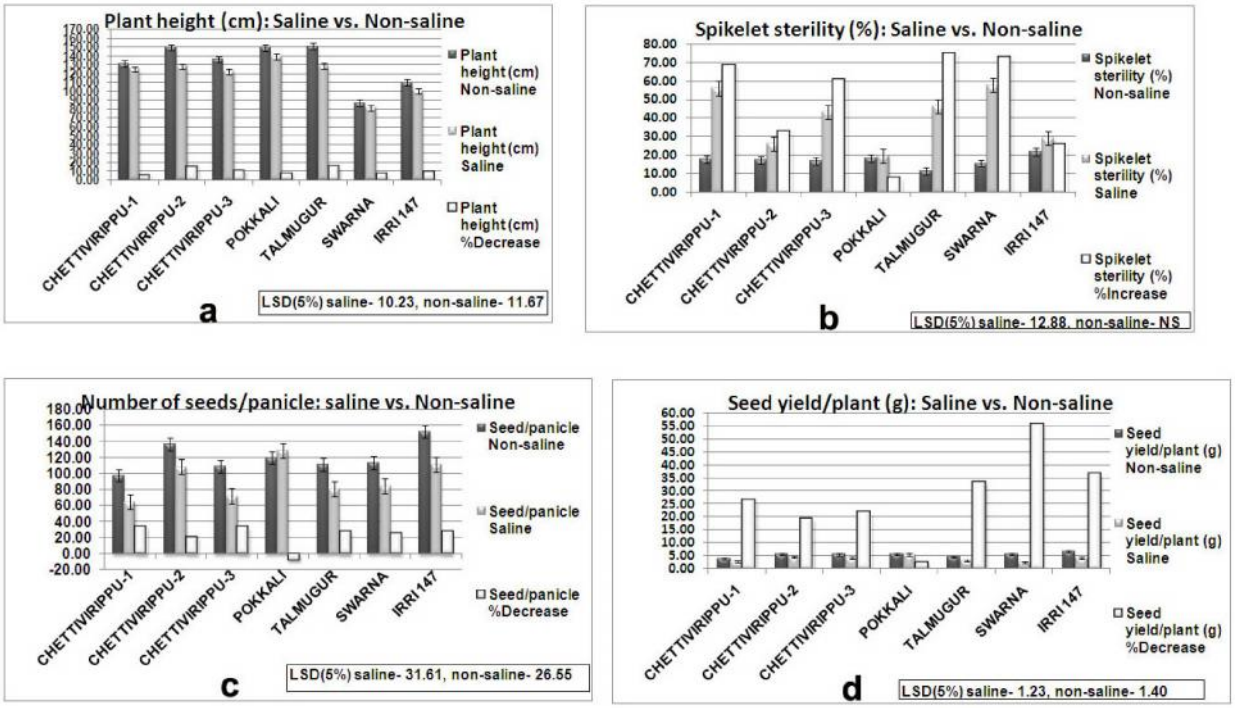

Fig. 1. Graphical representation of comparative performance of seven varieties based on: a. plant height, b. spikelet sterility, c. number of seeds/panicle and d. seed yield/plants under salinity stress situation

while least was recorded in Pokkali. Yield decline was also not statistically significant for Chettivirippu-2, followed by Chettivirippu-3 and Chettivirippu-1 (Fig. 1). In the present investigation, Pokkali could able to satisfy the most important component traits collectively responsible for salt-tolerance at flowering stage. Chettivirippu-2 and Chettivirippu-3 were also found moderately tolerant for this stage. UPGMA dendrogram (Fig. 2a) based on phenotypic data did not able to classify genotypes entirely based on their tolerance reaction. This could only identify distantly located tolerant and susceptible pairs such as Pokkali-Swarna (Dist coefficient = 1.90) and Chettivirippu-2-Swarna (Dist coefficient = 1.67).

Under present set of investigations we had employed polymorphic primers (Table 1) which were able to deliver clear and reproducible banding pattern in all assays. RM340 and RM7000 were found most informative on the basis of higher PIC values. A total of 102 bands were distributed in 29 amplicons. Except two, all of them were polymorphic. The highest similarity was observed between Pokkali and Chettivirippu-3 (0.59) and the least in genotype pairs such as Talmugur- Chattivirippu-2 (0.27), Chettivirippu-2-Swarna (0.32) and Pokkali-Swarna (0.32). Salt-tolerant and moderately tolerant genotypes were consolidated in a single cluster of the UPGMA dendrogram (Fig. 2b). This observation indicated the overall usefulness of these markers in identification of the tolerant genotypes. Salt tolerant and susceptible pairs such as Chettivirippu-2-Swarna and Pokkali-Swarna were identified as genetically distant based on polymorphism of microsatellite markers as well as diversity in yield and yield attributing traits under both stress and non-stress conditions. Amplicons identified in Pokkali were 136bp (RM212), 170bp (RM7000), 150bp (RM1022), 165bp (RM340), 125bp (RM11), 156 bp (RM206) and 120 bp (RM229). Among them a unique band (125 bp) was given by RM11 in Pokkali and a 150bp band given by RM1022 was only associated with Pokkali and Chettivirippu. Further marker-phenotype association analysis was done. Phenotypic data combining percent of reduction of plant yield and plant height and increase of spikelet sterility under salinity stress as compared to non-stress showed significant positive rank correlation with separate binary data sets delivered by RM7000 and RM229 [3, 5]. Therefore, these SSR markers could be tested further for their association with tolerance in a large number of germplasm set or in mapping population derived from tolerant parent Pokkali.

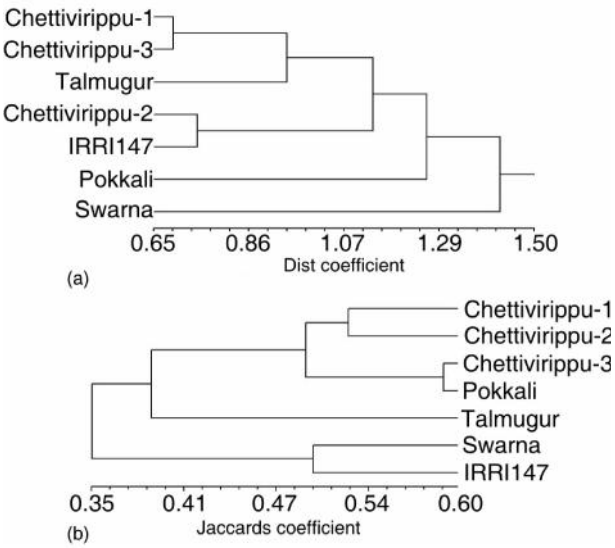

Fig. 2. Dendrograms derived by UPGMA method showing clustering among seven rice genotypes based on a) phenotypic variation and b) microsatellite polymorphism

A few studies revealed that salinity tolerance in rice genotypes was same in seedling stage, vegetative stage and reproductive stage, affecting more the yield and yield components at the flowering stage [10]. For developing mapping population for QTL analysis for salt tolerance the same Pokkali and Cherivirippu accessions were used as tolerant sources for both the stages [8]. The present experiment also pointed towards the fact that although separate set of genes/ QTLs might be responsible for seedling and reproductive stage salt-tolerance, they could be detected from the same genotype.

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References

1. **Zeng L., Kwon T. R., Xuan L., Wilson, C., Grieve, C. M. and Gregorio G. B.** 2004. Genetic diversity analysis by microsatellite markers among rice genotypes with different adaptation to saline soils. Plant Sci., **166**: 1275-1258.
2. **Surekha R., Mishra P. B., Gupta S. R. and Rathore A.** 2008. Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. Plant Breed., **127**: 256-261.
3. **Zang J., Sun Y., Wang Y., Yang J., Fang L. I., Zhou Y. L., Zhu L. H., Jessica R., Fotokian M., Jianlong X. U. and Zhikang L. I.** 2008. Dissection of genetic overlap of salt tolerance QTLs at the seedling and tillering stages using backcross introgression lines in rice. Sci. China, C, Life Sci., **51:** 583-591.
4. **Sabouri H. and Sabouri A.** 2008. New evidence of QTLs attributed to salinity tolerance in rice. Afr. J. Biotechnol., **7**: 4376-4383.
5. **Sabouri H. and Biabani A.** 2009. Towards the mapping of agronomic characters on a rice genetic map: quantitative trait loci analysis under saline condition. Biotechnol., **8**: 144-149.
6. **Rana P., Jain S.,Yadav S., Saini N. and Jain R. K.** 2009. Identification of SSR markers for salt-tolerance in rice variety CSR10 by selective genotyping. J. Plant Biochem. Biotechnol., **18**: 87-91.
7. **Mansuri S.M., Jelodar N. B. and Bagheri N.** 2012. Evaluation of rice genotypes to salt stress in different growth stages via phenotypic and random amplified polymorphic DNA (RAPD) marker assisted selection. Afr. J. Biotechnol., **11**: 9362-9372.
8. **Hossain H., Rahman M., Akhlasur M., Alam M.S., Sajise A. G., Vispo A. N., Thomson M., Ismail A., Gregorio G. and Singh R. K.** 2010. Identification of novel QTLs for salinity tolerance at reproductive stage in rice. *In*: Proc. Third International Rice Congress, Hanoi, Vietnam, 8-12 November, 2010.
9. **Zeng L., Shannon M. C. and Grieve C. M.** 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. Euphytica, **127**: 235-245.
10. **Blouch W. A., Soomro A. M., Javed M. A., Bughio H. R., Alam S. M., Bughio M. S., Mohammad T. and Mastio N. N.** 2003. Induction of salt tolerance on rice through mutation breeding. Asian J. Plant Sci., **2**: 273-276.