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MOLECULAR CHARACTERIZATION OF RICE (*ORYZA SATIVA* L) GENOTYPES FOR SALT TOLERANCE USING MICROSATELLITE MARKERS

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ABSTRACT
Salinity stress is the major constraint in rice production. Selection for salinity tolerance genotypes of rice based on phenotypic performance alone is less reliable and will delay in progress in breeding. Recent advent of molecular markers, microsatellites or simple sequence repeats (SSRs) were used to find out salt tolerant rice genotypes. In view of this, the mapping and marker-assisted selection for salt tolerance genes in rice have been conducted. The present study was carried out with the objective of evaluation of genetic diversity among 19 rice genotypes, representing highly tolerant as well as susceptible rice cultivars using SSR markers. Among 39 SSR markers used, 26 SSR marker loci generated polymorphic patterns and a total of 185 alleles were detected. From these 26 SSR markers, 16 SSR markers are located on the *Saltol* region on chromosome 1 of rice. The number of alleles per locus ranged from 3-11 with a mean of 7.1 alleles per locus. The PIC values for 26 SSR markers varied from 0.50 (RM6737) to 0.89(RM3412) with an average PIC of 6.7. Hence, from the present study, it can be proved that SSR markers can detect high polymorphism and are very useful in studying variation among different genotypes.

INTRODUCTION

Rice is one of the most important world food crops, serving as the staple food for over one-third of the world's population (Nejad *et al.*, 2010). It serves as the principal source of nourishment for over half of the global population and is the most important cereal crop. Rice breeders are increasingly challenged in the new century to meet the rapidly growing food demands of an increasing human population. Presently, growers in many regions have extended cultivation into marginal lands where salinity levels in soils are above thresholds affecting rice growth and yield. These instances, along with water conservation practices such as irrigating crops with marginal quality water have increased the need for genetic improvement of salt tolerance in rice. Unfortunately, rice is one of the most salt-sensitive cereal crops. Salinity ingress has lead to deterioration in the environment in the coastal areas with adverse implication on agriculture (Zeng *et al.*, 2004). Progress in salinity tolerance breeding is slow due to the following aspects as limited knowledge in the genetics of tolerance, complexity of the several tolerance mechanisms involved, inadequate screening techniques, low selection efficiency and poor understanding of salinity and environmental interactions (Bhowmik *et al.*, 2009; Lang *et al.*, 2008). Breakthrough in salinity tolerance breeding became feasible after the identification of major chromosomal regions (Qualitative trait loci, QTLs) underlying salinity (*Saltol)* stresses, the development and use of marker system for their speedy incorporation into modern high yielding and popular varieties through marker assisted backcrossing (Huyen *et al.*, 2012). With the recent development in the field of molecular marker analysis, it is now feasible to analyze both the simple inherited traits and the quantitative traits and then identifying the individual genes controlling salinity tolerance which could facilitate selection in rice for this low heritable trait (Aliyu *et al.*, 2011).

Looking into the above facts, the present investigation was carried out with the objective of assessing the presence and level of genetic diversity among *O. sativa* cultivars and for identifying microsatellite markers for salinity resistance genes.

MATERIALS AND METHODS

19 rice genotypes obtained from the Main Rice Research Station, Anand Agricultural University, Nawagam, Gujarat, India were used for molecular analysis. Total DNA was extracted from three weeks old seedlings by Cetyl Trimethyl Ammonium Bromide (CTAB) method with minor modifications (Ahmadikhah, 2008). The qualitative analysis of the isolated DNA was performed spectrophotometrically using Nanodrop N.D. 1000 (Software V.3.7.1).

Each polymerase chain reaction was carried out in 20μl reaction volume containing 50ng of DNA, 10X polymerase buffer (10x tris with 20mM $MgCl_2$), 25mM dNTPs, 0.5μl of each primer (10pmol), 1 unit of Taq polymerase (Fermentas), 0.5μl of 5% formamide and 1μl of 5mM spermidine using Eppendorf and Applied biosystem thermal cyclers. Thermal

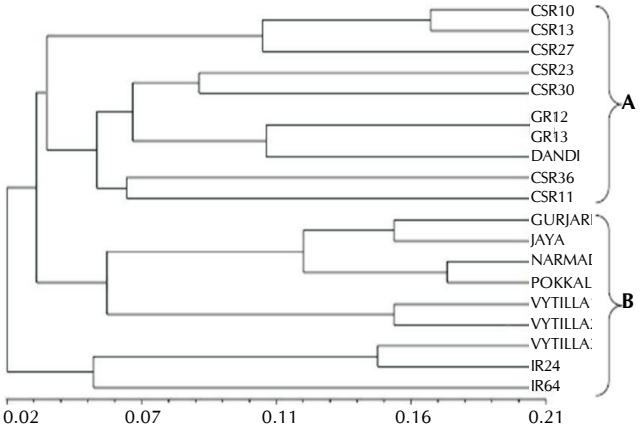

Figure 1: Dendrogram of genetic relationship among Rice genotypes based on SSR Markers

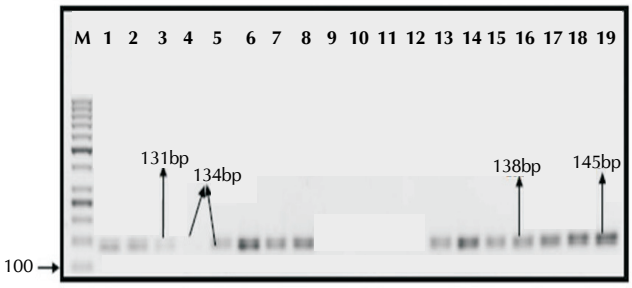

Figure 2: SSR Profile of RM315

cycler programme for PCR comprised 95ºC for 5 minutes for initial denaturation, followed by 36 cycles of 95ºC for 45 seconds, 55 to 65ºC for 45 seconds, 72ºC for 45 seconds and ending up with 7 minutes at 72ºC for the final extension. The annealing temperature was adjusted based on the specific requirements of different primer combinations. The PCR products were resolved by electrophoresis in 2.5% agarose gel containing 0.5 *i* g/mL of Ethidium Bromide prepared in 1X TBE buffer at a constant voltage of 80v for period of 2h. The gel was visualized in UV transilluminator and documented using SYNGENE GENESNAP G-BOX gel documentation system. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting module of the NTYSYSpc Version 2.02 (Rohlf , 1994). Identity software was also used for data analysis (Wagner and Sefc, 1999) which revealed information about expected heterozygosity, observed heterozygosity, number of alleles and allele frequency and standard deviation. Relationships among rice cultivars were graphically represented in the form of dendrograms.

RESULTS AND DISCUSSION

In the present investigation 19 rice genotypes were used for genetic diversity analysis with 10 SSR markers. An overall comparison of the markers and genotypes involved in genetic diversity analysis revealed that the markers could be distinguished between different genotypes (Table 2). A total of 10 SSR markers were used for analyzing genetic diversity among 19 rice genotypes. The allele length for this 10 SSR markers varied from 131-443bp, whereas the highest allele length was recorded for RM535 (443bp) for varieties Pokkali, Jaya, Vytilla1, IR24 and IR64. The allele frequency produced by different markers was observed in the range of 5.8-71.4%. The highest allele frequency (71.4%) was recorded by the marker RM315 for varieties Vytilla1, Vytilla 2 and IR64. Similar results were obtained by Singh *et al*. (2011) where RM535 showed a PIC of 0.74 in several elite varieties of rice. Expected heterozygosity amongst 19 rice genotypes was observed in the range of 0.50-0.86, where in the marker RM208 revealed the highest value of 0.86. The average expected heterozygosity was 0.78. The present result corroborates with the results of Liang *et al*. (2004) where marker assisted selection by several SSR markers including RM 208 on *O. rufipogon* was performed. It was observed that the primers used in this study could unravel allele frequencies maximum number of times for variety Vytilla1 followed by Vytilla2, Dandi, Jaya and CSR27. Regarding allele length, Vytilla1 and Vytilla3, followed by Vytilla2 and IR64 were repeated for maximum number of occasions.

For detecting the presence of salt tolerant genes among 19 rice genotypes, a total of 16 SSR markers were used (Table 3). The following details were obtained from it. The allele length for this 16 SSR markers varied from 67-307bp, where the highest allele length was observed for RM10890 (307bp) for variety IR64.The allele frequency produced by different markers was observed in the range of 5-71.4%. The highest

Table 1: List of genotypes used in the present study

Sr.No.	Genotypes	Pedigree /origin
1.	CSR10	M40-431-24-11× Jaya
2.	CSR13	(CSR1×Bas.370)×CSR5
3.	CSR23	(IR 64×IR4630)×IR964-45-2-2
4.	CSR27	NonaBokra×IR565-33-2
5.	CSR30	BR4-10×Pak. Basmati
6.	CSR36	(CSR13×Panvel 2)×IR36
7.	GR11	Z-31×IR-8-246
8.	GR12	GR4×IR64
9.	GR13	GR11×IET-14726
10.	DANDI	PNL-2×IET-8320
11.	GURJARI	Asha×kranti
12.	JAYA	TN1×T141
13.	NARMADA	TN1×Bas.370
14.	POKKALI	Local selection.
15.	VYTILLA1	PLS×Pokkali
16.	VYTILLA2	PLS× Cheuvirippu
17.	VYTILLA3	Vytilla1×TN1
18.	IR24	IRRI cultivar
19.	IR64	IRRI cultivar

Table 2: Details of SSR markers used for genetic diversity analysis

Sr.No	Name of the SSR marker	No. of Alleles	Range of allele size(bp)	PICValue
1.	RM 315	8	131-148	0.83
2.	RM 259	7	148-185	0.84
3.	RM 306	9	153-219	0.86
4.	RM 337	4	144-153	0.73
5.	RM 535	8	137-443	0.77
6.	RM 208	10	270-373	0.86
7.	RM 258	6	134-157	0.77
8.	RM 28050	8	162-210	0.85
9.	RM 6737	3	184-246	0.50
10.	RM 7102	7	146-172	0.83

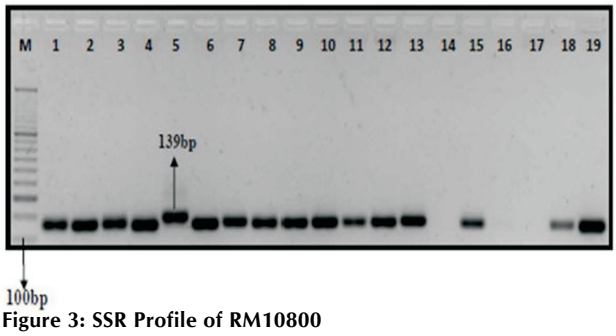

Figure 3: SSR Profile of RM10800

M.	MARKER	5.	CSR30	10.	DANDI	15.	VYTILLA1
1.	CSR10	6.	CSR36	11.	GURJARI	16.	VYTILLA2
2.	CSR13	7.	GR11	12.	JAYA	17.	VYTILLA3
3.	CSR23	8.	GR12	13.	NARMADA	18.	IR24
4.	CSR27	9.	GR13	14.	POKKALI	19.	IR 64

Table 3: Details of salt specific SSR markers

Sr.No.	Name of the SSR marker	No. of Alleles	Range of allele size(bp)	PIC(Polymorphism Information Content)Value
1.	RM 493	9	168-226	0.86
2.	RM 3412	11	94-139	0.89
3.	RM SC3	4	188-204	0.67
4.	RM 10655	6	198-268	0.80
5.	RM 10800	7	121-139	0.82
6.	RM 10701	6	67-75	0.80
7.	RM 10748	9	86-111	0.86
8.	RM 10720	4	174-207	0.72
9.	RM 10711	4	188-204	0.66
10.	RM 10713	8	132-159	0.84
11.	RM 10927	7	138-257	0.83
12.	RM 10890	4	262-307	0.83
13.	RM 10852	7	144-163	0.74
14.	RM 10843	9	131-159	0.86
15.	RM 10793	10	125-194	0.88
16.	RM 243	10	111-136	0.88

allele frequency (71.4%) was observed in RM10927 for varieties Dandi and GR11.Expected heterozygosity for 19 rice genotypes varied from 0.67-0.89, wherein the marker RM3412 revealed highest value. The average expected heterozygosity was 0.82. Among 16 specific primers utilized for detecting salt tolerant genes, study could unravel allele frequencies maximum number of times for GR13 and Dandi followed by Jaya and Gurjari. Regarding allele length, variety IR64 accounted for maximum number of times. Similar results were recorded by Lang *et al*. (2008) where 95% of SSR markers for genetic diversity were reported polymorphic with the IR64 variety. The above results are in accordance with the results reported by McCouch *et al.,* in 2002. The present work also corroborates with the work of Singh *et al*. (2011) where genetic diversity of rice genotypes were found by 20 SSR markers. They characterized 12 TGMS lines developed at DRR, GBPUAT and IRRI using morphological traits and quantified the level of genetic diversity based on their clustering pattern. Also, Thomson *et al*. (2010) worked on the genetic diversity of rice for salt tolerance with 100 SSR markers and confirmed the location of the *Saltol* QTL on chromosome 1 and identified additional QTLs associated with tolerance. Genetic diversity is fundamentally important for developing heterotic rice hybrids. Grouping based on SSR markers, in general, agreed with the parental pedigree information provides indispensable information regarding the genetic diversity among the genotypes. Varieties and lines sharing the common ancestry were clustered in to the same group, indicating the efficiency of SSR markers in detecting the genetic diversity in rice (Singh *et al*., 2011).

It was inferred from the genetic diversity analysis as well as salt-specific SSR markers study that neither the allele size, nor allele frequencies can be clearly used as criteria for detecting salt tolerant genes in rice genotypes. Characters like salt tolerance are controlled by large number of QTLs which may share homology between genes responsible for other abiotic stresses like temperature, drought, flood, submergence etc. The genotypes and primers used should complement each other to obtain perfect results. Refinement in primer sequences and increasing their specificity in relationship to characters under study can enhance the efficacy of microsatellite markers as a tool for tagging salt tolerance genes. Hence, from the present study, it can be proved that SSR markers can detect high polymorphism and are very useful in studying variation among different genotypes.

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