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Molecular characterization of rice genotypes using microsatellite markers

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ABSTRACT

Characterization of twenty rice genotypes using 20 SSR markers exhibited high polymorphism.The markers were distributed among 10 chromosomes of rice. A total of 101 alleles were amplified by twenty markers. An average of 5.05 alleles was produced. Average effective number of alleles was 3.77. Genetic diversity per locus for most of the selected markers was high. The primers RM12921, RM18384, RM23877, RM23744 RM257, RM25181, RM25735 and RM5479 were highly polymorphic. These microsatellites are useful in assessing the genetic diversity of rice. Cluster analysis performed by DARwin V. 5.1.153 using unweighted neighbor joining method clearly separated the genotypes into 3 main clusters with different sub-clusters within a cluster. Promising selections of parents for future hybridization program to generate desirable segregates has been suggested.

Key words:*rice, DNA extraction, primers, PCR amplification*

Rice is one of the principal cereal crops in India. The rice production in India has augmented significantly in the last 20 years largely because of availability of improved varieties and better crop management. However, to feed the ever increasing population of India, plant breeders need to focus on evolving varieties with higher productivity with desirable traits. The wide genetic resources available in rice offer greater scope for crop improvement program. Molecular marker technology provides useful information about markers which are linked to the traits of interest. The markers are in general, insensitive to environments and reproducible which turn the program towards more reliable predictable venture. Several molecular markers namely RFLP (Becker *et al*., 1995), RAPD (William *et al.,* 1990), Simple Sequence Repeat (Levinson and Gutman, 1987); AFLP (Mackill *et al.,* 1996) and Single Nucleotide Polymorphism (Vieux *et al*., 2002) are now available to determine genetic diversity of crop plants (Joshi *et al.,* 2000). Simple sequence repeat (SSR) or microsatellite markers are tandem repeats interspersed throughout the genome and can be amplified with apropos primers that belong to specific regions (Giovannani *et al.,* 1991). Being co-dominant and PCR based, SSRs are preferred over other markers for genetic study (Powell *et al.,* 1996). Moreover, SSRs are reproducible, abundant, locus specific and thus it is possible to identify chromosomal location of the gene (s) controlling the traits based on linked SSR markers (Neelu *et al.,* 2006).

The microsatellite markers are more polymorphic in rice than RFLP or RAPD (Wu and Tanksley 1993; Yang *et al.,* 1994) and are efficient in evaluating genetic diversity (Akai *et al.,* 1997). Wide genetic resources are available in the State of West Bengal as rice is the major cereal crop in this part of India. The present investigation was aimed at detecting genetic divergence within local genotypes of rice along with some other improved varieties through microsatellite markers.

MATERIALS AND METHODS

Twenty genotypes of rice (Table1) comprised the experimental materials. The genotypes were multiplied at Calcutta University Experimental Farm, Baruipur, West Bengal in 2010. DNA was extracted from young rice seedlings following the cetyl tri-methyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). Thirty rice microsatellite markers covering over

12 chromosomes were initially chosen from Gramene web site (www.gramene.org) using the simple rule: 20-25 nucleotides in length, 40-60 % G-C content and non complementary 3' nucleotides. Annealing temperature existed between 55^0C-65^0C. The primers were synthesized by Invitrogen (www.invitrogen.org). The motif for these markers is given in the website. Polymerase chain reaction (PCR) was carried out in 20 ul reaction volume containing 50 ng DNA, 10 uM of each primer pair, 10X PCR buffer, 50 mM $MgCl_2$, 2 mM dNTPs, 0.2 unit Taq polymerase (Bioline,UK). The reaction was carried out in Eppendorf thermocycler, model Super Pro (Germany). The PCR was programmed as an initial denaturation of 3 minute at 95^0C, 35 cycles of 1 min at 94^0C, 2 minute at the annealing temperature of a particular primer pair, 1 minute at 72^0C and a final extension for 5 mins at 72^0C.

The PCR products were run in a 2% agaorose gel (Thermo Fisher scientific, USA) in Bio-rad (USA) gel apparatus. The fragments were compared with marker DNA of Fermentas (USA). The fragments were calculated in software of UVP gel-documentation unit (UK).

Table 1. List of accessions of rice used in the present study

SNo.	Genotypes	Origin	Type	Seed source
1	Nayanmoni	West Bengal	Indica landrace	CU
2	Lemont	USA	Tropical Japonica	University of Aberdeen
3	Jajati	Orissa	Indica HYV	CRRI
4	Yugal	West Bengal	Indica landrace	CU
5	Radha	West Bengal	Indica landrace	CU
6	Gotra 2	West Bengal	Indica HYV	BCKV
7	Kalindi	West Bengal	Indica landrace	CU
8	Hatchal Patnai	West Bengal	Indica landrace	CU
9	Gotra 1	West Bengal	Indica HYV	BCKV
10	Rupali	West Bengal	Indica landrace	CU
11	Bakul Priya	West Bengal	Indica landrace	CU
12	Ganga	West Bengal	Indica landrace	CU
13	Kalingo II	Orissa, India	Indica HYV	CRRI
14	Saru Pankaj	West Bengal		CU
15	IR 36	Philippines	Indica HYV	IRRI
16	Khitish	West Bengal	Indica HYV	CU
17	Gajapati	Orissa, India	Indica HYV	CRRI
18	Ranjan	West Bengal	Indica landrace	CU
19	Patharea	Orissa, India	Indica HYV	CRRI
20	Khayersail	West Bengal	Indica landrace	CU

The size of intensively amplified band was only determined compared to standard marker. Amplified products were scored for presence and absence of marker allele as discrete variables, '1' for presence and '0' for absence. Effective allele per locus (A_{ep}) were calculated following Weir (1990) with the formula $1/(1-H_{ep})$, H_{ep} represents the genetic diversity for each locus and H_{ep} is equal to 1-"P_i^2 , P_i means the frequency of ith allele at the locus. A dendrogram was constructed to determine genetic diversity among the genotypes by applying un-weighted pair group method with arithmetic averages (UPGMA) clustering algorithm by software program DARwin 5.1 .153 (Perrier and Bonnot, 2003).

RESULTS AND DISCUSSION

Initially 30 SSR markers were selected for preliminary screening and the microsatellite markers displaying non-specific banding pattern, no polymorphism or without PCR products were discarded. Finally , 20 primers were selected (Table 2) and the primers produced multiple products in each genotype. Altogether 101 alleles were detected among the 20 genotypes with an average of 5.05 ± 1.64 alleles per locus . (Table 3) . The number of alleles ranged from 2 to 8 alleles. It was revealed from the data (Table 3) that the SSR primers RM25181 (Fig.1a) followed by RM5479 (Fig.1b) and RM23877 on chromosome 10, 12 and 8, respectively were more polymorphic while, SSR loci RM1369 (Fig.1d) on chromosome 6 showed a minimum of 2 alleles. Variable allelic diversity in microsatellite markers have been reported by several scientists (Akagi *et al.,*1999, McCouch *et al.,* 2001 and Ravi *et al.,* 2003). Ferreira and Grattapaglia (1998) concluded that microsatellite markers demonstrate high genetic diversity per locus because of their multi-allelism. In several SSR primers namely RM12921, RM1388, RM22306, RM23877, RM23744 (Fig.1c), RM25425, RM25181 and RM247, the number of alleles surpassed the average number of alleles. So, these primers are very useful in determining the genetic diversity of genotypes in rice. In the present finding the effective number of alleles was less than the observed alleles with mean being 3.77 (Table 3). No relationship was found between the number of alleles detected at a locus and the maximum number of simple repeats within the targeted microsatellite DNA (r=0.172). Ni *et al.,* 2002 obtained contradictory result as regards to repeat motif and they concluded that number of alleles detected in their finding was solely

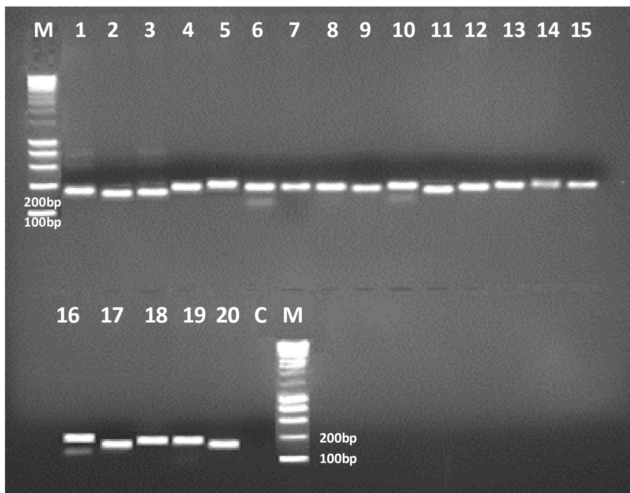

Fig. 1a. Agarose gel Electrophoresis picture of the rice genotypes with RM 25181

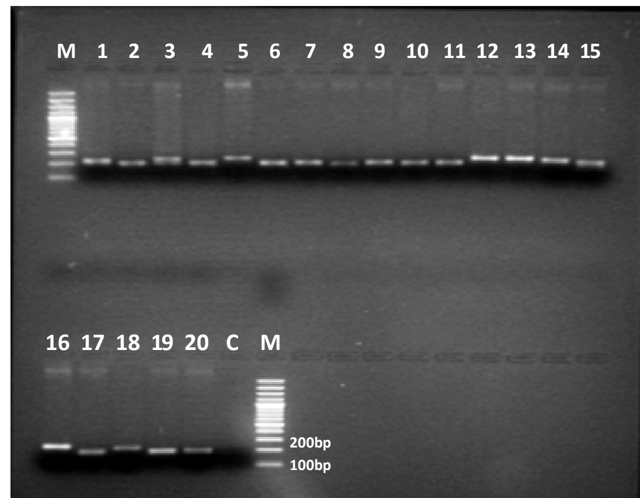

Fig. 1b. Agarose gel Electrophoresis picture of the rice genotypes with RM 5479

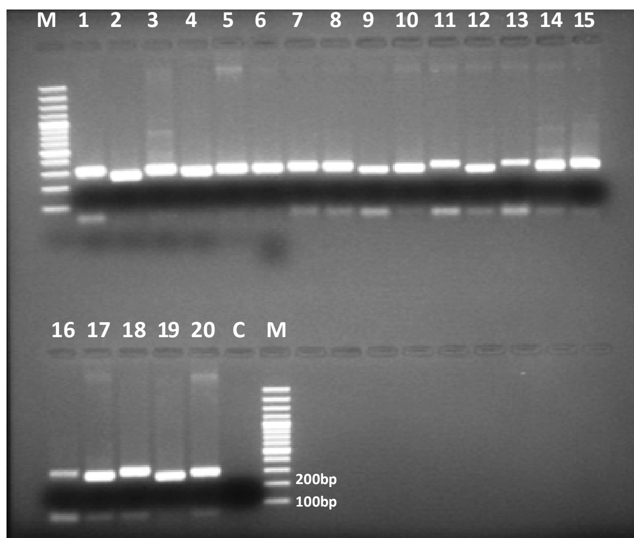

Fig. 1c. Agarose gel Electrophoresis picture of the rice genotypes with RM 23744

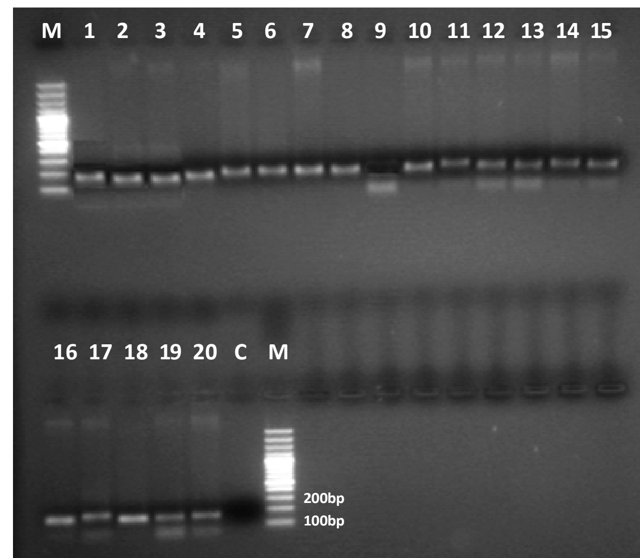

Fig. 1d. Agarose gel Electrophoresis picture of the rice genotypes with RM 1369

1 - Nayanmoni, 2 - Lemont, 3 - Jajati, 4 - Yugal, 5 - Radha, 6 - Gotra 2, 7 - Kalindi, 8 - Hatchal Patnai, 9 - Gotra 1, 10 - Rupali, 11 - Bakul Priya, 12 - Ganga, 13 - Kalingo II, 14 - Saru Pankaj, 15 - IR 36, 16 - Khitish, 17 - Gajapati, 18 - Ranjan, 19 - Patharea, 20 - Khayersail

dependent on maximum repeat number in microsatellite data. They worked with a large number of markers and perhaps that enabled them to get such correlation. However, Cuc *et al.,*2008 and Yu *et al.,* 1999 recorded insignificant correlation between repeat motif and PIC value.

Genetic diversity is defined as probability that two alleles in a population are different. Molecular marker technology is a reliable tool for assessing genetic variation in rice varieties Chakravarthi and Naravaneni (2006). Compared to morphological traits, molecular markers can disclose distinct difference at the DNA level, contributing efficient tool for germplasm profiling, which is insensitive to environmental influence. Among different markers, SSR markers are more popular in rice because they are highly informative, co-dominant,

Table 2. Simple sequence repeat primers used in rice

Name	Forward Primer	Reverse primer	Motif Repeat
RM13263	AAGATTGCACACTGGTGTTCTCC	AGAAGAGCCGGTCTTTGTCTCC	(CT)44
RM12921	TCGTATTTCCCGGTGTCTCAGG	ACTAGTACTCGGTGCAGGGAATCG	(CT)40
RM14076	GATTGTGGTGGCCGTACTGATACTCC	AGCTGCTCCTGCACTCTTCTGC	(GA)29
RM15251	CCTTACATTGGGATGCTTTAGTCC	CATCTCCCACCCTGTTGTCG	(CT)29
RM1388	TTCAATGAGGCAAAGGTAAG	CATCTCCCACCCTGTTGTCG	(AG)46
RM18384	GCAGCAGAAAGGGAGAGAGTATGG	CAGCAACGTACGTACCAACAGG	(CTT)22
RM20466	CTGCATCCTTGCGTAGGTTTAGG	TCTAAATAAAGCAGTCGCCAGTCG	(AG)27
RM19419	CCTCATGCACACCAACGACAGG	CATCTCCGAGCTCACACTCAAATCG	(CT)37
RM1369	AACCTGAGAGTGCCAATTGG	TCCCCTAGTAAAGCGGATTC	(AG)27
RM22306	AATAGCAGGATGCCGACTAGC	AGGTGTACATACCTAGACGACGTAGC	(TC)37
RM23877	TGCCACATGTTGAGAGTGATGC	TACGCAAGCCATGACAATTCG	(CA)30
RM23744	CTTAATACTCCGACGTAACAGTGG	CCTGACTAAATGGAGCTTCTTCC	(CT)37
RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	(CT)24
RM25425	CCAGCCCAAACAGCTCTTGC	GGGCACTGTTTGTCTTTCTGTGC	(CT)33
RM25181	AAAGAGCTTCCCTAATGGCTTCG	GAGAGAATGACCTCTCCCAAGACC	(TTC)27
RM25735	AGGCAGGCAAGCAGTAGTTTCG	ATCAAGATCAGGAGCCGCAAGG	(AAG)25
RM26255	AAGACAAGGAGGTTCCAGTGTCC	GCCCTAAACCCAAATAGAAGAACG	(AG)28
RM202	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA	(CT)30
RM247	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	(CT)16
RM5479	AACTCCTGATGCCTCCTAAG	TCCATAGAAACAATTTGTGC	(TC)21

and cost effective (Gracia *et al.,* 2004). The information on genetic diversity highlights the effectiveness of SSR loci information. In the present investigation genetic diversity per locus in general, was found to be high for most of the SSR loci and ranged from 0.37 to 0.85 (Table 3), average being 0.615. Such value was highly informative as values higher than 0.5 in general, indicate high polymorphism (De Woody *et al.,* 1995) In an

Table 3. Allele size, number of allele and genetic diversity among 20 SSR loci in rice genotypes

Primers	Chromosome	Range of allele (bp)	No. of allele (A)	Effective allele (Ae)	Genetic Diversity (He)
RM 13263	2	148-218	5	4.26	0.77
RM 12921	2	400-548	6	4.76	0.79
RM 14076	2	132-170	5	3.33	0.70
RM 15251	3	140-170	3	3.03	0.67
RM 1388	4	200-245	3	2.59	0.62
RM 18384	4	162-228	6	4.56	0.78
RM 20466	5	266-356	5	2.48	0.60
RM 19419	6	332-469	5	3.08	0.68
RM 1369	6	291-305	2	1.81	0.45
RM 22306	6	132-209	4	3.46	0.71
RM 23877	8	105-181	7	5.41	0.82
RM 23744	9	200-296	7	4.63	0.78
RM 257	9	286-410	6	3.85	0.74
RM 25425	9	176-302	5	4.26	0.77
RM 25181	10	152-341	8	6.58	0.85
RM 25735	10	170-206	6	4.88	0.80
RM 26255	10	130-162	5	2.63	0.62
RM 202	11	478-620	3	1.58	0.37
RM 247	11	168-189	3	2.43	0.59
RM 5479	12	143-246	7	5.88	0.83
MEAN	5.05±1.64	3.77±1.36	0.70±0.11		

earlier study Ravi *et al.,* (2003) reported PIC value of 0.578 among rice cultivars, landraces and wild races. The present investigation, distinctly delineated that primers were efficient enough to distinguish the rice genotypes. In the present finding, cluster analysis was used to group genotypes following unweighted neighbour joining algorithm based on 20 SSR loci (Fig. 2). The rice genotypes have been classified into 3 main clusters through radial branching. The clusters formed two sub clusters each. Cluster I contained 5 genotypes placed into two different sub-clusters. On the other hand, cluster II and cluster III contained 6 and 9 genotypes respectively. Interestingly, albeit the genotype Lemont belonged to tropical japonica type but it grouped with indica types namely Khitish and Baradhan in sub-cluster IIIa envisaging that all these three genotypes were genetically closer (Fig. 2). But the sub-cluster IIIb comprised of only local landraces except Jajati. In other sub-clusters both high yielding varieties and local landraces were intermixed. The dissimilarity index with only high magnitude are presented in Table 4. The maximum dissimilarity index was observed between the genotypes Yujal and Kalindi (Table 4). The genotypes Yujal belonging to local landrace was distinctly diverse with a number of genotypes namely IR 36, Kalinga II, Khitish, Baradhan and Gojopati. Similarly, the variety IR 36 was conspicuously diverse with Khitish and Baradhan. Likewise, the variety Kalinga II also showed a large distance with Khitish and Baradhan. The information on genetic diversity of rice genotypes for specific genomic regions aids a new dimension in rice breeding program. A major application of this work is to map genes along different types of rice apart from selection of diverse parents in any hybridization program. In the present finding, hybridization of Yujal with any of the diverse parents like IR36, Kalinga II, Khitish, Baradhan and Gojopati would like to generate desirable segregates in segregating generation.

Table 4. Dissimilarity Index between some selected genotypes

	Yujal	Lemont	IR 36	Kalinga II	Khitish	Baradhan	Gajapati
Yujal	-	0.91	0.90	0.89	0.89	0.89	0.89
Lemont		-	0.86	0.85	0.67	0.78	0.85
IR 36			-	0.78	0.85	0.84	0.77
Kalinga II				-	0.84	0.83	0.66
Khitish					-	0.76	0.83
Baradhan						-	0.80
Gajapati							-

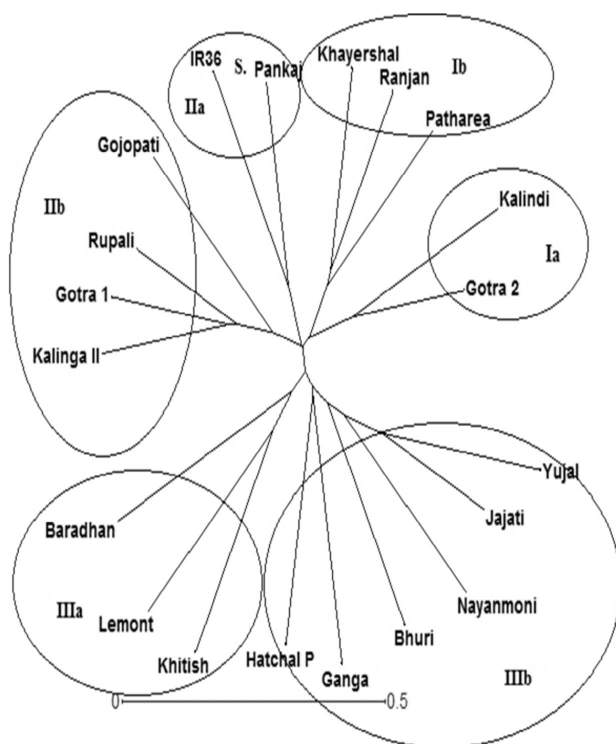

Fig. 2. Genetic diversity tree of rice genotypes based on SSR primers using neighbor-joining analysis

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