Plant Archives Vol. 19 No. 2, 2019 pp. 3777-3781 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR BREEDING FOR SALINITY TOLERANCE THROUGH SSR MARKERS IN RICE (*ORYZA SATIVA* L.)

A. Mary Roshni, K.R. Saravanan*, M. Prakash, S. Ranjith Raja Ram and M. Harikrishnan

Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar - 608002, Tamilnadu, India.

Abstract

The study was done to evaluate the molecular characteristics and diversity over the molecular level using SSR markers at seedling stage. This was followed by a molecular characterisation using 12 SSR markers which are linked to the saltol QTL. The diversity analysis grouped 10 genotypes under five clusters. The cluster 1 is occupied by three genotypes, CR 3437-1*200-83, JK-58, NDRK 11-20. The second cluster was occupied by IRLON GSR- 9, IRLON GSR 5. The third cluster was occupied by CSR 2016 IR 18- 18, CR 3878-245-2-4-1. The fourth cluster was occupied by a single genotype CSR 2748-44-195. The final fifth cluster was occupied by CSR-11-143, CARI Dhan7. The polymorphism information content ranged from 0.18 to 0.8 the highest being with the marker RM 312 (0.8) followed by RM562 (0.78). The lowest *pic* value was with the marker RM10825 (0.18). Thus the highly divergent parents can be used to create a better breeding line.

Key words: Rice, salinity, clusters, micro satellite markers.

Introduction

Rice is cultivated mostly in major parts of the world. It is regarded as the staple food for many communities. As the growing population enforces a threat on the major production of this staple food, we need to develop high yielding and tolerant lines to feed the world. Many parts of the world are imposed with a salt environment due to various reasons. Hence, developing a genotype tolerant to these conditions plays a major role in the progress of feeding the growing population. Nearly around one lakh hectare is affected by coastal salinity in Tamil Nadu.

Rice is grown in wide range of soils, from alluvium to impermeable heavy clay of central Thailand. Mostly river-line alluvium soil and clayey loam is best suited for rice cultivation. However, soil salinity affects its growth with impacts on its yield and susceptibility. Salinization might be naturally induced (primary salinization) or due to human activities (secondary salinization). Some natural calamities like tsunami, cyclones and sea water intrusion along with tidal overflow into rivers and aquifers can make the nearby areas vulnerable to soil salinity (Flowers, 1999; Tiaz & Zeiger, 2002).

***Author for correspondence:** E-mail : ds.saifdeen@uoanbar.edu.iq

A saline soil is a condition when it contains sufficient quantity of soluble salts that interferes with the growth of most crop species (Elphick *et al.,* 2001; Werner and Finkelstein, 1995). Salinity causes harmful effects on the growing plants which might be grouped into two, osmotic stress and ionic toxicity. High soil salinity increases the osmotic pressure of the soil when compared to the plant cell thus reducing the plants ability in water uptake along with other mineral nutrients such as K^+ and Ca^{2++} (Glenn, Brown & Khan, 1997; Munns, James & Lauchli, 2006). In some cases, root cells are dehydrated due the high soil salinity. This in turn affects in maintaining cell turgor and cell elongation which leads to wilting and death of the plants.

Plant tolerance towards salinity depends on various anatomical, physiological, biochemical and molecular adaptations to survive. These are broadly grouped into three categories namely osmotic tolerance, ion exclusion and tissue tolerance (Flowers *et al.,* 1997; Tuteja 2007; Munns and Tester 2008; Deinlein *et al.,* 2014; Roy *et al.,* 2014). It takes place at whole plant, cellular and molecular levels of organisation (Munns, 2002). It is therefore a cumulative effect of many physiological traits such as Na^+ and K^+ concentration in root and shoot; Na^+

exclusion, ion balance, tissue tolerance vacuolar sequestration etc. Screening of salinity tolerance at seedling stage in rice is appreciative mode to detect the genotype's tolerance towards salinity. If a plant can able to withstand the stress at seedling stage it can establish itself better to the adapted environment.

Molecular markers have shown a rapid advancement in recent years in the field of crop breeding programs. Now-a-days, SSR or microsatellite markers have showed to be ideal for making genetic maps and to study the genetic divergence among genotypes. With the reference from previous studies the markers which are associated with saline tolerance were found to be in the shorter arm of the chromosome and are commonly called as saltol regions and the markers as saltol markers. these findings can make the selection of markers easy.

Materials and methods

Plant materials

This diversity and expressional analysis study comprised of ten genotypes *viz.,* IRLON GSR-9 (G1), IRLON GSR-5 (G2), CSR-2016-IR-18-18 (G3), CR-3878-245-2-4-1 (G4), CSR-11-143 (G5), CARIDHAN-7 (G6), CSR-2748-441-195 (G7), CR-3437-1*200-83 (G8), JK-58 (G9), NDRK-11-20 (G10).

Molecular analysis

Table 1: Geographical indication of genotypes used.

Sl. No	Name of the Genotype	Origin Code	Genotype
1	IRLON GSR-9	International Rice research	
		Institute, Philippines.	G1
2	IRLON GSR-5	International Rice research	
		Institute, Philippines.	G2
3	CSR-2016-IR-18-18	Central Soil Salinity Research	
		Institute, Karnal, Haryana.	G3
4	CR-3878-245-2-4-1	Central Rice Research Institute,	
		Cuttack. G4	
5	CSR-11-143	Central Soil Salinity Research	
		Institute, Karnal, Haryana.	G5
6	CARI DHAN-7	Central Island Agricultural Research,	
		Andhaman and Nicobar Island.	G6
7	CSR-2748-441-195	Central Soil Salinity Research	
		Institute, Karnal, Haryana.	G7
8	CR-3437-1*200-83	Central Rice Research Institute,	
		Cuttack	G8
9	JK-58	Jawaharlal Nehru Krishi	
		Vishwavidyalaya, Jabalpur.	G9
10	NDRK-11-20	NarendraDev University of	
		Agriculture &Technology,	
		Kumarganj, Faizabad, Uttar Pradesh.	G10

Isolation and quantification

Cetyl Trimethyl Ammonium Bromide (CTAB) is a detergent, which is used along with other reagents to extract nucleic acids from the cell. This is an efficient method in isolating plant genomic DNA devised by Doyle, and Doyle. Leaf samples from 15 to 20 days were collected and used for the study. Add liquid nitrogen to about 2g of leaf sample and grind them using pre-chilled pestle and mortar. 2 ml of CTAB buffer after incubation at 65°C was used to macerate the ground samples. Transfer the sample to fresh 15 ml centrifuge tubes and incubate them at 65°C for 30 minutes with shaking in between. Keep them for 10 minutes at room temperature and add equal volume of Chloroform: isoamyl alcohol (24:1). Mix them and centrifuge them at 12000 rpm for 10 minutes. Collect the supernatant in a fresh centrifuge tube and add equal volume of ice-cold isopropanol and 0.6 volume of sodium acetate (pH 5.2) and incubate at -20°C for 20 minutes. Centrifuge the contents for 10 minutes at 12000 rpm and discard the supernatant. Wash the pellet with 70% ethanol and air dry the pellet. Dissolve the pellet in 100il TE buffer and add 3il RNase. Incubate it at 37°C for 30 minutes. The isolated DNA was quantified in nano spectrometer and also in gel electrophoresis.

A set of 20 SSR markers linked to saltol QTL was used to determine the molecular diversity. The list markers used are given in table. The DNA samples isolated were used in thermal cycler to perform Polymerase Chain Reaction with SSR markers. The temperature for amplification is given in table. The reaction volume of 15 µl containing 2 µl of genomic DNA 1X assay buffer, 200 mM of deoxyribo nucleotides, 2 µM of $Mgcl_2$, 0.2 µM of primer, 1 unit of Tag DNA polymerase and 6.6 µl of sterile water. Annealing temperature was standardized for each primer and adopted for all the primers used in the study as identified by their specific Tm requirement.

Statistical analysis

Each SSR bands were scored as presence or absence. To measure the informativeness of the marker, polymorphic information content was calculated as

$$PIC \quad 1 \quad \sum_{i\ 1}^{k} P_i^2 \quad \frac{2n}{2n\ \ 1} \quad \sum_{i\ 1\ j\ i\ 1}^{k\ 1\ \ k} 2P_i^2 P_j^2$$

Where P; was the estimated allele frequencies of k alleles (I = 1 to 1e) and n

Table 2: Description of SSR markers used

S. No.	Primer	Primer SequenceForward Primer/Reverse Primer	Product size(bp)	Anealing Temperature(°C)
1	RM451	GATCCCCTCCGTCAAACACCCCTTCTCCTTTCCTCAACC	207	55
2	RM 420	GGACAGAATGTGAAGACAGTACTAATCCACCAACGCATCC	197	55
3	RM 312	GTATGCATATTTGATAAGAGAAGTCACCGAGTTTACCTTC	97	55
4	RM8094	AAGTTTGTACACATCGTATACACGCGACCAGTACTACTACTA	209	55
5	RM3412	AAAGCAGGTTTTCCTCCTCCCCCATGTGCAATGTGTCTTC	211	55
6	RM493	TAGCTCCAACAGGATCGACCGTACGTAAACGCGGAAGGTG	211	55
7	RM483	CTTCCACCATAAAACCGGAGACACCGGTGATCTTGTAGCC	325-350	55
8	RM336	CTTACAGAGAAACGGCATCGGCTGGTTTGTTTCAGGTTCG	135-250	55
9	RM562	CACAACCCACAAACAGCAAGCTTCCCCCAAAGTTTTAGCC	230-260	55
10	RM8115	TATATAGTAAATTTGTTTGGTGTAGGACAGATGGATATTATAA GAAGTAACA	120-130	55
11	RM10825	GGACACAAGTCCATGATCCTATCCGTTTCCTTTCCATCCTTGTTGC	97	55
12	RM8046	AGTACGATTTCTGTCAGCGTTGCTTAGTGGATGAAAGTTGATGGA TGATCTACTTGTT	125-225	55

Table 3: PCR Temperature Profile.

S.No.	Step	Temperature(°C)	Time	Cycles
1.	Initial denaturation	94	3 minutes	1
2.	Denaturation	94	1 minute	
3.	Annealing	55-60	1 minute	30 cycles
4.	Extension	72	2 minutes	
5.	Final extension	72	10 minutes	1
6.	4°C		∞	

was the number of individuals sampled (Botstein D., White R.L Skolnik M and Davis R.W., 1980).

Genetic diversity of ten genotypes was determined from the polymorphic molecular marker pattern by estimating the genetic distance using DICE dissimilarity co-efficient.

Estimated using the following formula,

$dij = b + c / 2a + (b + c)$

where,

dij - dissimilarity between units I and j

a - both allele present

b and c - either allele present

D - both allele absent

Cluster analysis was performed on a dissimilarity matrix of simple matching coefficients using unweighted neighbour joining algorithm using DARwin version 5.0.158 (Perrier and Jacquemoud-Collet, 2006) with 7000 permutations.

Results and Discussion

Grouping of genotypes using SSR markers

The genetic distance was estimated using DICE dissimilarity coefficient. It indicated lowest dissimilarity coefficient between G 10(NDRK 11-20) and G 9JK-58) followed by genotype G10 (NDRK 11-20) and G8(CR 3437-1*200 - 83). The highest divergence was showed between G1(IRLON GSR-9) and G8(CR 3437-1*200 - 83). The dendrogram constructed using the DICE dissimilarity coefficient between genotypes exhibiting five apparent cluster based on marker allele distribution.

The first cluster consisted of three genotypes *viz.,* G8 (CR 3437-1*200-83), G9 (JK-58) and G10 (NDRK 11-20) the second cluster consisted of G1 (IRLON GSR-9) and G2(IRLON GSR 5). Third cluster consisted of G3 (CSR 2016 IR 18- 18) and G4 (CR 3878-245-2-4-1). Forth cluster considered of G7 (CSR 2748-44-195) alone. Fifth cluster considered of G5 (CSR-11-143) and G6(CARI Dhan 7). The first cluster accommodated about 30 percent of the total population based on allelic similarity.

Table 4: Polymorphic information content (PIC) value for the SSR primers.

S.No.	Name of the marker	Polymorphic Information Content (PIC)
1	RM 451	0.42
2	RM 420	0.493
3	RM 312	0.8
4	RM 8094	0.64
5	RM 3412	0.72
6	RM 493	0.7
7	RM 483	0.72
8	RM 336	0.64
9	RM 562	0.78
10	RM 8115	0.6
11	RM 8046	0.64
12	RM 10825	0.18

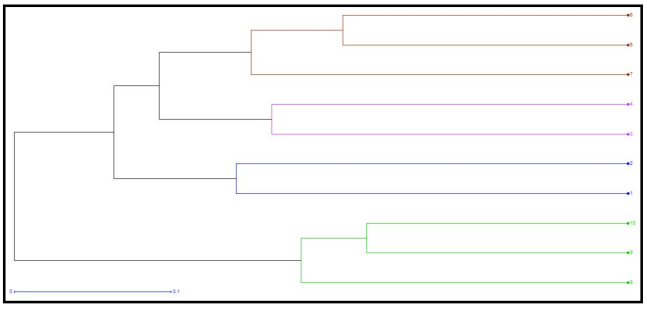

Fig. 1. Dendrogram showing the clustering pattern

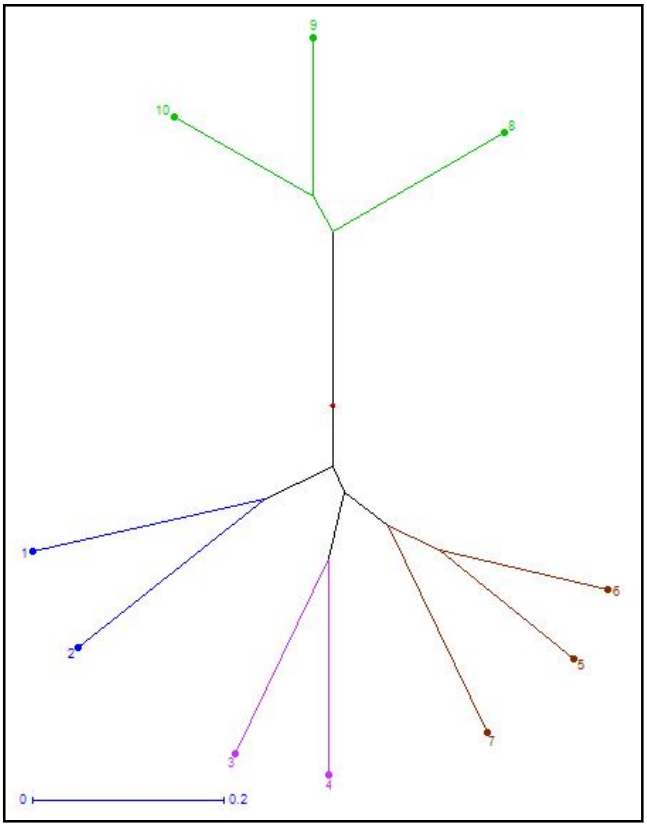

Fig. 2. Clustering pattern of genotypes

The second, third and fifth cluster contributed of two genotypes, having a membership density of 20per cent. The monogenotypic fourth cluster accounted for 10 per cent of the genotypic diversity. The similarity coefficients among 10 genotypes ranged from 0.1 to 0.5. Among all the pair-wise combinations, CR 3437-1*200-83, JK-58 and NDRK 11-20 showed (0.5) the highest similarity index, while genotypes *viz.,* CSR 11-143, CARI Dhan 7 and CSR 27488-44-195 showed the lowest similarity index.

Polymorphic information content (PIC Value) of SSR primers was calculated. In SSR markers the values ranged from 0.18 (RM 10825) to 0.8 (RM 312) with an average of 0.61088. High PIC value for SSR marker was observed for SSR (RM 312) 0.80 and for PIC values of all the genotypes are listed in the table 1. Percentage polymorphism was calculated. 100 percent polymorphism has been observed for all the markers used in the study. In the present study, the parental selection based on the genetic diversity is highly essential to develop a good variety. In future breeding programme, by utilizing the highly genetic divergent parents of CR 3437-1*200-83, JK-58, NDRK 11-20 and IRLON GSR- 9, IRLON GSR 5, CARI DHAN 7 may help to develop high yielding salt tolerant varieties. The key markers *viz.*, RM312, RM562, RM 3412, RM483, RM493, RM8115, RM8094 and RM8046 will be more useful for identification of salt tolerant and the salt susceptible genotypes.

References

Agrama, H. and G. Eizenga (2008). Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. *Euphytica*, **160(3):** 339-355.

Alam, R., M.S. Rahman, Z.I. Seraj, M.J. Thomas, A.M. Imail, E. Tumimbang-Raiz and G.B. Gregorio (2011). Investigation of seedling stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. *Plant Breed.,* **130:** 430-437.

Anonymous (2009). IRRI World Rice Statistics (WRS). IRRI, Manila, Philippines.

Beyene, Y., A.M. Botha and A.A. Myburg (2005). A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland Maize. *Afr. J. Biotechnol.*, **4:** 586-595.

Chao, D.Y., J.P. Gao and H.X. Lin (2007). Understanding the abiotic stress tolerance mechanisms: recent studies on stress response in rice. *J. Integrative plant Bio.,* **49(6):** 742-750.

Doyle, J.J., J.J. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin,* **19:** 11-15.

Fooland, M.R., L.P. Zhang and G.Y. Lin (2001). Identification and validation of QTLs for salt tolerance during vegetative growth in tomato by selective genotyping, *Genome,* **44:** 444 - 454.

Giarrocco, L.E., M.A. Marassi and G.L. Saleno (2007). Assessment of the genetic diversity in Argentina Rice Cultivars with SSR markers. *Crop Sci*., **47(2):** 853-858.

Hang-yong, Y., W. Xing-hua, W. Yi-ping, Y. Xiao-ping and T. Sheng-xiang (2004). Study on genetic variation of rice varieties derived from Aizizhan by using morphological traits, allozymes and SSR markers. *Chin. J. Rice Sci*., **18:** 477- 482.

Kabir, M.H., M.M. Islam, S.N. Begum and A.C. Manidas (2008). Application of SSR technique for the identification of markers linked to salinity tolerance in rice. *Progress. Agric.,* **19(2):** 57-65, ISSN 1017-8139.

Karp, A., O. Seberg and M. Buiatti (1996). Molecular techniques in assessment of botanical diversity. *Ann. Bot*., **78:** 143, doi:10.1006/anbo.1996.0106.

Khatun, S., C.A. Rizzo and T.J. Flowers (1995). Genotypic variation in the effect of salinity on fertility in rice. *Plant and soil,* **173:** 239-250.

G. Mohammadi-Nejad, A. Arzani, A.M. Rezail, R.K. Sing and G.B. Gregorio (2008). Assessment of rice genotypes for salt tolerance using microsatellite markers associated with *Saltol* QTL. *African Journal of Biotechnology,* **7(6):** 730-736.

Nasim, Ali, Md, Lucina Yeasmin, Saikat Gantait, Rupak Goswami, Somsubhra Chakraborty (2014). Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. *Physiology and Molecular biology of plants,* **20(4):** 411-423.

Perrier, X., Jaquemoud-collect, (2006). DARwin software.

Ram, S.G., V. Thiruvengadam and K.K. Vinod (2007). Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *J. Appl. Genet.,* **48(4):** 337-345.

Rathor, A.G., M.A. Zargar and F.A. Sheikh (2001). Genetic divergence in rice (*Oryza sativa* L.) under temperate conditions. *Indian J. Agr. Sci.*, **7(5):** 344-345.

Seetharam, K., S. Thirumeni and K. Paramasivam (2009). Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. *Afr. J. Biotech.,* **8(10):** 2050-2059.

Ganie, Karnakar, Mondal Chowdry and Dey (2014). Assessment of genetic diversity in salt tolerant rice and its wild relatives for ten SSR loci and one allele mining primer of Salt gene located on 1st chromosome. *Plant Systematics and Evolution*., **200(7):** 1741-1747.

Virk, P.S., J. Zhu, H.J. Newbury, G.J. Bryan, M.T. Jackson, Ford-lloyd (2009). Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa* L.) *germplasm. Euphytica.*, **112:** 275-284.