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Molecular characterization and diversity analysis of traditional and elite cultivars of rice using simple sequence repeat (SSR) markers

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ABSTRACT

Molecular characterization and diversity analysis of thirty-two rice genotypes using 11 SSR primers were undertaken in order to further utilize these lines in the breeding programmes. The results revealed that 7 primers showed distinct polymorphism among the genotypes consistently. A total of 33 amplification products were obtained with all the selected seven primers. The number of alleles varied from 3 to 7 with a mean of 4.71. The PIC values of 7 primers varied from 0.1986 to 0.3038 with a mean of 0.2625. Cluster analysis based on SSR data revealed that the genotypes MTU-2077 and JGL-1798 were clustered at the two extremes based on their similarity coefficients. This information could be used in hybridization programmes for the improvement of rice.

Key words: *rice, SSR markers, genetic diversity and molecular characterization*

Characterization of the elite and traditional cultivars in the working germplasm collections is imperative in order to choose the parents for initiation of a breeding programme. Diversity analysis based on phenological and morphological studies, though made a great impact in rice crop improvement usually vary with the environment and evaluation of these traits requires growing the plants to full maturity prior to identification. DNA based molecular markers are highly useful in this context since they are available in abundance and clearly allow the comparison of genetic material at juvenile phase avoiding any environmental influence on gene expression. Information regarding genetic diversity at molecular level could be used to identify and characterize genetically unique germplasm that compliments existing cultivars. Several types of molecular markers *viz*., Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) or microsatellite markers are available today. Microsatellite markers based on variation in the number of Simple Sequence Repeats (SSRs), have become the markers of choice due to ease of their use and co-dominant nature. Microsatellites or SSRs are PCR based markers that are both technically efficient and cost-effective and common in rice (Chen *et al.*, 1997). Compared to others, SSRs detect a significantly higher degree of polymorphism in rice (Wu and Tanksley, 1993) and are especially suitable for evaluating genetic diversity among closely related cultivars (Akagi *et al.*, 1997). Hence, in the present investigation 32 genotypes were characterized using SSR markers to study the level of diversity and to establish genetic similarities among themselves.

MATERIALS AND METHODS

Thirty-two genotypes of rice consisting of elite and traditional cultivars with diverse genetic origin were used in this study. The genotypes were sown in pots under sterile conditions in green house. At the age of 18 days old, healthy leaf samples were collected and used for DNA extraction.

DNA was extracted by the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980). The quality and quantity of DNA was estimated using spectrophotometer at 260 nm. Eleven SSR primers distributed among different chromosomes were selected randomly and used for

PCR amplification. PCR amplifications were performed in 25 ìl of reaction mixture containing 2.5 ìl of 10X buffer, 2.5 ìl of $MgCl_2$ (25mM L^{-1}), 0.2 ìl of dNTPs (20 m M L^{-1}), 2 ìl of 0.6 picomoles each of forward and reverse primers (Table 1), 0.06 ìl of Taq DNA polymerase (5 U L^{-1}), 1 ìl of template DNA (50 ng/ìl) and 14.74 ìl of ddH_2O. The polymerase chain reaction was performed by using Corbett Palm Cycler with the following temperature profile. The initial denaturation was 95°C for 2 minutes followed by 35 cycles of denaturing at 94°Cfor 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 2 minutes and ending up with 2 minutes at 72°C for the final extension. The annealing temperature was adjusted as per the specific requirement of each primer pair. The PCR products were electrophoresed in 3% agarose gel at 100V for 3 hours in 1X TBE buffer. The gels were next stained with ethidium bromide (10 mg/ml). A 1 kb ladder (Fermentas) was used for appropriate sizing of the products. The gel was photographed under UV light using Alpha Innotech Corporation gel doc system.

SSR alleles were scored sequentially from the largest to the smallest size band based on their position relative to the ladder. Each fragment that was amplified using each SSR primer was scored as 1 or 0 depending on its presence or absence. Polymorphic Information Content (PIC) values were calculated as follows (Chattopadhyay *et al.*, 2008):

PIC =1/n “2f(1-f)

Where, f = proportion of a particular allele among the genotypes

n = number of alleles recorded for a particular primer

The genetic associations among 32 genotypes of rice were evaluated using Jaccard’s similarity co-efficient and clustered with unweighted pair-group method of arithmetical averages (UPGMA) analysis. Statistical Package for Social Sciences (SPSS) package was used for the cluster analysis and subsequent dendrogram preparation.

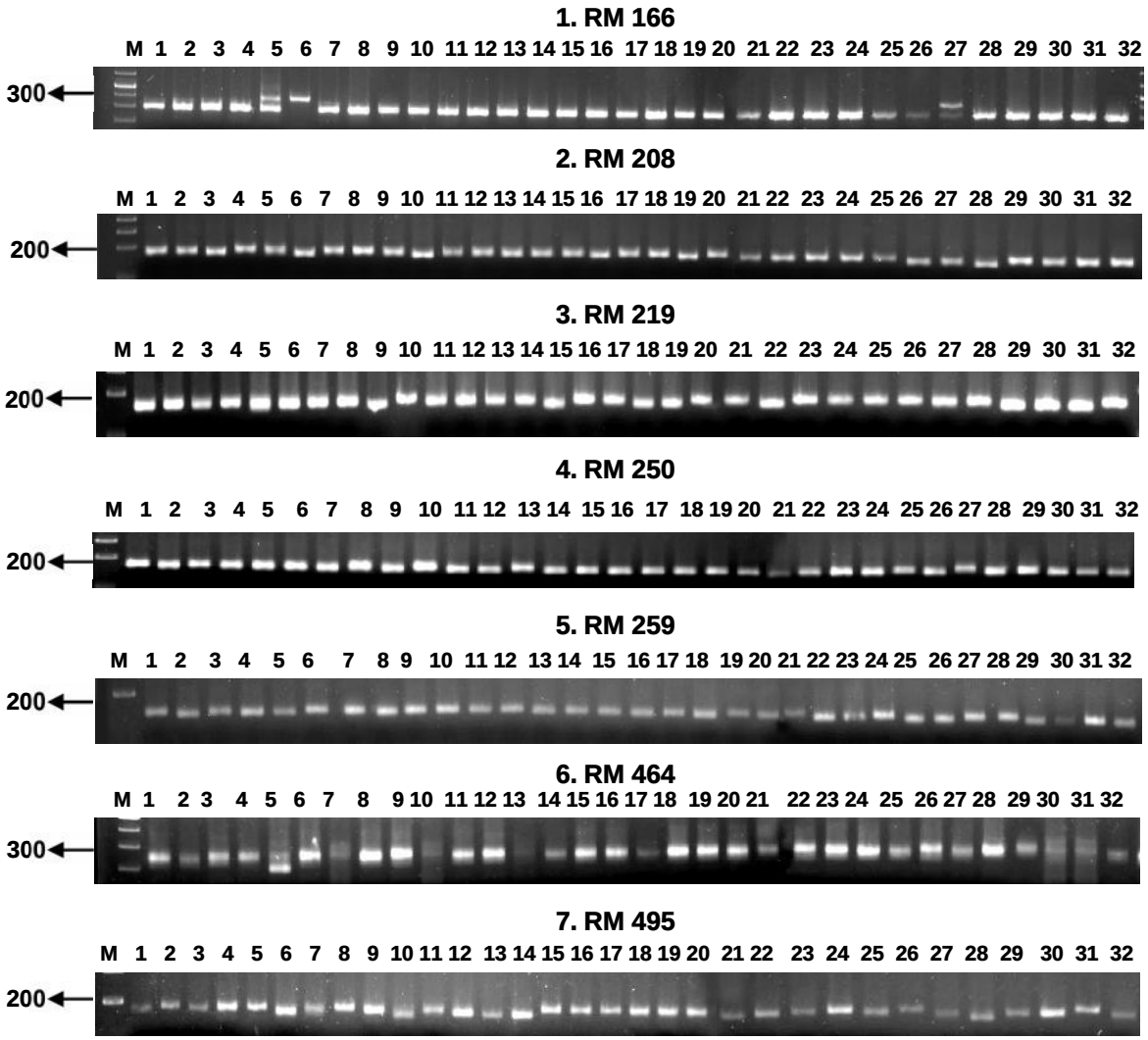
RESULTS AND DISCUSSION

Out of eleven SSR markers, seven markers that were amplified in all the genotypes consistently were selected to analyze the variation among the genotypes. These markers produced alleles ranging from 3 to 7 with an average of 4.7 alleles. A total of 33 alleles were obtained and all were polymorphic in nature (Table 1). A high degree of polymorphism was obtained with the marker RM 495 (7 bands) followed by RM 208, RM 259 and RM 464 (5 bands each) and RM 166 and RM 219 (4 bands each) and RM 250 (3 bands) indicating a greater magnitude of diversity among the genotypes included in the investigation (Fig 1). Ravi *et al*. (2003) and Chakravarthy and Rambabu (2006) also reported significant differences in allelic diversity among various microsatellite loci in rice. All these markers showed an average PIC value of 0.2625, which infers that yet some more markers that cover the entire genome were to be used to confirm the genetic divergence existing in the material to the maximum extent.

Jaccard’s similarity co-efficients among thirty-two genotypes were calculated to establish the genetic relationships. The similarity index values varied from 0.000 to 1.000, indicating the presence of wide range of genetic diversity at molecular level among the thirty-two genotypes. The genotypes MTU-2077 and MTU-1081 were closely related. Ganesh *et al*. (2007), Chakravarthy and Rambabu (2006) and Hee Chung *et al*. (2007) also noticed a wide range of genetic diversity by SSR markers at molecular level in germplasm lines of rice.

Table 1. List of SSR primer pairs selected along with their nucleotide sequence, PIC values and allelic information in rice

Primer pair	Nucleotide sequence (52 to 32)	PIC values	No. of alleles
RM 166	**F** GGT CCT GGG TCA ATA ATT GGG TTA CC**R** TTG CTG CAT GAT CCT AAA CCG G	0.2567	4
RM 208	**F** TCT GCA AGC CTT GTC TGA TG**R** TAA GTC GAT CAT TGT GTG GAC C	0.3038	5
RM 219	**F** CGT CGG ATG ATG TAA AGC CT**R** CAT ATC GGC ATT CGC CTG	0.2997	4
RM 250	**F** GGT TCA AAC CAA GCT GAT CA**R** GAT GAA GGC CTT CCA CGC AG	0.2876	3
RM 259	**F** TGG AGT TTGAGA GGA GGG**R** CTT GTT GCA TGG TGC CAT GT	0.2257	5
RM 464	**F** AAC GGG CAC ATT CTG TCT TC**R** TGG AAG ACC TGA TCG TTT CC	0.2655	5
RM 495	**F** AAT CCA AGG TGC AGA GAT GG**R** CAA CGA TGA CGA ACA CAA	0.1986	7



M = Marker

1. Velluthachera	2. MTU-1031	3. Lalnakanda	4. Vasundhara	5. MTU-1071
6. Plutikambani	7. MTU-2077	8. MTU-1081	9. MTU-9993	10. MTU-5249
11. MTU-1061	12. ADT-43	13. MTU-7029	14. Bhadraj	15. BPT-5204
16. Lunisree	17. RGL-2537	18. NLR-145	19. Triguna	20. Salivahana
21. Dular	22. TKM-6	23. Sasyasree	24. Mahsuri	25. MTU-1001
26. Intivadlu	27. ARC-5757	28. Accession no.11103	29. PR-106	30. TN-1
31. JGL-1798	32. MTU-2067			

Fig. 1. SSR gel profiles of thirty-two rice genotypes using following primers

The dendrogram (Fig.2) generated using pooled data divided the thirty-two genotypes into six clusters at about 30 % similarity level. Cluster I was the largest with 17 genotypes and cluster IV was the smallest with only 1 genotype. The generated dendrogram showed that the genotype MTU-2077 of cluster I and JGL-1798 of cluster VI were clustered at the two extremes based on their similarity co-efficients. The genotypes MTU-2077 and MTU-1081 of cluster I belonging to the same ecological region (Maruteru) exhibited maximum similarity based on the present set of markers used in the present investigation. The genotypes Bhadraj from Hazaribagh were closely associated to ADT-43 from Tamil Nadu and RGL-2537 from Ragolu. The tendency of accessions in clusters irrespective of geographic boundaries demonstrates that geographical

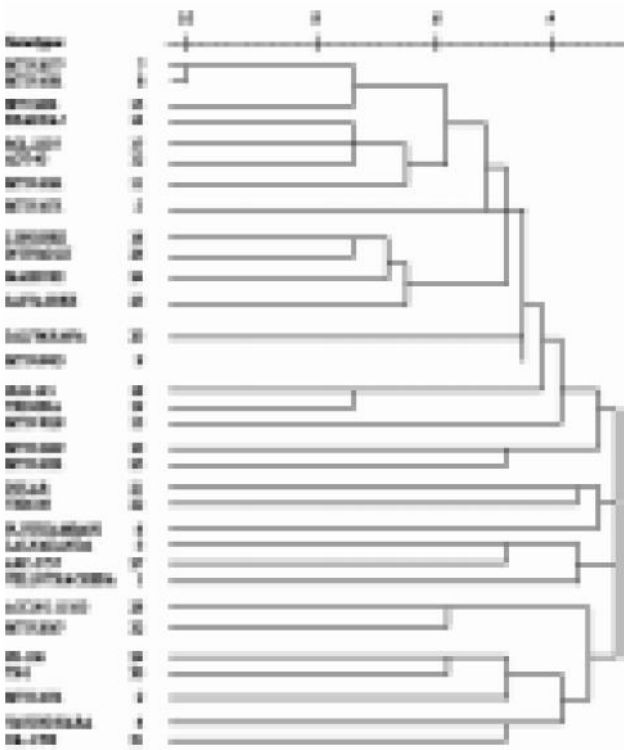


Fig.2. Dendrogram generated using UPGMA analysis showing relationship between 32 genotypes of rice using SSR markers

isolation is not the only factor causing genetic diversity. These results also showed that despite their common origin i.e. Maruteru, the genotypes MTU-2077 (cluster I), MTU-5249 (cluster II) and MTU-2067 (cluster VI) exhibited diversity at genetic level. Cluster IV comprised of only one genotype, Plutikambani, which exhibited less similarity with other genotypes and was genetically more distinct and diverse.

On the whole, based on molecular diversity analysis using SSR markers, hybridization programme may be initiated between the genotypes Bhadraj, MTU-1061 and NLR-145 from cluster I, Velluthachera from cluster V and/or Vasundhara from cluster VI in order to get transgressive segregants, since these genotypes showed maximum diversity among themselves. Besides this, the SSR polymorphism identified in the present cultivars that are differing in various qualitative and quantitative traits, would be highly useful in undertaking quantitative trait loci (QTL) mapping experiments as well as planned utilization of promising genotypes in breeding for various desired objectives for the rice crop improvement.

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