International Journal of Agriculture, Environment and Biotechnology
Citation: IJAEB: 9(4): 493-497 August 2016
DOI Number: 10.5958/2230-732X.2016.00065.6

GENETICS AND PLANT BREEDING

Genetic diversity associated with nutritive and grain quality traits using microsatellite markers in traditional land races and improved cultivars in rice (*Oryza sativa* L.)

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Paper No. 468 Received: 18-1-2016 Accepted: 11-8-2016

Abstract

Genetic diversity underlies the improvement of crops by plant breeding. Land races of rice (*Oryza sativa* L.) can contain some valuable alleles not common in modern germplasm. The aim here was to measure genetic diversity and its effect on nutritive and grain quality traits among rice land-race genotypes grown in Tamil nadu and Kerala. The experimental material consists of six high yielding ruling rice varieties *viz.,* IR 72, ADT 43, ADT 45, ASD 16, TPS 4 and ADT 39 were utilized as female parent. Four nutritive and medicinal landraces in rice consumed by the people in different parts of Tamil Nadu and Kerala *viz.,* Veeradangan, Kavuni, Kathanellu and Navara were collected and used as male parents. In molecular marker diversity analysis using SSR markers, the similarity indices for all the pair wise combinations among the 10 parents were computed. The similarity index was highest (0.93) between ADT 39 and ADT 43 along with TPS 4 and IR 72. The least similarity index (0.37) was observed between Kavuni and Veeradangan. On the basis of cluster analysis the 10 parents were grouped in to 5 clusters at 71 per cent similarity levels. Cluster 1 was constituted by Navara, Kathanellu, ADT 39 and ADT 43. Cluster 2 was constituted by IR 72, ASD 16 and TPS 4. Cluster 3, cluster 4 and cluster 5 consisted of single genotype *viz.,* Veeradangan, ADT 45 and Kavuni respectively. It inferred that landraces have diverse genetic bases and can be utilized in future breeding programs. The results showed the potential of SSR markers for genetic diversity assessment.

Highlights

- The high PIC value indicates that all these markers were highly informative and capable of distinguishing between cultivated varieties and land races.
- The SSR markers are neutral and co-dominant and could be powerful tool to assess the genetic variability among cultivated varieties and land races.
- SSR markers are more informative and can be useful for marker assisted selection for nutritive rice land races and cultivars.

Keywords: Rice, genetic diversity, land races, cultivated varieties, molecular markers.

Rice (*Oryza sativa*), one of the agronomically and nutritionally important cereal crop in the grass family (Poaceae), is the principal staple food in developing countries like India. Landraces are precious genetic resources, because they contain huge genetic variability which can be used to complement and broaden the gene pool of advanced genotypes. The landraces maintained by farmers are endowed with tremendous genetic variability as they are not subjected to subtle selection over a long period of time. Further crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and the use of new biotechnological tools. Microsatellites

(also known as simple sequence repeats) are simple; tandemly repeated 5-20 fold; often ditto tetra-nucleotide; sequence motifs; each flanked by unique sequences. They are valuable as genetic markers because: they are co-dominant in nature; show high allelic diversity; are easily and economically assayed by PCR; and their use may be automated. The present study has been conducted to evaluate the pattern and extent of genetic variability and relatedness among some rice landraces based on important nutritive traits using SSR markers. DNA marker analysis will help the identification and differentiation of landraces with different genetic make-up. The information will enable maximized selection of diverse parents and assist in broadening the germplasm base and good heterosis of future rice breeding programs. These microsatellite markers could serve as a powerful tool in selecting genetically diverse accessions to execute efficient selection in highly segregating generations (Singh *et al.* 2015).

Meterials and Methods

The present study was carried out in the Department of Plant Breeding and Genetics at Agricultural College and Research Institute, Madurai during *kharif* 2012-2014. The experimental material consisted of six female parents *viz.,* IR 72, ADT 39, ADT 45, ADT 43, ASD 16 and TPS 4 and four medicinal landraces of rice *viz.,* Veeradangan, Kavuni, Navara and Kathanellu as male parents. Young leaves at seedling stage were harvested for the isolation of genomic DNA. Fresh leaves from 5 individuals of each line/variety were bulked together and the DNA was extracted by following the protocol of Dellaporta *et al*. (1983). Quantification of DNA is necessary to check the quality and concentration of DNA for carrying out PCR reaction. Genomic DNA was quantified using the spectrophotometer. The concentration of nucleic acids is usually determined by measuring the sample OD at 260 nm against blank. The ratio A260/A280 is used to estimate the purity of nucleic acid. Pure DNA should have a ratio approximately 1.8. The quality of the DNA was also checked using 0.8 % agarose gel electrophoresis.

A total of 10 primers were used for PCR amplification. Amplification reactions were carried out in 25uL reaction volumes containing 50ng genomic DNA, 100uM each of dATP, dCTP, dGTP and dTTP, 30ng primer, 1 unit of Taq DNA Polymerase (Fermentas), 1X Taq Polymerase Buffer and 2.5mM $MgCl_2$. DNA amplification was performed in DNA Thermal Cycler (Eppendorf) programmed as follows: an initial denaturation of 5 min at 94°C; 35 cycles of 94°C for 45 seconds (denaturation), 56-59°C for 1 minute (annealing), and 72°C for 1.5 minutes (extension). One additional cycle of 72°C for 7 minutes was used for final extension. Amplification products were resolved by electrophoresis on 3.5% agarose gels run in 10X TBE. The amplified products were observed under UV transilluminator after stained with ethidium bromide (4ug/mL). The amplification profile of all the used varieties for any given primer were compared with each other and presence of each band were scored as "1" and the absence of the same band of the same size in other varieties were scored as "0". In this way all the amplified profiles from all the primers used were scored and integrated together to form a data matrix for the estimation of genetic distance between the lines. The genetic similarity coefficients were calculated using Nei & Li method (1979). Cluster analysis was performed based on the similarity coefficient between genotypes using unweighted pair group of arithmetic means (UPGMA).

Results and Discussion

The information on genetic diversity helps in choosing parents for developing new varieties which needs continuous evaluation of germplasm for useful characters, which in earlier days was purely based on the available morphological data. Morphological traits/markers reflects not only the genetic composition of the cultivar, but also the interaction of the genotype with the environment in which it is expressed and hence the description based on morphological information for the calculation of genetic distance. Advance in molecular biology has provided description based on DNA markers. Simple Sequence Repeats (SSR) has shown great promise in genetic diversity analysis because it is technically simple, co-dominant, highly reproducible and can be assayed by PCR. The PCR reaction was performed using ten SSR primers and they are listed in Table 1. Some of the SSR markers are very informative and can be useful foe marker assisted selection in future breeding programme (Ankita *et al.* 2014).

Table 1: Particulars of SSR Primers used in the study

Nutrients	SSR Marker	Sequence
Fe	RM 300	Forward - GCTTAAGGACTTCTGCGAACC Reverse – CAACAGCGATCCACATCATC
Fe	RM 53	Forward – ACGTCTCGACGCATCAATGG Reverse – CACAAGAACTTCCTCGGTAC
Zn	RM 34	Forward – GAAATGGCAATGTGTGCG Reverse – GCCGGAGAACCCTAGCTC
Zn	RM 237	Forward – CAAATCCCGACTGCTGTCC Reverse – TGGGAAGAGAGCACTACAGC
Mg	RM 243	Forward – GATCTGCAGACTGCAGTTGC Reverse – AGCTGCAACGATGTTGTCC
Mg	RM 312	Forward – GTATGCATATTTGATAAGAG Reverse – AAGTCACCGAGTTTACCTTC
Ca	RM 317	Forward – CATACTTACCAGTTCACCGCC Reverse – CTGGAGAGTGTCAGCTAGTTGA
Protein	RM 18	Forward – TTCCCTCTCATGAGCTCCAT Reverse - GAGTGCCTGGCGCTGTAC
Protein	RM 234	Forward – ACAGTATCCAAGGCCCTGG Reverse – CACGTGAGACAAAGACGGAG
Waxy content	RM 190	Forward- CTTTGTCTATCTCAAGACAC Reverse – TTGCAGATGTTCTTCCTGATG

Table 2: Similarity matrix of ten parents using SSR markers in rice

Parents	Veeradangan	Kavuni	Navara	Kathanellu	IR 72	ADT 39	ADT 45	ADT 43	ASD16	TPS 4
Veeradangan	1.00									
Kavuni	0.37	1.00								
Navara	0.48	0.52	1.00							
Kathanellu	0.59	0.52	0.70	1.00						
IR 72	0.67	0.56	0.52	0.59	1.00					
ADT 39	0.67	0.48	0.78	0.74	0.63	1.00				
ADT 45	0.59	0.48	0.48	0.56	0.67	0.63	1.00			
ADT 43	0.74	0.48	0.74	0.74	0.70	0.93	0.70	1.00		
ASD 16	0.67	0.44	0.50	0.56	0.74	0.70	0.70	0.78	1.00	
TPS 4	0.70	0.56	0.52	0.63	0.93	0.70	0.74	0.70	0.78	1.00

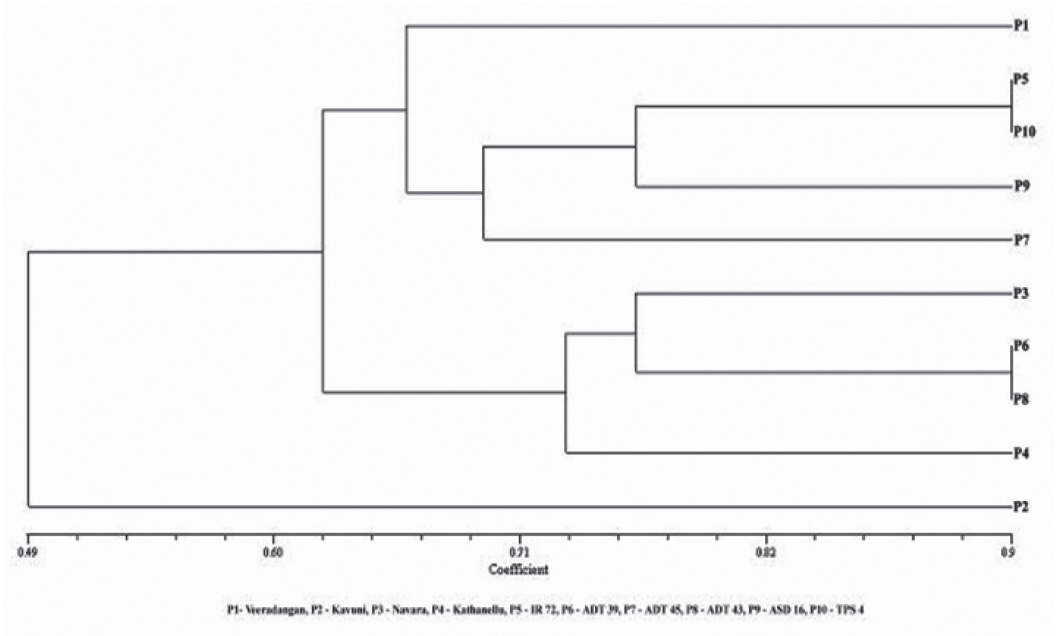
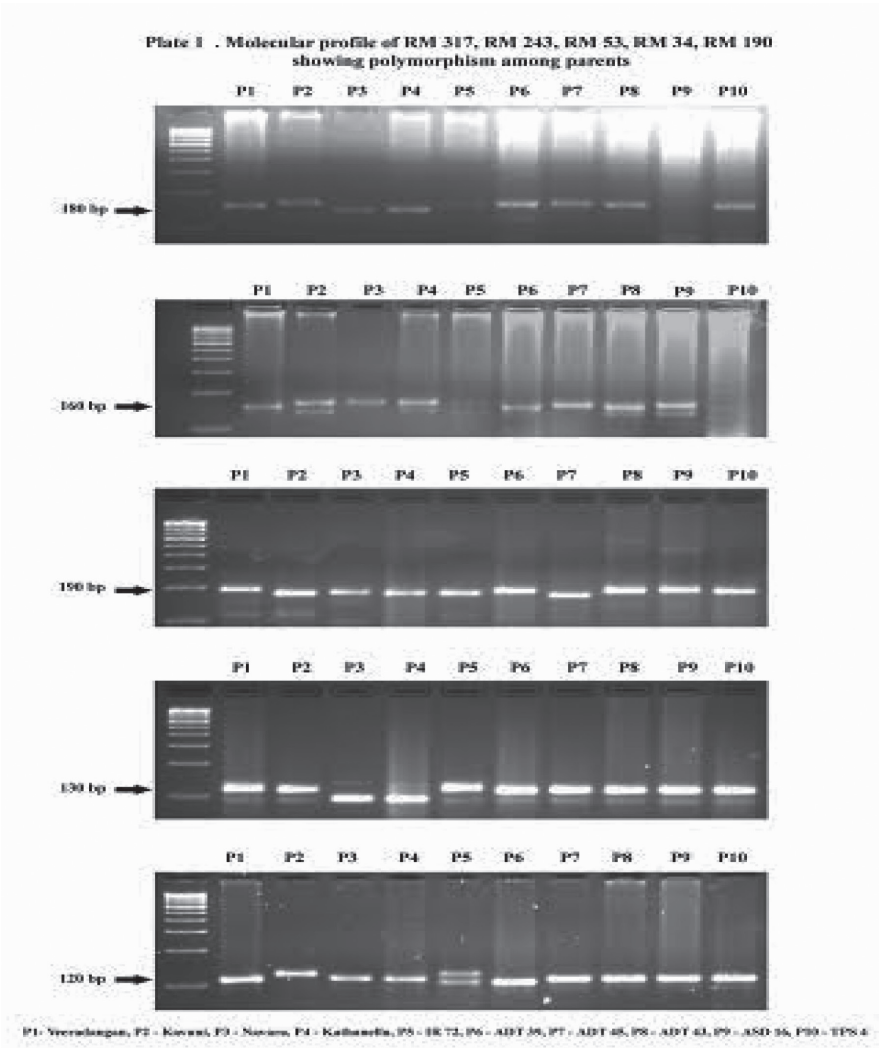


Fig. 1: Dendrogram of parental polymorphism based on SSR markers



The markers were selected based on nutritive value of the rice for calcium, magnesium, iron and zinc contents. A total of 10 primers RM 300 and RM 53 for iron content, RM 34 and RM 237 for zinc content, RM 312 for magnesium content, RM 317 for calcium content, RM 18 and RM 234 for protein content and RM 190 for waxy gene content. Out of 10 primers, 9 were polymorphic primers for parents. In this investigation the extent of genetic diversity of 10 parents of rice was estimated using SSR markers. The markers were selected based on nutritive value of the rice for calcium, magnesium, iron and zinc contents. A total of 20 polymorphic alleles were generated by ten primers. Out of 10 primers, 9 were polymorphic primers (Plate 1).

Similarity indices for all pair-wise combinations among the ten parents were presented in Table 2. The data of SSR markers was analysed using Sequential Hierachial and Nested (SHAN) clustering methods of the NTSYS – pc program (Rolf, 2001) based on Jaccard's similarity coefficient with an unweighted pair group method with arithmetic average (UPGMA). The similarity index values were computed as a ratio of number of similar bands to the number of bands in each pair wise comparisons of all the ten parents. The similarity index was highest (0.93) between ADT 39 and ADT 43 along with TPS 4 and IR 72. The least similarity index (0.37) was observed between Kavuni and Veeradangan. The cluster analysis was performed similarity coefficient matrices calculated from SSR marker to generate a dendrogram of ten parents (Figure 1). The similarity ranged from 0.49 to 0.93. As a whole the ten genotypes were grouped into 5 clusters at 71 per cent similarity levels. Cluster 1 was constituted by Navara, Kathanellu, ADT 39 and ADT 43. Cluster 2 was constituted by IR 72, ASD 16 and TPS 4. Cluster 3, cluster 4 and cluster 5 consisted of one genotypes respectively *viz.*, Veeradangan, ADT 45 and Kavuni. So, the diverse parents ADT 39, ADT 43, IR 72 and TPS 4 can be used for crossing programme. Similar result was reported by Rekha *et al.* (2011) and Karpagam (2011).

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