

Research Article**Molecular characterization of rice land races using SSR markers**

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Abstract

Genetic improvement mainly depends on the extent of genetic variability present in the population. The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. The objective of this study was to evaluate the genetic divergence of 12 rice land races using five SSR markers. A total of 11 alleles were detected in 12 land races and the number of alleles per locus ranged from 2 to 3 with an average of 2.2 per locus. Among the primers used RM 481 identified more number of alleles and average PIC was 0.43. The dendrogram based on SSR marker analysis grouped the 12 rice accession into six clusters, where cluster VI was the largest with three accessions. The similarity coefficient through Jaccard's revealed that Anna samba and Chettivirippu were ascertained to be the genetically diverse from the other land races. The study also highlighted use of more number of markers for efficient characterizing the land races used for the present study.

Key words: Genetic divergence, Rice land races, SSR markers, Dendrogram

Introduction:

Rice (*Oryza sativa* L.) is the principal staple food for more than half of the world's population. Landraces of rice played a very important role in the local food security and sustainable development of agriculture, in addition to their significance as genetic resource for rice genetic improvement (Tang *et al.*, 2002). Landraces provided "adaptability genes" for specific environmental conditions. Incorporation of adaptability genes from landraces only could ensure optimum grain yield for the region. According to Food and Agricultural Organization (FAO, 1997), about three quarters of original varieties of agricultural crops have already been lost from the farm fields between 1950 and 1995. Therefore, to maintain crop diversity, collection, characterization and conservation of traditional landraces are vital.

Genetic diversity is a ubiquitous feature of all species in nature. Narrow genetic base in rice cultivars continues to limit the productivity of

rice which has been cultivated for more than 9000 years. Genetic divergence among the genotypes plays an important role in the selection of parents having wider variability for different characters.

Genetic diversity can be evaluated with morphological traits, seed proteins, isozymes and DNA markers. Molecular marker technology is the powerful tool for determining genetic variation in rice varieties (Xu and Wang, 1974). In contrast to morphological traits, molecular markers can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation, management, and untouched by environmental influence. Among various PCR based markers, SSR markers are more popular in rice because they are highly informative, mostly monolocus, codominant, easily analyzed and cost effective (Gracia *et al.*, 2004). SSR markers are class of repetitive DNA sequences usually 2.6 kb that are distributed through out whole genome and are flanked by highly conserved region. (Chambers and Avoy 2000). The objective of this present study was to

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evaluate genetic divergence of 12 rice land races with five SSR markers.

Materials and methods

A total of 12 rice land races were used in the study (Table 1). DNA was extracted from seed using CTAB method (Dellaporta *et al.*, 1983). Sequence of microsatellite primer pairs were downloaded from genome database, Rice Genome Microsatellite Markers (<http://www.gramene.org/db/markers.html>). Five primers were chosen randomly covering all the chromosomes or enomic regions (Table 2) PCR reaction was carried out using a Programmable Thermal Cycler (MJ research Inc.USA). The reaction volume was 15µl containing 2µl of genomic DNA, 1.5µl of 1X PCR buffer, 1.5µl of 200µM dNTPs mix, 0.3µl of 2µM MgCl₂, 2µl of each primer and 1 unit of *Taq* polymerase. The temperature cycles were programmed as 95°C for 2 minutes, 94°C for 45 seconds, 55°C for 1 minute, 72°C for 1 minute and 30sec, for 34 cycles and additional temperature of 72°C for 10 min for final extension and 4°C indefinitely for cooling and storage. The PCR products were electrophoresed in agarose gels. The gel was then stained in ethidium bromide and observed on a UV transilluminator.

Diversity Analysis

Clearly resolved unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the presence and absence of bands in each variety respectively. Polymorphic information content (PIC) values were calculated for each of the SSR loci using the formula developed by Nei (2002).

$$PIC=1-\sum x_k^2/n$$

where, x_k^2 represents the frequency of the k^{th} allele, n represents the number of genotypes. The data of microsatellite markers were analyzed using NTSYS-pc statistical package, version 2.1 (Exeter software, Setauket, NY).

Results and discussion

A total of five microsatellite markers were used to assess the extent of genetic diversity across the 12 genotypes. All the five SSR markers generated polymorphic patterns. A total of 11 alleles were detected among the genotypes. The number of alleles per locus ranged from 2 (RM 590, RM274, RM 443, RM234) to 3 (RM 481) with an

average of 2.2 per locus. In a study conducted by Ram *et al.* (2007), the number of alleles per locus varied from 3 to 8, with average number of alleles per locus at 4.86, indicating a less magnitude of diversity with reference to the 5 markers among the plant materials in this present investigation.

The PIC values derived from allelic diversity and frequency among the genotypes were not uniform for all the SSR loci tested. The PIC value for 5 primers varied from 0.28 (RM 443) to 0.57 (RM 481) with a mean of 0.43 (Table 3). Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. Low PIC values for some other primers were earlier reported by Juneja *et al.*, (2006). Among the primers used in the present study, RM 481 is highly informative since it recorded high PIC value (0.57). The markers showed an average PIC value of 0.43 which indicated that SSR markers used in this study were not highly informative because only PIC values higher than 0.5 indicate high polymorphism.

The multivariate nature of SSR markers has the unambiguous advantage of discriminating genotypes more precisely. The UPGMA analysis could reveal allelic richness of six clusters (Table 4) for various sizes at a similarity coefficient level of 0.74. Among them, Annasamba and Chettivirippu may be chosen as a parent for hybridization with any of the land races from other divergent cluster involving land races.

The use of more number of markers would be efficient to characterize the land races than used for the present study, which highlighted the presence of diversity at genomic level among the genotypes studied.

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Table 1: List of rice land races used in the present study

SL. No	Rice land races
1.	Anna samba
2.	Chettivirippu
3.	Chikoor
4.	Gopal Bhag
5.	Koorgood
6.	Anakkodan
7.	Red Thriveni
8.	Pokkali
9.	Poompalai
10.	Rupsail
11.	Saket
12.	Socnan

Table 2: Details of SSR primers used for PCR amplification

Oligo Name	Repeat Motif	Sequence (5'-3')	Product size (bp)
RM 590	(TCT) ₁₀	CATCTCCGCTCTCCATGC* GGAGTTGGGGTCTTGTTTCG**	137
RM 481	(CAA) ₁₂	TAGCTAGCCGATTGAATGGC* CTCCACCTCCTATGTTGTTG**	169
RM 443	(GT) ₁₀	GATGGTTTTTCATCGGCTACG* AGTCCCAGAATGTCGTTTTTCG**	124
RM 274	(GA) ₁₅₋₇ , (CGG) ₅	CCTCGCTTATGAGCTTCG* CTTCTCCATCACTCCCATGG**	160
RM 234	(CT) ₂₅	ACAGTATCCAAGGCCCTGG* CACGTGAGACAAAGACGGAG**	156

* Forward primer, ** Reverse primer, bp (base pairs).

Table 3: Allele variation and PIC values for SSR markers identified in 12 genotypes

Oligo/Markers	Chromosome location	Number of Alleles	Product size (bp)	PIC values
RM 481	7	3	169	0.49
RM 274	5	2	160	0.37
RM 590	1	2	137	0.57
RM234	7	2	156	0.45
RM443	1	2	124	0.28

Table 4: Distribution of genotypes to different clusters based on UPGMA method

Cluster	Number of genotype(S)	Name of genotype(S)
I	2	Anna Samba, Chettivirippu.
II	1	Pokkali
III	2	Chikoor, Rupsail.
IV	2	Saket, Socnan.
V	2	Gopal Bhag, Red Thriveni.
VI	3	Koorgood, Anakkodan, Poompalai.