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Research Article

Molecular diversity analysis of rice (*Oryza sativa* L.) genotypes using RAPD and SSR marker

Vipul G. Baldaniya, Ajay V. Narwade, Pathik B. Patel, Nilima Karmakar

Abstract

Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) molecular markers were used for detecting the genetic variability of 20 rice genotypes using 79 SSRs and 30 RAPD primers. Among primers used, a set of 16 SSR and 14 RAPD markers showed polymorphism, and banding patterns were scored as 1 (present) or 0 (absent) in the datasheet which was further analyzed by using Jaccard's similarity coefficient and SAHN clustering. The number of alleles, PIC value, and heterozygosity for individual 16 SSR and 14 RAPD markers were used to assess the degree of polymorphism among the rice genotypes. A total of 36 polymorphic loci were found in 16 polymorphic SSR markers and 77 polymorphic loci were observed in 14 polymorphic RAPD markers. The number of alleles produced per locus ranged from 2 (RM447, RM21, RM171, RM237, RM25, RM283, RM510, RM259, RM334, RM433, RM489, and RM212) to 3 (RM263, RM152, RM413, and RM3331) in 16 SSRs markers and 3 (OPR-05) to 9 (OPR-02 and OPC-06) in 14 RAPD. In SSRs, the PIC value diverse from 0.30 (RM447) to 0.58 (RM152) with an average of 0.38 per locus and an average value of heterozygosity (0.49). RAPD analysis showed an average PIC value of 0.78. Based on the information generated, the 20 rice genotypes were grouped into two main clusters in both the analysis of the marker. In SSR markers analysis, cluster I comprised 4 genotypes and cluster II comprised 16 genotypes and in RAPD cluster I was shown 2 genotypes and cluster II show 18 genotypes.

Keywords molecular diversity, polymorphism, RAPD, rice, SSR

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Introduction

One-third of the world's largest population relies on the primary food rice (*Oryza sativa* L., Poaceae, 2n = 12). There are 25 different species in the genus *Oryza*, but only two of them, *Oryza sativa* and *Oryza glaberrima*, are grown commercially. The crop provides more than 65% of India's population with their primary source of nutrition, accounting for 43% of all food grains produced and 47% of all cereals produced. Rice contributes nearly 20% of the world's dietary energy. Rice has considerable cultural and social importance in rice-consuming countries, in addition to its nutritional value. Furthermore, rice is the main food supply for half of the world's population. Rice output will need to rise by 21% by 2025 and it is one of the world's most essential crops, feeding more than half of the world's population [1]. Rice is grown on about 162

million hectares (ha) all around the globe, with 755 million tonnes harvested every year. Asia was responsible for 90% of global rice production and consumption. With 44.0 million acres of rice-growing land, India ranks as the second-largest world producer of rice after just China [2]. Rice cultivars with different genetic backgrounds hold a lot of potential for future rice crop development. Every year, a huge number of rice varieties with improved yield, quality, and nutritional challenges, and the ability to adapt to changing farming practices depending on user needs are published and notified in India.

Depending on the number of near ancestors and their phylogenetic location, Molecular Marker Technology may assist determine the distinctiveness of germplasm and their ranking. The quick advancement of DNA marker technology offers fantastic prospects to improve the nutritional qualities of traditionally grown grains and crops. Recent progress in molecular biology, such as PCR, DNA sequencing, and data analysis technologies, have made a major contribution to rice genome analysis and evaluation. PCR has emerged as a valuable and popular technique for analyzing the rice genome, notably in the assessment of rice genetic diversity, among the aforementioned technologies. Simple Sequence Repeat (SSR) is a crucial technique for identifying genetic differences in germplasm [3]. As a result, it is widely used in analyses of germplasm diversification, the detection of heterosis, the genetic link between species, etc. Microsatellites are more effective at detecting variation within genotypes [4].

DNA markers were predicted to just be available in the next decade for finding genetic diversity in rice crops in a less expensive and time-consuming technique [5]. SSR and RAPD markers were used to investigate genetic variation in 20 rice types in this experiment. The study's main goal was to evaluate the genetic diversity of different kinds to determine the number of genotypic variances, and genetic relationships, and to aid in expanding the germplasm base of future rice breeding efforts.

Methodology

In the current work, rice seeds were obtained from the main rice research center, Navsari Agricultural University, Navsari, and a field trial were conducted at N. M. College of Agriculture Farm, NAU, Navsari, Gujarat, India. A total of 20 rice genotypes (GNR-4, GR-15, Lalkada, NVSR-6158, IET-25453, IET-25453, IET-25470, IET-24336, IET-27170, IET-26375, Chittimuthyalu, IET-28690, IET-28691, IET-28694, IET-28695, IET-28696, IET-28701, IR-64, BPT-5204, IET-28704 and IET-28705) were grown in the field randomized block design with three replications and further used for molecular diversity analysis.

DNA extraction

Genomic DNA extraction from plants was collected at the four-leaf stage from rice plants with 20 genotypes and DNA was isolated using the cetyltrimethylammonium bromide (CTAB) protocol [6]. The isolated genomic DNA was quantified by a spectrophotometer at 260 and 280 nm. Agarose gel electrophoresis (0.8 percent) was used to assess the DNA's quality and quantity.

PCR analysis and gel electrophoresis

A set of sixteen SSR and fourteen RAPD primers were used. The PCR reaction for SSR markers was carried out using genomic DNA (1 µl) with master mix (5 µl), forward primer (1 µl), reverse primer (1 µl), and sterile distilled water (2 µl) for one sample while the PCR reaction for RAPD markers was carried out genomic DNA (1 µl) with master mix (5 µl), primer (2 µl), and sterile distilled water (2 µl) for one sample. DNA replication using SSR markers was accomplished by polymerase chain reaction using a BIO-RAD thermocycler machine under the following conditions: initial denaturation (5 min at 94°C), followed by 35 cycles of 30 sec at 94°C, 30 sec at 55°C for annealing, 45 sec at 72°C for extension, for final extension (10 min at 72°C) and ∞ time at 4°C for a halt. DNA replication using

RAPD markers was accomplished by polymerase chain reaction using a BIO-RAD thermocycler machine under the following conditions: initial denaturation (5 min at 95°C), followed by 35 cycles of 30 sec at 95°C, 45 sec at 38°C for annealing, 60 sec at 72°C for extension, final extension (10 min at 72°C) and ∞ time at 4°C for a halt until samples were collected. Amplification products were resolved by 3% agarose gel using a DNA ladder (50 bp) for the SSR-PCR product and 100 bp DNA ladder with 1.8% agarose gel used for the RAPD-PCR product.

Data analysis

For each marker allele genotype combination, the amplified product from the SSR and RAPD marker analyses was scored quantitatively for presence (1) or absence (0). The similarity was calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by an agglomerative technique using the UPGMA method by SAHN clustering function of PAST version 3.23. Polymorphism information content (PIC) was estimated as per the below equation [7].

$$PIC = 1 - \left(\sum_{i=1} P_i^2\right) - \left(\sum_{i=0}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2\right)$$

Where P_i and P_j are the frequencies of i^{th} and j^{th} marker

Results and Discussion

Molecular SSR and RAPD markers were used in a genomic study for the characterization of genetic resources and to assess the genetic variability in genotypes used in a breeding program. In the current work, SSR and RAPD primers used diversity greatly in their capacity to resolve variability between 20 rice genotypes. Among 79 SSR markers and 30 RAPD markers used to generate markers profiles, 16 SSR markers and 14 RAPD markers were found polymorphic and suitable for analysis.

The majority of the SSR loci had noticeably different allele counts. From 79 SSR markers screened, 16 SSR markers, which displayed clear and repeatable polymorphic bands (Table 1), were selected for analysis, and some polymorphic markers figures show here (Figures 1 to 4). In SSR, all of the markers produced 16 polymorphic bands, and SSR markers analysis of all sixteen primers series viz., RM263, RM447, RM21, RM152, RM171, RM237, RM413, RM25, RM3331, RM283, RM510, RM259, RM334, RM433, RM489, and RM212 generated total 36 polymorphic bands.

Table 1. Details of polymorphic SSR markers

SN.	Markers	Forward sequence	Reverse sequence
1.	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
2.	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
3.	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
4.	RM152	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
5.	RM171	ACGCGAGGCACACGTACTTAC	ACGAGATACGTACGCCTTTG
6.	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
7.	RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
8.	RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
9.	RM3331	CCTCCTCCATGAGCTAATGC	AGGAGGAGCGGATTTCTCTC
10.	RM283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
11.	RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
12.	RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT
13.	RM334	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG
14.	RM433	TGCGCTGAACTAAACACAGC	AGACAAACCTGGCCATTCAC
15.	RM489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
16.	RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG

Polymorphism of SSR markers

Polymorphic information content (PIC) result is a reflection of allelic multiplicity and regularity among the genotypes. The percentage of polymorphism shown in 16 markers was 100%. The polymorphism information content (PIC) value fluctuated between 0.30 to 0.58 with an average of 0.38. High PIC values were obtained for the primers RM152 (0.58) and RM3331 (0.55), while primer RM447 (0.30) showed lower values of PIC. Calculation of heterozygosity (HI) in 16 SSR polymorphic markers, the heterozygosity value ranged from RM447 (0.36) to RM153 (0.65) with an average of 0.48 while the highest (0.65) heterozygosity was found in RM153 followed by RM3331 (0.63), and lowest (0.36) heterozygosity observed in primer RM447 (Table 2).

In 16 primers, a total 36 number of loci were found and several polymorphic loci ranged from 2 to 3 with an average of 2.25. The highest number of amplified alleles (03) was exhibited by the SSR primer RM263, RM152, RM413, and RM3331 whereas; the lowest numbers of amplified alleles (02) were exhibited by the primer viz., RM447, RM21, RM171, RM237, RM25, RM283, RM510, RM259, RM334, RM433, RM489, and RM212.

The PIC values and the number of alleles at SSR loci were correlated. In contrast, a modest mean PIC value (0.38) may be the result of (A) a small number of genotypes that have adapted to the environment successfully (B) A smaller number of differences in DNA regions (C) Gene pool with thin genetic base (D) Di/Tri repeat mutation rate is lower Ashraf et al., [8].

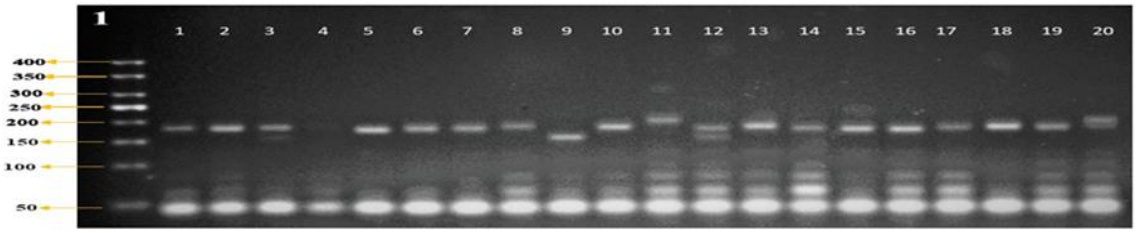

Figure 1. Polymorphism among accessions using SSR marker RM263 (50 bp ladder)

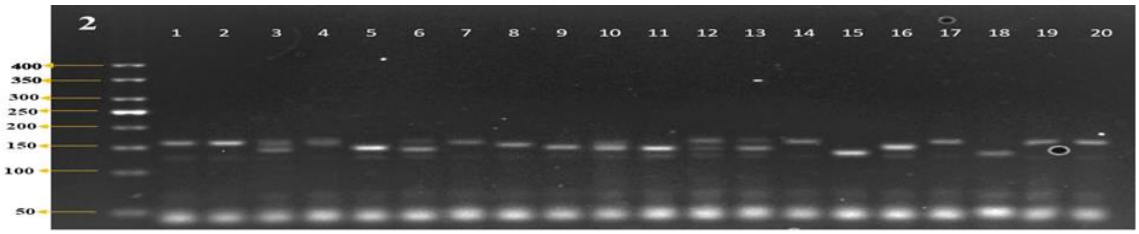

Figure 2. Polymorphism among accessions using SSR marker RM152 (50 bp ladder)

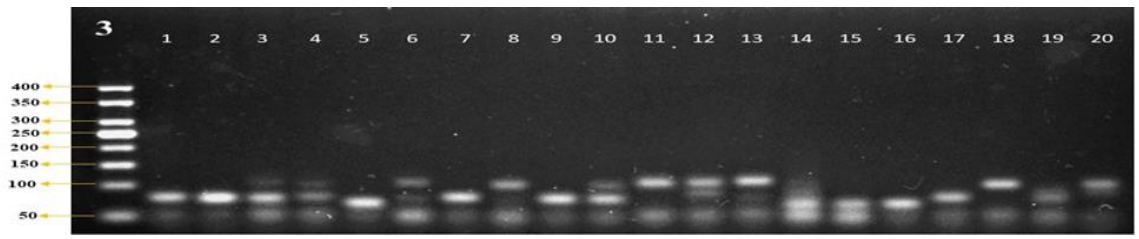

Figure 3. Polymorphism among accessions using SSR marker RM431 (50 bp ladder)

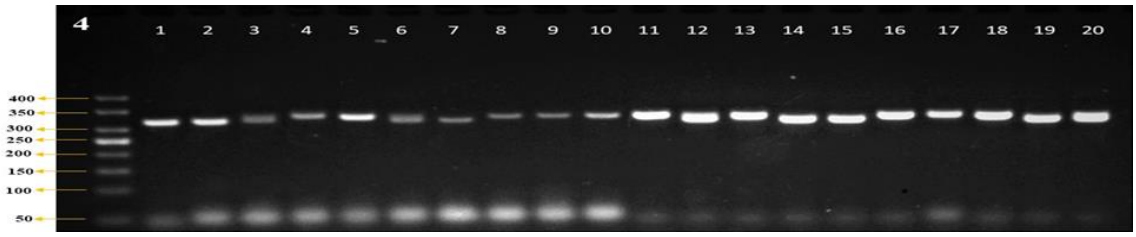

Figure 4. Polymorphism among accessions using SSR marker RM171 (50 bp ladder)

Table 2. Polymorphism obtained with different SSR primers in twenty rice genotypes

SN.	Primer	Total number of loci	No. of polymorphic loci	Polymorphism percentage	PIC value	HI
1.	RM263	3	3	100.00	0.36	0.39
2.	RM447	2	2	100.00	0.30	0.36
3.	RM21	2	2	100.00	0.37	0.49
4.	RM152	3	3	100.00	0.58	0.65
5.	RM171	2	2	100.00	0.38	0.50
6.	RM237	2	2	100.00	0.35	0.46
7.	RM413	3	3	100.00	0.38	0.50
8.	RM25	2	2	100.00	0.35	0.46
9.	RM3331	3	3	100.00	0.55	0.63
10.	RM283	2	2	100.00	0.35	0.44
11.	RM510	2	2	100.00	0.35	0.44
12.	RM259	2	2	100.00	0.37	0.48
13.	RM334	2	2	100.00	0.35	0.46
14.	RM433	2	2	100.00	0.35	0.44
15.	RM489	2	2	100.00	0.33	0.41
16.	RM212	2	2	100.00	0.37	0.49
TOTAL		**36**	**36**	**100.00**	**6.09**	**7.60**
AVERAGE					**0.38**	**0.48**

Cluster analysis and genetic diversity pattern based on SSR marker

For SSR markers alleles at various loci, the input matrix for the genetic diversity/relatedness among the genotypes was created, resulting in the development of closeness values among the genotypes under study. The twenty rice genotypes were divided into two major clusters, A and B, which had four and sixteen genotypes, respectively (Figure 5).

Major cluster A was further split into the A1 (1 genotype) and A2 sub-clusters (3 genotypes). A maximum number of accessions were grouped in sub-cluster A2, indicating high genetic similarity between genotypes of this group. Out of 4, 3 genotypes viz., IET-28696, IET-25453, and IET-28701 were grouped in sub-cluster A2. One genotype viz., IET-26375 was grouped in sub-cluster A1. Main cluster B included 16 genotypes viz., IET-25470, Lalkada, IET-28691, GR-15, NVSR-6158,

Chittimuthyalu, IET-28694, IET-28690, IET-28695, IET-24336, GNR-4, IR-64, IET-28704, BPT-5204, IET-28705 and IET- 27170. Out of 16 genotypes, nine genotypes viz., IET-25470, Lalkada, IET-28691, GR-15, NVSR-6158, Chittimuthyalu, IET-28694, IET-28690, and IET-28695 were grouped in Sub Cluster B1 and another seven viz., IET-24336, GNR-4, IR-64, IET-28704, BPT-5204, IET-28705, and IET-27170 genotypes included in sub-cluster B2.

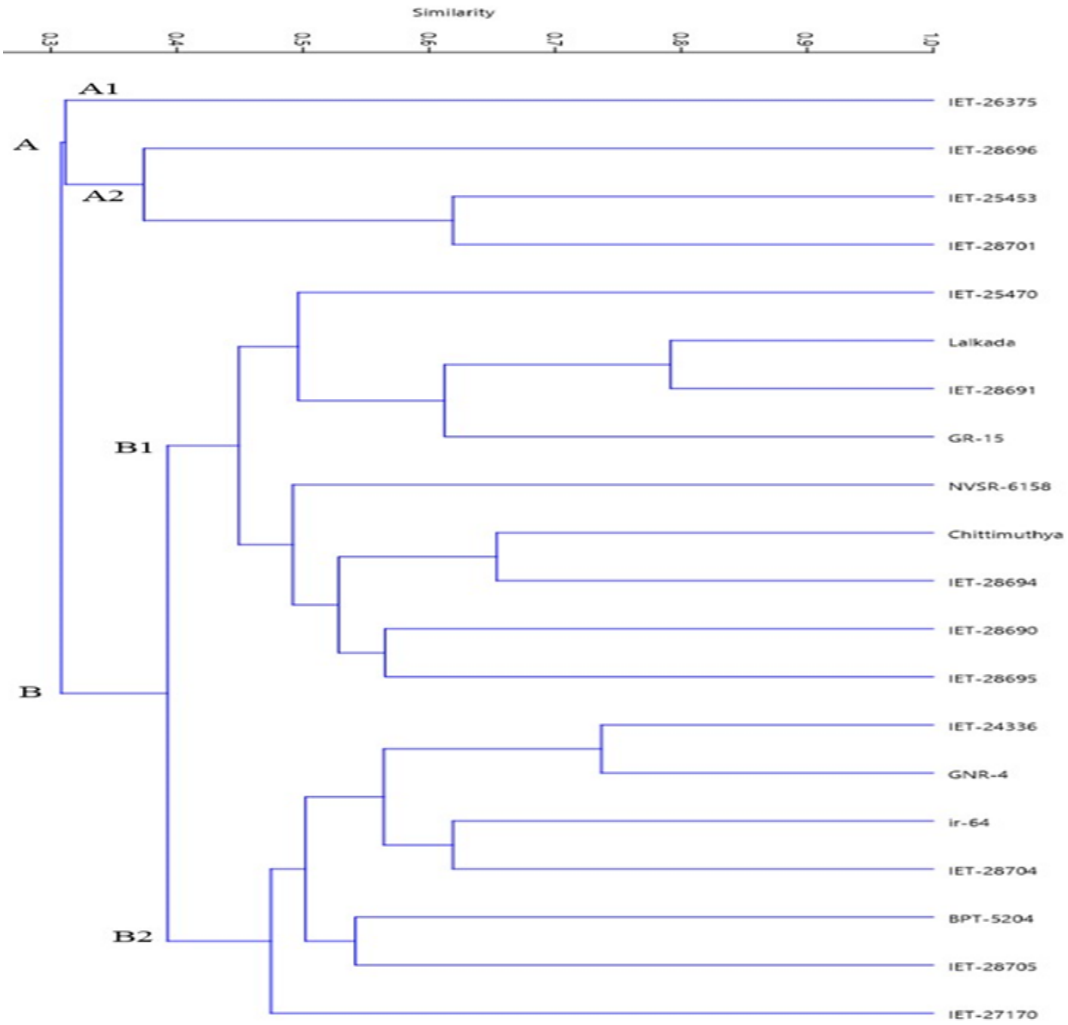

Figure 5. Dendrogram showing the genetic association between 20 rice genotypes based on SSR data using UPGMA

The genotype-to-genotype genetic similarity coefficient varied from 0.30 to 1.00, with an average similarity coefficient of (0.65) (Table 3). The IET-28691 and Lalkada genotypes had the maximum genetic distance (0.79), indicating that these two genotypes differ significantly at the genomic level and can be used to develop bi-parental mapping populations as well as in rice development projects to enlarge the genetic background of different rice genotypes. The lowest (0.17) genetic distance was observed between genotypes IET-25453 and GR-15 also similar distance was recorded between genotypes IET-27170 and IET-25453. Indicating that these genotypes may share a common gene pool. The PIC range of markers gives an estimation of their discriminating power in a set of accessions by taking not only the number of alleles but also the frequencies of

each allele. As a result, the markers RM152 and RM3331 may be used to identify genetic differences between rice genotypes and investigate their evolutionary connection. The SSR markers are co-dominant and could be a strong tool to assess the genetic variability among cultivated varieties and genotypes and more informative and can be useful for marker-assisted selection for nutritive rice cultivars. The high PIC value shows that all these markers were extremely informative and able to differentiate between cultivated varieties and genotypes. The outcome of the current study was in agreement with some scientists, including Sabouri et al., [9], Mehmood et al., [10], and Raza et al., [11].

Table 3. Jaccard's similarity coefficient for 20 rice genotypes based on SSR molecular marker

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.67	1.00																		
3	0.52	0.63	1.00																	
4	0.57	0.36	0.42	1.00																
5	0.27	0.17	0.28	0.23	1.00															
6	0.57	0.37	0.60	0.46	0.46	1.00														
7	0.74	0.48	0.37	0.45	0.23	0.40	1.00													
8	0.46	0.57	0.39	0.42	0.17	0.32	0.48	1.00												
9	0.28	0.22	0.29	0.35	0.24	0.21	0.29	0.32	1.00											
10	0.43	0.46	0.57	0.44	0.22	0.45	0.39	0.58	0.46	1.00										
11	0.35	0.33	0.39	0.55	0.26	0.37	0.31	0.38	0.32	0.46	1.00									
12	0.50	0.60	0.79	0.41	0.23	0.52	0.46	0.48	0.28	0.55	0.48	1.00								
13	0.37	0.41	0.52	0.50	0.24	0.44	0.38	0.46	0.25	0.65	0.65	0.62	1.00							
14	0.46	0.44	0.39	0.48	0.21	0.42	0.48	0.38	0.22	0.41	0.57	0.48	0.58	1.00						
15	0.25	0.33	0.30	0.26	0.31	0.28	0.36	0.38	0.32	0.37	0.33	0.38	0.46	0.50	1.00					
16	0.25	0.24	0.26	0.31	0.62	0.37	0.26	0.29	0.38	0.32	0.29	0.25	0.31	0.33	0.44	1.00				
17	0.48	0.46	0.27	0.38	0.32	0.24	0.65	0.59	0.45	0.38	0.30	0.34	0.32	0.35	0.46	0.46	1.00			
18	0.48	0.30	0.36	0.38	0.38	0.50	0.57	0.46	0.33	0.54	0.40	0.34	0.48	0.35	0.30	0.35	0.48	1.00		
19	0.48	0.35	0.23	0.27	0.38	0.33	0.65	0.40	0.23	0.29	0.21	0.34	0.23	0.35	0.35	0.35	0.62	0.36	1.00	
20	0.48	0.36	0.32	0.33	0.24	0.39	0.57	0.46	0.30	0.43	0.36	0.40	0.38	0.36	0.41	0.31	0.54	0.54	0.54	1.00

Genotypes Name: 1) GNR-4 2) GR-15 3) Lalkada 4) NVSR-6158 5) IET-25453 6) IET-25470 7) IET-24336 8) IET-27170 9) IET-26375 10) Chittimuthyalu 11) IET-28690 12) IET-28691 13) IET-28694 14) IET-28695 15) IET-28696 16) IET-28701 17) IR-64 18) BPT-5204 19) IET-28704 20) IET-2870

RAPD analysis

The genetic variability among the 20 rice genotypes was studied in an initial screening using thirty RAPD primers among them 14 primers produced informative, polymorphic products resolvable by agarose gel electrophoresis, and some of them are shown here (Figures 6 and 7). Polymorphic primers, the total number of loci, number of polymorphic loci, polymorphism percentage, and PIC value data are recorded in Table 4.

There we found a total of 85 loci in the study and 77 (90.59%) of which were polymorphic, with PIC values ranging from 0.63 to 0.87 on average and values for several polymorphic loci from 3 to 9. Seven primers (OPC-06, OPC-08, OPC-11, OPC-16, OPC-20, OPD-07, and OPR-05) out of 14

polymorphic primers exhibit 100% polymorphism. The lowest PIC value was detected in the marker OPR-05 (0.63) and the highest found in marker OPC-06 (0.87). The lesser PIC value in this work could be attributed due to the low genotypic diversity of 20 rice genotypes. The high PIC value and huge number of alleles per marker can also be qualified to the nature of the materials studied. The average PIC value (0.87) is higher when compared with the previous study by Mursyidin et al., [12] and Rajani et al., [13].

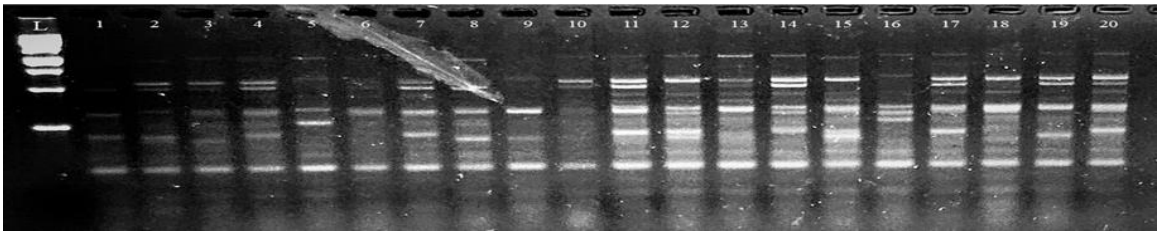

Figure 6. Polymorphism among accessions using RAPD marker OPC-08 (100 bp ladder)

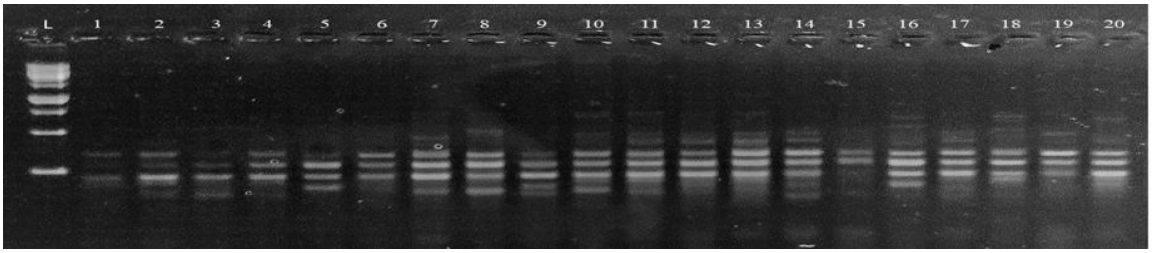

Figure 7. Polymorphism among accessions using RAPD marker OPC-16 (100 bp ladder)

Table 4. Polymorphism obtained with different RAPD primers in twenty genotypes

SN.	Primer	Sequence	Total number of loci	No. of polymorphic loci	Polymorphism percentage	PIC value
1.	OPC-04	CCGCATCTAC	8	7	87.5	0.86
2.	OPC-06	GAACGGACTC	9	9	100.00	0.87
3.	OPC-08	TGGACCGGTG	6	6	100.00	0.78
4.	OPC-11	AAAGCTGGGG	6	6	100.00	0.83
5.	OPC-12	TGTCATCCCC	7	6	85.71	0.84
6.	OPC-16	CACACTGGAG	6	6	100.00	0.85
7.	OPC-18	TGAGTGGGTG	5	4	80.00	0.69
8.	OPC-20	ACTTCGCCAC	5	5	100.00	0.79
9.	OPD-05	TGAGCGGACA	5	3	60.00	0.71
10.	OPD-07	TTGGCACGGG	5	5	100.00	0.71
11.	OPR-02	CACAGCTGCC	9	8	88.89	0.86
12.	OPR-03	ACACAGAGGG	7	6	85.71	0.81
13.	OPR-04	CCCGTAGCAC	4	3	75.00	0.71
14.	OPR-05	GACCTAGTGG	3	3	100.00	0.63
TOTAL			**85**	**77**	**90.59**	**10.94**
AVERAGE			**6.07**	**5.5**	**90.60**	**0.78**

Cluster analysis and genetic diversity pattern based on RAPD marker

A dendrogram create based on the similarity values generated by the RAPD, was depicted by using the UPGMA cluster shown in Figure 8. Twenty rice genotypes were divided into two main groups (A and B) using the phylogenetic tree. Two sub-clusters, A1 (1 genotype) and A2 (1 genotype), were created from the main cluster A. Sub-cluster A1 had the genotype GNR-4, whereas sub-cluster A2 contained the genotype GR-15. Second key cluster B was the largest with 18 rice genotypes. Key cluster B was further separated into two sub-clusters B1 (3 genotypes) and B2 (15 genotypes). Sub-cluster B2 was the largest with 15 genotypes viz., IET-28696, IET-28705, IR-64, IET-28694, IET-28695, IET-28691, IET-28690, IET-28704, BPT-5204, Chittimuthyalu, IET-27170, IET-24336, IET-25470, NVSR-6158 and Lalkada.

The genotypes had a genetic similarity score between 0.45 and 0.97. The IET-25470 and NVSR-6158 genotypes had the maximum genetic distance (0.83), indicating that these two genotypes are quite different at the genomic level and can be used in bi-parental mapping populations and rice development projects to expand the genetic diversity of different rice genotypes. The lowest (0.40) genetic distance was observed between genotypes GNR-4 and Chittimuthyalu (Table 5).

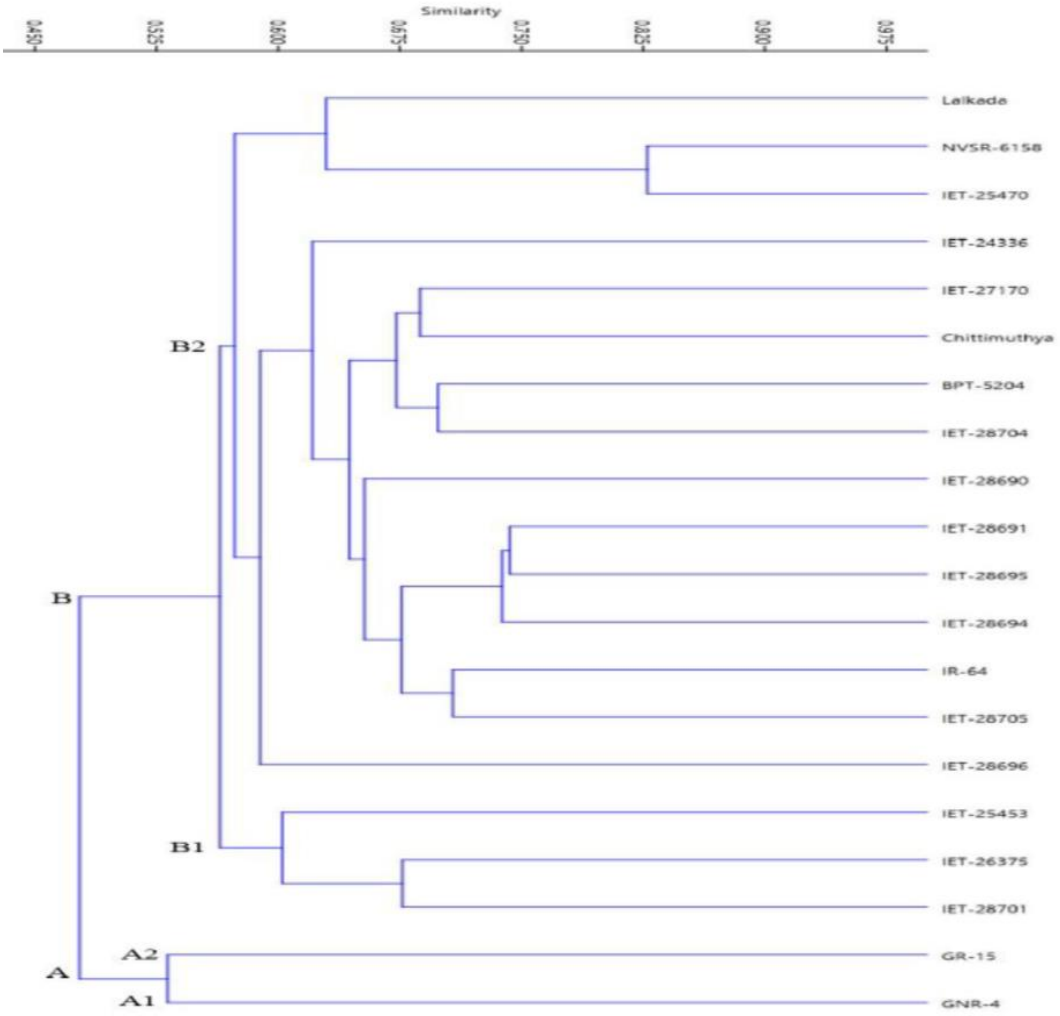

Figure 8. Dendrogram showing the genetic association between 20 rice genotypes based on RAPD data using UPGMA

Table 5. Jaccard's similarity coefficient for 20 rice genotypes based on RAPD molecular marker

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.53	1.00																		
3	0.52	0.62	1.00																	
4	0.56	0.59	0.66	1.00																
5	0.43	0.47	0.53	0.59	1.00															
6	0.59	0.51	0.60	0.83	0.54	1.00														
7	0.44	0.43	0.52	0.57	0.53	0.57	1.00													
8	0.47	0.52	0.56	0.59	0.55	0.57	0.65	1.00												
9	0.44	0.48	0.52	0.57	0.58	0.59	0.66	0.58	1.00											
10	0.40	0.46	0.60	0.58	0.54	0.53	0.59	0.69	0.54	1.00										
11	0.48	0.43	0.60	0.61	0.50	0.58	0.64	0.61	0.53	0.60	1.00									
12	0.44	0.41	0.51	0.63	0.57	0.54	0.61	0.61	0.59	0.62	0.67	1.00								
13	0.41	0.46	0.51	0.67	0.53	0.61	0.57	0.64	0.57	0.65	0.58	0.74	1.00							
14	0.45	0.50	0.54	0.59	0.53	0.52	0.62	0.71	0.55	0.68	0.68	0.74	0.74	1.00						
15	0.45	0.44	0.52	0.60	0.58	0.51	0.52	0.59	0.57	0.51	0.62	0.64	0.62	0.67	1.00					
16	0.35	0.46	0.42	0.59	0.63	0.51	0.52	0.56	0.68	0.59	0.49	0.61	0.66	0.62	0.61	1.00				
17	0.47	0.53	0.50	0.58	0.52	0.58	0.64	0.64	0.64	0.58	0.68	0.65	0.63	0.73	0.55	0.61	1.00			
18	0.47	0.50	0.52	0.59	0.53	0.59	0.56	0.70	0.60	0.66	0.59	0.63	0.66	0.69	0.59	0.60	0.66	1.00		
19	0.60	0.47	0.53	0.69	0.55	0.64	0.63	0.67	0.60	0.66	0.64	0.66	0.59	0.69	0.61	0.58	0.69	0.70	1.00	
20	0.49	0.48	0.56	0.63	0.53	0.61	0.70	0.67	0.60	0.63	0.66	0.65	0.68	0.73	0.56	0.58	0.71	0.69	0.64	1.00

Genotypes Name: 1) GNR-4 2) GR-15 3) Lalkada 4) NVSR-6158 5) IET-25453 6) IET-25470 7) IET-24336 8) IET-27170 9) IET-26375 10) Chittimuthyalu 11) IET-28690 12) IET-28691 13) IET-28694 14) IET-28695 15) IET-28696 16) IET-28701 17) IR-64 18) BPT-5204 19) IET-28704 20) IET-2870

Conclusion

Any breeding effort should include genetic variation as a key element in crop improvement programs. Among 20 rice genotypes, genotypes (IET-28691 and Lalkada) and (IET-25470 and NVSR-6158) were found genetically highest distant in the main cluster based on molecular diversity analysis using SSR and RAPD markers, respectively.

In the sub-cluster, genotypes (GNR-4 and IET-24336) and (IET-28695 and IR-64) were found genetically highest distant based on molecular diversity analysis using SSR and RAPD markers, respectively, these can be used as a genetically distant parent for breeding programs. The PIC values determined the polymorphism between genotypes for a marker locus helped in linkage analysis and identified the most effective SSR markers namely RM152 and RM3331 and in the case of RAPD markers namely OPC-06, OPC-04, OPR-02, OPC-16, OPC-12, OPC-11 and OPR-03. The use of SSR and RAPD markers used in this experiment exhibits the usefulness of these markers for the assessment of genetic diversity.

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