UDC 575.630
https://doi.org/10.2298/GENSR2501063K
Original scientific article

MOLECULAR PROFILING AND GENETIC DIVERSITY ASSESSMENT OF RICE (*Oryza sativa* L.) USING SSR MARKERS

Shruti KUMARI[1], S. K. CHAKRABARTY[1], Praloy KUMAR BHOWMICK[2], Paul DEBASHIS[3]

[1]Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India
[2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India
[3]ICAR-Central Institute for Cotton Research, Regional Station, Sirsa, Haryana, India

Kumari S., S. K. Chakrabarty, P. Kumar Bhowmick, P. Debashis (2025). *Molecular profiling and genetic diversity assessment of rice (Oryza sativa L.) using SSR markers*- Genetika, Vol 57, No.1, 63-75.

Evaluating genetic resources for seed vigour traits is crucial for identifying genotypes and traits suitable for enhanced variety development. Simple sequence repeat (SSR) markers have been undertaken for the evaluation of genetic diversity among 44 Indian rice varieties. Among the 41 SSR markers used, 38 markers were found to be 100% polymorphic and three markers, RM 204, RM 20, and RM 125 were found to be monomorphic for one allele but another allele was polymorphic. Out of a total of 141 alleles detected 138 were polymorphic. The number of alleles per locus varied from 2 to 5 with an average of 3.43 allele per locus. The overall size of amplified products varied from 50 base pair (bp) (RM 240) to 600 bp (RM 13). The Polymorphic Information Content (PIC) value of each marker ranged from 0.013 to 0.967, with an average of 0.430. A UPGMA dendrogram based on SSR polymorphism rice varieties were classified into five major clusters (I to V) based on their genetic similarity values and maturity periods, ranging from early to very late. Cluster I contained three early-maturing varieties,

Corresponding author: Dr. S. K. Chakrabarty, Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India (E-mail: shyamal kumar chakrabarty@icar.org.in;Phone No.: 91- 9968279444), ORCID: 0000-0001-7519-0169, Shruti Kumari ORCID: 0009-0006-7077-8848, Praloy Kumar Bhowmick ORCID: 0000-0002-0467-2192, Debashis Paul ORCID: 0000-0001-9358-9536

cluster II 21 varieties of early and mid-maturity groups, and cluster III 18 late and very late-maturing group varieties. Clusters IV and V had single variety of mid and very late-maturity group. The results can accelerate the creation of high-performing varieties with enhanced seed vigour, promoting improved crop establishment and overall yield potential while enhancing the adaptability of plant varieties to changing environmental conditions.

Key words: Genetic diversity, SSR markers, seed vigour, rice

INTRODUCTION

Estimation of genetic diversity is a prerequisite to select genetically diverse parents for any crop improvement programme because it helps in the development of superior recombinants (HAUSILA PRASAD SINGH *et al*., 2022), through selection of diverse parents which provide an opportunity to plant breeders to obtain desirable recombinants during hybridization programme (SRIVASTAVA *et al*., 2020) and improved cultivars by introgression of desirable traits (KHODADADI *et al*., 2011)

Estimating genetic diversity among genotypes typically involves assessing physiological and morphological variations in quantitative and economically significant traits. Ensuring robust seed germination and vigor is crucial for achieving increased and sustainable rice yields across various cultivation environments. Genetic diversity among inter and intra plant populations can be estimated by morphological, biochemical and DNA markers (OGWU *et al*., 2014). Traditional morphological markers often fall short in effectively distinguishing genetic diversity due to their susceptibility to environmental influences and the complex inheritance patterns of many traits. This limitation has led researchers to favor molecular markers, such as SSRs, which offer greater reliability and are less affected by external factors (SINGH SALINI *et al*., 2022). The molecular markers are promising and effective tools for measuring genetic diversity in plant germplasm and elucidating their evolutionary relationship, because molecular markers are more reliable and remain unaffected across different growth stages, seasons, locations and agronomic practices. Molecular characterization of genotypes offers detailed insights into genetic diversity, aiding in the formulation of appropriate breeding programs. SSR markers are widely preferred for estimating genetic diversity because they are abundant, highly polymorphic, multiallelic, reliably scored, and evenly distributed throughout the genome (SALGOTRA *et al*., 2015). Numerous microsatellite markers have been developed and characterized in rice research, with their chromosomal locations and polymorphism levels determined. Estimation of molecular genetic diversity represents variations at the allelic level in the form of polymorphic information content (TANG *et al*., 2021), molecular dendrogram (SUVI *et al*., 2019; NAHAR *et al*., 2020), and genetic distance (DONDE *et al*., 2019) among the selected genotypes and shows the contribution of specific traits towards the total divergence. Therefore, the present investigation aimed to assess the nature and magnitude of genetic divergence present in the 44 rice genotypes and to select suitable genotypes for further utilization in crop improvement program.

MATERIALS AND METHODS

Seed materials

The description of 44 rice varieties used in this study is presented in Table 1. The seeds of all varieties of different maturity groups (from sowing to harvesting) i.e., early (<120 days),

medium (121-140 days), late (141-150 days), and very late maturity (>160 days) were produced following the recommended package of practices in the field at ICAR-IARI, New Delhi during Rainy season, 2020.

Table 1.A list of rice genotypes (44) and maturity duration

S. no.	Varieties	Place of origin	Maturity duration (days)	Maturity groups	S. no.	Varieties	Place of origin	Maturity duration (days)	Maturity groups
1.	JD-6	New Delhi	119	Early	23.	Satabdi	West Bengal	132	Medium
2.	VL-Dhan 221	Uttarakhand	119	Early	24.	Vikramarya	Andhra Pradesh	137	Medium
3.	VL-Dhan 81	Uttarakhand	119	Early	25.	Jaya	Karnataka	140	Late
4.	Remaya	Kerala	118	Early	26.	Shyamala	Madhya Pradesh	140	Late
5.	Poornima	Madhya Pradesh	118	Early	27.	Mandyavijaya	Karnataka	141	Late
6.	Pant Dhan 11	Uttar Pradesh	118	Early	28.	Dudheswar	West Bengal	141	Late
7.	JD-13	New Delhi	118	Early	29.	Budiluchai	Madhya Pradesh	141	Late
8.	Govind	Chhattisgarh	118	Early	30.	Naveen	West Bengal	141	Late
9.	IR 688 97 B	IRRI	118	Early	31.	Vasumati	Odisha	143	Late
10.	Rasi	Telengana	118	Early	32.	CRD-204	Odisha	143	Late
11.	Annada	West Bengal	118	Early	33.	IR-64	IRRI,Philippines	140	Late
12.	Jyoti	Kerala	119	Early	34.	Basmati-370	New Delhi	146	Late
13.	Pusa 834	New Delhi	119	Early	35.	Indirasugandhit	Chhattisgarh	160	Very Late
14.	Pusa 33	New Delhi	119	Early	36.	Phalguna	Andhra Pradesh	160	Very Late
15.	NDR 97	Uttar Pradesh	123	Medium	37.	MTU1010	Andhra Pradesh	164	Very Late
16.	Vadana	Odisha	123	Medium	38.	Kranti	TamilNadu	162	Very Late
17.	Ravi	Andhra Pradesh	120	Medium	39.	Swarna	Andhra Pradesh	164	Very Late
18.	Lochit	Assam	126	Medium	40.	Krishnaveni	Andhra Pradesh	162	Very Late
19.	Satyabhama	Odisha	126	Medium	41.	Salivahana	Odisha	172	Very Late
20.	Bidhan Gontra-2	West Bengal	126	Medium	42.	CRD-300	Odisha	164	Very Late
21.	PNR-381	New Delhi	126	Medium	43.	Surajone	Chhattisgarh	164	Very Late
22.	PantDhan-12	Uttar Pradesh	132	Medium	44.	Ranikajar	Chhattisgarh	164	Very Late

Isolation of genomic DNA, PCR and SSR markers selection

DNA was isolated from the fresh plant tissue by CTAB method (DOYLE, 1990). For polymerase chain reaction (PCR), the program comprises of initial denaturation at 94ºC for 4 min. which was further followed by 35 cycles of 94℃ for 1 min, 55℃ for 30 sec. and 72℃ for 1 min. Final extension was carried out at 72℃ for 7 min. The amplified products were resolved on 3.5% metaphor Agarose gel. A Sample of 50 bp ladder was also loaded. 41 SSR markers were

selected (Table. 2) based on its association with seed germination and seedling vigour related traits. The sequence information for the respective primer pairs was obtained from the *https://archive.gramene.org/markers/microsat/all-ssr.html*. Selection of these markers was based on the positions across the 12 chromosomes for estimation of diversity and to identify the polymorphic loci. The PCR reactions, electrophoresis and gel documentation were performed following previous publications (BARIK *et al*., 2016; PRADHAN *et al*., 2019b; MOHAPATRA *et al*., 2021).

Table 2.List of SSR markers, sequences, chromosome number and QTLs associated with seed vigour traits

S.No.	Marker name		Primer sequences(5' to 3')	Chromosome No.	Seed Vigour Traits
1.	RM 6	F	GTCCCCTCCACCCAATTC	2	SDW
		R	TCGTCTACTGTTGGCTGCAC		
2.	RM 9	F	GGTGCCATTGTCGTCCTC	1	GR, SDW, SFW
		R	ACGGCCCTCATCACCTTC		
3.	RM 13	F	TCCAACATGGCAAGAGAGAG	5	SL, RL, DW, GR, SDW
		R	GGTGGCATTCGATTCCAG		
4.	RM 16	F	CGCTAGGGCAGCATCTAAA	3	SL, SDW,RL, GR
		R	AACACAGCAGGTACGCGC		
5.	RM 19	F	CAAAAACAGAGCAGATGAC	12	GR, SDW, SL
		R	CTCAAGATGGACGCCAAGA		
6.	RM 20	F	ATCTTGTCCCTGCAGGTCAT	12	SL, SR, SDW, SEV
		R	GAAACAGAGGCACATTTCATTG		
7.	RM 26	F	GAGTCGACGAGCGGCAGA	5	TDW,SDW, RDW, VI, SL,GR
		R	CTGCGAGCGACGGTAACA		
8.	RM 85	F	CCAAAGATGAAACCTGGATTG	3	GR, SL, RL, SEV, FW
		R	GCACAAGGTGAGCAGTCC		
9.	RM 87	F	CCTCTCCGATACACCGTATG	5	SDW
		R	GCGAAGGTACGAAAGGAAAG		
10.	RM 125	F	ATCAGCAGCCATGGCAGCGACC	7	GR, GP, GI
		R	AGGGGATCATGTGCCGAAGGCC		
11.	RM 148	F	ATACAACATTAGGGATGAGGCTGG	3	GR,SL, SEV,FW
		R	TCCTTAAAGGTGGTGCAATGCGAG		
12.	RM 168	F	TGCTGCTTGCCTGCTTCCTTT	3	SL, DW, GR,RL, SEV, FW
		R	GAAACGAATCAATCCACGGC		
13.	RM 204	F	GTGACTGACTTGGTCATAGGG	6	TDW, GR, SDW,
		R	GCTAGCCATGCTCTCGTACC		
14.	RM 213	F	ATCTGTTTGCAGGGGACAAG	2	SDW, RDW
		R	AGGTCTAGACGATGTCGTGA		
15.	RM 214	F	CTGATGATAGAAACCTCTTCTC	7	RL
		R	AAGAACAGCTGACTTCACAA		
16.	RM 218	F	TGGTCAAACCAAGGTCCTTC	3	SL, FY
		R	GACATACATTCTACCCCCGG		
17.	RM 221	F	ACATGTCAGCATGCCACATC	2	SEV, SDW, SL, RL, GP, GR, VI, FGC
		R	TGCAAGAATCTGACCCGG		
18.	RM 223	F	GAGTGAGCTTGGGCTGAAAC	8	SW, GR, SL, SEV
		R	GAAGGCAAGTCTTGGCACTG		
19.	RM 224	F	ATCGATCGATCTTCACGAGG	11	GR, SDW, SL
		R	TGCTATAAAAGGCATTCGGG		
20.	RM 228	F	CTGGCCATTAGTCCTTGG	10	SS, SL, FW, WS
		R	GCTTGCGGCTCTGCTTAC		
21.	RM 229	F	CACTCACACGAACGACTGAC	11	GI, RL
		R	CGCAGGTTCTTGTGAAATGT		
22.	RM 230	F	GCCAGACCGTGGATGTTC	8	GR, SL, RL
		R	CACCGCAGTCACTTTTCAAG		
23.	RM 234	F	ACAGTATCCAAGGCCCTGG	7	GR, GI, SL
		R	CACGTGAGACAAAGACGGAG		
24.	RM 240	F	CCTTAATGGGTAGTGTGCAC	2	GR, SDW, WS, SR
		R	TGTAACCATTCCTTCCATCC		
25.	RM 250	F	GGTTCAAACCAAGCTGATCA	2	SDW
		R	GATGAAGGCCTTCCACGCAG		
26.	RM 263	F	CCCAGGCTAGCTCATGAACC	2	SL, SDW
		R	GCTACGTTTGAGCTACCACG		
27.	RM 264	F	GTTGCGTCCTACTGCTACTTC	8	GR, SL, RL
		R	GATCCGTGTCGATGATTAGC		

28.	RM 270	F	GGCCGTTGGTTCTAAAATC	12	SDW
		R	TGCGCAGTATCATCGGCGAG		
29.	RM 306	F	CAAGGTCAAGAATGCAATGG	5	GI, FV, FGC, EGC
		R	GCCACTTTAATCATTGCATC		
30.	RM 315	F	GAGGTACTTCCTCCGTTTCAC	1	SL, SDW,LA
		R	AGTCAGCTCACTGTGCAGTG		
31.	RM 334	F	GTTCAGTGTTCAGTGCCACC	5	SDW
		R	GACTTTGATCTTTGGTGGACG		
32.	RM 336	F	CTTACAGAGAAACGGCATCG	7	GR, SDW, SL
		R	GCTGGTTTGTTTCAGGTTCG		
33.	RM 341	F	CAAGAAACCTCAATCCGAGC	2	GR
		R	CTCCTCCCGATCCCAATC		
34.	RM 427	F	TCACTAGCTCTGCCCTGACC	7	GI
		R	TGATGAGAGTTGGTTGCGAG		
35.	RM 480	F	GCTCAAGCATTCTGCAGTTG	5	SL, RL, SDW
		R	GCGCTTCTGCTTATTGGAAG		
36.	RM 528	F	GGCATCCAATTTTACCCCTC	6	GP, SL, SDW
		R	AAATGGAGCATGGAGGTCAC		
37.	RM 1339	F	ATCAAAGCATGTAAACCAGC	1	SL
		R	CGTAAGATCTCCCTACCACC		
38.	RM 1353	F	ATGAGGTTCAAAATGAGACG	7	GI
		R	TTAAGCTACTGTCTGCCTCC		
39.	RM 3428	F	ATTCATGCTTCCTTTCAGTG	11	GP, GI, NL, SS
		R	GATTACTGGTTTGCCATTTG		
40.	RM 5389	F	TCTTGCATGAGAGCCAACAC	1	SL, RL, SDW, TFW
		R	GCTATTGCGCGAGATTATCC		
41.	RM 5609	F	CGCCAGTGTCGAATATGATG	12	SL, GR, SDW
		R	TCTTGGTGCAGTAGGTGCAC		

F-Forward, R- Reverse, GI-Germination index; SL-Shoot length; SDW-Shoot dry weight, SW- Seedling weight; GP-germination percentage; GR: germination rate; RL: root length; SL-Shoot length; RA: root activity; RDW-Root dry weight; SFW-Seedling fresh weight; DW-Dry weight; LA- Leaf Area; FW-Fresh weight; SR: seed reserve utilization efficiency; SEV: seedling early vigour; TDW: Total dry weight; WS-Weight of mobilized seed reserve; SR-Seed reserve utilization efficiency; FV-Field vigour; FGC-First germination count; EGC-Ending germination count; VI-Vigour index; TFW-Total fresh weight; SS-Seed size; FW-Fresh weight; NL-Number of leaves respectively

Allele scoring and SSR markers analysis

For microsatellite markers, the size of the most prominently amplified band can be determined by comparing its electrophoretic mobility to a molecular weight marker of 50 base pairs. This technique allows for accurate determination of the size of the amplified DNA fragments. Each genotype was assessed for the presence of the SSR band, which was assigned a score of '1', or the absence of the band, which was assigned a score of '0'. To determine the level of polymorphism in the samples, the polymorphism information content (PIC) was calculated using the method outlined by ANDERSON *et al*. in 1993:

$PICi = 1- \sum^{k} P^2$

Where, $P_{ij}$is the frequency of the j^{th} allele for i^{th}marker and summation extends over k alleles.

The Unweighted Pair Group Method with Arithmetic Averages (UPGMA) was used to cluster the rice cultivars based on the obtained similarity coefficients. The NTSYS-pc version 2.02 software was utilized for the analysis and 'Dendrogram' construction.

RESULTS AND DISCUSSIONS

DNA amplification

The amplification profile revealed that all the 41 SSR markers were polymorphic in nature, with a range of polymorphism percentage from 50% to 100%. RM 204, RM 20, and RM 125 showed a polymorphism percentage of 80%, 80%, and 50%, respectively, while the

remaining 38 SSR markers exhibited 100% polymorphism across all 44 rice genotypes (Table 3). The QTL, qDW-5 located in chromosome number 5 was analysed with RM315 SSR marker that showed polymorphism in PRH-10 and its parental lines (KUMARI *et al*., 2020). These findings suggest a high level of genetic diversity among the rice genotypes studied. SSR markers have been widely used for genetic diversity analysis in various crops, including rice (SINGH *et al*., 2017). The high polymorphism percentage observed in the present study is consistent with previous studies on rice using SSR markers (CHOUDHURY *et al*., 2013). Polymorphism in SSR markers arises from their ability to detect variations in the number and sequence of simple sequence repeats, which differ greatly among individuals. SSR is the most popular DNA marker used in genetic diversity analysis due to its high level of polymorphism, reproducibility, genome wide distribution and co-dominant nature (MC COUCH *et al*., 2008; ABDELLATIF and KHIDR, 2010; SALGOTRA *et al*., 2015; LIYANAGE *et al*., 2020)

Table 3. Details of SSR markers used indicating total alleles, allele size range, number of polymorphic alleles and polymorphism information content (PIC)

S.No.	Marker	Chromosomes no.	Expected band size	Range of band size observed	Total no. of allele	No. of polymorphic alleles	% polymorphic alleles	PIC
1	RM 6	2	163	160-180	3	3	100	0.869
2	RM 9	1	136	90-140	4	4	100	0.152
3	RM 13	5	141	140-600	3	3	100	0.770
4	RM 16	3	181	170-200	4	4	100	0.886
5	RM 19	12	226	220-250	4	4	100	0.920
6	RM 20	12	140	130-160	5	4	80	0.722
7	RM 26	5	112	115-130	3	3	100	0.826
8	RM 85	3	107	80-100	4	4	100	0.761
9	RM 87	5	151	140-150	2	2	100	0.603
10	RM 125	7	127	130-140	2	1	50	0.483
11	RM 148	3	129	130-150	3	3	100	0.843
12	RM 168	3	116	90-120	3	3	100	0.853
13	RM 204	6	169	50-160	5	4	80	0.801
14	RM 213	2	139	130-180	4	4	100	0.127
15	RM 214	7	112	90-100	2	2	100	0.659
16	RM 218	3	148	130-160	4	4	100	0.911
17	RM 221	2	192	190-200	2	2	100	0.521
18	RM 223	8	165	100-200	5	5	100	0.510
19	RM 224	11	157	150-170	4	4	100	0.905
20	RM 228	10	154	90-130	3	3	100	0.913
21	RM 229	11	116	120-140	3	3	100	0.253
22	RM 230	8	257	240-260	3	3	100	0.800
23	RM 234	7	156	140-200	4	4	100	0.728
24	RM 240	2	132	50-120	4	4	100	0.753

25	RM 250	2	153	150-170	3	3	100	0.773
26	RM 263	2	199	190-230	5	5	100	0.750
27	RM 264	8	178	150-190	5	5	100	0.936
28	RM 270	12	108	110-120	2	2	100	0.464
29	RM 306	5	155	180-190	4	4	100	0.803
30	RM 315	1	133	138-140	2	2	100	0.416
31	RM 334	5	182	180-230	5	5	100	0.680
32	RM 336	7	154	150-200	4	4	100	0.911
33	RM 341	2	172	140-180	5	5	100	0.908
34	RM 427	7	185	180-200	2	2	100	0.603
35	RM 480	5	225	180-210	3	3	100	0.775
36	RM 528	6	232	200-210	2	2	100	0.742
37	RM 1339	1	144	145-170	4	4	100	0.210
38	RM 1353	7	193	190-200	2	2	100	0.123
39	RM 3428	11	156	140-160	3	3	100	0.836
40	RM 5389	1	132	110-140	4	4	100	0.880
41	RM 5609	12	158	140-160	3	3	100	0.856

Number of alleles and allele size

The level of polymorphism was evaluated by calculating allele number and polymorphic information content (PIC) value for each of the 41 SSR marker loci evaluated (Table 3). For marker RM 204, RM 20, and RM 125, one allele was found to be monomorphic while the other allele was polymorphic. Among the 41 polymorphic markers, nine amplified two alleles each, twelve amplified three alleles each, thirteen amplified four alleles, and seven amplified five alleles each. (SHAH *et al*. 2012) evaluated the genetic diversity of 22 rice genotypes, including restorer, maintainer, and male sterile lines, using SSR markers. Out of the 30 SSR markers tested, 25 produced polymorphic patterns, resulting in the amplification of 231 alleles in total. The results showed high levels of polymorphism with all but two markers exhibiting more than one allele in the genotypes tested. Similarly, the present result was higher than the results reported by (PACHAURI *et al*., 2013) with the mean number of alleles per locus detected as 2.79 in molecular and morphological characterization of Indian farmers rice varieties. The average number of alleles per locus was 3.43. A similar number of alleles (2–5) for SSR markers were reported in 141 Basmati rice accessions of North Western Himalaya. (SALGOTRA *et al*., 2015). It was also found that the higher the PIC value of a locus, the higher the number of alleles detected (WONG *et al*., 2009). The alleles per locus ranged from 2 to 5, with amplified product sizes varying from 50 bp (RM 240) to 600 bp (RM 13).

Polymorphic information content (PIC) value of 41 SSR markers

PIC value is a reflection of allele diversity and the frequency of genotypes and each marker can be evaluated on the basis of its allele and it varied greatly for the SSR loci tested. The level of polymorphism was evaluated by calculating the PIC value of each of the SSR loci. The PIC ranged from a minimum of 0.152 (RM 9) to a maximum of 0.936 (RM 264) (Table 3).

The average PIC value considering each band generated by the 41 SSR primers was 0.617. Among the set of SSR markers tested, 16 SSR markers were found to be more informative in comparison to other SSR markers as they would reveal more polymorphic information content (PIC) (Fig.1). PIC values of the SSR markers namely, RM229, RM 1353, RM 223, RM 1339, RM 315, RM 270, RM 213, RM 9, RM 204, and RM 125 were low and therefore, these were excluded or less preferred from the analysis of genetic diversity. Additionally, three markers (RM 223, RM 9, and RM 204) were considered non-polymorphic or monomorphic in nature. The high PIC values observed in this study indicate that the SSR markers used are highly informative and suitable for assessing genetic diversity in rice (WONG *et al*., 2009). This confirms that SSR markers used in the study were highly informative because PIC values higher than 0.50 indicate high polymorphism (BRONDANI *et al*., 2008, RAJENDRA KUMAR *et al*., 2009 and MATIN *et al*., 2012). The findings of this study are consistent with previous research that also showed that SSR markers are highly polymorphic and useful for genetic diversity analysis in rice (ZHANG *et al*., 2016). Excluding markers with low PIC values from genetic diversity analyses is a standard practice in population genetic studies due to their limited contribution to genetic variability within the population (POWELL *et al*., 1996). Polymorphic Information Content values indicate that the SSR markers used are highly informative and well-suited for thoroughly assessing genetic diversity within the studied rice population.

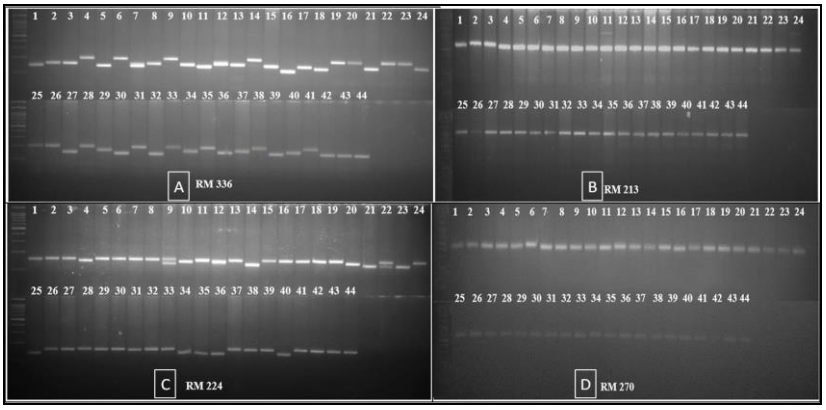

Figure 1. Banding patterns of 44 genotypes by polymorphic SSR markers; A: RM 336, B: RM 213, C: RM 224, D: RM 270

Cluster Analysis

A dendrogram was generated by UPGMA using genetic similarity values among the varieties to show the genetic relationship of the rice varieties studied. The cluster analysis showed a significant genetic variation among the rice cultivar with a similarity coefficient ranging from 0.58 to 0.94. Cluster diagram grouped 44 rice varieties into 5 major clusters I, II, III, IV, and V (Fig. 2).

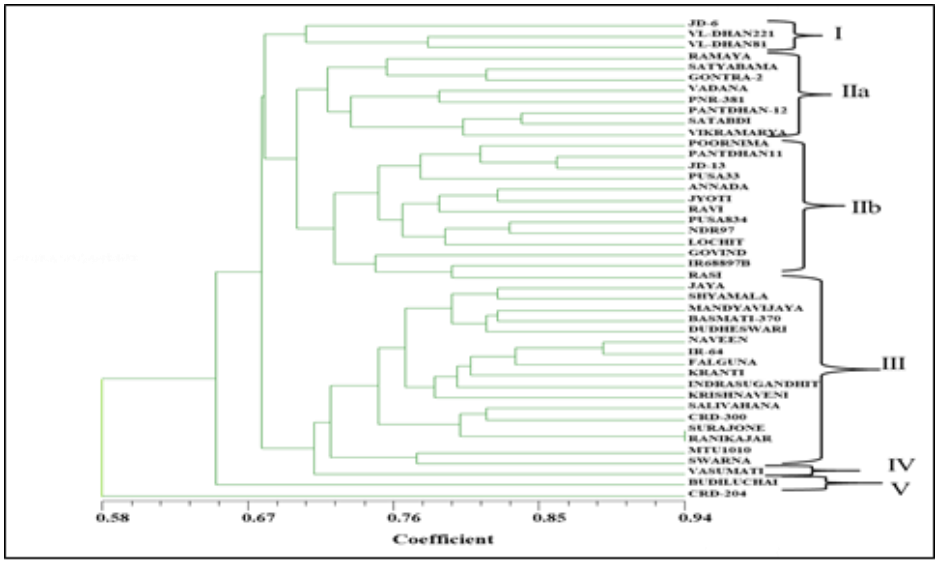

Figure 2. Dendrogram of 44 Indian rice varieties of different maturity groups derived from UPGMA cluster analysis using Jaccard coefficient based on 41 SSR markers

Cluster I comprises three rice varieties (JD-6, VL-DHAN 221, and VL-DHAN 81) with early maturity periods, sharing a similarity coefficient of 0.70. Cluster II consists of 21 rice varieties encompassing both early and mid-maturity varieties. At a similarity coefficient of 0.72, Group II was subdivided into two sub-clusters, IIA and IIB. Sub-cluster IIA, with a similarity coefficient of 0.74, includes eight rice varieties (Ramaya, Vadana, Satyabama, Gontra-2, PNR-381, PantDhan-12, Satabdi, and Vikramarya), primarily comprising medium-maturity varieties except for Ramaya. Sub-cluster IIB, at a similarity coefficient of 0.76, comprises thirteen rice varieties (Poornima, Pant Dhan11, JD-13, Govind, IR 68897B, Rasi, Annada, Jyoti, Pusa834, Pusa33, NDR97, Ravi, and Lochit), predominantly early-maturity varieties except for NDR97, Ravi, and Lochit, which are medium-maturity varieties.

Cluster III comprises 18 rice varieties, with 8 (Jaya, Shyamala, Mandyavijaya, Dudheswari, Naveen, Vasumati, IR-64, and Basmati-370) belonging to the late maturity group and 10 to the very late maturity group (Indira sugandhit, Falguna, MTU 1010, Kranti, Swarna, Krishnaveni, Salivahana, CRD 300, Surajone, and Ranikajar), at a similarity coefficient of 0.72. Cluster IV consists of a single variety, Buduluchai, categorized as a mid-maturity group variety, while Cluster V also contains a single variety, CRD 204, classified under the very late maturity group. The study results indicate significant genetic variation among the 44 rice varieties. The findings were consistent with those of (UPADHYAY *et al*., 2011), who reported the clustering of 29 rice genotypes into major groups during their study on the development of molecular markers in rice. Similarly, (RAJENDRAN *et al*., 2013) observed distinct clustering of maintainer and restorer lines into two separate groups while analyzing genetic diversity and DNA fingerprinting among hybrid rice parental lines (*Oryza sativa* L.). In a related study, (SONKAR *et al*., 2016) examined

the molecular diversity of 36 rice cultivars using four SSR primers and reported comparable results, with the germplasms being classified into four distinct clusters. Clustering based on genetic similarity values offers valuable insights for crop breeding and the selection of parental lines in hybridization programs, as well as for improving seed vigour in subsequent generations. Moreover, the observed genetic diversity among the studied rice varieties may be exploited in crop improvement initiatives aimed at developing high-yielding, stress-tolerant, and disease-resistant rice varieties (ELLUR *et al*., 2016). The identification of the early, mid, late, and very late maturity groups within the clusters is important for rice breeders, as it provides information on the appropriate growing conditions and management practices for each variety. Early maturity varieties are suitable for areas with short growing seasons, while late maturity varieties are suitable for areas with long growing seasons (FAZILY *et al*., 2021).

CONCLUSIONS

Genetic diversity among 44 rice genotypes using 41 SSR markers associated with seed germination and seedling vigor traits. The study revealed a high degree of polymorphism across the SSR markers, with 38 out of 41 markers displaying 100% polymorphism. The average number of alleles per locus was 3.43, and the polymorphic information content (PIC) values ranged from 0.152 to 0.936, with an average of 0.617. Thus, the selected SSR markers were highly informative and effective for evaluating the genetic diversity within the rice germplasm.

The UPGMA cluster analysis grouped the genotypes into five major clusters, reflecting significant genetic divergence among the varieties. The clusters also revealed clear differentiation based on maturity duration, with distinct groupings for early, medium, late, and very late maturing varieties. By identifying clusters of varieties with dissimilar genetic profiles and seed vigour traits, breeders can strategically select parents for hybridization and do further selection to enhance seed vigour in subsequent generations. Moreover, this information is also essential to the plant breeders aiming to develop high-yielding, stress-tolerant, and disease-resistant rice varieties.

Thus, observed genetic variation and clustering patterns underscore the potential of utilizing molecular markers for precise and efficient identification of diverse rice genotypes. The identification of polymorphic SSR markers associated with key agronomic traits enhances the selection efficiency in marker-assisted breeding programs.

ACKNOWLEDGEMENT

The research is part of a Ph.D. thesis of the first author submitted to the Post-Graduate School, ICAR-IARI, New Delhi, India. The first author gratefully acknowledges the University Grants Commission (UGC), GOI for providing Senior Research Fellowship during the study period.

Received, May 05th, 2024

Accepted February 16th, 2025

REFERENCES

ABDELLATIF, K. F., Y. A KHIDR (2010): Genetic diversity of new maize hybrids based on SSR markers as compared with other molecular and biochemical markers. J. Crop Sci. Biotechnol., *13*(3): 139-145.

ANDERSON, A., G.A. CHURCHILL, J. E. AUTRIQUE, S. D. TANKSLEY, M. E SORRELLS (1993): Optimizing parental selection for genetic linkage maps. Genome, *36*(1): 181-186.

BARIK, S. R., PANDIT, E., MOHANTY, S. P., D. K. NAYAK, S. K. PRADHAN, T MOHAPATRA (2016): Parental polymorphism survey and phenotyping of recombinant inbred lines for reproductive stage drought tolerance parameters in rice. Oryza, *53*(4), 374-384.

BRONDANI, C., K. DA SILVA CALDEIRA, T.C.O. BORBA, P.N. RANGEL, O.P. DE MORAIS, J. CAI, G. ZHANG (2014): The novel methods of development of the maintainer and cytoplasmic male sterile lines with different cytoplasm based on chromosome single-segment substitution lines in rice. Turk. J. Agric. For., *38*(4): 441-446.

CHOUDHURY, B., M. L. KHAN, S.DAYANANDAN (2013): Genetic structure and diversity of indigenous rice (*Oryza sativa*) varieties in the Eastern Himalayan region of Northeast India. Springer plus, *2*(01): 1-10.

DONDE, R., J. KUMAR, G. GOUDA, M.K. GUPTA, M. MUKHERJEE, S.Y. BAKSH, P. MAHADANI, K.K. SAHOO, L. BEHERA, S.K DASH (2019): Assessment of Genetic Diversity of Drought Tolerant and Susceptible Rice Genotypes Using Microsatellite Markers. Rice Sci., *26*(4): 239–247.

DOYLE, J. J. (1990): A rapid total DNA preparation procedure for fresh plant tissue. Focus, *12*(01): 13-15.

ELLUR, R. K., A. KHANNA, A. YADAV, S. PATHANIA, H. RAJASHEKARA, V. K. SINGH, S. G. KRISHNAN, P. K. BHOWMICK, M. NAGARAJAN, K. K. VINOD, G. PRAKASH (2016): Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci., *242* (1):330-341.

FAZILY, T. (2021): Effect of sowing dates and seed rates on growth and yield of different wheat varieties: a review. Int. J. Agric. Sci. Technol, *8*(03): 10-26.

KHODADADI, M., M.H. FOTOKIAN, M. MIRANSARI (2011): Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. Aust. J. Crop Sci. *5* (1): 17–24.

LIYANAGE, N.M.N., RANAWAKE, A.L., P.C.G BANDARANAYAKE (2020): Cross–pollination effects on morpho logical molecular and biochemical diversity of a selected cinnamon (*Cinnamomum zeylanicum* Blume) seedling population. J. Crop Improv., *35*(1): 21–37.

MATIN, S., M. ASHRAFUZZAMAN, M.MD. ISLAM, S.U. SIKDAR, N. ZOBAYE (2012): Molecular marker based (SSR) genetic diversity analysis in deepwater rice germplasms of Bangladesh. Int. J Biosci., *2*(10): 64-72

MCCOUCH, S.R., S. TEMNYKH, A. LUKASHOVA, J, COBURN, G. DECLERCK, S. CARTINHOUR, S. HARRINGTON, M. THOMSON, E. SEPTININGSIH, M. SEMON, P. MONCADA, J.LI (2008): Microsatellite markers in rice: abundance, diversity, and applications. Rice Genetics *4*(01): 117–135.

MOHAPATRA, S., A. K. PANDA, A. K. BASTIA, A. K. MUKHERJEE, P. SANGHAMITRA, J. MEHER, S. K. PRADHAN (2021): Development of submergence-tolerant, bacterial blight-resistant, and high-yielding near isogenic lines of popular variety, 'Swarna'through marker-assisted breeding approach. Front. Plant Sci, *12*(03): 672618.

NAHAR, S., L. LAHKAR, M.A. ISLAM, D. SAIKIA, Z.M. SHANDILYA, L.R. VEMIREDDY, L. SAHOO, TANTI (2020): Genetic diversity based on osmotic stress tolerance-related morpho-physiological traits and molec ular markers in traditional rice cultivars. Biologia, *75*(3): 669–679.

OGWU, M.C., M.E. OSAWARU, C.M AHANA (2014): Challenges in conserving and utilizing plant genetic resources (PGR). Int. J. Genet. Mol. Biol, *6*(1): 16–23.

PACHAURI, V., N. TANEJA, P. VIKRAM, N.N. SINGH, S. SINGH (2013): Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). Aust. J. Crop Sci., *7*(7): 923-932.

PRADHAN, S. K., E. PANDIT, S. PAWAR, B. BHARATI, K. CHATOPADHYAY, S. SINGH, P. DASH, J. N. REDDY (2019a): Association mapping reveals multiple QTLs for grain protein content in rice useful for bio fortification. Mol. Genet. Genomics, *294*(02): 963-983.

RAJENDRA, KUMAR P., A.K. BISWAL, K. SAKTHIVEL, M.S. MADHAV, C. NEERAJA, S.M. BALACHANDRAN, R.M. SUNDARAM (2009): Development and validation of class I SSR markers targeting (GATA) n repeat motifs in rice. Euphytica, *169*(2): 263-271.

RAJENDRAN, N., L. MUKHERJEE, K.K. REDDY, H.E. SHASHIDHAR (2013): DNA fingerprinting and estimation of genetic diversity among hybrid rice parental lines (*Oryza sativa* L.) using simple sequence repeats (SSR) markers. Int. J. Adv. Res., *1*(1): 001-006.

RAM, S. G., V. THIRUVENGADAM, K.K. VINOD (2007): Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. J. Appl. Genet., *48*(01): 337-345.

SALGOTRA, R. K., B. B. GUPTA, J. A. BHAT, S. SHARMA (2015): Genetic diversity and population structure of Basmati rice (*Oryza sativa* L.) germplasm collected from North Western Himalayas using trait linked SSR markers. Plos one, *10*(7): e0131858..

SHAH, G., N. SASIDHARAN, S. CHAKRABORTY, R., R. RAVIKIRAN, D. DAVLA (2012): Genetic diversity and molecular analysis for fertility restorer genes in Rice (*Oryza sativa* L.) for wild abortive (WA) cytoplasm using microsatellite markers. J. Agric. Technol. *8*(1):261-271.

SHRUTI K., S. K. CHAKRABARTY, P. K. BHOWMICK, V. J. SINGH, A. H. PRASAD (2020): Validation of hybrid rice seed vigour traits using SSR marker (*Oryza sativa* L.). Indian J. Genet. Pl. Br., *80*(02): 204-208.

SINGH, SHALINI, B. SINGH, V.R. SHARMA, M. KUMAR, U SIROHI (2022): Assessment of Genetic Diversity and Population Structure in Pea (*Pisum sativum* L.) Germplasm based on Morphological Traits and SSR Markers. Legume Research, *45*(6): 683-NAYAK688.

SINGH, U. M., S. YADAV, S. DIXIT, P. J. RAMAYYA, M. N. DEVI, K. A. RAMAN, A. KUMAR (2017): QTL hotspots for early vigor and related traits under dry direct-seeded system in rice (*Oryza sativa* L.). Front. Plant Sci., *8*(2): 286.

SINGH, H. P., O. P. RAIGAR, R. K. CHAHOTA (2022): Estimation of genetic diversity and its exploitation in plant breeding. Bot. Rev., *88*(3), 413-435.

SONKAR, S., S.K. SINGH, P.R. VENNELA, D.K. SINGH (2016): Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using SSR markers. Int. J. Agric. Environ. Biotechnol., *9*(1): 45.

SRIVASTAVA, A., S. GUPTA, K. SHANKER, N. GUPTA, A.K. GUPTA, R.K. LAL (2020): Genetic diversity in Indian poppy (*Papaver somniferum* L.) germplasm using multivariate and SCoT marker analyses. Ind. Crops Prod., *144*(1): 112050.

SUVI, W. T., H. SHIMELIS, M. LAING, I. MATHEW, A. I. T. SHAYANOWAKO (2020): Assessment of the genetic diversity and population structure of rice genotypes using SSR markers. Acta Agric. Scand. Sect. B - Soil Plant Sci., *70*(1), 76-86.

TANG, R., D. CUI, J. ZHOU, W. LI, X. MA, B. HAN, X. GUO, Z. ZHAO, L. HAN (2021): Comparative analysis of genetic diversity of rice (*Oryza sativa* L.) varieties cultivated in different periods in China. Genet. Resour. Crop Evol., *68*(1): 1439–1451.

UPADHYAY, P., V.K. SINGH, C.N. NEERAJA (2011): Identification of genotype specific alleles and molecular diversity assessment of popular rice (*Oryza sativa* L.) varieties of India. Int. J. Plant Breed. and Genet., *5*(2): 130-140.

WONG, S. C., P. H YIU., S. T. W. BONG., H. H. LEE., P. N. P. NEOH., A. RAJAN (2009): Analysis of Sarawak barrio rice diversity using microsatellite markers. Am. J. Agric. Bio. Sci., 4(02):298–304.

ZHANG, X., H. ZHANG, L. LI, H. LAN, Z. REN, D. LIU, S. GAO (2016): Characterizing the population structure and genetic diversity of maize breeding germplasm in Southwest China using genome-wide SNP markers. BMC genomics, *17*(01):1-16

MOLEKULARNO PROFILISANJE I PROCENA GENETSKE RAZNOLIKOSTI PIRINČA (*Oryza sativa* L.) KORIŠĆENJEM SSR MARKERA

Shruti KUMARI[1], S. K. CHAKRABARTY[1], Praloy Kumar BHOWMICK2, Paul DEBASHIS[3]

[1]Odeljenje za nauku i tehnologiju semena, ICAR - Indijski institut za poljoprivredna istraživanja, Nju Delhi, Indija
[2]Odeljenje za genetiku, ICAR - Indijski institut za poljoprivredna istraživanja, Nju Delhi, Indija

Izvod

Procena genetskih resursa za osobine vigora semena je ključna za identifikaciju genotipova i osobina pogodnih za poboljšani razvoj sorti. Za procenu genetske raznolikosti između 44 indijske sorte pirinča korišćeni su SSR markeri. Od 41 SSR markera 38 markera je 100% polimorfno, a tri markera, RM 204, RM 20 i RM 125, su monomorfna za jedan alel, ali je drugi alel bio polimorfan. Od ukupno 141 detektovanih alela, 138 je bilo polimorfno. Broj alela po lokusu varirao je od 2 do 5 sa prosekom od 3,43 alela po lokusu. Ukupna veličina amplifikovanih proizvoda varirala je od 50 baznih parova (bp) (RM 240) do 600 bp (RM 13). Vrednost polimorfnog informacionog sadržaja (PIC) svakog markera kretala se od 0,013 do 0,967, sa prosekom od 0,430. Na osnovu UPGMA dendrograma zasnovanpog na SSR polimorfizmu, sorte pirinča su klasifikovane u pet glavnih klastera (I do V) na osnovu njihovih vrednosti genetske sličnosti i perioda zrelosti, u rasponu od ranih do veoma kasnih. Klaster I je sadržao tri sorte ranog zrenja, klaster II 21 sortu iz grupe ranog i srednjeg zrenja, a klaster III 18 sorti kasnog i veoma kasnog zrenja. Klasteri IV i V su imali po jednu sortu iz grupe srednjeg i veoma kasnog zrenja. Rezultati mogu ubrzati stvaranje visokoproduktivnih sorti sa poboljšanom energetskom snagom semena, uz povećanje prinosa, uz istovremeno poboljšanje prilagodljivosti biljnih sorti promenljivim uslovima okoline.

Primljeno 05.V.2024.
Odobreno 16. II. 2025.

How to cite this article: Kumari S., S. K. Chakrabarty, P. Kumar Bhowmick, P. Debashis (2025). *Molecular profiling and genetic diversity assessment of rice (Oryza sativa L.) using SSR markers*- Genetika, Vol 57, No.1, 63-75.