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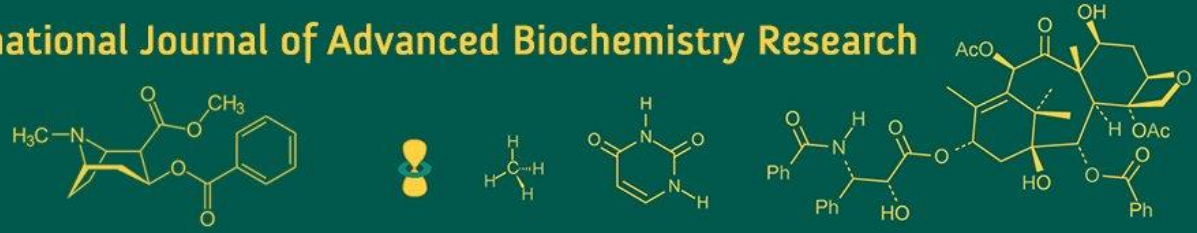

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Molecular diversity of rice (*Oryza sativa* L.) landraces using SSR markers

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Abstract
In the present study, 49 rice genotypes, including 45 landraces and 4 checks, were characterized using eight Simple Sequence Repeat (SSR) markers to assess molecular diversity. Genomic DNA extracted from 21-day-old seedlings was amplified using standard PCR protocols, generating clear and reproducible banding patterns. A total of 16 alleles were detected across the markers, indicating measurable genetic variability. Polymorphism information content (PIC) values ranged from 0.04 to 0.49, with RM11307 showing the highest discriminatory power, consistent with earlier reports highlighting the effectiveness of SSRs in rice diversity studies (McCouch *et al*., 2002) [13].
Cluster analysis using UPGMA based on Jaccard's similarity coefficients grouped the genotypes into 24 distinct clusters at a 0.1 similarity level. Cluster VI contained the largest number of genotypes, while several clusters with only one or two entries reflected unique or rare allelic patterns, a characteristic frequently observed in traditional landraces (Roy *et al*., 2015) [16]. Overall, the findings reaffirm that SSR markers are highly efficient tools for detecting genetic variation in rice and highlight the importance of landraces as valuable resources for breeding programmes (Zeng *et al*., 2019) [22].

Keywords: Rice landraces, SSR markers, molecular diversity, genetic variability, cluster analysis

Introduction
Rice (*Oryza sativa* L.) is one of the most important staple food crops worldwide, feeding more than half of the global population and serving as a foundation for global food security (Khush, 2013) [9]. Traditional rice landraces, which have evolved over centuries through natural selection and farmer-driven selection pressures, hold a rich repository of genetic variation essential for sustaining rice improvement programmes (Roy *et al*., 2015; Thomson *et al*., 2007) [16, 20]. These landraces often possess unique combinations of traits including high yield potential, superior grain and cooking quality, improved nutritional value, and resilience to biotic and abiotic stresses and are well adapted to diverse agro-ecological regions (Glaszmann, 1987; Upadhyay & Singh, 2011) [5, 21]. Because of their long evolutionary history and environmental adaptation, rice landraces are considered valuable genetic resources for developing climate-resilient and nutritionally enhanced rice varieties (Henry, 2014) [7] Historically, genetic diversity in rice has been assessed through morphological and agronomic traits. Although informative, such descriptors are strongly influenced by environmental conditions, management practices, and genotype × environment interactions, making them unreliable for precise diversity assessment (Chakravarthi & Naravaneni, 2006; Smith & Smith, 1989) [2, 18]. Morphological traits also exhibit limited variability and often require multi-season and multi-location evaluations, making phenotypic characterization time-consuming and insufficient for distinguishing closely related genotypes (Jain *et al*., 2004; Cuevas *et al*., 2016) [8, 3].
The development of molecular marker technologies has overcome many limitations of traditional characterization methods. DNA-based markers allow direct assessment of genetic variation and remain unaffected by environmental fluctuations or plant developmental stages (Reddy & Sarla, 2005; Henry, 2014) [15, 7]. Among them, Simple Sequence Repeats (SSRs) are particularly valued for their abundance, multi-allelic nature, co-dominant inheritance, reproducibility, genome-wide distribution, and high polymorphism (McCouch *et al*., 2002; Panaud *et al*., 1996) [13, 14].

SSRs have consistently proven effective in detecting subtle genetic differences among closely related landraces and are widely used in genetic diversity analysis, varietal identification, germplasm characterization, and marker-assisted breeding (Thomson *et al*., 2007; Singh *et al*., 2013) [20, 17]. Molecular diversity assessments using SSR markers enable the classification of genotypes into distinct genetic groups, identification of divergent parents for hybridization, and detection of rare alleles valuable for enhancing grain quality, nutritional attributes, and stress tolerance (Zeng *et al*., 2019; Das *et al*., 2013) [22, 4]. They also help identify genetically rich or threatened landrace populations, which is essential for effective germplasm conservation and management (Ali *et al*., 2011) [10]. Furthermore, molecular markers reveal population structure and evolutionary patterns within rice germplasm, offering insights into domestication and diversification processes (Londo *et al*., 2006) [11] Given the importance of rice landraces and the limitations of morphological descriptors, molecular characterization has become an indispensable approach for understanding genetic diversity. Hence, the present study aims to evaluate the genetic variation of 49 rice genotypes including 45 landraces and 4 checks using a standardized panel of eight SSR markers. The study also seeks to classify these genotypes through cluster analysis, providing insights that will support breeding strategies aimed at improving yield, grain quality, nutritional properties, and resilience to environmental stresses (Tanksley & McCouch, 1997; Brar & Khush, 1997) [19, 1].

Materials and Methods

Plant Material

A total of 49 rice genotypes comprising 45 landraces and 4 checks were selected for molecular characterization. These genotypes represent a diverse set of traditional rice varieties maintained across different agro-ecological regions. The study was conducted at the Indian Institute of Rice Research (IIRR), Hyderabad, where uniform nursery conditions were maintained to obtain seedlings for DNA extraction. Young, healthy leaves from 21-day-old seedlings were collected to ensure high-quality genomic DNA suitable for molecular analysis.

List of genotypes

Genotypes				
CRPAB-5	CRPAB-389	CRPAB-429	CRPAB-480	CRPAB-613
CRPAB-237	CRPAB-394	CRPAB-430	CRPAB-481	CRPAB-614
CRPAB-238	CRPAB-399	CRPAB-431	CRPAB-484	CRPAB-615
CRPAB-240	CRPAB-416	CRPAB-432	CRPAB-518	CRPAB-618
CRPAB-241	CRPAB-417	CRPAB-433	CRPAB-571	CRPAB-633
CRPAB-244	CRPAB-422	CRPAB-434	CRPAB-574	ISM
CRPAB-245	CRPAB-425	CRPAB-435	CRPAB-575	BPT5204
CRPAB-246	CRPAB-426	CRPAB-456	CRPAB-576	DRR DHAN-42
CRPAB-387	CRPAB-427	CRPAB-463	CRPAB-588	DRR DHAN-75
CRPAB-387-1	CRPAB-428	CRPAB-473	CRPAB-612	

2.2 Genomic DNA Extraction and SSR Assay

Genomic DNA was isolated using the standardized method described by Prabhu *et al*. (1998), which ensures consistent and efficient extraction from young rice tissues. Following extraction, DNA quantity and purity were assessed using a spectrophotometer, and working concentrations were adjusted to 30 ng/µL for PCR amplification. A panel of eight SSR markers RM562, RM11307, *OsASN*1, *OsLHT*1, RM3233, RM6120, RM23556, and RM6209—was selected based on their distribution across the rice genome and their reported polymorphism levels.

PCR amplification was carried out in a reaction mixture containing template DNA, primers, dNTPs, PCR buffer, $MgCl_2$, and Taq DNA polymerase. The thermal cycling profile consisted of an initial denaturation step at 94 °C, followed by 35 cycles of denaturation, 55^0C annealing temperature, and a final extension at 72 °C. Amplified PCR products were electrophoresed on 3.5% agarose gel to achieve high-resolution separation of alleles. Bands were visualized under a UV transilluminator and documented for scoring.

2.3 Data Analysis

Allelic data obtained from SSR banding patterns were converted into binary matrices based on the presence or absence of bands. PIC values were calculated using the formula proposed by Nei (1973) to determine marker informativeness. Genetic similarity among genotypes was estimated using Jaccard's coefficient, and the resulting similarity matrix was subjected to UPGMA clustering using NTSYS-pc software to construct a dendrogram illustrating genetic relationships.

3. Results

The eight SSR markers used in this study successfully amplified genomic DNA across all 49 rice genotypes and produced clear, reproducible banding patterns. A total of 16 alleles were detected, indicating measurable molecular variation within the population. The polymorphism information content (PIC) values varied considerably among markers, ranging from 0.04 to 0.49, reflecting differences in their discriminatory capacity. RM11307 exhibited the highest PIC value of 0.49, demonstrating its strong effectiveness in distinguishing the genotypes, while *OsLHT*1 also showed relatively high informativeness with a PIC value of 0.46. In contrast, RM6209 displayed the lowest PIC value of 0.04, indicating minimal polymorphism at this locus. Although none of the markers exceeded a PIC of 0.50, the marker set collectively revealed moderate diversity and allowed reliable differentiation among the landraces.

Cluster analysis using Jaccard's similarity coefficients and the UPGMA method provided further insight into the genetic structure of the genotypes. The analysis grouped the 49 genotypes into 24 distinct clusters at a 0.1 similarity level, highlighting substantial genetic divergence within the germplasm. Cluster I comprised three genotypes and was separated into two subclusters, Ia and Ib, suggesting fine-level genetic variation among its members. Cluster II contained two genotypes, which also formed two subclusters, indicating subtle but detectable differences.

Cluster VI emerged as the largest cluster with seven genotypes that shared comparatively higher similarity, suggesting a closer genetic relationship among them. In contrast, clusters III to XII consisted predominantly of smaller groups with one to five genotypes, revealing the presence of unique or less-related landraces.

The dendrogram further arranged the clusters into three major groups, illustrating broader patterns of molecular divergence and potential ancestral differentiation among the landraces. The clear differences in banding profiles, particularly in markers such as RM11307 and RM562, supported the clustering results and confirmed the reliability of SSR-based diversity assessment. Overall, the results indicate that the rice landraces possess a rich spectrum of genetic variation, making them valuable resources for breeding programmes aimed at improving grain quality, nutritional traits, and stress resilience.

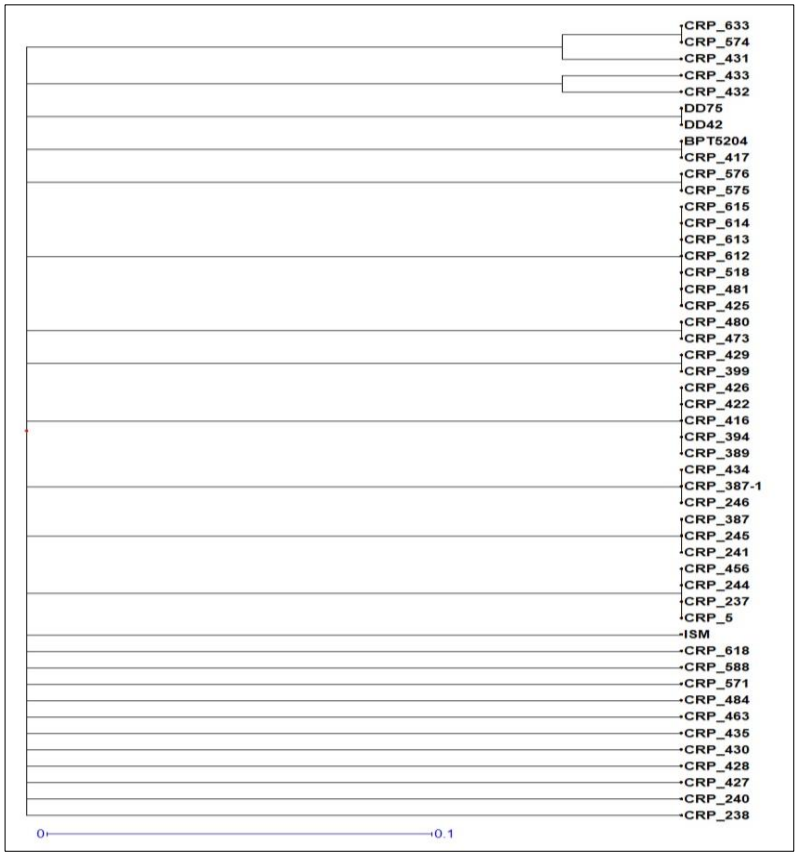
4. Discussion

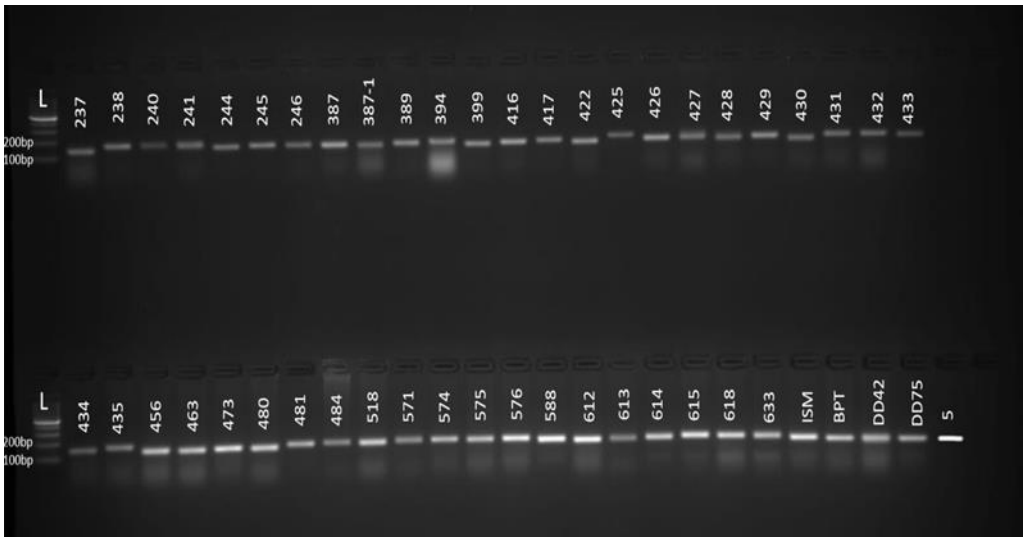
The molecular characterization of 49 rice genotypes using eight SSR markers revealed significant diversity within the evaluated germplasm, demonstrating the value of SSR markers for assessing genetic variation among rice landraces. The detection of 16 alleles across the eight markers indicates a moderate degree of polymorphism, which is expected in traditional landrace populations that have evolved under localized cultivation and natural selection pressures. The variation in PIC values, ranging from 0.04 to 0.49, further reflects the differential ability of the markers to resolve genetic differences. RM11307, with the highest PIC value of 0.49, proved to be the most informative marker, while OsLHT1 also demonstrated considerable discriminatory power. The lower PIC values observed for markers such as RM6209 highlight the presence of genomic regions that may be more conserved across these genotypes. Such variability in marker informativeness is consistent with previous studies examining SSR-based diversity in rice germplasm.

The clustering pattern generated through UPGMA analysis provides deeper insights into the genetic relationships among the genotypes. The formation of 24 distinct clusters at a 0.1 similarity threshold clearly points to substantial molecular divergence among the landraces. The distribution of genotypes across a high number of clusters suggests that the landraces represent a broad genetic pool rather than a few closely related groups. Cluster VI, containing seven genotypes, represents the only relatively large cluster, indicating some level of shared ancestry or genetic similarity among those accessions. In contrast, the presence of numerous small clusters, some containing only one or two genotypes, highlights the existence of unique or rare allelic patterns that may have developed due to geographical isolation, farmer selection, or adaptation to specific micro-environments.

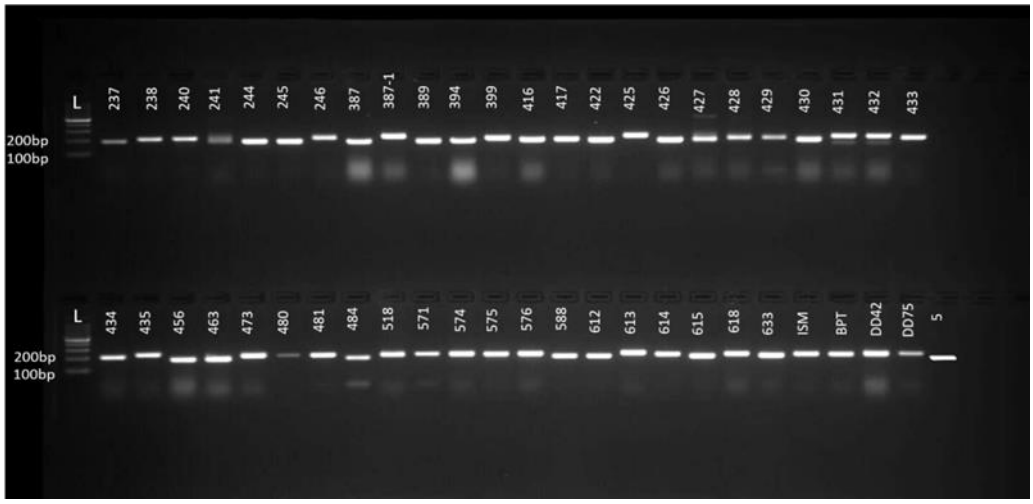
The separation of clusters into three major groups in the dendrogram further supports the presence of distinct genetic lineages within the landraces. This broad genetic structuring is advantageous for breeding programs, as genotypes from divergent clusters are more likely to generate heterotic combinations and contribute novel alleles for traits such as grain quality, nutritional properties, and stress tolerance. The clear banding differences observed in markers like RM11307 and RM562 reinforce the reliability of the molecular analysis and validate the clustering patterns obtained.

Overall, the results demonstrate the rich genetic diversity present in rice landraces and underscore their importance as valuable genetic resources for crop improvement. The identified diversity patterns can guide breeders in selecting genetically contrasting parents, thereby enhancing the efficiency of varietal development programs.





Marker RM11307



Marker RM562

5. Conclusion

The molecular characterization of 49 rice genotypes using eight SSR markers revealed substantial genetic variation, demonstrated by the detection of 16 alleles and moderate PIC values ranging from 0.04 to 0.49. The formation of 24 distinct clusters and the clear separation of genotypes into three major groups highlight the broad genetic diversity present among these landraces. Such diversity is crucial for identifying genetically distinct parents for breeding programs aimed at improving grain quality, nutritional traits, and stress resilience. The results confirm the effectiveness of SSR markers as reliable tools for assessing genetic relationships in rice, supporting their continued use in germplasm conservation and varietal development (McCouch *et al*., 2002; Roy *et al*., 2015) [16].

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