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Full Length Research Paper

Assessment of genetic diversity of rice (*Oryza sativa*) cultivars using simple sequence repeat (SSR) markers

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A set of 36 polymorphic simple sequence repeat (SSR) primers well distributed on all the 12 rice chromosomes have been used to assess the genetic diversity among the rice varieties. A total of 98 alleles were detected with an average of 2.78 alleles per locus across 39 genotypes. The number of alleles varied from two to four of these SSR loci. Among the primers, RM 401, RM 20A, RM 536, RM 7575 and RM 5862 produced maximum number of alleles (4). Out of the 98 alleles, 86 were polymorphic and this exhibited 87.76% polymorphism. SSR marker analysis differentiated the genotypes into two distinct groups. The polymorphism information content (PIC) value of the markers ranged between 0.28 and 0.50 with a mean value of 0.45. The highest PIC value (0.50) was observed for ten primers that is, RM 2, RM 24, RM 411, RM 104, RM 266, RM 125, RM 4674, RM 247, RM 3476 and RM 3351 while the lowest was 0.28 for RM 29. The genetic similarity coefficients for 39 genotypes obtained with SSR markers ranged from 23.8 to 78.4%.

Key words: Rice, genetic diversity, simple sequence repeat (SSR) markers.

INTRODUCTION

For the application of marker assisted selection (MAS) within a subspecies, it is important to obtain information on the genetic diversity within a rice subspecies over different genomic regions. Accurate identification keys based only on morphological and physiological parameters with clear cut features of distinctness are not always possible. Thus, other characteristics obtained with the help of new laboratory based technologies, supplement the need for precise description. Numerous genes of economic importance having quantitative nature are repeatedly transferred from one varietal background to another by plant breeders through conventional breeding methodologies. Phenotypic observations of many traits are time consuming and expensive, if such genes can be tagged with DNA markers, time and money can be saved when transferring them from one varietal background to another. The application of molecular marking techniques may be applied as the first step towards efficient conservation, maintenance and utilization of such genetic diversity of rice.

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. Simple sequence repeats markers (microsatellites) are co-dominant, hypervariable, abundant and well distributed throughout the rice genome (Temnykh et al., 2001). The

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application of microsatellites markers in rice include characterization of the genetic structure of the cultivated rice *O. sativa* at both the inter and intra-varietal level, genetic diversity and/or evolutionary analyses of landraces, weedy and wild rice germplasm, determination of the purity of breeding material or seed stocks, prediction of hybrid performance and heterosis and the analyses and tagging of valuable quantitative trait loci (QTL) and genes (Gao, 2005).

DNA marker technology has provided an efficient tool to facilitate plant genetic resource conservation and management. A wide variety of DNA markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Insertion/Deletion markers (INDEL) etc. have been extensively used in rice for genetic diversity analysis, phylogenetic and evolutionary studies, mapping and tagging genes for quantitative traits of agronomic importance and marker assisted selection (Patel et al., 2014). Kibria et al. (2009) has screened several rice varieties for studying the genetic diversity by using SSR and RAPD markers. SSR markers can estimate genetic diversity between cultivars for example between parents of gene pool or between plants extracted from a population or between populations. Microsatellites are more powerful for the identification of within cultivar variation (Lapitan et al., 2007). Thus, in the present investigation 39 genotypes were characterized using SSR markers to study the level of diversity and to establish genetic similarities among themselves.

MATERIALS AND METHODS

A total of 39 rice genotypes were used in this study (Table 1). The plants were raised in Randomized Block Design with three replications during September 2008 at Paddy Breeding Station, Coimbatore. Total genomic DNA isolated from the genotypes was described by Porobreski et al. (1997) method with a slight modification. Mercaptoethanol (1%) was added to the extraction buffer to remove the phenolics. Approximately 1.0 g of leaf tissue was ground with liquid nitrogen and to this powder 750 µL of preheated (65°C) CTAB buffer was added. It was then incubated at 65°C in a water bath for 1 h. After bringing the tubes to room temperature, equal volume (750 µL) of chloroform: isoamyl alcohol (24:1) was added and the contents were mixed well for 10 min to form an emulsion. It was then centrifuged at 10000 rpm for 15 min. The supernatant was transferred to a fresh tube and the chloroform: isoamyl alcohol step was repeated. The aqueous phase was transferred to a new tube and $2/3^{rd}$ volume of ice cold isopropanol was added and incubated in a freezer overnight. The contents were then centrifuged at 10000 rpm for 10 minutes at 4°C. The pellet was now saved by discarding the solution. The pellet was washed with 70% ethanol and air dried. It was finally dissolved in TE buffer (0.2 M EDTA+1M Tris) and stored at – 20°C.

A total of 50 random primers were utilized for SSR analysis, out of which 36 primers were selected on the basis of the clarity of banding patterns and polymorphism and the list of primers surveyed are listed in Table 2. The PCR reaction was conducted in a reaction volume of 15 µL containing nine ng of genomic DNA, 1.5 µL of 10X PCR buffer (including 15 mM $MgCl_2$), 0.6 µl of 10mM each of dATP, dTTP, dGTP and dCTP, 2 µL of forward and reverse SSR primer, 0.3 µl of Taq DNA polymerase (Bangalore Genei Pvt Ltd, Bangalore) and sterile water. Amplifications were performed in Bio-Rad (MyCycler thermal cycler) and Corbett PCR machine. The thermal cycler was programmed as follows. one cycle of 94°C for 1 min followed by 35 cycles of 94°C for 1 min, 55/61°C for 1 min., and 72°C for 2 min., and finally 1 cycle of 72°C for 5 min. PCR products were kept at 4°C until further use.

The PCR products were subjected to electrophoresis in 3% Agarose. Data were scaled as 1 (present) and 0 (not present) for all the alleles of each of the SSR locus. Polymorphism information content (PIC) was computed according to the method of Wilkie et al. (1997).

$$PICi = 1-\sum_{j=1}^{n} Pij^2$$

Where, Pij is the frequency of the jth allele at the ith locus summed over the number of alleles (n). A similarity coefficient matrix was calculated from binary data using Jaccards coefficient, followed by clustering from the estimated genetic distance and the phylogenetic tree topology was inferred with the clustering method of the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The cluster analysis and subsequent dendrogram construction was performed using NTSYS-PC VERSION 2.02 (Rohlf, 1999).

RESULTS AND DISCUSSION

Out of 50 SSR markers, 36 markers that were polymorphic in all the genotypes consistently were selected to analyze the variation among the genotypes (Table 2). The random primers produced a total of 98 alleles with an average of 2.78 alleles per primer across 39 genotypes. The number of alleles varied from two to four of these SSR loci. Among the primers, RM 401, RM 20A, RM 536, RM 7575 and RM 5862 produced maximum number of alleles (4) Out of the 98 alleles, 86 were polymorphic and this exhibited 87.76 per cent polymorphism. The band size ranged from 100 bp to 500 bp. Shefatur Rahman et al. (2009) who recorded 6.33 alleles per locus using a small set of three SSR markers on 34 varieties. Tabkhkar et al., (2012) reported that the overall size of amplified products ranged from 93bp in locus RM276 to 169bp in locus RM584. PIC is a quantification of the number of alleles that a marker has and the frequency of each of the alleles in the subset of germplasm tested. Since, a marker with fewer alleles has less power to distinguish several samples, and alleles present at low frequency have less power to distinguish, a higher PIC is assigned to a marker with many alleles and with alleles present at roughly equal proportions in the population (Jiang et al., 2010). The PIC (Polymorphism Information Content) ranged between 0.28 and 0.50 with a mean value of 0.45. The highest PIC value (0.50) was observed for ten primers *viz.,* RM 2, RM 24, RM 411, RM 104, RM 266, RM 125, RM 4674, RM 247, RM 3476 and RM 3351 while the lowest was 0.28 for RM 29. Our results both for number of alleles and PIC values are comparable to those reported by Thomson et al. (2007) and Lapitan et al. (2007). Patel et al., (2014) reported that among 9 SSR markers and 12 INDEL markers highest PIC value was obtained for INDEL marker R9M10 0.81.

Dendrogram resulting from cluster analysis of SSR data

Table 1. Materials used in the present study.

S/N	Genotypes	Pedigree	Source
1	IR 72081	IR 58025A/6*IR 65493-67-3-2-2-4-10-6-9-1/ IR 65493-67-3-2-2-4-10-6-9-1	IRRI, Philippines
2	IR 75596	D297A/7*IR 68897B/ IR 68897B	IRRI, Philippines
3	IR 75601	G46A/7*IR 68897B/ IR 68897B	IRRI, Philippines
4	IR 75608	KRISHNA A/7* 69627B/IR 69627B	IRRI, Philippines
5	IR 80154	IR 75603A/4* IR73329-80-2-3/ IR73329-80-2-3	IRRI, Philippines
6	IR 80559	IR 73328A/6*IR 73330-25-2-2/ IR 73330-25-2-2	IRRI, Philippines
7	CRMS 32	KALINGA/MIRAI	CRRI, Cuttack
8	APMS 6	-	APRRI, Maruteru
9	IR 62037-93-1-3-1-1	IR 48563-123-5-5-2/IRRI 104	IRRI, Philippines
10	IR 62036-222-3-3-1-2	IR 48563-123-5-5-2/IR 42068-22-3-3-1-3	IRRI, Philippines
11	IR 63881-49-2-1-3-2	IR 52280-117-1-1-3/IR 53294-65-1-1-3	IRRI, Philippines
12	IR 62124-83-3-2-1	IR 50358-102-2-3-3/IR 52280-117-1-1-3	IRRI, Philippines
13	IR 72865-94-3-3-2	IR 63868-2-3-2—2/IR 67023-30-3-3-2-2	IRRI, Philippines
14	IR 62030-83-1-3-2	IR 48525-65-2-1 / IR 52280-117-1-1-3	IRRI, Philippines
15	IR 59673-93-2-3-3	IR 48566-22-3-2-3/IR 28239-94-2-3-6-2	IRRI, Philippines
16	IR 68427-8-3-3-2	IR 58029-180-2-3-3/IR 44624-127-1-2-2-3	IRRI, Philippines
17	IR 68926-61-2	IR 34686-179-1-2-1R/IR 40750-82-2-2-3R	IRRI, Philippines
18	AD 01260	CR 1009 / JEERAGASAMBA	Aduthurai
19	MDU 5	RESELECTION FROM IR 10476 – 28-2-2 (*O. glaberrima* x pokkali)	Madurai
20	ACK 99017	INDUCED MUTANT OF WHITE PONNI AT 35 KR	Killikulam
21	AD 01259	CR 1009/JEERAGASAMBA	Aduthurai
22	TP 1021	ADT 43/JEERAGASAMBA	Thirupathisaram
23	RR 363-1	GAURAV / KALINGA III	CRRI, Cuttack
24	RR 361-3	SNEHA / GAURAV	CRRI, Cuttack
25	RR 354-1	RR 20-2-10 / RR 158-327	CRRI, Cuttack
26	RR 347-1	RR 19-2 / RR 149 – 1129	CRRI, Cuttack
27	RR 286-1	RR 165 – 1160 / RR 145 -22	CRRI, Cuttack
28	RR 348-6	CR 143-2-2/ANNADA	CRRI, Cuttack
29	RR 434-3	CR 222MW10 / BROWN GORA	CRRI, Cuttack
30	RR 433-1	IRAT 112 /CR 222 MW10	CRRI, Cuttack
31	ASD 06-2	ADT 36/BPT 5204	Ambasamudram
32	RR 166-645	C22/CR 289-1208	CRRI, Cuttack
33	ASD 06-3	ADT 36/TKM 9	Ambasamudram
34	ASD 06-4	ADT 39/BPT 5204	Ambasamudram
35	ASD 06-8	IR 50/TKM 9	Ambasamudram
36	ASD 06-7	IR 50/ASD 16	Ambasamudram
37	ASD 06-5	CO 43/ASD 16	Ambasamudram
38	ASD 06-6	CO 43/ADT 36	Ambasamudram
39	ASD 06-1	ASD 16/ADT 36	Ambasamudram

obtained from 39 rice genotypes is presented in Figure 1. The Jaccard's similarity coefficients for 39 genotypes obtained with SSR markers were calculated to establish the genetic relationships and the similarity index values ranged from 23.8 to 78.4%, this indicating the presence of wide range of genetic diversity at molecular level among thirty nine genotypes. Untung Sustano et al. (2008) reported parents of the mapping populations with 37%of similarity representing the maximum diversity, on the basis of 183 SSR markers. Lestari et al. (2009) observed that as nucleotide differences among genotypes are a major source of heritable variation, molecular markers derived from them should provide an effective measure of genotypic variation and hence phenotypic differences among varieties. Thirteen genotypes *viz.,* IR 80559 A, APMS 6 A, IR 72081 A, IR 75601 A, IR 75596 A, IR 80154 A,

Table 2. Number of alleles, their chromosomal locations and PIC value across 39 rice genotypes.

S/N	Marker	Number of alleles	Number of polymorphic bands	Percentage of poly morphism	Polymorphism information content (PIC)	Amplified fragment size range (bp)	Chromosome number	Annealing temperature (°C)
1	RM 216	3	2	66.67	0.44	100-200	10	57
2	RM 2	2	2	100.00	0.50	120-150	7	59
3	RM 174	2	2	100.00	0.40	180-200	2	66
4	RM 492	3	3	100.00	0.42	230-260	2	59
5	RM 24	3	1	33.33	0.50	150-500	1	59
6	RM 411	2	1	50.00	0.50	100-110	3	57
7	RM 132	2	1	50.00	0.49	100-150	3	65
8	RM 104	3	3	100.00	0.50	150-300	1	65
9	RM 570	3	3	100.00	0.44	250-320	3	59
10	RM 401	4	4	100.00	0.41	300-380	4	59
11	RM 584	3	2	66.67	0.46	150-200	6	59
12	RM 20 A	4	4	100.00	0.43	100-400	12	57
13	RM 147	3	3	100.00	0.39	100-210	10	66
14	RM 266	2	2	100.00	0.50	150-180	2	53
15	RM 536	4	4	100.00	0.49	150-500	11	59
16	RM 144	3	3	100.00	0.36	100-220	11	64
17	RM 7575	4	4	100.00	0.41	120-300	2	61
18	RM 17	2	2	100.00	0.49	120-180	12	59
19	RM 125	3	2	66.67	0.50	180-220	7	66
20	RM 248	2	1	50.00	0.48	100-130	7	59
21	RM 4674	3	2	66.67	0.50	180-210	5	51
22	RM 247	2	2	100.00	0.50	150-160	6	54
23	RM 214	3	2	66.67	0.46	100-200	7	55
24	RM 244	2	1	50.00	0.40	180-200	10	57
25	RM 9	3	3	100.00	0.45	100-210	1	60
26	RM 341	2	2	100.00	0.48	150-180	2	55
27	RM 589	2	2	100.00	0.48	150-180	6	55
28	RM 7653	3	2	66.67	0.45	100-140	5	57
29	RM 3476	2	1	50.00	0.50	120-180	5	51
30	RM 289	3	3	100.00	0.43	100-200	5	55
31	RM 29	2	2	100.00	0.28	200-220	2	59
32	RM 257	2	2	100.00	0.37	150-180	9	57
33	RM 440	3	3	100.00	0.44	180-250	5	55
34	RM 239	3	3	100.00	0.46	100-150	10	55
35	RM 3351	2	2	100.00	0.50	180-200	5	57

Table 2. Contd.

36	RM 5862	4	4	100.00	0.40	120-400	2	50
	Mean	2.72	2.36	85.65	0.45	-	-	-
	SD	0.70	0.93	21.74	0.05	-	-	-

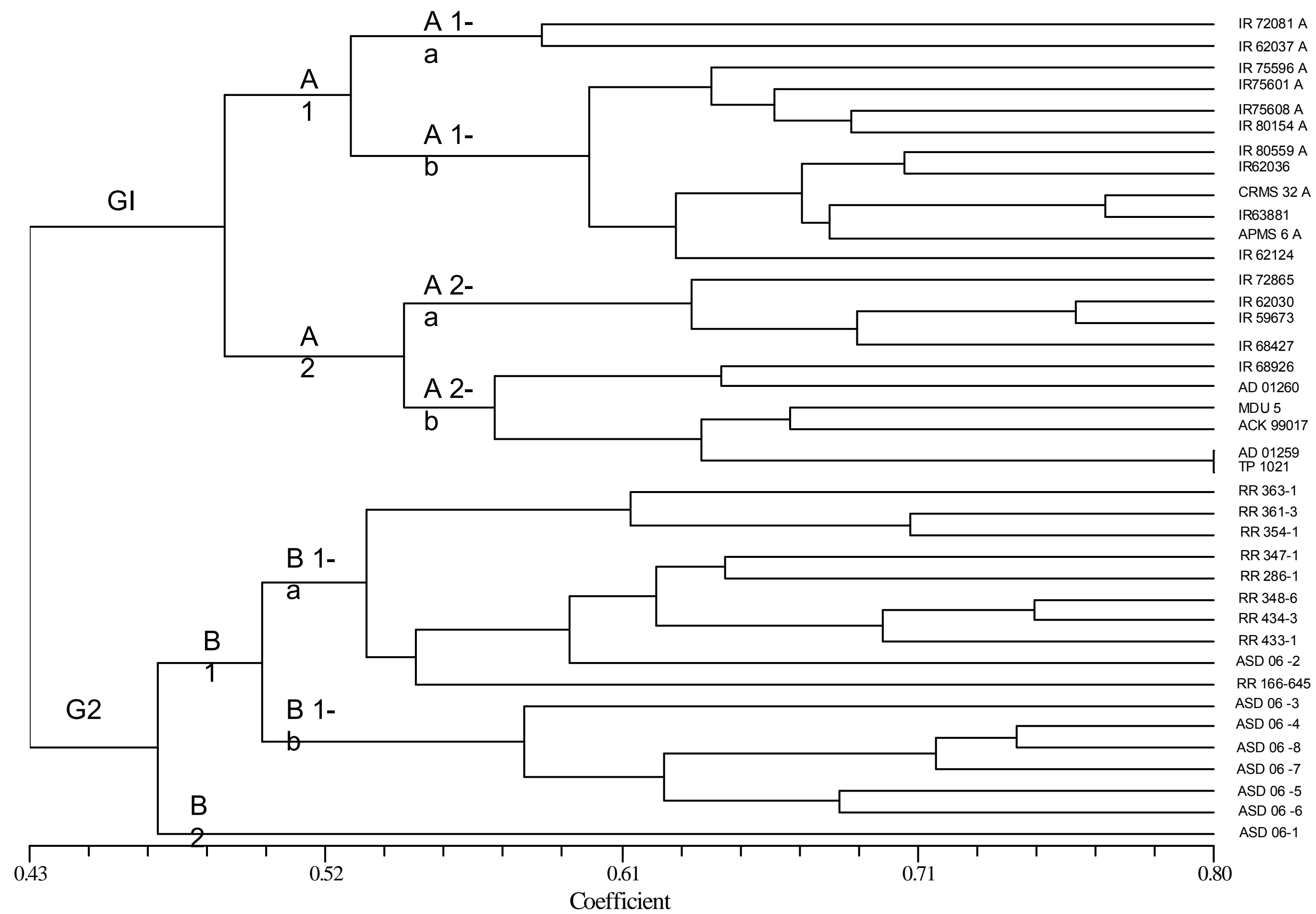

Figure 1. Dendrogram depicting genetic diversity among 39 genotypes using SSR markers.

CRMS 32 A, IR 75608 A, IR 62124-83-3-2-1, IR 62036-222-3-3-1-2, IR 63881-49-2-1-3-2, IR 72865-94-3-3-2 and IR 62030-83-1-3-2 were clustered in Group I, thirteen genotypes *viz.,* RR 354-1, RR 347-1, RR 348-6, RR 166-645, RR 433-1 RR 434-3, ASD 06-2, ASD 06-3, ASD 06-4, ASD 06-5, ASD 06-6 ASD 06-7 and ASD 06-8 were clustered in Group II, three genotypes *viz.,* RR 363-1, RR 361-1 and RR 286-1 clustered in Group II-B1a and three genotypes *viz.,* MDU 5, ACK 99017 and AD 01259 in Group I-A2b based on Jaccards similarity coefficient and these also confirmatory with D^2 values. Among the 39 genotypes, all the IR lines were clustered in cluster II (eleven) and all the RR lines (six) and ASD lines (six) were clustered in cluster I by dendrogram and also confirmed Mahalonobis D^2 statistics.]

The results indicate that geographical distribution and the source of the genotypes played major role in clustering along with the similarity and differences in their adaptation, selection criteria, selection pressure and environmental conditions. The above results provided an overview of the genetic diversity of the rice genotypes. This study provided a platform for identifying most appropriate parents for developing mapping populations to study the genetics of the biometrical and quality traits. The parents of the mapping populations were chosen keeping in mind maximization of genetic diversity estimates available from the present study. For example, parents of the mapping populations represent with >24% level of similarity representing the maximum diversity, on the basis of 50 SSR markers used in the present study. Mapping populations are currently being field evaluated and would be phenol typed for biometrical and quality related characters and genotyped with polymorphic SSR markers available from the present study for carrying out QTL mapping in the near future. The present work will be a boon for plant breeders in choosing the varieties for generating a new hybrid.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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