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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF RICE VARIETIES AND LANDRACES BASED ON SSR MARKERS

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ABSTRACT

Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. In the present study twenty rice cultivars (ten mega rice varieties and ten landraces) of India were evaluated for genetic diversity using 50 microsatellite markers. A total of 98 alleles were detected by 34 polymorphic markers with an average of 2.88 alleles per locus. A maximum of five alleles were observed with the primer pair OSR13 and RM474 and the minimum of two alleles were observed in as many fifteen primer pairs. PIC values ranged from 0.71 with the SSR markers RM 1 and OSR13 and 0.18 with the marker RM124. The Jaccard's similarity indices obtained for each pair wise comparison among the 20 genotypes, revealed highest similarity index of 0.74between the genotypes Chinikamini and Acharmati;andChinisakkar and Chinikamini, while the lowest similarity index of (0.13) was observed between MTU1010 and PR-113. UPGMA cluster dendogram created in this study identified 2 clusters at ~23 % similarity levels. At ~30% similarity levels, the cluster I produced two sub clusters and at ~34% similarity level, the sub-cluster Ia was further divided into two sub-clusters.

KEY WORDS
Genetic Diversity Landrace Molecular Characterization
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INTRODUCTION

Rice (*Oryza sativa*) is the staple food crop for more than 60% of the world's population and about 90% of all rice grown in the world is produced and consumed in Asia (http://www.rice-trade.com/world-wide-rice-production.html). Plant uniformity, which can be resulted by the use of modern plant breeding techniques, can produce plants, which are more efficient by means of different goals including enhanced resistance under stress, however much more research must be performed to indicate the most optimized methods that can be used for the production of efficient plants. This is of significance for the production of food forth world increasing population (Fu & Somers, 2009; Khodadadi*et al.,* 2011). Accordingly, the increased attention to the production of resistant plant species for prolonged food production under different conditions indicates the necessity of performing breeding experiments (Martin *et al*., 2008; Khodadadi et al., 2011). One of the important approaches to rice breeding is hybridization and subsequent selection. Parents choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary. It has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size of all major crop plants of 430 MB (Dipali *et al*., 2014), 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 world wide) and the availability of widely collected, compatible wild species.In India, rice is grown in diverse ecological niches and occupies about 45 million hectares. With the advent of neo- approaches of plant breeding and careful utilization of the traditional techniques, several high yielding varieties (HYVs) were developed and released. The impact HYVs on food production was dramatic, which led to the green revolution in the 1960s. A critical analysis of the genetic variability is a prerequisite for initiating any crop improvement programme and for adopting of appropriate selection techniques (Dhanwani *et al*., 2013). Therefore, molecular characterization of the released varieties and landraces helps in developing the database based on which new varieties developed can be distinguished and the characterization. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of 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. Among PCR based markers in rice, microsatellites are abundant and well distributed throughout the genome (Akagi, 1996; McCouch, 1997; Wu and Tanksley, 1993). They are valuable tools for assessing the allelic diversity and are assayed efficiently by the PCR (Mc Couch *et al*., 1997). Therefore, it has been applied widely in the identification, registration of plant variety and in monitoring the seed purity and the authenticity with high accuracy, high reliability and low cost. The current study was undertaken to characterization of the popular rice varieties and aromatic landraces of India based on the molecular fingerprints and genetic diversity analysis of aromatic landraces to measure the extent of genotypic differences, genetic relation ship and to assist in broadening the germplasm baseof future aromatic rice breeding programs.

MATERIALS AND METHODS

Plant materials

A total of 20 rice varieties (ten mega rice varieties and ten landraces) (Table 1) were characterized using the standard panel of 50 SSR markers (Table 2 and 2a) in the present study. These cultivars were obtained from the Division of Genetics Indian Agriculture Research Institute, New Delhi and work was done in Molecular laboratory of Genetics division (Rice), IARI, New Delhi.

Genomic DNA extraction and SSR assay

DNA was extracted from 21-days-old seedling leaves collected from at least 2-3 seedling in each cultivar, according to the protocol described by (Prabhu *et al.,* 1998) and was quantified using a spectrophotometer. The final DNA concentration was adjusted to 30ng/μL.

The PCR mixture contained, 25–30 ng template DNA, 5 pmol of each primer, 0.05 mMdNTPs (MBI, Fermentas, Lithuania, USA), 10×PCR buffer (10 mMTris, pH 8.4, 50 mMKCl,1.8 mM MgCl2) and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) in a reaction volume of 10 l. Template DNA wasinitially denatured at 94ºC for 5 min followed by 35 cycles of PCRamplification with the following parameters: 1 min denaturationat 94ºC, 1 min annealing at 55ºC and 2 min of primer extension at72ºC followed by final extension of 72ºC for 7 min. The amplifiedproducts were resolved in 3.5% agarose. The resolvedamplified products were visualized under UV transilluminatoranddocumented in gel documentation system (Bio-Rad LaboratoriesInc., USA).

Polymorphism information content

Polymorphism information content (PIC) or expected heterozygosity scores for each SSR marker was calculated based on the formula

$H_j = 1-\Sigma Pi^2$

where Pi is the allele frequency for the i^{th} allele (Nei, 1973).

Cluster analysis

The binary data scored was used to construct a dendrogram. The genetic associations between varieties were evaluated by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by primers (Jaccard, 1908). Similarity matrix was generated by using the NTSYS-pc software (Rohlf, 1994). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the unweighted pair-group method with arithmetic average (UPGMA) (Mathew *et al.,* 2000).

RESULTS AND DISCUSSION

SSR Polymorphism and allele number

The 50 SSR markers used across 20 rice genotypes for their characterization, Among these 34 markers showed polymorphism with a total of 98 alleles identified across cultivars. The number of alleles ranged from 2 (RM495, RM431, RM452, RM124, RM507, RM161, RM334, RM510, RM408, RM284, RM433, RM447, RM105 and RM271) to 5 (RM154, OSR13 and RM474) (Table 3). The number of alleles detected (2-5) in the present study is in consistent with the earlier reports (2-11 alleles per microsatellite locus) of Panaudet*et al.,* (1996). Earlier Yang *et al.,* (1994) observed an allelic range of 3-25 per SSR locus using different rice landraces and cultivars of both *japonica* and *indica* origin which is larger than that of Panaudet *et al.,* (1996). Saghaiet*et al.,* (1994) observed 37 alleles in one SSR locus of barley, which was significantly a higher number when compared to the present study. In the present investigation, the average number of alleles observed was 2.88. This average allelic number is much smaller when compared with the reports of Saghai Maroof *et al.,* (1994) (average of 17.7 alleles in barley and 11 alleles in wheat). Of the 50 SSR markers utilized in the current study, 34 SSR markers proved to be highly informative which was in accordance with the result of Saghai-Maroofet *et al.,* (1994) and Russell *et al.,* (1997) in barley, Djeet *et al.,* (2000) in sorghum, Prasad *et al.,* (2000) in wheat and Ravi (2000) in rice.

PIC Value

The PIC values, a reflection of allele diversity and frequency among the cultivar, also varied from one locus to another. The average PIC value for all 34 microsatellite loci in the present study was 0.59, with a range from 0.18 (RM124) to 0.71 (RM1 and OSR13) (Table 4). The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The higher the PIC value of a locus the higher the number of alleles detected. The current PIC

Table 1: List of 20 rice varieties and landraces used for molecular characterization

S.No.	Rice varieties	S .No.	Aromatic landraces
1	Swarna Sub-1	11	PeeliBadam
2	Swarna	12	Jaiphula
3	Pusa Basmati-1	13	Acharmati
4	Improved Pusa Basmati-1	14	Chinikamini
5	PR113	15	Bindli
6	Pusa Basmati-1121	16	TilakChandan
7	Pusa Sugandh-5	17	CRRI Black Aroma
8	Pusa 1401	18	ChiniSakkar
9	IR64	19	JeeragSambha
10	MTU1010	20	Dhama Prasad

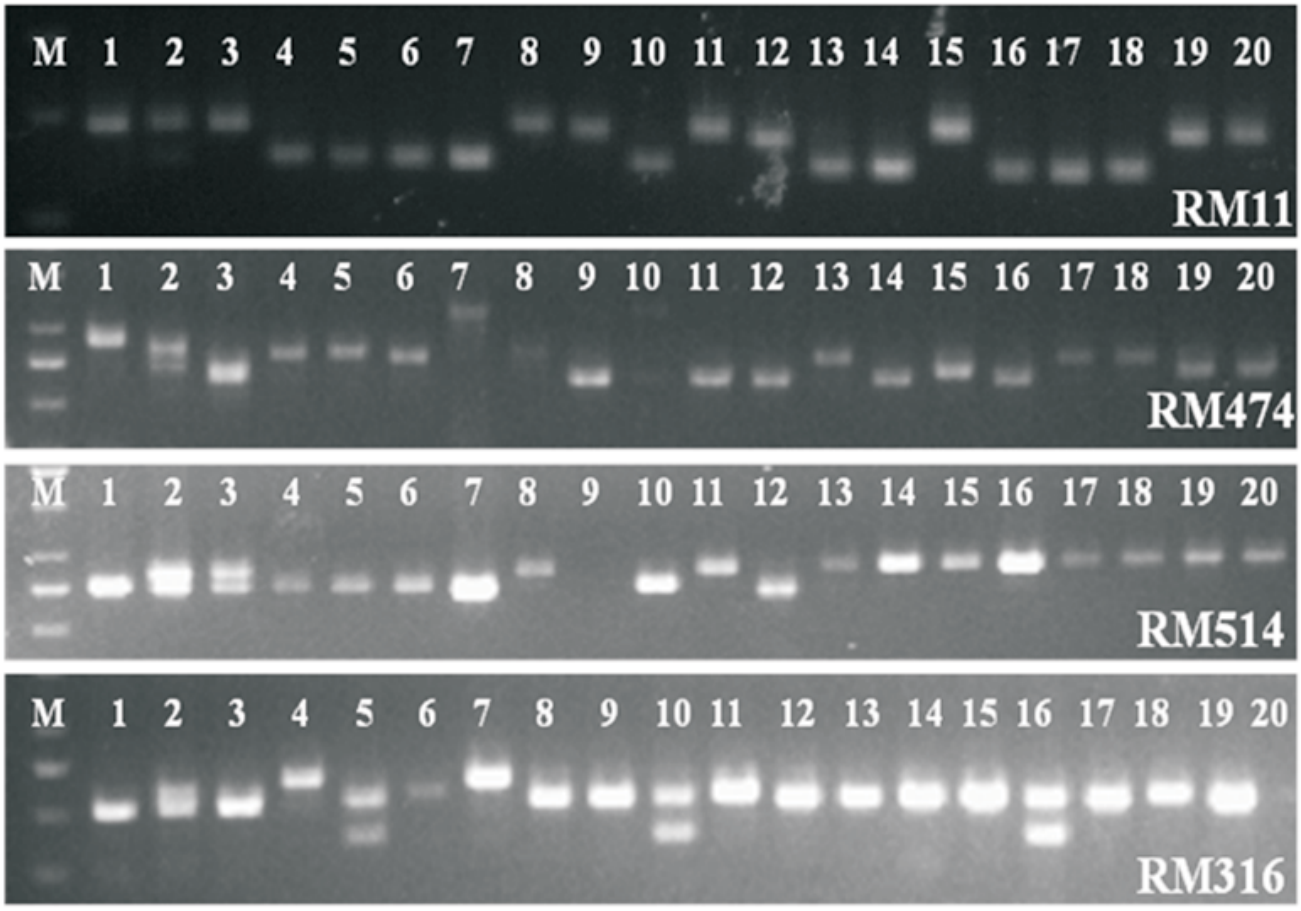

Figure 1: The molecular profile generated in 20 genotypes using the polymorphic SSR markers RM11, RM474, RM514 and RM316

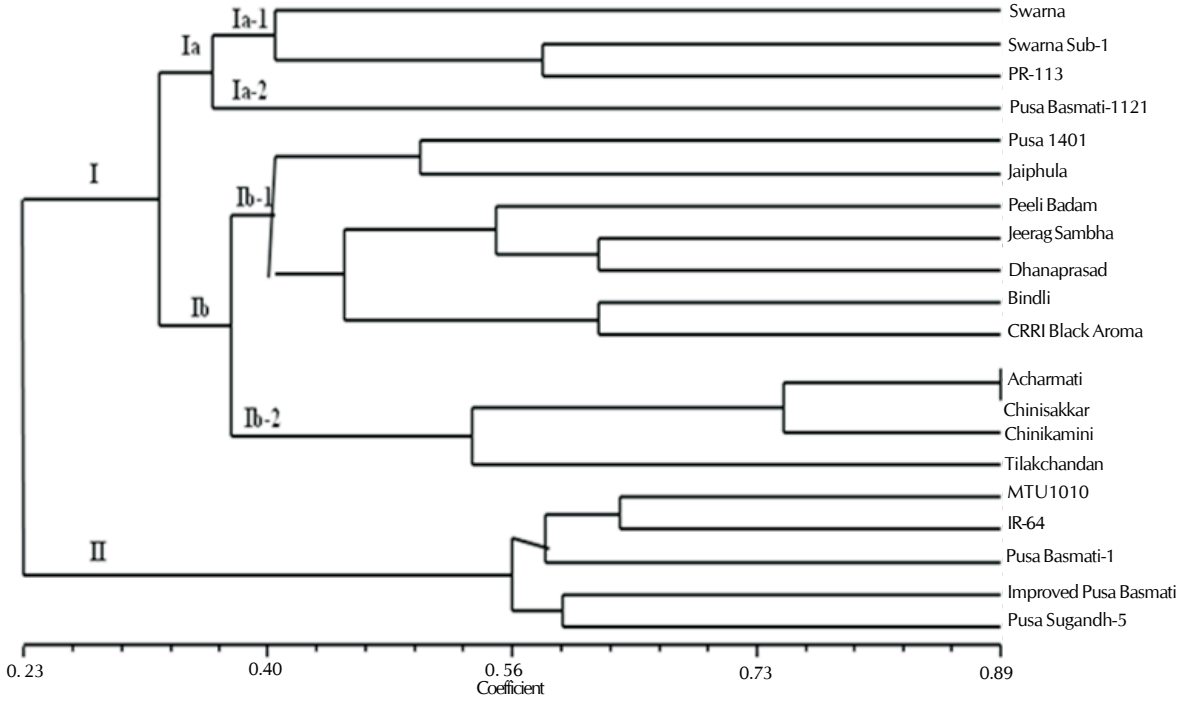

Figure 2: The Dendogram generated using the 34 SSR markers in 20 Genotypes

Table 2: List of 50 standard SSR primers used for molecular characterization

Marker	Chr.	Physical Position (Mb)	Forward Primer	Reverse Primer	Anneal temp.
RM495	1	213775	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	55
RM1	1	4633595	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	55
RM283	1	4883717	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG	61
RM259	1	7443424	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	55
RM312	1	14890771	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC	55
RM5	1	23952076	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	57
RM237	1	26795365	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC	55
RM431	1	38874702	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	55
RM154	2	1083895	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	61
RM452	2	9564377	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	61
RM489	3	4316348	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	55
OSR13	3	7109988	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC	53
RM338	3	13210600	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	55
RM55	3	29002484	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	55
RM514	3	35229618	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG	55
RM307	4	12979675	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC	55
RM124	4	34485157	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	67
RM507	5	71397	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC	55
RM413	5	2181391	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	53
RM161	5	20714463	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG	61
RM178	5	24923084	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	69
RM334	5	28285978	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	55
RM133	6	226944	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC	63
RM510	6	2831513	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	57

value is higher than the previous reports of Panaud *et al.,* (1996) and Olufowote *et al.,* (1997)**.** The high PIC value obtained in the present investigation might be due to high genetic diversity **(**Garland, 1999) among the genotypes under study. Example of SSR alleles as resolved with the PCR assay for RM11, RM 474, RM 316 and RM 514 are illustrated in figure 1.

Pairwise Genetic Dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the cultivars studied. The dissimilarity index values obtained for pair wise comparison among 20 genotypes based on SSR markers ranged from 0.13 to 0.74. The pairwise genetic dissimilarity indices (Table 4) indicated that the highest genetic dissimilarity (0.74) was observed between the genotypes Chinikamini and Achrmati and between Chinisakkar and Chinikamini and lowest dissimilarity index (0.13) was observed between MTU 1010 and PR-113

Cluster analysis based on SSR markers

Table 2a.List of 50 standard SSR primers used for molecular characterization.

Marker	Chr.	Phy. (Mb)	Forward Primer	Reverse Primer	Anneal temp.
RM454	6	23336824	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG	55
RM162	6	23991705	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	61
RM125	7	5478776	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC	63
RM11	7	19256213	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	55
RM455	7	22349919	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	57
RM118	7	26635903	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG	67
RM408	8	119935	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	55
RM152	8	677616	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	53
RM25	8	4372113	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	53
RM44	8	11753077	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	53
RM284	8	21012223	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC	55
RM433	8	25691233	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC	53
RM447	8	26416867	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC	55
RM316	9	1022645	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	55
RM105	9	12496919	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	63
RM215	9	20837148	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	55
RM474	10	1798783	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG	55
RM271	10	16202474	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	55
RM171	10	18614310	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	55
RM484	10	20630348	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	55
RM552	11	4836203	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	55
RM536	11	8963471	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC	55
RM287	11	16733868	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	55
RM144	11	28158704	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	57
RM19	12	2432429	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	55
RM277	12	18286130	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	55

Table 3: SSR markers, their allelic status and PIC observed among the genotypes studied

S. No.	Polymorphic marker	No. of Alleles amplified	PIC
1	RM495	2	0.42
2	RM1	3	0.71
3	RM5	4	0.67
4	RM287	3	0.61
5	RM431	2	0.26
6	RM154	5	0.64
7	RM452	2	0.48
8	RM489	3	0.4
9	OSR13	5	0.71
10	RM55	3	0.54
11	aRM514	3	0.58
12	RM307	3	0.34
13	RM124	2	0.18
14	RM507	2	0.26
15	RM413	4	0.57
16	RM161	2	0.42
17	RM334	2	0.38
18	RM510	2	0.26
19	RM162	3	0.56
20	RM125	3	0.52
21	RM11	3	0.49
22	RM408	2	0.46
23	RM152	3	0.49
24	RM25	3	0.61
25	RM284	2	0.5
26	RM433	2	0.32
27	RM447	2	0.46
28	RM316	3	0.47
29	RM105	2	0.46
30	RM474	5	0.65
31	RM271	2	0.5
32	RM552	3	0.34
33	RM144	4	0.69
34	RM19	4	0.69

Genetic similarity values among the rice varieties used led to the construction of dendogram presented in Figure 2. The similarity matrix was computed using SSR markers based on Jaccard's coefficient following the UPGMA method using SHAN programme of NTSYS-pc. The Jaccard's similarity coefficient for the SSR data set varied from 0.13 to 0.74. The SSR marker profiles resulted in 2 clusters. The 20 genotypes formed 2 clusters at nearly 23 % similarity levels. The cluster I consisted of 15 genotypes, while cluster II consisted of 5 genotypes (MTU1010, IR-64, Pusa Basmati 1, Improved Pusa Basmati 1 and PusaSugandh 5). At ~30% similarity levels, the cluster I produced two sub clusters Ia and Ib. The sub-cluster Ia consisted of four genotypes namely, Swarna, Swarna*Sub1*, PR113 and Pusa basmati 1121. At ~34% similarity level, the sub-cluster Ia was further divided into two sub-clusters namely Ia-1 and Ia-2. The genotype Pusa Basmati 1121 being long grain Basmati rice variety was separately clustered as against the genotypes Swarna, Swarna*Sub1* and PR113, which are medium grained *indica* rice varieties. Similarly, the sub-cluster Ib was divided into two more sub-clusters namely Ib-1 and Ib-2 at ~35% similarity level. The cluster Ib-1 consisted of 7 genotypes namely, Pusa 1401, Jaiphula, PeeliBadam, JeeragSambha, Dhanaprasad, Bindli and CRRI Black Aroma. While, the sub-cluster Ib-2 consisted of Acharmati, Chinisakkar, Chinikamini and Tilakchandan. The potentiality of microsatellite markers have very well been demonstrated in determining the relationship between closely related genotypes (Ravi *et al.,* 2003; Saker *et al.,* 2005; Ganeshram *et al.,* 2007). Ghatge and Kadu (1993) studied 48 rice genotypes from different eco-geological regions of India and grouped into seven clusters.

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Table 4: Eucledian distances computed for the twenty genotypes using 34 polymorphic SSR markers.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	1																			
G2	0.39	1																		
G3	0.42	0.58	1																	
G4	0.19	0.15	0.13	1																
G5	0.16	0.18	0.16	0.63	1															
G6	0.18	0.18	0.22	0.56	0.61	1														
G7	0.18	0.18	0.12	0.60	0.57	0.57	1													
G8	0.33	0.39	0.36	0.19	0.29	0.18	0.22	1												
G9	0.40	0.29	0.40	0.24	0.20	0.25	0.16	0.34	1											
G10	0.13	0.19	0.13	0.51	0.60	0.52	0.60	0.26	0.24	1										
G11	0.33	0.30	0.33	0.17	0.21	0.21	0.19	0.38	0.55	0.25	1									
G12	0.37	0.29	0.34	0.24	0.29	0.27	0.22	0.37	0.50	0.24	0.55	1								
G13	0.45	0.33	0.36	0.39	0.31	0.31	0.26	0.36	0.43	0.28	0.38	0.37	1							
G14	0.42	0.26	0.31	0.28	0.20	0.26	0.24	0.28	0.37	0.26	0.41	0.34	0.74	1						
G15	0.39	0.24	0.28	0.17	0.14	0.14	0.16	0.42	0.43	0.17	0.50	0.40	0.36	0.51	1					
G16	0.24	0.26	0.24	0.36	0.31	0.37	0.37	0.31	0.34	0.42	0.28	0.24	0.51	0.51	0.31	1				
G17	0.28	0.15	0.17	0.26	0.24	0.20	0.24	0.36	0.26	0.28	0.35	0.31	0.45	0.55	0.62	0.28	1			
G18	0.42	0.28	0.33	0.42	0.31	0.31	0.29	0.36	0.40	0.31	0.38	0.34	0.89	0.74	0.36	0.58	0.45	1		
G19	0.36	0.21	0.26	0.24	0.22	0.24	0.29	0.33	0.37	0.28	0.57	0.34	0.33	0.42	0.51	0.24	0.33	0.36	1	
G20	0.45	0.31	0.39	0.17	0.20	0.20	0.22	0.36	0.40	0.24	0.53	0.43	0.39	0.48	0.58	0.31	0.42	0.39	0.62	1

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