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Genetic Diversity in Rice (Oryza sativa L.) Genotypes Using SSR Markers

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Abstract: ***Genetic diversity is a critical factor in understanding how different genotypes adapt to changing environments. To investigate this phenomenon, a study was conducted on 23 rice landrace genotypes during the late Rabi season of 2021 - 2022. The genetic diversity was assessed using SSR markers, revealing intriguing insights into the diversity among these rice genotypes. The analysis of SSR markers identified that the 23 rice landrace genotypes could be grouped into three distinct clusters based on their genetic profiles, as indicated by the dendrogram. This clustering suggests that these genotypes exhibit varying degrees of genetic relatedness and divergence, highlighting the rich genetic diversity present among these landraces.***

Keywords: Rice, SSR markers, diversity and land races

1. Introduction

Rice (Oryza sativa. L) stands as one of the world's most essential staple crops, providing sustenance to over half of the global population. This cereal grain is not merely a dietary staple but also a crucial economic and cultural resource in many parts of the world sajib *et al*., (2012). The diversity within rice varieties plays a vital role in ensuring food security, as different varieties exhibit varying levels of resistance to pests, diseases, and environmental stressors, making them adaptable to a wide range of agroecological conditions pathaichindachote *etal*., (2019). Understanding the genetic diversity of rice is essential for crop improvement and breeding programs aimed at developing more productive, resilient, and sustainable rice varieties. One of the powerful tools used for assessing genetic diversity in rice and other crops is the utilization of Simple Sequence Repeat (SSR) markers. SSR markers, also known as microsatellites, are short, repetitive DNA sequences scattered throughout an organism's genome Brondani *et al*., (2006). They are highly polymorphic, meaning that they exhibit variations in the number of repeat units among individuals, making them ideal for assessing genetic diversity. SSR markers have gained popularity in the field of molecular genetics due to their co - dominant inheritance, multiallelic nature, and relative ease of use Rashmi *et al*., (2017). Diversity analysis using SSR markers involves genotyping a diverse set of rice accessions or varieties to assess the allelic variations present at specific SSR loci. This analysis helps researchers and breeders identify unique alleles, estimate genetic distances, and construct genetic maps. By studying the genetic diversity within rice populations, researchers can identify valuable genetic resources, understand the population structure, and develop breeding strategies to improve rice yield, quality, and resistance to biotic and abiotic stresses Singh *et al*., (2016).

2. Materials and methods

Plant material: 23 rice varieties collected from Karnataka, Andhra Pradesh, and Odisha. The experimental trials were conducted in a randomized block design with three replications. Each replication involved the collection of fresh and young leaf tissues from each plot for DNA extraction.

Table 1: Genotypes, Type and their Source:

S. No.	Rice Genotypes	Type	Source and contribution of genotypes
1	Siddasanna	Seed	Karnataka
2	Gandhasale	Seed	Karnataka
3	NMS2	Seed	Karnataka
4	HMT	Seed	Karnataka
5	Jeeregaesanna	Seed	Karnataka
6	Kempu mundugae	Seed	Karnataka
7	Dodda byranallu	Seed	Karnataka
8	Raja mundi	Seed	Karnataka
9	Ratana choodi	Seed	Karnataka
10	Barma black	Seed	Karnataka
11	Kalabatti	Seed	Andhra Pradesh
12	Madimurangi	Seed	Andhra Pradesh
13	Kala jeera	Seed	Andhra Pradesh
14	Navara	Seed	Andhra Pradesh
15	Akshaya ponni	Seed	Andhra Pradesh
16	Asubhajana	Seed	Odisha
17	Sundermani	Seed	Odisha
18	Balibajana	Seed	Odisha
19	Bibisal	Seed	Odisha
20	Kujei	Seed	Odisha
21	Namal kathi	Seed	Odisha
22	Asu	Seed	Odisha
23	Pooja	Seed	Odisha

Isolation of genomic DNA

Young leaves were collected from 12 - day - old rice seedlings, snap - frozen with liquid nitrogen (N2), and immediately stored at - 20°C until further processing. Genomic DNA isolation was carried out using the CTAB buffer (Tris 1M, NaCl 5M, EDTA 0.5M, CTAB 2%, β - mercaptoethanol 2%). Briefly, 200 mg of the stored leaves were combined with 1 ml of pre - warmed CTAB buffer for DNA isolation. Subsequently, the extracted DNA was dissolved in Tris EDTA (TE) buffer and stored at - 20°C.

For DNA purification and removal of any RNA contamination in the isolated genomic DNA, 10 µl of RNase enzyme was added to the tubes and incubated at 37°C for 30 minutes. The purification steps were then executed following the procedures detailed in appendix no. (1.1). Finally, the purified DNA was dissolved in 50 µl of TE buffer and stored at - 20°C.

DNA Quality check

The quality and quantity of the purified DNA were assessed through agarose electrophoresis. A 0.8% (w/v) gel was utilized, and the DNA samples were loaded into the wells and run to evaluate their quality. Upon completion of the gel run, the gel was visualized under a transilluminator, revealing distinct DNA bands. The gel was then photographed using a gel documentation system. By comparing with the molecular standard, the quality and quantity of the extracted DNA were estimated.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was conducted to selectively amplify a specific segment of the total genomic DNA. A microsatellite or simple sequence repeat (SSR) - based PCR was employed for the current study. A total of 23 rice genotypes were analysed in the PCR. The reaction was carried out with a final volume of 25 µl under the following conditions: initial denaturation at 94°C for 5 mins, followed by 35 cycles of denaturation, annealing, and extension with specific conditions set at 92°C for 1 min, 42 - 54°C (depending on the primer used) for 1 min, and 72°C for 1 min, respectively. The reaction concluded with a final extension at 72°C for 10 mins.

Marker analysis

A total of 10 SSR markers (RM211, RM153, RM303, RM273, RM319, RM463, RM589, RM588, RM461, and RM31) were used for the molecular analysis. The details of SSR primers used are listed in Table, no 2

S. No.	Marker	Forward / Reverse	Sequence	Annealing Temp.
1	RM 153	Forward Reverse	GCCTCGAGCATCATCATCAG ATGAAGCTGCAGTTGCCTGG	51
2	RM 211	Forward Reverse	CCGATCTCATCAACCAACTG CTTCACGAGGATCTCAAGG	48
3	RM273	Forward Reverse	GAAGCCGTCGTGAAGTTACC GTTCCCTACCTGATCGCGAC	50
4	RM 319	Forward Reverse	ATCAAGGTACCTAGACCACCAC TCCTGGTGCAGCTATGTCTG	50
5	RM 303	Forward Reverse	GCATGGCCAAATATTAAAGG GGTTGGAATAGAAGTTCGGT	45
6	RM463	Forward Reverse	TTCCCCTCCTTTTATGGTGC TGTTCTCCTCAGTCACTGCG	51
7	RM589	Forward Reverse	ATCATGGTCGGTGGCTTAAC CAGGTTCCAACCAGACACTG	51
8	RM588	Forward Reverse	GTTGCTCTGCCTCACTCTTG AACGAGCCAACGAAGCAG	50
9	RM 461	Forward Reverse	GAGACCGGAGAGACAACTGC TGATGCGGTTTGACTGCTAC	45
10	RM 31	Forward Reverse	GATCACGATCCACTGGAGCT AAGTCCATTACTCTCCTCCC	50

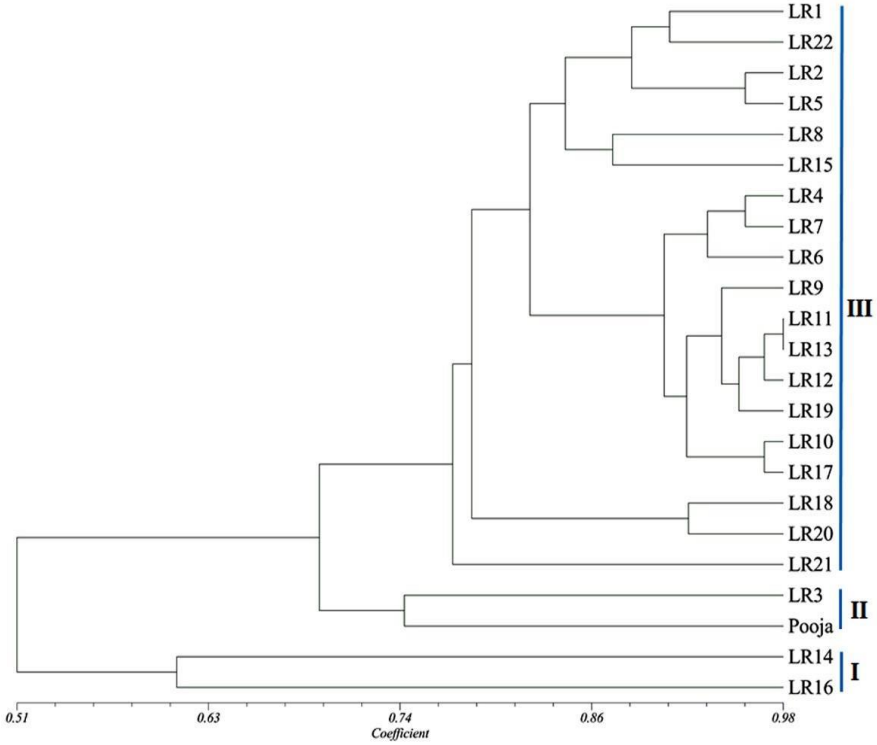
Variability parameter

Scoring of the band position for all genotype and primer combinations was done from the respective gel images. SSR profiles of those particular genotype × primer combinations, which provided constant amplification for all the genotypes and did not have any blank lane per unclear bands, were considered in the current study. The amplified fragments were scored as '1' if present and '0' if they were absence, generating a 0 and 1 matrix. This binary data matrix was then utilized to harbor genetic similarity data among all the considered 23 lines of rice genotypes by using the software NtSYSPc v.2.0. From the results, the molecular diversity among the rice genotypes were concluded from the similarity matrix data and from the obtained dendrogram.

Molecular diversity using SSR markers Molecular diversity study has been assessed by using chemically designed SSR molecular markers matin *et al*., (2012). These sequences are the complimentary sequences of DNA which lie close to a particular gene or QTL. Through primer annealing, we can amplify our target gene Shakil *et al*., (2015). In current investigation the study of diversity among twenty - three rice genotypes was done by using 10 rice SSR markers. All the selected SSR markers showed polymorphism and scoring of alleles showed linkage dendrogram by jackard coefficient analysis method. Polymorphic Information Content (PIC) was used for each locus to estimate the information of each marker and its discriminatory ability. The calculation of PIC (Wright, 1996) for the ith marker is PIC = 1 – ΣPij (j= 1, 2. . ., n) where Pij is the frequency of the jth pattern for the ith marker and the summation extends over (n) patterns. The PIC value depicts the diversity and frequency among the varieties. The PIC value of each marker can also be determined on the basis of its alleles and the value is diverse for all SSR loci. The largest PIC value was observed for locus RM 303 (0.915) followed by RM 273 (0.889), RM211 (0.843), RM153 (0.912), RM319 (0.911), RM463 (0.721), RM589 (0.682), RM588 (0.521), RM31 (0.803) and lowest PIC value was recorded by RM 461 (0.41). The PIC value for the present set of markers ranged from 0.91 to 0.41.

Dendrogram analysis A dendrogram based on Jackard's similarity coefficient was constructed using UPGMA. The genetic divergence was assessed based on D2 statistics Nirubana *et al*., (2020). In similarity matrix index highest similarity index found in LR11 (Ratanachoodi), LR12 (Asubhajana) and lowest similarity found in pooja check variety (0.26). The most diverse genotypes are therefore, important in order to select desirable genotypes for utilizing in breeding programs. Twenty - three rice genotypes were grouped into three main clusters i. e. cluster I, cluster II and cluster III. Genotypes belonging to each cluster is detailed in the table no 3.

3. Results



L1 - Siddasanna
L2 - NMS
L3 - ASU
L4 - HMT
L5 - Balibajana
L6 - Kempu munduga
L7 - Namal kathi
L8 - Navara
L9 - Kalabatti
L10 - Akshayaponni
L11 - Ratanachoodi
L12 - Asubhajana
L13 - Kujei
L14 - Rajamundi
L15 - Gandhasale
L16 - Doddabyrannallu
L17 - Sundermani
L18 - Barmablack
L19 - Kalajeera
L20 - Madimurangi
L21 - Jeeregaesanna
L22 - Bibisal
POOJA

Figure 1: JACCARD COEFFICIENT ANALYSIS METHOD

Table 3: Grouping of Twenty - three genotypes of rice into three clusters on the basis of dendrogram analysis

Sl. No.	Cluster	Number of genotypes	Genotypes
1	I	2	Rajamundi, Doddabyranallu
2	II	2	Asu, Pooja
3	III	19	Siddasanna, NMS2, HMT, Balibajana, Kempu munduga, Namal Kthi, Navara, Kalabatti, Akshaya ponni, Ratana choodi, Asubhajana, Kujei, Gandhasale, Sundermani, Barmablack, Kalajeera, Madimurangi, Jeeregaesanna, Bibisal

Table 4: Alleles and polymorphism information content (PIC) of SSR primers used in the present study.

Markers	Allele	PIC
RM153	94	0.912
RM211	23	0.843
RM273	76	0.889
RM319	94	0.911
RM303	72	0.915
RM463	60	0.721
RM589	50	0.682
RM588	40	0.521
RM461	30	0.411
RM31	70	0.803

Table 5: Similarity index

	LR1	LR2	LR3	LR4	Pooja	LR5	LR6	LR7	LR8	LR9	LR10	LR11	LR12	LR13	LR14	LR1S	LR16	R17	LR18	LR19	LR20	LR21	LR22
LR1	1																						
LR2	0.89	1																					
LR3	0.74	0.68	1																				
LR4	0.83	0.91	0.69	1																			
Pooja	0.77	0.74	0.75	0.67	1																		
LR5	0.9	0.95	0.74	0.89	0.78	1																	
LR6	0.85	0.86	0.71	0.91	0.69	0.84	1																
LR7	0.87	0.91	0.71	0.95	0.69	0.89	0.95	1															
LR8	0.85	0.86	0.69	0.79	0.71	0.86	0.79	0.84	1														
LR9	0.78	0.84	0.69	0.91	0.67	0.82	0.89	0.91	0.82	1													
LR10	0.83	0.89	0.71	0.89	0.64	0.86	0.89	0.89	0.82	0.89	1												
LR11	0.79	0.85	0.68	0.92	0.61	0.8	0.9	0.9	0.78	0.94	0.94	1											
LR12	0.84	0.85	0.68	0.92	0.63	0.83	0.92	0.92	0.83	0.94	0.92	0.95	1										
LR13	0.82	0.87	0.66	0.94	0.61	0.83	0.92	0.92	0.8	0.94	0.94	0.98	0.98	1									
LR14	0.54	0.55	0.52	0.62	0.54	0.55	0.57	0.62	0.69	0.69	0.57	0.63	0.66	0.63	1								
LR15	0.84	0.83	0.72	0.78	0.72	0.83	0.83	0.83	0.87	0.83	0.78	0.82	0.84	0.82	0.63	1							
LR16	0.33	0.39	0.45	0.44	0.26	0.41	0.41	0.41	0.48	0.46	0.48	0.47	0.47	0.47	0.61	0.4	1						
LR17	0.82	0.9	0.68	0.97	0.66	0.87	0.87	0.9	0.83	0.9	0.97	0.91	0.91	0.93	0.59	0.77	0.47	1					
LR18	0.77	0.76	0.72	0.71	0.7	0.78	0.74	0.71	0.76	0.76	0.8	0.77	0.79	0.77	0.49	0.77	0.54	0.77	1				
LR19	0.8	0.86	0.64	0.93	0.62	0.82	0.89	0.91	0.79	0.93	0.91	0.94	0.94	0.97	0.62	0.78	0.46	0.94	0.76	1			
LR20	0.83	0.82	0.71	0.75	0.71	0.84	0.79	0.77	0.79	0.79	0.86	0.8	0.83	0.8	0.51	0.8	0.48	0.83	0.92	0.79	1		
LR21	0.75	0.78	0.68	0.78	0.72	0.78	0.71	0.76	0.78	0.83	0.74	0.79	0.79	0.79	0.66	0.84	0.43	0.75	0.77	0.78	0.74	1	
LR22	0.91	0.85	0.77	0.78	0.77	0.9	0.8	0.83	0.83	0.76	0.8	0.75	0.79	0.77	0.52	0.86	0.38	0.79	0.84	0.76	0.85	0.82	1

The study of molecular diversity is an indispensable component in germplasm characterization and conservation. Results derived from analyses of genetic diversity at the DNA level can be used to plan effective breeding programs aimed at broadening the genetic bases of commercially grown varieties Srivastava *et al*., (2019). The analysis of the rice plant is conducted using chemically designed molecular markers, which are complementary DNA sequences occurring close to specific genes or Quantitative Trait Loci (QTL). By annealing the primer, we can amplify our target gene. In the present investigation, diversity among twenty - three rice cultivars was assessed using ten polymorphic rice SSR markers. All SSR markers showed polymorphisms in scoring alleles. The UPGMA - based dendrogram was constructed from binary data deduced from the DNA profiles of the samples analysed, wherein genotypes derived from genetically similar types clustered simultaneously. The evaluation of even larger germplasm requires many primers from different chromosomes to obtain reproducible facts. These facts can be used as a set of references by concerned breeders, variety registration authorities, and seed production agencies. However, based on the observations in the current investigation, the best SSR marker identified is RM303 (0.915). Twenty - three rice genotypes were grouped into three main clusters: Cluster I, Cluster II, and Cluster III. These clusters suggest the genetic relatedness of the rice genotypes selected in this study.

Ten randomly selected SSR markers, all demonstrating polymorphism, were employed to assess the molecular diversity among 23 distinct rice genotypes. The PIC values varied across the markers, with the highest reported in the RM303 locus (0.915) and the lowest in the RM461 locus (0.41). The overall PIC values ranged from 0.91 to 0.41, indicating a significant range of genetic diversity. Polymorphism was consistently observed across all primers. The 23 rice genotypes were classified into three main clusters: Clusters I, II, and III. The RM303 marker exhibited the highest PIC value (0.912), and the majority of genotypes clustered in Cluster III. Cluster I included two genotypes, while Cluster II comprised two genotypes, including the reference variety.

4. Conclusion

The analysis of molecular diversity using SSR markers revealed substantial genetic variation among the 23 rice genotypes. The PIC values provided insights into the informativeness of each marker, with RM303 standing out as particularly informative. The clustering pattern indicated distinct genetic relationships, with genotypes in Cluster III showing higher similarity and those in Clusters I and II forming separate groups.

These findings contribute valuable information for rice breeding programs, allowing for the selection of genotypes with diverse genetic backgrounds. The high PIC values, especially in RM303, suggest the utility of these markers in characterizing and conserving rice germplasm. This study underscores the importance of employing diverse molecular markers for comprehensive germplasm characterization and informs future breeding strategies aimed at enhancing the genetic base of commercially grown rice varieties.

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