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Full Length Research Paper

SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L)

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The genetic diversity and DNA fingerprinting of 15 elite rice genotypes using 30 SSR primers on chromosome numbers 7-12 was investigated. The results revealed that all the primers showed distinct polymorphism among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. Cluster analysis grouped the rice genotypes into 10 classes in which japonica types DH-1 (Azucena) and Moroborekan clustered separately from indica types. Principal component analysis was done to visualize genetic relationships among the elite breeding lines. The results were similar to UPGMA results. Based on this study, the larger range of similarity values for related cultivars using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize 9 varieties using 18 different RM primers. This information can be used in background selections during backcross breeding programs.

Key words: PCR, polymorphism, DNA fingerprinting, dendogram.

INTRODUCTION

Rice, *Oryza sativa* (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals. It is grown under diverse cultural conditions and over wide geographical range. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. Approximately 11% of the world's arable land is planted annually to rice, and it ranks next to wheat. The world's rice production has doubled during last 25 years, largely due to the use of improved technology such as high yielding varieties and better crop management practices (Byerlee, 1996). Further scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and use of new biotechnological tools. There is wide genetic variability available in rice among and between wild relatives and varieties leaving a wide scope for future crop improvement.

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Moreover, rice is also an ideal model plant for the study of grass genetics and genome organization due to its diploid genetics, relatively small genome size 430 Mb (Causse et al., 1994; Kurata et al., 1994), significant level of genetic polymorphism (McCouch et al., 1998; Tanksley, 1989; Wang et al., 1992), large amount of well-conserved genetically diverse material (approximately 100,000 accessions of rice germplasm worldwide) and the availability of widely collected, compatible wild species.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains.

Diversity based on phenological and morphological characters usually vary with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. Protein or isozyme marker studies are also influenced by environment and reveal low

polymorphism. Now, the rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of plants.

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidationof genetic relationships within and among species. Several molecular markers viz. RFLP (Becker et al., 1995; Paran and Michelmore, 1993;), RAPD (Tingey and Deltufo, 1993; Williams et al., 1990), SSRs (Levinson and Gutman, 1987), ISSRs (Albani and Wilkinson, 1998; Blair et al., 1999), AFLP (Mackill et al., 1996; Thomas et al., 1995; Vos et al., 1995; Zhu et al., 1998) and SNPs (Vieux,et al., 2002) are presently available to assess the variability and diversity at molecular level (Joshi et al., 2000). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars. In the present investigation the following objectives were studied; assessment of genetic variability and diversity at molecular level among 15 elite rice genotypes and DNA fingerprinting of 15 elite rice genotypes using SSR markers on chromosomes 7-14.

Table 1. SSR primers used to amplify the *O. sativa* in this study.

Primer code	Primer sequence (5′ to 3′)
RM 21	F ACAGTATTCCGTAGGCACGG
	R GCTCCATGAGGGTGGTAGAG
RM 216	F GCATGGCCGATGGTAAAG
	R TGTATAAAACCACACGGCCA
RM 171	F AACGCGAGGACACGTACTTAC
	R ACGAGATACGTACGCCTTTG
RM 286	F GGCTTCATCTTTGGC GAC
	R CCGGATTCACGAGATAAACTC
RM 536	F TCTCTCCTCTTGTTTGG CTC
	R ACACACCAACACGACCACAC
RM R	F ACAGTATCCAAGGCCC T
	R CACGTGAGACAAAGACGGAG
RM 206	F CCCATGCGTTTAACTAT TCT
	R CGTTCCATCGATCCGTATGG
RM 19	F CAAAAACAGAGCAGATGAC
	R CTCAAGATGGACGCCAAGA
RM 20	F ATCTTGTCCCTGCAGGTCAT
	R GAAACAGAGGCACATTTCATTG
RM R	F ACGAGCTCTCGATCAGCCT A
	R TCGGTCTCCATGTCCCAC
RM 167	F GATCCAGCGTGAGGAA CACGT
	R AGTCCGACCACAAGGTGCGTTGTC
RM 333	F GTACGACTACGAGTGTCACCAA
	R GTCTTCGCGATCACRCGC
RM 264	F GTTGCGTCCTACTGCTACTTC
	R GATCCGTGTCGATGATTAGC
RM 125	F ATCAGCAGCCATGGCAGCGACC
	R AGGGGATCATGTGCCGAAGGCC

Table 1. contd.

RM 566	F ACCCAACTACGATCAGCTCG
	R CTCCAGGAACACGCTCTTTC
RM 320	F CAACGTGATCGAGGATAGATC
	R GGATTTGCTTACCACAGCTC
RM 247	F TAGTGCCGATCGATGTAACG
	R CATATGGTTTTGACAAAGCG
RM 544	F TGTGAGCCTGAGCAATAACG
	R GAAGCGTGTGATATCGCATG
RM 346	F CGAGAGAGCCCATAACTACG
	R ACAAGACGACGAGGAGGGAC
RM 547	F TAGGTTGGCAGACCTTTTCG
	R GTCAAGATCATTCTCGTAGCG
RM 10	F TTGTCAAGAGGAGGCATCG
	R CAGAATGGGAAATGGGTCC
RM 286	F GGCTTCATCTTTGGCGAC
	R CCGGATTCACGAGATAAACTC
RM 519	F AGAGAGCCCCTAAATTTCCG
	R AGGTACGCTCACCTGTGGAC
RM 149	F GCTGACCAACGAACCTAGGCCG
	R GTTGGAAGCCTTTCCTCGTAACACG
RM 210	F TCACATTCGGTGGCATTG
	R CGAGGATGGTTGTTCACTTG
RM 346	F CGAGAGAGCCCATAACTACG
	R ACAAGACGACGAGGAGGGAC
RM 144	F TGCCCTGGCGCAAATTTGATCC
	R GCTAGAGGAGATCAGATGGTAGTGCATG
RM 561	F GAGCTGTTTTGGACTACGGC
	R GAGTAGCTTTCTCCCACCCC
RM 47	F ACTCCACTCCACTCCCCAC
	R GTCAGCAGGTCGGACGTC
RM 222	F CTTAAATGGGCCACATGCG
	R CAAAGCTTCCGGCCAAAAG

MATERIAL AND METHOD

DNA extraction

Fifteen elite rice genotypes constituted the experimental material. List of Rice Genotypes Selected, DH1 (Azucena), DH2 (IR 64), Sambamashuri (Tall), Sambamashuri (Dwarf), IR 24, CO-39, DM 360, Moroborekan, IR 64, IR 91-1591-3, 25 A, 29 A, AJAYA-R and Thellahamsa. These genotypes are successively being used in MRF rice breeding programmes. The DNA was isolated from the leaves by modified PVP method that is regularly being followed at MRF biotechnology laboratory. It was found that DNA isolated by modified Dellaporta method was of high purity and the yield was also substantial (Dellaporta et al., 1983). Hence the DNA isolated for all the samples by this method was used for further analysis.

Polymerase chain reaction (PCR)

The polymerase chain reaction was carried out in a DNA DYAD Engine Peltier Thermal Cycler using primers listed in Table 1. The

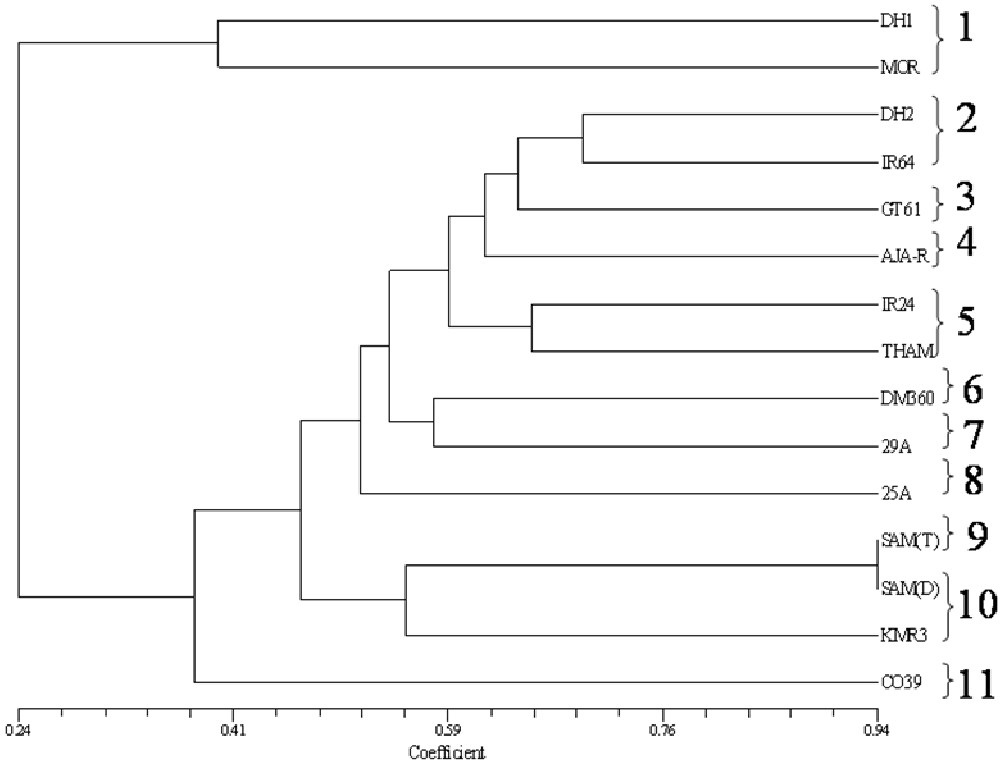

Figure 1. Clustering of 15 *O. sativa* species based on pooled SSR markers.

PCR reaction mix includes the following: DNA, 10 ng/µl; 10X buffer; 10 mM dNTPs; 50 mM $MgCl_2$; 10 µM each of forward and reverse primers. The PCR profile starts with 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min extension at 72°C for 2 min. A final extension 72°C for 7 min was included. The PCR products were electrophoresed in a 2% agarose gels (for SSRs) at 100 V for 2-3 h. The gel was then stained in ethidium bromide for 30 min, de-stained for 15-30 min and then observed on a UV transilluminator. The PCR products of SSR primers were run on 4% polyacrylamide gels to achieve better resolution of the bands. After the gel was dried completely, it was scanned using BioRad Model GS-700 Imaging Densitometer and the individual bands were scored for further analysis.

Data analysis

All the genotypes were scored for the presence and absence of the SSR bands. And the data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02J. The 0/1 matrix was used to calculate Similarity as DICE coefficient using SIMQUAL subroutine in SIMILARITY routine. The resultant similarity matrix was employed to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method with Arithmetic Means (UPGMA) to infer genetic relationships and phylogeny. In addition, Principal Component Analysis (PCA) based clustering was also done using the subroutine EIGEN.

RESULTS AND DISCUSSION

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Results obtained in genetic diversity studies of *O. sativa* cultivars with RFLP and RAPD markers indicate that more genetic diversity exists in *indica* and *japonica* gene pools (Fuentes et al., 1999; Qjan et al., 1995). Classical breeding affects genetic diversity with in breeding programs. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao et al., 1998). In the present investigation microsatellites (Rice microsatellites) or SSR markers (Simple Sequence Repeats) from chromosome numbers 7-12 were used to characterize and to assess genetic diversity among 15 breeding lines of rice.

A total of 30 RM primers were utilized to provide genetic diversity among 15 elite breeding lines of rice belonging to *indica* and *japonica* types. All 30 RM primers showed polymorphism between 15 rice cultivars. A total of 462 bands were scored and of which no bands were found to be monomorphic. The study revealed that the primer RM20 on chromosome No.12 have seven alleles

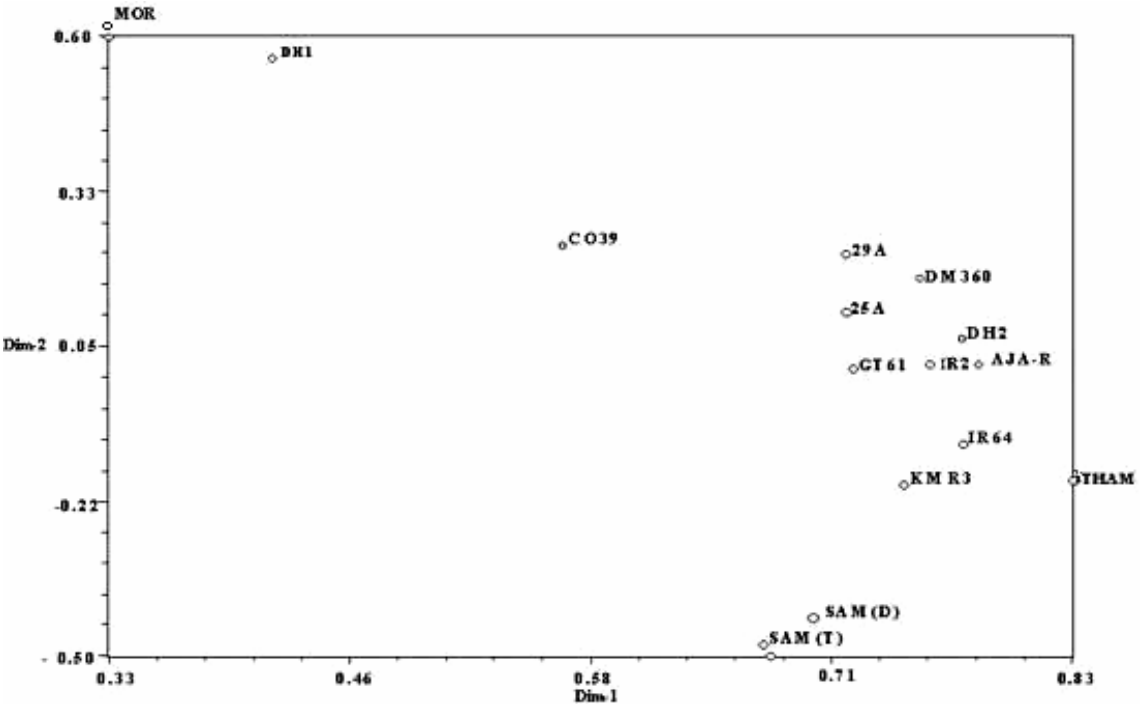

Figure 2. Principle Component Analysis grouping of 15 *O. sativa* species based on pooled SSR markers.

compared to primers RM536, RM320, RM544, RM536, RM519, RM346, RM561 and RM47 belonging to different chromosomes with two allele levels. Similar observations were made by Akagi et al. (1997) that RM1 and RM3 and four other RM prefixed markers to be less polymorphic. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch et. al., 2001).

Rice similarity ratio revealed that high degree of similarity to the extent of 94% exists between Sambamashuri (Tall) and Sambamashuri (Dwarf) whereas very low level of similarity of 12% exists between Moroborekan and Thellahamsa. It is important to note here that Sambamashuri (Dwarf) is a mutant line of Sambamashuri (Tall) hence shows highest similarity co-efficient indicating their belonging to similar genetic background. Whereas Moroborekan is a *japonica* type variety showing the least similarity with *indica* type variety Thellahamsa. Similar studies were made by different authors using SSR markers (Panaud et al., 1996).

Cluster analysis was used to group the varieties and to construct a dendogram. The similarity matrix representing the DICE Co-efficient was used to cluster the data using the UPGMA algorithm. The UPGMA based dendogram obtained from the binary data deduced from the DNA profiles of the samples analysed adds a new dimension to the genetic similarity perspectives generated. A total of 11 distinct groups resulted out of analysis of pooled SSR marker data (Figure 1). This dendogram revealed that the genotypes that are derivatives of genetically similar type clustered more together. The *japonica* types DH-1 (Azucena) and Moroberekon clustered in to a separate group. *Indica* cultivars clustered at more than 50% similarity co-efficient compared to *japonica* types (similarity co-efficient less than 25%). Ko et al. (1994) found three *indica* types to cluster at 80% similarity to a selection of Australian, U.S, Japanese, Italian cultivars. In this study, the larger range of similarity values for cultivars revealed by micro satellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. Principle component analysis was also done to visualize genetic relationships among the elite breeding lines (Figure 2). The results were similar to UPGMA results.

Based on study the large range of similarity values for related cultivars using microsatellites provides greater confidence for the assessment of genetic diversity and relationships. The practical approach developed in the study is useful in DNA fingerprinting also. Among the 30 SSR RM primers studied, 19 primers spread over chromosome numbers 7-12 were found to be useful in fingerprinting of nine genotypes of the present study. We observed that Moroborekan fingerprinting can be done by 7 different primers followed by IR64 with 5 primers. Fingerprinting of the remaining cultivars can be done using a minimum of one or two RM primers. This fingerprinting makes identification and characterization of genotype very easy and further it will be of greater help in background selections during back cross breeding programs.

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