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Assessment of genetic diversity of rainfed lowland rice genotypes using microsatellite markers

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Abstract

Genetic diversity in rainfed lowland rice genotypes constituting landraces, high yielding varieties and improved lines was assessed using microsatellite markers distributed over 12 chromosomes of rice. A total of 143 alleles were amplified, of which 136 were polymorphic. The polymorphism information content (PIC) and discriminating power of microsatellite markers ranged between 0 and 0.91 with an average of 0.66 per locus. Genetic similarities among genotypes varied from 0.37 to 0.89 with an average of 0.6. The UPGMA cluster analysis grouped the rice genotypes into two major clusters at 50% level of genetic similarity. All the genotypes studied could be uniquely distinguished from each other at probability of 2.2×10^{-13} of identical match by chance. The high level of genetic variability was detected among the lowland rice genotypes which would enable for selection of diverse donors in the development high yielding rice varieties suitable for rainfed lowland ecosystem. Seven genotype with specific unique alleles were identified, which can be used as molecular tags. A basic molecular database was created for rainfed lowland genotypes which will be useful for future reference and to protect this unique rice under IPR regime.

Key words: Genetic diversity, low land rice, microsatellite marker

Introduction

Rice production environment in India is characterized by extreme diversity and disparity in many ways. It has been estimated that about 38 percent are rainfed lowland and 14 percent are flood-prone. Ninety two per cent of rainfed lowland rice (including deepwater) is distributed in eastern India (Assam, Bihar, West Bengal, Eastern U P and Chhattisgarh) and north-eastern states [1]. Despite the large area coverage by the rainfed low-land rice, its productivity is very low accounting only 17% of the total rice production due to abiotic stresses such as flood, stagnant water and low soil fertility [1]. Not much progress has been made in the development of suitable high yielding cultivars to effectively combat these abiotic constraints despite many years of sustained efforts by conventional breeders. Furthermore, narrow genetic base may be reasons for the lack of significant yield increase during varietal developmental process. With little scope for expansion of the irrigated rice lands, the future increase in rice production has to come from this neglected and fragile ecosystem to meet the food demands of ever increasing rice consuming population. It is, therefore, necessary to develop rice varieties with higher yield potential and better tolerance to abiotic stresses. It is important to broaden the genetic base of low land rices so as to permit new allelic combination through use of diverse germplasm and biotechnological tools in breeding programs.

Accurate assessment of genetic diversity and relationships within and among cultivars holds great importance for the effective utilization and protection of plant genetic resources. Molecular marker technology is considered superior over morphological and biochemical markers for assessment of genetic diversity among cultivars, identification of cultivars, and thus protection of plant genetic resources [2]. Morphological and biochemical markers are less reliable because many characters of interest have low heritability, genetically complex in nature, stage specific expression and hence, genetic diversity estimates are less reliable. Moreover, at times there

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may be little morphological or isozyme diversity among cultivars. Among molecular markers such as RFLP, RAPD, microsatellite, ISSR, AFLP etc., used for assessment of genetic diversity both in cultivated and wild rices [3-5], the microsatellite or simple sequence repeat (SSR) markers are considered to be the best because of their co-dominant, multi-allelic, highly polymorphic nature and distribution throughout the genome. These markers have been used for identification and purity checking of rice varieties [6], characterization of genetic diversity in cultivated [7-10] and wild rices [5], and also more distantly related grass species [11].

Farmers in eastern India are growing different low land rice cultivars which constitute an important source of genetic variation. These cultivars can be utilized in breeding programs to broaden the genetic base of lowland rice varieties with tolerance to flood, stagnant water and other biotic and abiotic stresses. Hence, conservation, characterization and documentation of these rice genotypes are important step forward in view of Intellectual Property Rights (IPR) regime. The present study was undertaken to evaluate the extent of genetic diversity and relatedness among 48 rainfed lowland rice genotypes employing microsatellite markers.

Materials and methods

Plant materials and genomic DNA isolation

The seeds of 48 rainfed lowland rice genotypes used in this study (Table 1). The seeds of these genotypes were obtained from Gene Bank of Central Rice Research Institute, Cuttack. For precise understanding of the extent of genetic diversity among genotypes, they were further grouped as a) shallow low land, b) semi deep and c) deep water. Fifteen seeds per genotypes were germinated in petridishes and seedlings were transplanted in individual pots in green house. Four weeks after transplanting, the young leaves were harvested from 10 young plants and bulked for each genotype. Genomic DNA was isolated from 3-4gm of bulked leaf samples following Cetyl Trimethyl Ammonium Bromide (CTAB) method [12]. The quantity and quality was estimated using spectrophotometer and agarose gel electrophoresis using known concentration of Lambda DNA. The samples were diluted in $T_{10}E_1$ buffer to get final concentration of 20ng/µl for amplification.

Table 1. List of rainfed lowland rice genotypes used in study

S.No.	Genotypes	Parentage	Ecology*
1	Varsadhan	IR 31432-3-3-2 / IR31406-3-3-3-1 // IR26940-3-3-3-1	SD
2.	Sabita	Selection from land race, Boyan	SD
3	Dinesh	Jaladhi 2/ IR 5-114-3-1-2	DW
4	Purnendu	Patnai 23/Jaladhi 2	SD
5	Chakaakhi	CR149-5010-228/ T242	SD
6	Jal Lahari	Pankaj//Mahsuri/ TKM6	DW
7	Panikekoa	Land race	DW
8	Vaidehi	Selection from land race, Beldar	SD
9	Padmanath	Pankaj/Jagannath// Negeri Bao	SD
10	Panidhan	CR 151-79/CR1004	SD
11	FRI3A	Land race	SL
12	Jitendra	Land race	SL
13	Katakipateli	Land race	SL
14	Khoda	Land race	SL
15	Atiranga	Land race	SL
16	Bazail 65	Land race	SL
17	Ramaboita	Land race	SL
18	NDR402	T 100 / Pankaj	SL
19	Rangbao	Land race	DW
20	Amulya	Selection from land race, Najani	SL
21	Dhulia	Land race	SL
22	Kalaputia	Land race	SL
23	RD19	IR 262-43-8-11/ Pingaew 56	DW
24	Salivahan	RP 5-3-2/Pankaj	SL
25	TKM6	Co 18/GEB 24	SL
26	CR2242-3-2-1-1	Ambika/Utkalprava	DW
27	CR2251-1-1-1-1	CR661-236/Swarna	DW
28	CR2285-6-6-3-1	Ravana/Mahsuri	DW
29	CR2078-91-6-2	Sam son polo/ LPR 8	DW
30	CR2244-4-5-1-1	Ambika/Savitri	DW
31	CR2285-7-1-2-1	Ravana/Mahsuri	DW

32	CR2308-1-1-1-1	RD 19/Chaka akhi	DW
33	CR2076-180-1-1	Daeng Laem/ Jalnidhi	DW
34	CR2282-1-2-5-1	Panikekoa/Ambika	DW
35	CR2244-2-1-1-1	Ambika/Savitri	DW
36	CR2078-91-3-1	Sam son polo/ LPR 8	DW
37	CR2080-169-1-1	Sam son polo/ Jalnidhi	DW
38	CR 780-2211-1-1	IR 32/IR 13246	SL
39	CR661-236-2-1-1	CR 563-1014/ BG90-2 // IR42	SL
40	CR2286-3-3-1-1	Sabita/Hatipanjari	DW
41	CR2259-8-2-1-1	CR778-95/Gayatri	SL
42	CR2242-6-3-1-1	Ambika/Utkalprava	DW
43	CR661-236-1-1	CR 563-1014/ BG90-2 // IR42	SL
44	CR2246-1-2-1-1	ARC 14403/ ARC 6579	DW
45	CR780-1937-1	Savitri/IR44	SL
46	CR2285-5-6-1-1	Ravana/Mahsuri	DW
47	CR662-2211-2-1	IR 32/IR 13246	SL
48	CR 2309-1-2-1-1-1	RD 19/Durga	DW

*SL-Shallow lowland, SD-Semi deep, DW-Deep water

PCR amplification and electrophoresis

A set of 51 microsatellite markers (http:// www.gramene.org) distributed over 12 chromosomes of rice were used (Table 2). The PCR amplification was carried out in a 20µl reaction mixture volume. The PCR was performed in a thermal cycler (Thermal Cycler, Perkin Elmer, Cetus) as per following cycling parameters: initial denaturation at 94^{o}C for 3 min followed by 36 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 55-67^{o}C (depending upon primer) for 1 min and extension at 72^{o}C for 1.5 min and final extension at 72^{o}C for 7 min. The amplified products were separated on 2.5% agarose gels using 1X TBE buffer and stained with ethidium bromide (0.5µg/ml). The gels were visualized under UV radiation and photographed using a gel documentation system (Fluor Chem^{TM} 5500, Alpha Innotech, USA) to detect polymorphism. The size of amplified bands was determined based on the migration relative to molecular weight size markers (50 bp DNA ladder, MBI Fermentas, Lithuania).

Data analysis

The amplified bands (alleles) were scored as present (1) or absent (0) for each genotype and primer combination. The data was entered into a binary matrix and subsequently analyzed using the computer package, NTSYS-pc (Version 2.02) [13]. The total number of alleles per locus, percentage of polymorphic alleles, low frequency alleles, high frequency alleles, rare alleles and polymorphism information content (PIC) were calculated to assess diversity of alleles of marker locus. Dice genetic similarity coefficients were calculated and used to assess the genetic relationship among 48 low land rice genotypes [14]. The dendrogram was constructed using unweighted pair group method using arithmetic averages (UPGMA) and sequential agglomerative hierarchal nested (SHAN) cluster analysis. The Cophenetic correlation coefficient [15] was calculated to measure the goodness of fit of clusters.

The polymorphism information content (PIC) was calculated using the formula, PIC = 1-ΣPij^2, where Pij is the frequency of ith allele for the jth locus and summation extends over n alleles [16]. In order to find the efficiency of SSR markers for differentiation of genotypes, the discriminating power (D) of each marker loci was calculated following formula, Dj = 1-Cj = 1-Σ Pi (NPi-1)/(N-2), where Dj is discriminating power of jth locus, Pi is frequency of ith allele, Cj confusion probability of jth locus [17]. Further, in order to know minimum number of marker loci required to identify and differentiate genotypes from each other, total number of non-differentiated pairs(Xj) of genotypes were calculated for the jth locus using formula, Xj = {N(N-1)/2}Cj.

Results and discussion

The accurate assessment of genetic diversity is important not only for crop improvement but also for efficient management and protection of the germplasm. Further, this will maximize the probability of transgressive segregation and accumulation of positive alleles from different cultivars. Microsatellites are considered to be appropriate for assessment of genetic diversity, fingerprinting for variety identification and assessment of seed purity because of their ability to detect large numbers of discrete alleles accurately and efficiently [18].

Allelic diversity of microsatellite markers

Forty four out of 51 microsatellite primers revealed

Table 2. Data on chromosome number, repeat motif, number of total alleles, number of polymorphic alleles, rare alleles, low frequency alleles, high frequency alleles and polymorphism information content (PIC) obtained using 51 microsatellite primers in 48 rice genotypes

Microsatellite locus (RM)	Chromo-some	Repeat motif	TA	PA	RA	LFA	HFA	Size (bp)	PIC	DP
9	1	(GA)15GT(GA)2	5	5	0	1	4	130-220	0.831	0.836
212	1	(CT)24	3	3	0	1	2	120-150	0.870	0.874
259	1	(CT)17	2	2	0	1	1	165-170	0.664	0.668
428	1	(AG)15	1	0	0	0	1	260	0	0
488	1	(GA)17	3	3	0	1	2	170-205	0.867	0.871
154	2	(GA)21	4	4	1	2	1	180-250	0.855	0.858
263	2	(CT)34	5	5	1	2	2	160-250	0.886	0.862
475	2	(TATC)8	2	2	0	0	2	180-200	0.722	0.727
530	2	(GA)23	2	2	0	1	1	160-180	0.682	0.686
16	3	(TCG)5(GA)16	2	2	0	1	1	190-230	0.635	0.638
218	3	(TC)24ACT5(GT)11	2	2	0	0	2	150-180	0.734	0.739
203	3	(AT)21	4	4	0	1	3	200-500	0.854	0.858
426	3	(CA)10	2	2	0	1	1	200-250	0.624	0.627
321	3	(GA)59	2	2	0	0	2	230-250	0.715	0.72
307	4	(AT)14(GT)21	4	4	0	3	1	130-300	0.808	0.859
335	4	(CTT)25	3	3	0	0	3	100-160	0.782	0.788
401	4	(CT)15	4	4	1	2	1	230-360	0.868	0.871
470	4	(CTT)14	2	2	0	1	1	100-135	0.609	0.612
164	5	(GT)16TT(GT)4	3	3	0	2	1	250-300	0.832	0.836
334	5	(CTT)20	3	3	1	1	1	175-225	0.751	0.753
336	5	(CTT)18	4	4	0	2	2	130-200	0.894	0.897
440	5	(CTT)22	6	6	4	1	1	60-700	0.851	0.853
30	6	(AG)9A(GA)12	3	3	0	2	1	75-120	0.793	0.796
225	6	(CT)18	2	2	0	0	2	230-250	0.715	0.72
253	6	(GA)25	3	3	1	1	1	180-350	0.725	0.727
276	6	(AG)8A3(GA)33	2	2	0	1	1	60-80	0.677	0.68
432	7	(CATC)9	2	2	0	1	1	140-150	0.652	0.656
478	7	(AG)12	2	2	0	1	1	230-240	0.697	0.702
5752	7	(ACT)13	1	0	0	0	1	90	0	0
8044	7	(CTT)55	2	2	0	0	2	250-280	0.731	0.736
152	8	(GGC)10	1	0	0	0	1	140	0	0
337	8	(CTT)4-19-(CTT)8	2	2	0	0	2	150-200	0.739	0.744
433	8	(AG)13	2	2	0	0	2	230-240	0.729	0.734
8271	8	(AG)32	3	3	0	1	2	175-230	0.815	0.819
189	9	(AG)11	1	0	0	0	1	175	0	0
201	9	(CT)17	1	0	0	0	1	200	0	0
219	9	(CT)17	2	2	0	0	2	200-230	0.746	0.751
444	9	(AT)12	5	5	2	1	2	170-340	0.898	0.901

216	10	(CT)18	3	3	0	1	2	135-150	0.876	0.881
269	10	(GA)17	4	4	1	1	2	180-750	0.499	0.500
330	10	(CAT)5	4	4	0	2	2	180-350	0.746	0.743
333	10	(TAT)19(CTT)19	2	2	0	1	1	200-230	0.665	0.668
21	11	(GA)18	4	4	1	0	3	60-170	0.905	0.909
144	11	(ATT)11	3	3	0	1	2	230-250	0.782	0.786
202	11	(CT)30	4	4	0	2	2	170-230	0.898	0.902
224	11	(AAG)8(AG)13	4	4	1	1	2	130-165	0.868	0.871
17	12	(GA)11	3	3	0	1	2	170-200	0.872	0.877
20	12	(ATT)14	6	6	1	3	2	220-320	0.821	0.824
235	12	(CT)24	2	2	0	0	2	120-150	0.729	0.734
511	12	(GAC)7	1	0	0	0	1	130	0	0
519	12	(AAG)8	1	0	0	0	1	150	0	0
TOTAL			143	136	15	45	83		33.91	34.09
Mean			2.8	2.67	0.29	0.88	1.63		0.66	0.67
%				95.1	10.49	31.47	58.04			

TA = Total number of alleles, PA = Polymorphic alleles, UA= Unique alleles, LFA- Low frequency allele, HFA = High frequency alleles, DP – Discriminating power

polymorphism between genotypes. A total of 143 reproducible bands (alleles) were amplified of which 136(95.1%) were polymorphic. The number of alleles per locus ranged from 1 (RM152, RM189, RM201, RM428, RM511, RM519 and RM5752) to 6 (RM20, RM440) with an average of 2.8 (Table 2). The number of alleles per microsatellite locus detected in the present study corresponded well with earlier reports [5, 8, 9]. However, Jain *et al*. [8] obtained higher number of alleles (3 to 22) as compared to present study, because of inclusion of *Basmati* as well as non-*Basmati* varieties and use of fluorescent techniques in their study. The number of alleles detected by a single SSR locus varied from 1 to 31 depending upon the fingerprinting techniques and kind of germplasm used in the studies [4, 7, 19-22]. Microsatellite loci with complex mixed repeats generated highest number of alleles (average: 3.1, n = 10), followed by those with simple tri-nucleotide (average: 2.85, n = 13), di-nucleotide (average: 2.76, n = 26) and tetra-nucleotide repeat motifs(average: 2, n = 2) (Table 2). Similar to the present findings, Kaushik *et al*. [21] found that SSR loci with complex mixed repeats detected highest number of alleles followed by those with simple tri-nucleotide, di-nucleotide and tetra-nucleotide repeat motifs. However, Cho *et al*. [23] and Jain *et al.* [8] observed that SSR loci with di-nucleotide repeats detected greater number of alleles than those with tri-nucleotide repeats. Non significant correlation (r = 0.121, P > 0.10) was found between number of alleles amplified per locus and number of repeats in simple motif of a SSR locus. Similarly direct correlations between number of repeats and number of alleles detected in aromatic and wild rice germplasm have been reported earlier, however, Ni *et al*. [7] found positive correlation between number of alleles amplified and number of repeats within a microsatellite marker.

The size of alleles varied from 60bp (RM21, RM276, RM440) to 750bp (RM269). The size difference between the smallest and largest allele at a given SSR locus varied from 5 (RM259) to 570bp (RM269). The number of alleles per cultivar varied from 52 (Panidhan) to 64 (CR2076-180-1-1) with an average on 57,5 alleles per cultivar. As many as 15 rare alleles (10.49%) were observed at 11 loci. Rare alleles are important source of genetic diversity. Occurrence of rare alleles in rice cultivars may have resulted from unequal crossing-over, translocations or other type mutations.

Eighty three (58.04%) high frequency/common alleles and 45 (31.47%) of low frequency alleles were identified among 48 rice low land rice genotypes. All the 51 primers amplified at least one high frequency/ common allele. The primer RM9 amplified highest number of common alleles (i.e., 4) while three primers, RM21, RM203 and RM335 amplified 3 common alleles each. On an average, 67.78% of the 48 rice genotypes shared common alleles at any of 51 loci. The number

of alleles amplified ranged from 31.25% (RM224) to 100% (RM152, RM189, RM201, RM269, RM428, RM511, RM519 and RM5752). Seventy eight alleles were amplified at least in one genotype from all the three ecologies. The frequency of common alleles vary from 2/19 (at one allele) to 19/19 (at eight alleles) in shallow lowland genotypes, 1/7 (at eight alleles) to 7/7 (at fourteen alleles) in semi deep genotypes and 5/22 (at three alleles) to 22/22 (at eight alleles) in deep water genotypes. Five alleles (i.e. $RM17_{200}$, $RM212_{120}$, $RM216_{150}$, $RM224_{150}$ and $RM335_{120}$) amplified in shallow lowland and deep water genotypes are not amplified in semi deep genotypes. Thirty two primers amplified 45 low frequency alleles. On an average, 11.47% of 48 rice genotypes shared low frequency alleles at any of 32 loci. Jain *et al*. [8] observed that 53.6% of 69 rice genotypes shared common alleles at any locus. Thompson *et al*. [21] indicated that on an average, 62% of the 190 rice accessions of Indonesian origin shared a common allele at any given of SSR locus. Similar results were also reported by others [22, 24].

Polymorphism information content (PIC)

The PIC value provides an estimate of discriminating power of a marker locus. The PIC values for 51 SSR loci in our study varied from 0 (RM152, RM189, RM201, RM428, RM511, RM519 and RM5752) to 0.91 (RM 21) with an average of 0.66 (Table 2). Fig. 1 shows DNA amplification pattern of 48 low land rice genotypes with highly polymorphic microsatellite loci, RM21. All the loci except eight (RM152, RM189, RM201, RM 269, RM428, RM511, RM519 and RM5752) showed high PIC values (> 0.50). The estimated PIC values are relatively higher and thus might be due to higher genetic diversity present in selected low land rice genotypes. Moreover, the SSR markers used in the study were selected on the basis of their high PIC values reported earlier. Similar to our findings higher PIC values for SSRs were also reported in the literature [9, 10, 20, 25, 26]. Microsatellite loci with complex mixed repeats detected higher polymorphism (mean: 0.77, n = 10) followed by those with simple di-nucleotide motifs (mean: 0.69, n = 26) and tri-nucleotide (mean: 0.53, n = 13), (Table 2). A positive correlation (r = 0.7, P < 0.01) was evident between number of alleles amplified and PIC values. Jain *et al.* [18] had also observed that PIC values showed a positive correlation with total number of alleles at SSR locus (P = 0.01). Similarly, Kaushik *et al*. [22] observed a positive correlation between PIC and number of repeat motifs.

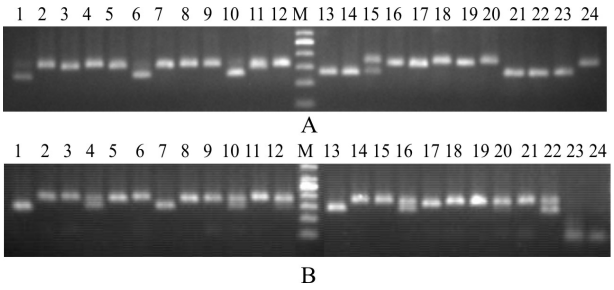

Fig. 1. DNA amplification pattern of 48 rainfed lowland rice genotypes (A and B) obtained with highly polymorphic microsatellite locus, RM 21. The lane number corresponds to a rice genotype as given in the Table 1, M-50bp DNA ladder

Genetic diversity and relationship among lowland rice genotypes

Genetic similarity coefficients of pair-wise comparisons estimated on the basis of all the 51 microsatellite loci ranged from 0.27 to 0.89 with an average of 0.6, indicating a wide range of genetic variation present in low land rice genotypes. The genotype, CR 780-2211-1-1 showed the highest similarity with CR661-236-2-1-1 (i.e. 0.89) while Kalaputia showed the least similarity with CR661-236-2-1-1 (i.e. 0.27). High level of similarity was also found among Sabita and Purnendu (0.88) as well as between CR661-236-2-1-1 and CR780-1937-1 (0.84). Similar to our observations, other studies using SSR markers revealed varying degrees of genetic similarity among the accessions of cultivated and wild species of rice [5, 7, 9, 10, 22].

UPGMA cluster analysis based on genetic similarity values provided a clear resolution of relationships among all the 48 rice genotypes. The Cophenetic correlation coefficients (r = 0.80) revealed the reliability and stability of clustering. Two major clusters were observed at 50% of genetic similarity coefficient (Fig. 2). First major cluster contained 45 low land rice genotypes with an average similarity index of 0.62. Further, it was sub-grouped into five clusters, IA, IB, IC, ID and IE. The cluster IA included 3 shallow lowland (Bazail65, Jitendra and CR2286-3-3-1-1), three semi deep (Varsadhan, Sabita and Purnendu) and three deep water (Jla Lahari, CR2080-169-1-1 and CR2259-8-2-1-1) genotypes. The similarity coefficients between any two rice genotypes in this cluster ranged from 0.58 to 0.88 with an average of 0.68 (Table 3). All the genotypes except Jitendra included in this subgroup are high yielding varieties and improved lines. Second cluster, IB was more diverse than first cluster,

IA. Twenty out of 22 genotypes (15 deep water and 5 shallow lowland) included in this cluster are promising/ elite cultures identified in the station trials and developed from different crosses. Two landraces Kataki Pateli and Ramboita are also included in this group. The similarity coefficients between any two genotypes varied from 0.48 to 0.89 with an average of 0.67. Third cluster, IC contained one shallow lowland (Salibahan), one semi deep (Chakkaakhi) and one deep water(Panikekoa) genotypes with similarity coefficients from 0.66 to 0.70 with an average of 0.67. Fourth cluster, ID included two shallow lowland (NDR402 and Amulya), three semi deep water (Padmanath, Vaidehi and Panidhan) and two deep water (Dinesh and Rangbao) genotypes with an average genetic similarity of 0.68. Fifth cluster, IE included three shallow lowland (Khoda, TKM6 and Dhulia) and one deep water (RD19 genotypes). The second major cluster consists of only three genotypes, FR13A, Kalaputia and Atiranga. These genotypes have high level of tolerance to submergence and suitable for shallow lowland ecosystem. Though genotypes are not clustered according to their ecology; genotypes from different ecology included in the same cluster might be due to similar yield potential, morphology, tolerance to submergence and similarity at genome level. Upadhyay *et al*. [10] also found that popular varieties grouped together according to their development rather than ecology.

The morphological and biochemical characters, especially isozymes have often been utilized for classification of rice varieties by the rice geneticists and breeders. Kato *et al*. [27] classified rice varieties as *indica* and *japonica* based on the morphological, serological and intervarietal hybrid fertility characters. Based on the morphogeographical characters, Matsuo *et al*. [28] classified rice varieties into 3 types A, B and C. Subsequently, these were referred as *indica*, *japonica* and *javanica* and has been used extensively by plant breeders [29]. Glaszmann [30] surveyed 15 polymorphic isozyme loci among 1688 traditional rices from Asia and identified 6 varietal groups with two major groups I and VI, two minor groups II and V, and two satellite groups, III and IV. Group I consisted the typical *indica* varieties from whole tropical Asia. It

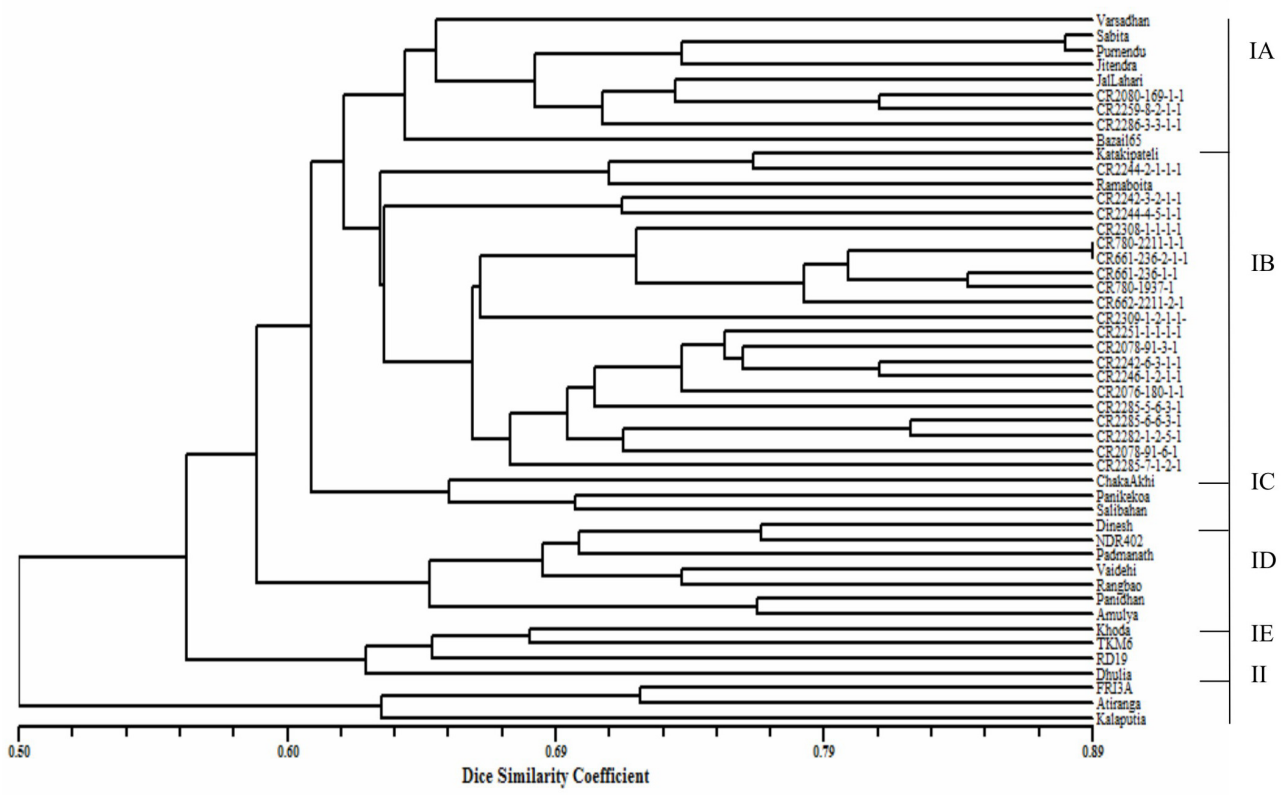

Fig. 2. UPGMA dendrogram showing genetic relationship among rainfed lowland rice genotypes based on Dice similarity matrix derived from 143 alleles at 51 microsatellite loci. The major groups and sub-clusters are indicated on right margin

Table 3. Clustering pattern of rainfed lowland genotypes

Major groups	Sub groups	No. of genotypes	Av. similarity	Genotypes
I	IA	9	0.684	Varsadhan, Sabita, Purnendu, Jitendra, Jal Lahari, CR2080-169-1-1, CR2286-3-3-1-1, CR2259-8-2-1-1, Bazail65
	IB	22	0.67	Kataki Pateli, CR2244-2-1-1-1, Ramboita, CR2242-3-2-1-1, CR2244-4-5-1-1, CR2308-1-1-1-1, CR 780-2211-1-1, CR661-236-2-1-1, CR661-236-1-1, CR780-1937-1, CR662-2211-2-1, CR 2309-1-2-1-1-1, CR2251-1-1-1-1, CR2078-91-3-1, CR2242-6-3-1-1, CR2246-1-2-1-1, CR2076-180-1-1, CR2285-5-6-1-1, CR2285-6-6-3-1, CR2282-1-2-5-1, CR2078-91-6-2, CR2285-7-1-2-1
	IC	3	0.671	Chakakhi, Panikekoa, Salibahan
	ID	7	0.681	Dinesh (DW), NDR402 (SL), Padmanath (SD), Vaidehi (SD), Rangbao (DW), Panidhan (SD), Amulya (SL)
	1E	4	0.621	Khoda, TKM6, RD19, Dhulia
II		3	0.662	FR13A, Atiranga, Kalaputia

comprises most of the Aman ecotype and all lowland varieties. Group VI consists of temperate and tropical *japonic*a varieties found in temperate areas and high elevation areas in Southeast Asia. Groups II, III, IV and V are found along the Himalayas and usually considered as *indica* rices. Many of high quality rices, such as Sadri rices from Iran, Basmati rices from Pakistan, India, Napal and Burma belong to group V.

Identification of genotype and ecology specific markers

Seven genotypes with specific (unique) alleles/markers were identified (Table 3). The genotypes, Varsadhan, FR13A, NDR402, CR2285-7-1-2-1, CR2080-169-1-1, CR2242-6-3-1-1 and CR 2309-1-2-1-1-1 amplified one unique allele each, which may be used as molecular tags (diagnostic marker) (Table 4). Davierwala *et al*. [4] identified many alleles specific to elite cultivars of India using microsatellite markers. Saini *et al*. [30] identified 58 unique alleles (36.2%) among Basmati and non-Basmati rice varieties. Similarly, others also detected unique alleles both in cultivated and wild rices [22, 25, 31]. Upadhyay *et al*. [13] identified 14 genotype specific alleles from 29 popular varieties of India using 20 microsatellite primers.

Five microsatellite primers, RM20, RM154, RM212, RM444 and RM470 amplified shallow lowland genotype specific six alleles/markers. Similarly, thee primers, RM307, RM336 and RM401 amplified deep water genotype specific alleles (Table 5). No allele/markers specific to semi deep genotypes could be identified. Nagaraju *et al.* [32] identified 16 microsatellite loci which differentiated traditional Basmati genotypes from non-basmati genotypes and 10 microsatellite loci differentiated traditional Basmati genotypes from evolved Basmati genotypes.

Table 4. Unique alleles amplified by microsatellite loci in different rice genotypes

Microsatellite locus (RM)	Chromo-some No.	Unique allele(bp)	Name of rice genotypes*
263	2	250	CR 2309-1-2-1-1-1(DW)
440	5	60	FR 13A(SL)
440	5	190	NDR402(SL)
440	5	340	CR2242-6-3-1-1(DW)
440	5	700	CR2080-169-1-1 (DW)
269	10	180	CR2285-7-1-2-1 (DW)
224	11	130	Varsadhan(SD)

*SL-shallow lowland, SD-Semi deep, DW-deep water

Differentiation of lowland rice genotypes

All the rice genotypes used in the present study could be distinguished precisely from each other at the level of 44 to 136 polymorphic alleles between individuals in pair wise comparison over all the 51 microsatellite loci. A number of microsatellite markers were identified that distinguished between different low land rice genotypes. However, none of the microsatellite locus could differentiate all the genotypes. The discriminating power of microsatellite loci vary from 0 (RM152, RM189, RM201, RM428, RM511, RM519, RM5752) to 0.91 (RM21) with an average of 0.67 (Table 2). On the basis

Table 5. Microsatellite loci amplifying ecology specific alleles

Sl No	Microsatellite locus	Chrom#	Size of allele/ marker (bp)	SL*	SD*	DW*
1	RM212	1	150	+ (11,15,22,38,39,45, 47)	-	-
2	RM154	2	180	+ (15, 24)	-	-
3	RM307	4	300	-	-	+ (6,28,34,46)
4	RM401	4	360	-	-	+ (28,46)
5	RM470	4	470	+ (11,15,21,24,25)	-	-
6	RM336	5	130	-	-	+(3,29,30)
7	RM444	9	180	+ (21,43)	-	-
8	RM444	9	340	+ (11,25)	-	-
9	RM20	12	320	+ (16,22)	-	-

*SL-shallow lowland, SD-semi deep, DW-deep water, parenthesis shows the number of genotypes, + shows presence of allele, -shows absence of allele; Parenthesis shows the genotypes given in the Table 1

of discriminating power, the minimum number of microsatellite loci required to differentiate all the genotypes in the present study was found to be six (i.e. RM9, RM21, RM202, RM203, RM336 and RM444) (Table 6). These six SSR loci amplified a total of 26 alleles, all being polymorphic. The frequency of these alleles ranged from 2/40 to 30/48. Six well chosen SSLPs were found to be sufficient to discriminate between 71 related lines of rice [33]. Rahman *et al.* [34] were able to unambiguously identify and discriminate twenty eight rice varieties which included thirteen high yielding, fourteen local and a wild rice cultivars using only three microsatellite markers. Similar findings in Basmati and non-Basmati rice genotypes using different DNA markers were reported earlier [24, 35].

The combination of all the polymorphic and non-polymorphic alleles obtained with all the 51 SSR loci enabled development of DNA fingerprints/profiles (data

Table 6. Efficiency of microsatellite markers in distinguihing rainfed lowland genotypes

S.No.	Microsatellite marker combination	No. of (theoretical) indistinguisble pairs/groups	Actual No. of indistinguisble pairs/groups*	Genotypes actually indistinguisble**
1	RM21	102.648	-	-
2	RM21+ RM202	10.0595	-	-
3	RM21+ RM202+RM444	0.9959	-	-
4	RM21+ RM202+RM444+RM336	0.1026	7(1.0)	(1,3), (6,14), (2,4,20,32), (9,26), (10,37), (18,35), (38,39)
5	RM21+ M202+RM444+RM336+RM216	0.0122	4(1.0)	(2,4); (10,37); (18,35); (38,39)
6	RM21+ M202+RM444+RM336+RM224	0.0132	3(1.0)	(2,4); (9,26); (38,39)
7	RM21+ M202+RM444+RM336+RM401	0.0132	4(1.0)	(1,31); (6,14); (2,4,20); (38, 39)
8	RM21+ M202+RM444+RM336+RM263	0.0142	3(1.0)	(2,4,20, 32), (38,39); (18, 35)
9	RM21+ M202+RM444+RM336+RM203	0.0146	3(1.0)	(2,20); (9,16); (38;39)
10	RM21+ M202+RM444+RM336+RM9	0.016	1(1,0)	(2,4)
11	RM21+ M202+RM444+RM336+RM9+ RM224	0.0021	1(1,0)	(2,4)
12	RM21+ M202+RM444+RM336+RM9 +RM263	0.0022	1(1.0)	(2,4)
13	RM21+ M202+RM444+RM336+RM9+RM203	0.0023	0	-

*Parenthesis shows the values of Dice similarity index between/among any indistinguishble pairs/groups of genotypes; **Parenthesis shows actual genotypes that are not distingushable as evident from UGMA dendrogram (figure not shown) . The numbers in the parenthesis corresspond to genotypes given in the Table 1.

Table 7. Analysis of DNA fingerprints using different sets of microsatellite loci

Items	6* SSR	10** SSR	51 SSR
Average no. of alleles for each variety ± SD	7.75 ± 1.02	12.25 ± 1.36	57.48 ± 2.3
Average similarity ('X_D) ± SD	0.639 ± 0.1	0.406 ± 0.14	0.602 ± 0.08
Probability of identical match by chance ('X_D)^n	3.1×10^{-2}	1.58×10^{-5}	2.2×10^{-13}

*6 highly discriminating microsatellite markers, RM9, RM21, M202, RM203, RM336 and RM444; **10 highly discriminating microsatellite markers, RM9, RM21, M202, RM203, RM216, RM224, RM263, RM336, RM401 and RM444

not shown) for 48 lowland rice genotypes, which can serve as a guide for easy visual comparison of any additional genotype as and when become available. The probability of identical match was found to be 3.1×10^{-2} based on the six most discriminating microsatellite loci (i.e., RM9, RM21, RM202, RM203, RM336 and RM444), suggesting that 10^2 rice genotypes can be distinguished by using these four loci. However, by including four additional highly discriminating microsatellite loci, RM216, RM224, RM263 and RM401, the efficiency of marker system was increased to the extent that 10^5 genotypes could be distinguished. Inclusion of all the 51 microsatellite loci provided a very high resolution power enabling nearly 10^{13} genotypes to be precisely identified (Table 7). Several workers have demonstrated the utility of microsatellite markers in establishing distinction of rice varieties [31, 32, 36].

The present study indicates the high level of genetic variability present among the rainfed lowland rice genotypes which would be useful for the selection of donors in the development of high yielding rice varieties suitable for lowland and deep water ecosystems. The study also clearly indicated that microsatellite markers are useful in assessing genetic diversity in lowland rices and all the genotypes analyzed could be distinguished from each other. Seven genotype specific, two deep water and seven shallow lowland specific alleles/markers were identified. A basic molecular data set was created for the rainfed lowland rice genotypes which now can be used for variety registration, preventing misappropriation and protecting the plant breeders as well as farmers' rights. We suggest a wider survey and collection of low rice genotypes from different lowland rice ecosystems of India in order to conserve maximum diversity, and utilize this valuable resource in breeding programs to benefit of farmers.

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