Indian J. Genet., 74(2): 243-247 (2014)
DOI: 10.5958/0975-6906.2014.00167.9

Short Communication

Assessment of genetic diversity in rice genotypes for salinity tolerance using *Saltol* markers of Chromosome 1

S. L. Krishnamurthy*, S. K. Sharma, V. Kumar, S. Tiwari[1], V. Batra and N. K. Singh[1]

Division of Crop Improvement, Central Soil Salinity Research Institute, Karnal 132 001; [1]National Research Center on Plant Biotechnology, Pusa Campus, New Delhi 110 012

(Received: June 2013; Revised: February 2014; Accepted: March 2014)

Abstract

The present study was conducted to assess the genetic diversity using *Saltol* markers among 57 rice genotypes. The material was screened in hydroponics using salinized (EC~10 dS/m) nutrient solution. Based on seedling stage salinity tolerance, the genotypes were grouped as highly tolerant, tolerant, moderately tolerant, sensitive and highly sensitive. Eight out of 21 SSR markers used were polymorphic. The PIC values ranged from 0.52 to 0.82 with an average of 0.74. The genotypes namely, FL 478, Pokkali, Trichi 1 and Trichi 2 were grouped in cluster I and were highly salinity tolerant. Clusters II, III, IV and VIII had single genotype each, which showed moderate tolerance to salinity stress. The genotypes in cluster VII exhibited moderate to high tolerance, while cluster VI had genotypes with mixed response to salinity. Salt tolerance in IR 64 could be improved by inter-crossing with salt-tolerant genotypes. Information generated from the present study would be useful in selection of parents and for marker-aided salinity breeding programs in rice.

Key words: *Oryza sativa*, *Saltol* markers, genetic diversity

Rice (*Oryza sativa* L.) is a one of the most important cereal crops and serves as the staple food for over one-third of the world's population [1]. The tremendous variation for salt tolerance within *Oryza* species [2] provide opportunities to improve rice for salt-stress tolerance through genetic means. The response of rice to salinity varies with growth stage. Rice is very sensitive during early seedling stage (2-3 leaf stage), pollination and fertilization [3]. Screening of germplasm at seedling stage is readily acceptable as it is based on a simple criterion of selection as the rapid screening is difficult at vegetative and reproductive stages. Breeding for salinity tolerance requires selection of parents with a wider genetic diversity. A narrow genetic base in breeding materials limits genetic gains in breeding, hence sufficient knowledge about genetic diversity in the gene pool is a prerequisite to adopt an efficient and variable breeding approach. Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level [4]. The SSR markers have been proved to be an ideal for studying genetic diversity in germplasm [5]. A major *Saltol* QTL was identified on chromosome 1 for salt tolerance in Pokkali [9]. The *Saltol* region flanked with 21 SSR markers. A newly developed line FL478 derived from IR29/Pokkali was used as a novel source of salinity tolerance at seedling stage [6]. The aim of the present study was to screen the collected rice genotypes in salinized hydroponic conditions and to assess the structure of genetic diversity among them using *Saltol* markers of chromosome 1.

A total of 57 genotypes were selected based on the performance for salinity tolerance at seedling stage (previous studies), which included advanced breeding lines, landraces, released varieties and wild species of rice (Table 1). These genotypes were screened for salt tolerance at seedling stage in a hydroponic system using Yoshida nutrient solution [7]. The nutrient solution was salinized by adding NaCl to obtain the desired

*Corresponding author's e-mail: kmurthy@cssri.ernet.in; krishnagene@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

EC (10 dS/m). Salinity (EC ~ 10 dS/m) was induced at the seedling stage (14 days after sowing) and the desired level of salinity was maintained for the next 21 days. The nutrient solution was renewed weekly and the pH maintained. The standard evaluation system (SES) was used in rating from 1-9 based on the visual symptoms of salt toxicity [8]. Score 1-2 indicates highly tolerant, 3-4 tolerant, 5-6 tolerant, 7-8 sensitive and score 9 indicates highly sensitive. Each genotype was scored after three weeks of salinization. Genomic DNA was isolated from leaf samples of 25 days old seedlings by using CTAB method [9]. Twenty one markers located between 10.4 to 15.3 Mb Saltol region [10] on rice chromosome 1 were used to analyze and assess the genetic diversity of the rice genotypes for salt tolerance on chromosome 1 [10]. The map position, original source and repeat motifs for these markers can be found in Rice Genes database (http://www.gramene.org). The Polymerase Chain Reaction (PCR) was conducted in a reaction mixture of 10 µl containing 50ng of template DNA. The polymorphism information content was determined according to Anderson *et al*. [11] based on allele pattern. The 0/1 matrix was used to calculate similarity genetic distance using 'simqual' sub-program of software NTSYS-PC version 2.02i.

The results of screening of genotypes are given in Table 1. Eleven genotypes *viz*., FL 478, BMZ 6, BMZ 10, BMZ 14, Azgo, Heera, IRRISTN-04-75, Panvel-16, Pokkali, Trichi 1 and Trichi 2 had salt injury score of 1 and were rated highly tolerant. Seven genotypes were tolerant, 20 moderately tolerant, 14 sensitive and 5 were highly sensitive. The most salt sensitive genotypes were VSR 156, IR 29, MI 48, Wild 1 and Wild 7, which had injury score of 9 (Table 1). Most of the landraces and traditional varieties cultivated in saline-prone regions were found to be salt tolerant whereas most of the released varieties were moderately tolerant to susceptible. The level of salinity tolerance was in accordance with that determined in previous reports which used similar growing conditions and evaluation system [12, 13].

Only eight out of 21 SSR markers *viz*., RM 10793, RM 10748, RM 3412, RM 493, RM 10852, RM 10713, RM 10871 and RM 10843 showed polymorphism among the genotypes. The highest number of alleles was found in RM 10793 (4), followed by RM 493 and RM 10843 (3) and remaining were 2 alleles each and the average was 2.5. The PIC values ranged from 0.52 (RM 3412) to 0.82 (RM 10843 and RM 10871) with an average of 0.74. The highest PIC value (0.82) was found for the RM 10843 and RM 10871 locus followed by RM 10852 (0.81), RM 10713 (0.78), RM 10793 (0.74), RM 10748 (0.73) and RM 493 (0.71) and RM 3412 (0.52). These results are in accordance with the results of earlier workers [14] who reported polymorphic information content (PIC) values ranging from 0.54 to 0.89. Molecular characterization of germplasm may help in knowing the magnitude of genetic diversity among parental genotypes prior to making crosses. The information generated may also minimize the efforts in the screening either for direct selection in traditional breeding or indirect selection. Another important aspect of the rationale utilizing molecularly characterized germplasm for salt tolerance breeding is that it will lead to know the genetic nature of the genotype with respect to salt tolerance, a complex trait in rice.

The cluster analysis using UPGMA based on Jacquard's similarity coefficient grouped 57 rice genotypes into 8 major clusters with a dissimilarity value of 55% between the clusters (Fig. 1). Similarity coefficient ranged from 0.25-0.99. Ren *et al*. [15] studied 45 accessions of AA-genome *Oryza* species and reported similarity coefficients ranging from 0.36 to 0.96 suggesting a wider range of genetic variability. The genotypes, namely, FL 478, Pokkali, Trichi 1 and Trichi 2 were grouped in cluster I and were highly salinity tolerant and these were from the same geographical origin. The clusters II, III, IV and VIII consisted one genotype each *viz*., Vasumati, Akshaydhan, T 23 and Prasanna, respectively which had moderate tolerance to salinity stress. The cluster V had 11 genotypes and was subdivided into two sub clusters, namely, V (A) and V (B). Sub cluster V (A) had three genotypes *viz*., IR 29, VSR 156 and MI 48 which possessed 64% similarity within the sub cluster. Sub cluster V (B) consisted eight genotypes, namely, HBC 19, Wild 9, Wild 8, Wild 11, Wild 13, Wild 18, Bulk 43 and Wild 10 with 56% similarity within the sub cluster. Highest number of genotypes (36) were grouped in Cluster VI and subdivided into four sub clusters i. e., VI (A), VI (B), VI (C) and VI (D). The sub cluster VI (A) comprised of nine genotypes, (Heera, IR 24, Wild 25, Azgo, Pantdhan 4, Pushpa, P-1176-91-1-2-3, NDR 359 and Bas 385) which showed 53% similarity within the sub cluster. Sub cluster VI (B) had four genotypes (Bundu, IR 84649-275-3-2-B, Wild 3 and Wild 7) with 55% similarity within the sub cluster. Twenty one genotypes showed 55% similarity within the sub cluster VI(C) and 45% dissimilarity with other sub clusters.

Table 1. Pedigree/location/sources and performance of 57 rice genotypes at seedling stage (35 days after sowing including 21 days of salinization) in EC ~ 10 dS/m

S.No.	Genotype	Pedigree/location/sources	Tolerance	Vigour score
1	FL478	IR66946-3R-178-1-1	HT	1-2
2	IR 29	IR833-6-2-1-1///IR1561-149-1///IR24*4/*O. nivara*	HS	9
3	Pushpa	Cultivated variety in India	MT	5-6
4	MI 48	Pelita//H4//H501	HS	9
5	Prasanna	Cultivated variety in India	MT	5-6
6	Akshyadhan	Cultivated variety in India	MT	5-6
7	Vasumati	PR 109/Pakistani Basmati	MT	5-6
8	Pokkali	Kerala India	HT	1-2
9	VSR 156	Not available	HS	9
10	HBC 19	Pureline selection from Basmati	MT	5-6
11	Heera	CR 408-48/CR 289-1208	HT	1-2
12	Trichi 2	IET 6238/IR 36	HT	1-2
13	Trichi 1	IR 578-172-2-2/BR-1-2-B-1	HT	1-2
14	Bundu	Landrace	MT	5-6
15	Billi Kagga	Landrace	MT	5-6
16	Bas (S)	Basmati 320/IR 661	S	7-8
17	Azgo	Landrace from Goa	HT	1-2
18	IRRISTN-04-75	IR64426-4B-11-1	HT	1-2
19	Bas 385	Cultivated variety in India	MT	5-6
20	IR 24	IR8 x centum Ratna231 x SLO x sedges	MT	5-6
21	Panvel-16	IR8 x Bhurarata 4-10	HT	1-2
22	Pant Dhan 4	IR 262/Remadja	T	3-4
23	P-1176-91-1-2-3	IR8 x Bhurarata 4-10	MT	5-6
24	NDR-359	BG-90-2-4x08677	S	7-8
25	IR 64	IR 5657-33-2-1/IR2061-465-1-5-3	MT	5-6
26	T-23	Selection from Kala Sukhdas	MT	5-6
27	PR 13	IR8/RP 2151-173-18/IR8*4	S	7-8
28	PRR-2	IR54 x PR 106	S	7-8
29	PR 4141	IR8/BJ//IR22	S	7-8
30	BMZ 1	IR 85212-56-10	T	3-4
31	BMZ 5	IR 84649-61-1	MT	5-6
32	BMZ 6	IR 84649-61-25	HT	1-2
33	BMZ 10	IR 84649-280-20	HT	1-2
34	BMZ 14	IR 84649-81-4-B-B	HT	1-2
35	BMZ 15	IR 84649-81-5-B-B	S	7-8
36	BMZ 17	IR 84649-95-1-1-B	T	3-4
37	BMZ 20	IR 84649-275-3-2-B	S	7-8
38	BMZ 24	IR 84649-305-6-1-B	S	7-8
39	BMZ 28	IR 84649-320-3-1-B	T	3-4
40	BMZ 29	IR 84649-320-21-1-B	T	3-4
41	Bulk 43	CSR 11 x CSR 27	MT	5-6
42	BMZ 61	IR67075-2B-2-2	MT	5-6
43	wild 1	IC 338204 (*Oryza spontanea)*	HS	9
44	wild 2	IC 330484	T	3-4
45	wild 3	IC 330485	S	7-8
46	wild 4	IC 330486	S	7-8
47	wild 5	IC 330487	MT	5-6
48	wild 6	IC 330488	S	7-8
49	wild 7	IC 330489	HS	9
50	wild 8	IC 330490 — All *Oryza nivara*	S	7-8
51	wild 9	IC 330491	MT	5-6
52	wild 10	IC 330492	MT	5-6
53	wild 11	IC 330493	T	3-4
54	wild 13	IC 330496	MT	5-6
55	wild 18	IC 330602	S	7-8
56	wild 19	IC 330612	MT	5-6
57	wild 20	IC 330616	S	7-8

HT-Highly tolerant, T-Tolerant, MT-Moderately tolerant, S-Susceptible, HS-Highly susceptible

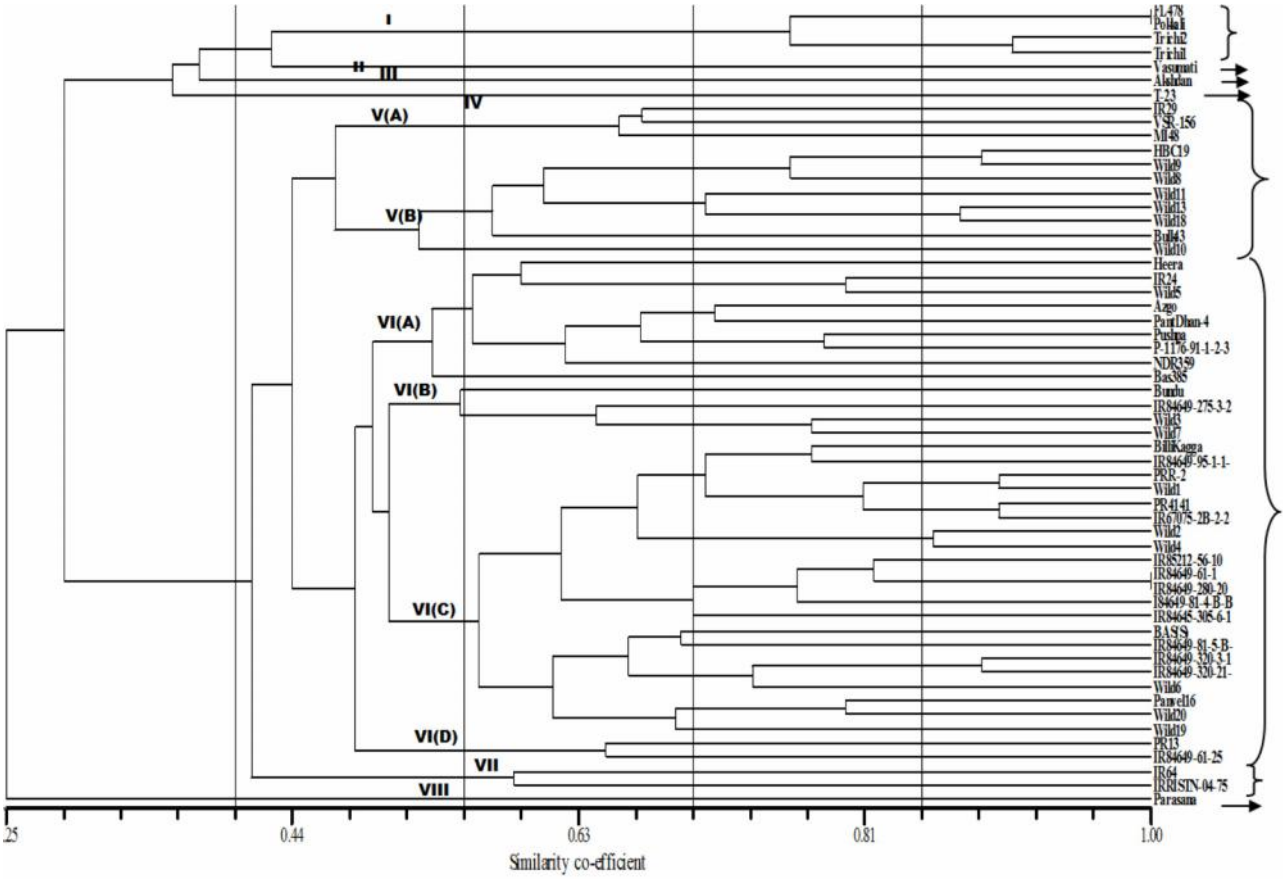

Fig. 1. UPGMA dendogram of 57 rice genotypes constructed based on *Saltol* markers

Two genotypes namely, PR 13 and IR 84649-61-25 with 64% similarity within the sub cluster VI(D) showed 36% dissimilarity with other sub clusters. The cluster VII consisted two genotypes *viz*., IR 64 and IRRISTN-04-75 which showed 58% similarity within the sub cluster and 42% dissimilarity with other sub cluster.

The present study used only 21 SSR markers distributed in chromosome 1. The other chromosomes certainly have genes for salinity tolerance and hence the clustering based *Saltol* markers could not resolve rice genotypes into distinct salt tolerant and salt susceptible groups, perfectly. Based on eight polymorphic *Saltol* markers the rice genotypes studied could be clustered according to five levels of salinity tolerance *i.e*. tolerant (T), moderately tolerant (MT), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). The results from this study provide some useful implications for salt tolerance breeding programs. The evaluation of genetic distance together with salt tolerance ability provides some useful information for assisting plant breeders in selecting suitable genetically diverse parents for hybridization. Salt tolerance of IR 64 could be improved by crossing it with salt-tolerant genotypes, namely, Pokkali, FL 478, Trichi 1, Trichi 2, Heera, Azgo, Panvel 16, IR 84649-61-25, IR 84649-280-20 and IR 84649-81-4-B-B, which have relatively, low genetic similarity with IR 64 and placed in different clusters from IR 64. IRRISTN-04-75 could not be used to improve the salt tolerance because both the genotypes (IR 64 and IRRISTN-04-75) were in the same cluster and may not diverse. The Indian variety Pokkali has frequently been used as donor for salt tolerance genes but limited success was obtained because this traditional landrace possesses too many undesirable traits often linked to salinity tolerance [16]. The cross between the elite cultivars, which were clustered in different groups could be used with a goal of creating IR 64 derived progenies that possess better salinity tolerance.

Acknowledgements

Authors express sincere thanks to the Indian Council of Agricultural Research, New Delhi for funding the Network Project on Transgenic Crops (NPTC) – Functional Genomics in Rice.

References

1. **Khush G. S.** 1997. Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol., **35**: 25-34.
2. **Sabouri H. and Biabani A.** 2009. Toward the mapping of agronomic characters on a rice genetic map, Quantitative Trait Loci analysis under saline condition. Biotechnol., **8**: 144-149.
3. **IRRI** (International Rice Research Institute), 1967. Annual Report, Los Baños, Laguna, Philippines. pp. 308.
4. **Weir B. S.** 1990. Genetic Data Analysis, Methods for Discrete Population Genetic Data. Sinauer Associates, Inc. Publishers. Sunderland, Massachusetts.
5. **Jayamani P., Negrão S., Martins M., Maçãs B. and Oliveria M. M.** 2007. Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci., **47:** 879-884.
6. **Walia H., Wilson C., Condamine P., Liu X., Ismail A. M., Zeng L., Wanamaker S. I., Mandal J., Xu J., Cui X. and Close T. J.** 2005. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol., **139**: 822-835.
7. **Yoshida S., Forno D. A., Cook J. H. and Gomez K. A.** 1976. Laboratory manual for physiological studies of rice. International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. 61-66.
8. **IRRI** (International Rice Research Institute). 1997. Rice Almanae. IRRI-WARDA-CIAT, Los Baños, Laguna, Philippines.
9. **Doyle J. J. and Doyle J. L.** 1990. Isolation of plant DNA from fresh tissue. Focus, **12**: 13-15.
10. **Niones J. M.** 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near isogenic lines, M. S dissertation, College, Laguna, Philippines: University of the Philippines Los Baños, Laguna.
11. **Anderson J. A., Churchill G. A., Autrique J. E., Tanksley D. S. and Sorrells M. E.** 1993. Optimizing parental selection for genetic linkage maps. Genome, **36**: 181-186.
12. **Suriya-Arunroj D., Supapoj N., Toojinda T. and Vanavichit A.** 2004. Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress. Science Asia, **30**: 411-415.
13. **Theerakulpisut P., Bunnag S. and Kong-Ngern K.** 2005. Genetic diversity, salinity tolerance and physiological responses to NaCl of six rice (*Oryza sativa* L.) cultivars. Asian J. Plant Sci., **4**: 562-573.
14. **Islam M. R., Gregorio G. B., Salam M. A., Collard B. C. Y., Singh R. K. and Hassan L.** 2012. Validation of *SalTol* Linked Markers and Haplotype Diversity on Chromosome 1 of Rice, Molecular Plant Breed., **10**: 103-114.
15. **Ren F., Lu B-R., Li S., Huang J. and Zhu Y.** 2003. A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. Theor. Appl. Genet., **108**: 113-120.
16. **Gregorio G. B., Senadhira D., Mendoza R. D., Manigbas H. L., Roxas J. P. and Guerta C. Q.** 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Res., **76:** 91-101.