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Estimation of Genetic Diversity among the Rice (*Oryza sativa* L.) Genotypes using Microsatellite Markers

Megha Joshi[1], PK Singh[1*], Pallavi[2], Showkat A Waza[1] and Aparajita Singh[1]
[1]*Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India*
[2]*Department of Botany, Institute of Science, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India*

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Sixteen microsatellite markers were used to study the genetic diversity among 73 genotypes of rice from geographically diverse locations. Thirteen markers revealed polymorphism, while three were observed to show the monomorphic amplicons. The polymorphic markers produced total of 34 alleles with an average of 2.62 alleles per locus. Amplification products ranged from 100 bp produced by marker K39512 to 220 bp produced by marker RM536. Polymorphism Information Content (PIC) value varied from 0.225 to 0.743 and the marker RM336 was observed to be most suitable for discriminating the genotypes owing to its highest PIC value. The UPGMA cluster diagram grouped the genotypes into eight major clusters. The Jaccard's similarity coefficients ranged from 0.16 to 1.00.

Key Words: Dendrogram, Diversity, Microsatellite markers, PIC value, Rice

Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world population and is one of the most important food crops grown worldwide. It is also a model monocot plant for genetic and genomic studies. However, the annual increase in rice yield has decreased from 2.4% (in the late 1980s) to 1.0% at present (Fischer *et al.*, 2014). One of the possible causes in low genetic gains of rice crop is the continuous use of genetically related elite germplasm in breeding programmes. Exploitation of genetic diversity among parental genotypes is one of the major approaches to maximize genetic gains in rice breeding programmes. Genetic diversity study helps in estimating and establishing the genetic relationship in germplasm collections for identifying diverse parental combinations to create segregating progenies with maximum genetic variability. It helps in the identification of superior recombinant lines for further selection and introgressing desirable genes from diverse germplasm (Thompson *et al.,* 1998; Islam *et al.,* 2012).

Genetic diversity is commonly estimated by genetic distance or similarity analyses, both of which entail that there are either differences or similarities at the genetic level (Weir, 1990). It can be evaluated using morphological traits, biochemical and molecular markers. However, DNA polymorphism based genetic variations are abundant and independent of environmental factors. Molecular Marker based Genetic Diversity Analysis (MMGDA) also has potential for assessing changes in genetic diversity over time and space (Duwick, 1984). Among various types of molecular markers, simple sequence repeats (SSRs) are efficient in detecting genetic polymorphisms and discriminating among genotypes from germplasms of various sources. These markers can detect finer levels of variation among closely related breeding lines even within a same variety (Lapitan *et al.,* 2007).

A number of studies have been carried out to study genetic diversity within the rice genotypes belonging to *indica* and *japonica* types using a set of SSR markers (Ravi *et al.,* 2003; Herrera *et al.,* 2008; Singh *et al.,* 2014; Waza *et al.,* 2013). The results revealed that the SSR markers showed distinct polymorphism among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. In the present study, an attempt has been made to classify and understand the nature and magnitude of genetic diversity among various genotypes of rice using SSR markers.

Materials and Methods

The research material consisted of 73 morphologically diverse rice genotypes from geographically variable locations (Table 1). DNA was extracted from the leaves

**Author for Correspondence: Email- pksbhu@gmail.com*

Table 1. List of genotypes evaluated in the present study

S.No.	Genotype	Source	S.No.	Genotype	Source
1.	IC 260937	DBT, Networking project, BHU, Varanasi	37.	IC 356431	DBT, Networking project, BHU, Varanasi
2.	IC 260961		38.	IC 356432	
3.	IC 260964		39.	IC 356448	
4.	IC 267416		40.	IC 362206	
5.	IC 278777		41.	IC 382604	
6.	IC 280466		42.	IC 383396	
7.	IC 281785		43.	IC 383404	
8.	IC 281786		44.	IC 383441	
9.	IC 282418		45.	IC 383559	
10.	IC 282443		46.	IC 384176	
11.	IC 282463		47.	IC 384190	
12.	IC 282471		48.	IC 384260	
13.	IC 282514		49.	IC 391524	
14.	IC 282808		50.	IC 418382	
15.	IC 282815		51.	IC 426012	
16.	IC 282816		52.	IC 426013	
17.	IC 282822		53.	IC 426017	
18.	IC 282824		54.	IC 426058	
19.	IC 331196		55.	IC 426060	
20.	IC 334180		56.	IC 426061	
21.	IC 337051		57.	IC 426137	
22.	IC 337367		58.	IC 438644	
23.	IC 337558		59.	IC 446975	
24.	IC 337578		60.	EC 545051	
25.	IC 337582		61.	EC 545061	
26.	IC 337588		62.	EC 545165	
27.	IC 337593		63.	Koshihikari	IRRI, Philippines
28.	IC 341351		64.	Mars	
29.	IC 346002		65.	Sang khla	
30.	IC 346004		66.	Swarna	
31.	IC 346813		67.	HUR-W-1	BHU, Varanasi
32.	IC 346880		68.	S-183	CRRI, Cuttack
33.	IC 356117		69.	Sarjoo-52	NRCPB, New Delhi
34.	IC 356419		70.	S-148	CRRI, Cuttack
35.	IC 356422		71.	S-155	
36.	IC 356429		72.	Nirboi	IRRI, Philippines
			73.	Tetep	

of 15 days old seedlings according to CTAB method with little modifications (Doyle and Doyle, 1987). DNA quality was evaluated by electrophoresis in 0.8% agarose gel and quantification was accomplished using spectrophotometer.

For molecular analysis, 16 randomly selected SSR markers were used (Table 2). DNA amplification was carried out in 10 µl reaction mixtures containing 5 ng /10µl template DNA, 1x PCR buffer, 2.4 mM $MgCl_2$, 0.12 mM dNTPs, 0.7 pM of each primer (forward and reverse) and 0.6 U of Taq DNA Polymerase. Polymerase chain reaction (PCR) was carried out in a thermal cycler (Eppendorf, USA) with the following temperature cycle profile: Initial denaturation at 94°C for 4 min, 40 cycles each of 1 min denaturation at 94°C followed 30 sec annealing at 55°C to 65°C (depending on the primer used) and 1 min extension at 72°C, and finally 4 min at 72°C for the final extension.

Table 2. Allele size (bp) and polymorphism information content (PIC) of the SSR primers used in the present study

S.No.	Primer	PIC	No. of alleles amplified	Approximate size of amplified product (bp)
1.	Sbq1	0.00	1	210 (monomorphic)
2.	sbq 11	0.42	2	150-160
3.	RM 3691	0.26	2	130-160
4.	RM 3428	0.24	2	140-170
5.	K 39512	0.23	2	100-110
6.	RM 536	0.34	2	210-220
7.	RM5481	0.00	1	100 (monomorphic)
8.	RM 224	0.66	3	125-160
9.	RM 306	0.52	3	150-210
10.	RM 338	0.00	1	180 (monomorphic)
11.	RM 257	0.32	2	110-150
12.	RM 210	0.64	4	110-210
13.	RM 202	0.36	2	160-180
14.	RM 209	0.69	3	110-150
15.	RM 336	0.74	4	140-210
16.	RM 251	0.67	3	140-190

The amplified products were separated in 2.5 percent agarose gel prepared in 1x TAE (Tris-acetate-EDTA) buffer and stained with ethidium bromide. The gels were run in 1x TAE buffer at constant voltage of 65 V for 3 hours. They were visualized and photographs taken using gel documentation instrument (BioRad). Clearly resolved and unambiguous bands for each primer were scored in the form of matrix as 1 (presence) and 0 (absence) for each genotype.

Statistical analysis was carried out using the software NTSYS-pc version 2.1 (Rohlf, 1998). The data from all the markers were analyzed to estimate similarity based on the number of shared bands. Similarity was assessed with SIMQUAL module of NTSYS that calculates various types of similarity and dissimilarity coefficients for qualitative data. The similarity matrix estimates based on Dice coefficient of similarity were estimated. The dendrogram based on Unweighted Pair Group Method based on Arithmetic Average (UPGMA) was generated using the similarity matrix. Polymorphic information content (PIC) was estimated using the formula proposed by Nei (1973).

$$PIC = 1-\sum x^2_k$$

Where, x^2_k represents the frequency of the k^{th} allele.

Results and Discussion

Seventy three rice genotypes were analysed using SSR markers to assess genetic diversity. Out of the sixteen primers used, thirteen produced reproducible and polymorphic DNA banding pattern, while three primers (sbq1, RM5481 and RM338) were found to be monomorphic. The thirteen polymorphic primers produced total of 34 fragments, whose size varied from 100bp (marker K39512) to 220bp (marker RM536). Among the polymorphic markers, 7 revealed 2 alleles each, 4 showed 3 alleles each and 2 produced a maximum of 4 alleles each. Maximum of four fragments were produced by primers RM336 and RM210. An average of 2.6 fragments were produced by the primers showing polymorphic amplification. The value is lower than that of 5.89 and 5.66 per microsatellite locus as reported by Lapitan *et al.* (2007) and Hoque *et al.* (2014), respectively. Gel images showing SSR banding profiles obtained by primer RM224 are presented in Fig. 1.

Polymorphism information content (PIC) provides information about allele diversity and frequency among the genotypes. Its value for each marker can be evaluated on the basis of alleles, which varied among the SSR loci tested. Higher the PIC value of a locus, higher the number of alleles detected. The PIC value of SSR markers in present study ranged from 0.225 to 0.743 with an average value of 0.47. Markers RM336, RM209, RM251 and RM224 were most informative on the basis of high PIC value of 0.743, 0.694, 0.668 and 0.656, respectively. SSR marker K39512 showed least PIC value of 0.225. The early studies on PIC values ranged from 0.24 to 0.92 with an average of 0.61 (Jain *et al.,* 2004), 0.19 to 0.90 with an average of 0.75 (Borba *et al.,* 2009), 0.356 to 0.798 with an average of 0.543 (Hoque *et al.,* 2014), which is relatively higher

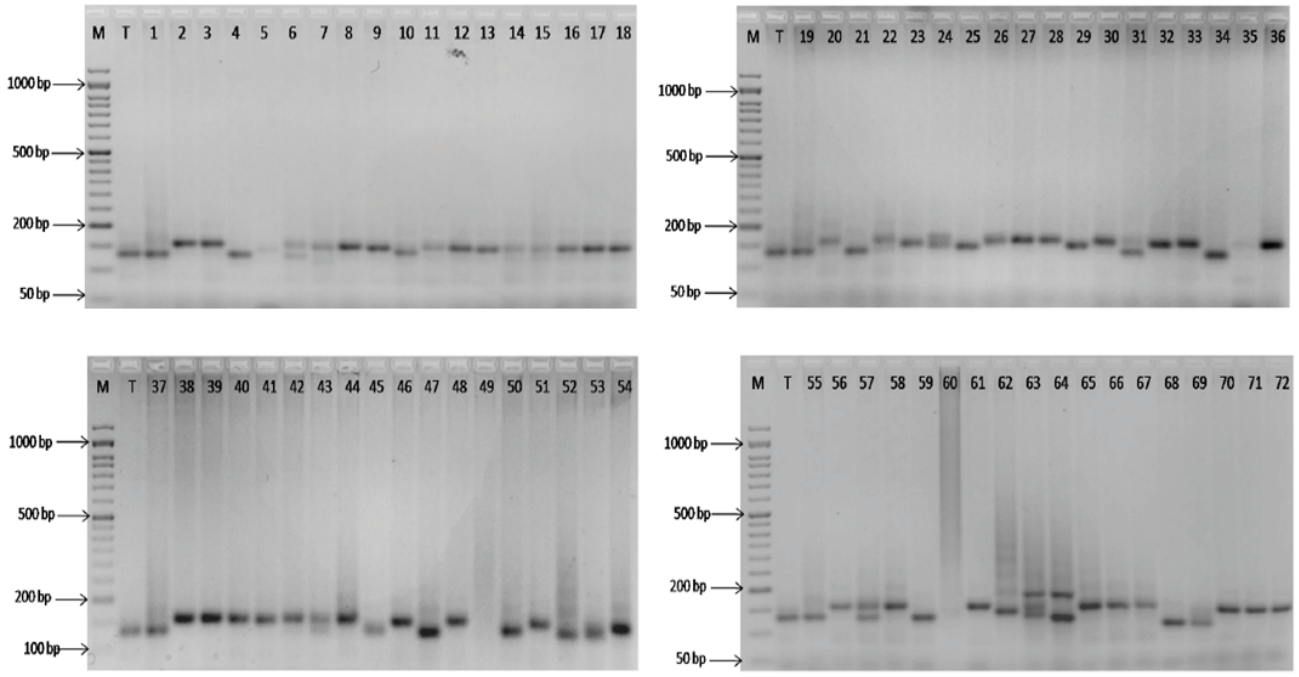

Fig. 1. Gel images showing SSR banding profile obtained by primer RM224. Lanes 1-72 represents the rice accessions as listed in Table 1; M = 100bp DNA size marker and T = Tetep variety (73rd rice accession in Table 1)

than that of present study. RM336 was found to be the most appropriate marker for discriminating various rice genotypes owing to its highest PIC value of 0.743.

A UPGMA cluster diagram obtained by all the 34 alleles (amplification products) generated by 13 polymorphic SSR markers grouped the genotypes into eight major clusters (Fig. 2). Cluster I, consisted of 38 genotypes, which were again subdivided into two sub clusters, *viz.*, Ia (14 genotypes) and Ib (24 genotypes). Cluster II includes only 3 genotypes. Cluster III contained twelve genotypes which were further divided into two sub clusters IIIa (6 genotypes) and IIIb (6 genotypes). Two genotypes were present in Cluster IV and six in Cluster V. Cluster VI includes five genotypes, whereas cluster VII consisted of six. The cluster VIII was monogenic containing only one genotype. Diversity studies involving cluster analysis have also been revealed by the earlier studies of Pervaiz *et al.* (2010), Tabkhkar *et al.* (2012) and Hoque *et al.* (2014). Kumar *et al.* (2014) reported that the genotypes studied were divided into five clusters, each consisting of accessions with distinct features. El-Malky *et al.* (2007) employed SSR makers for diversity studies and observed that genotypes evaluated were divided into two groups, one included the *indica* varieties and the other consisting of *japonica* types. Zeng *et al.* (2004) reported that the genotypes studied were grouped into two major branches in the dendrogram, one branch represents *japonica* rice and another represents the indica or the hybrids between *japonica* and *indica*.

The Jaccard's similarity coefficients in the present study ranged from 0.16 to 1.00. Hoque *et al.* (2014) observed the similarity coefficient range of 0.21 to 1.00 in the genotypes they evaluated. The pairwise similarity indices revealed 100% genetic similarity between the genotypes IC 282822 and IC 282824. These genotypes were found to be duplicate with respect to the loci studied. Sajib *et al.* (2012) also reported Deepa and Patnai-23 as duplicate accessions. Moreover, findings of the present study suggested that variety Tatep has least similarity with the genotype IC 267416 followed by IC 334180, IC 337578, IC 384176, IC 391524, IC 418382 and IC 446975.

The information regarding genetic diversity by microsatellite markers provides greater confidence for assessment of distinctness and relationships among various genotypes, which can be exploited in rice breeding programmes. With the help of microsatellite makers and clustering data, various distantly related rice genotypes may be combined by intercrossing to get hybrid varieties with highest heterosis and to screen

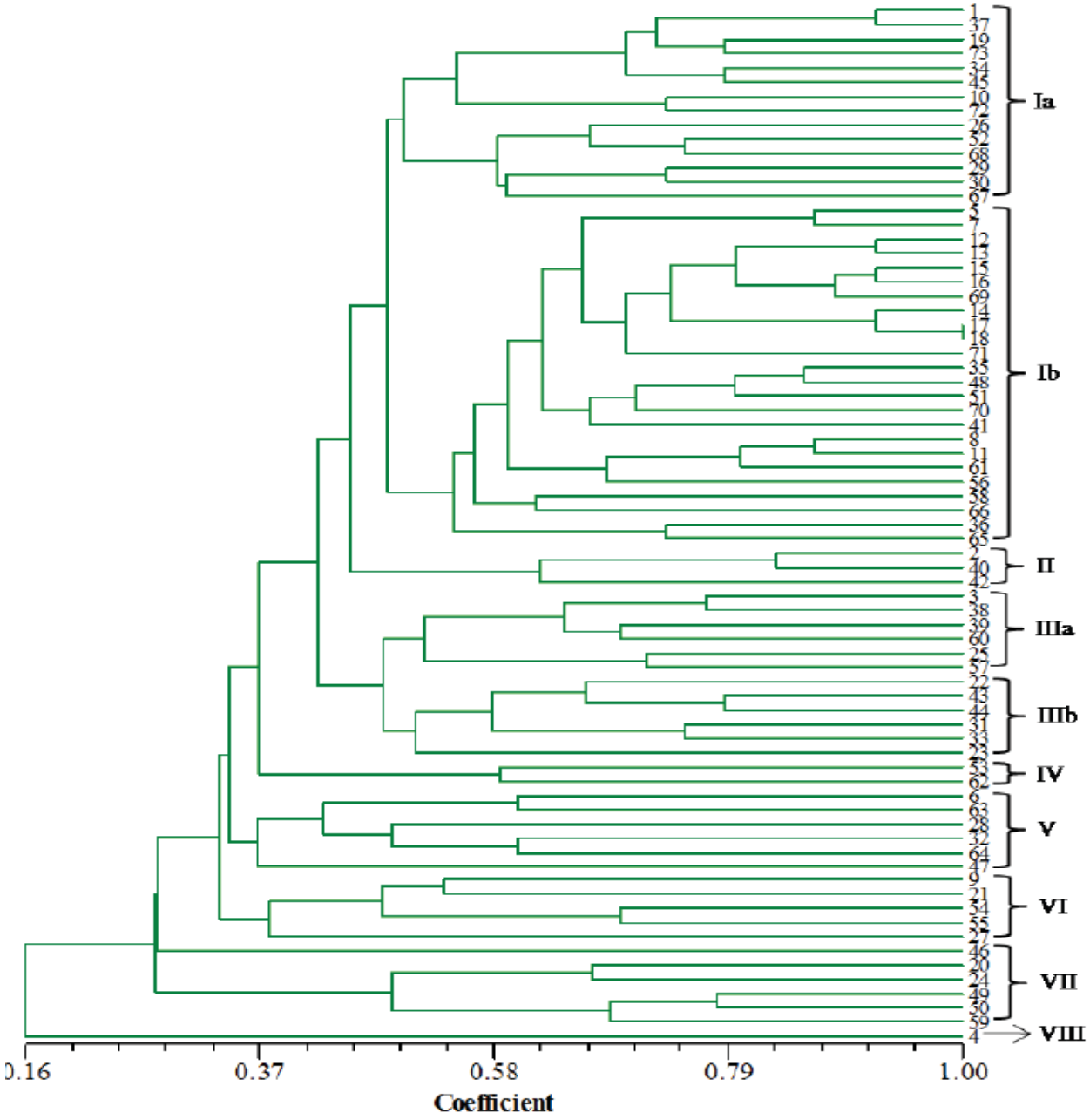

Fig. 2. UPGMA cluster dendrogram showing genetic relationship among various rice genotypes

out desirable genotypes from segregating generations. Moreover, the results can be used for fingerprinting and purity testing of genotypes.

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