



## MICROSATELLITE MARKERS BASED GENETIC DIVERSITY ASSESSMENT OF INDICA RICE (*ORYZA SATIVA L.*) GERMPLASM OF THE TUNGABHADRA COMMAND AREA OF KARNATAKA INDIA

Raghavendra N.R.<sup>1\*</sup>, Mohammed Ibrahim<sup>2</sup>, Shailaja Hittalamani<sup>3</sup> and Lokesha R.<sup>3</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, College of Agriculture,

University of Agricultural Sciences, Raichur, India

<sup>2</sup>Department of Genetics and Plant Breeding, Agricultural Research Station, Gangavati, India

<sup>3</sup>Bengaluru, India

\*Corresponding author E-mail: [raghavnrsp@gmail.com](mailto:raghavnrsp@gmail.com)

(Date of Receiving : 22-09-2025; Date of Acceptance : 03-12-2025)

The Tungabhadra Command Area of Karnataka, comprising the districts of Raichur, Koppal, Ballari, and Vijayanagara, harbours a rich diversity of locally adapted rice germplasm accessions that represent an important genetic reservoir for traits related to biotic and abiotic stress tolerance as well as grain quality. In the present study, twenty-four rice germplasm accessions were evaluated along with four check varieties BPT-5204, GGV-05-01, IET-19251 (Gangavati Emergency), and P27PO4 to assess genetic diversity using selected 14 simple sequence repeat (SSR) markers, of which 13 were found to be polymorphic, with an average polymorphism information content (PIC) value of 0.578, ranging from 0.076 (RM408) to 0.800 (RM212). The dendrogram constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Nei's genetic distance grouped the twenty-four rice accessions into ten distinct clusters, with Cluster III, IV, V exhibiting the highest heterogeneity. The SSR marker-based molecular profiling proved to be an effective tool for identifying genetically distinct accessions suitable for use as parental lines in hybridization programs, thereby enhancing heterosis, genetic gain, and the utilization of region-specific adaptive traits suited to the Tungabhadra agro-climatic conditions.

### ABSTRACT

**Keywords:** Rice, Germplasm, PIC value, Polymorphism, SSR markers, Dendrogram

### Introduction

Rice (*Oryza sativa L.*) is the most important food crop in the world and feeds over half of the global population. India, the second largest rice producing country, grows rice in an area of about 51.4 mha and produces around 150 mt which accounts for 28 percent of global production (Statista, 2024). However, India's average rice productivity is approximately 3.4 t/ha hectare, which is significantly lower than the global average of around 5.6 t/ha (Statista, 2024).

The effectiveness of crop improvement programs largely hinges on the precise identification and strategic utilization of genetic diversity, encompassing modern and newly developed cultivars, traditional landraces, wild species, and germplasm collections

(Swarup *et al.*, 2021). However, genetic diversity in crops has been assessed using morphological and physiological traits. Such phenotypic evaluations alone may not fully reflect the underlying genetic differences among genotypes (Temnykh *et al.*, 2000). Molecular markers provide a more accurate and efficient means of assessing genetic variation (Mukta *et al.*, 2024; Siwach *et al.*, 2004). Simple Sequence Repeat (SSR) markers are markers of choice due to highly informative, co-dominant, PCR-based, cost-effective, genome wide, and easy to analyse (Akagi *et al.*, 1996; McCouch *et al.*, 1997; Temnykh *et al.*, 2000).

The SSR markers are well-suited for assessing genetic diversity and elucidating genetic relationships among plant species, populations, and individuals (Kostova *et al.*

al., 2006; Rashmi *et al.*, 2017). SSR marker-based genetic divergence has been effectively used for parental selection in various crop species (Karakousis *et al.*, 2003).

The present investigation was undertaken to assess the underlying genetic diversity at the molecular level among twenty-four rice genotypes, which can be utilized in designing effective breeding programs aimed at broadening the genetic base of commercially grown cultivars.

## Materials and Methods

### Plant materials

A total of twenty-four rice genotypes (1 landrace, 10 Germplasm accessions, 4 Purelines, 1 hybrid and 8 Breeding lines) constituted the experimental material (Table 1 and Figure 1), were collected from the Agricultural Research Station, Gangavati, University of Agricultural Sciences, Raichur, India. The rice genotypes were raised under normal field conditions.

### Genomic DNA isolation

DNA was isolated from young leaf tissue of each rice genotype using a modified cetyl tri-methyl ammonium bromide (CTAB) protocol (Dellaporta *et al.*, 1983). The DNA obtained through this method was of high purity and yielded substantial quantities. The DNA concentration and quality were assessed using an Eppendorf biophotometer. The purified DNA samples were subsequently diluted to a working concentration of 20 ng/µL with double-distilled water. For SSR analysis, marker sequences, annealing temperature and chromosomal locations are obtained from Gramene database (<http://www.gramene.org/microsat/ssr.html>). that were highly polymorphic, trait-specific, and reproducible. a total of 14 SSR markers pairs obtained from Research Genetics Inc. (USA) were used for PCR amplification (Table 2).

### SSR Analysis

PCR amplifications were performed following the protocol described by (Panaud *et al.*, 1996). Each PCR reaction was carried out in a total volume of 20 µL, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 µM each of deoxynucleotide triphosphates (dNTPs), 0.2 µM each of forward and reverse primers, 1 U of Taq DNA polymerase, and 20 ng of template DNA. Amplifications were performed using a Bio-Rad thermocycler.

The thermal cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at

55–65°C for 1 min (depending on the primer pair used), and extension at 72°C for 2 min. A final extension step was carried out at 72°C for 7 min, after which the amplified products were stored at -20°C until further use.

The reproducibility of the amplification products was confirmed by repeating each reaction twice for every primer pair. Following amplification, a 15 µL aliquot of each PCR product was mixed with 3 µL of loading buffer (0.4% bromophenol blue, 0.4% xylene cyanol, and 5 mL glycerol) and electrophoresed on 3% agarose gels in 1× TBE buffer (10 mM Tris-borate, 1 mM EDTA) containing 0.5 µg/mL ethidium bromide. A 100 bp DNA ladder was used as a molecular size marker. After electrophoresis, gels were visualized and documented using an UV transilluminator, Alpha Gel Documentation System (Alpha Innotech).

### Data Analysis

Only clear and unambiguous polymorphic bands were scored visually based on their presence or absence across all genotypes. The data were recorded in a binary matrix format, where '1' indicated the presence and '0' indicated the absence of a band for each genotype. To measure the informativeness of the markers, the polymorphism information content (PIC) for each SSR locus was calculated according to the formula (Anderson *et al.*, 1993).

$$PIC_i = 1 - \sum_{j=1}^n (P_{ij})^2,$$

where  $PIC_i$  is the polymorphism information content for the  $i^{\text{th}}$  marker,  $n$  is the total number of alleles detected for that specific locus and  $P_{ij}$  is the frequency of the  $i^{\text{th}}$  allele of the  $j^{\text{th}}$  in the set of genotypes investigated.

The PIC or expected heterozygosity for each SSR marker was calculated using PowerMarker version 3.0 (Liu & Muse, 2005). PIC is also an estimate of the discriminatory power of a SSR marker locus. Pairwise comparisons among genotypes were performed based on the proportion of shared and unique amplification products (alleles) to estimate genetic similarity using the Dice coefficient.

The Dice similarity coefficients were computed using the SIMQUAL sub-program under the similarity routine of NTSYS-pc version 2.2 (Rohlf, 1988). The estimation of genetic similarity followed the method described by (Nei & Li, 1979). The resulting similarity matrix was subjected to Sequential Agglomerative Hierarchical Nesting (SAHN) clustering using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to generate a dendrogram depicting genetic

relationships and phylogeny among the rice genotypes (Sneath *et al.*, 1973). All computations were carried out using NTSYS-pc version 2.2 software (Rohlf, 1988).

## Results and Discussion

In the present study, twenty-four indica rice genotypes were analysed using fourteen SSR markers to assess genetic diversity. SSR are widely used DNA markers for identifying genotypes and studying genetic diversity. (Joshi & Behera, 2007; Nachimuthu *et al.*, 2015; Singh *et al.*, 2004). Out of fourteen SSR markers used, thirteen markers produced polymorphic bands, while one marker found to be monomorphic. A total of 29 alleles were detected, out of which 27 were polymorphic across twenty-four genotypes. Polymorphism percentage was 96.96. The number of alleles detected per primer pair ranged from 1 to 3 with an average of 2.074. Several previous reports have indicated the number of alleles per locus, polymorphic information content (Ram *et al.*, 2007). The maximum number of amplified products was observed in the profiles of the primer RM212 and RM315 (Figure 2). The minimum number of amplified products was observed in the profiles of primer RM408 and RM11 (Figure 3). The primers RM408 and RM11 were proved to be less polymorphic and rest were polymorphic. The SSR products size ranged from 148 to 302 bp.

### Polymorphic information content (PIC)

The polymorphic information content (PIC), was employed for each locus to assess the information of each marker and its potential for discrimination. Higher the PIC value of a locus, higher the number of alleles detected. PIC values ranged from 0 (monomorphic) to 1 (very high discriminative) with many alleles in equal frequencies (Nagy *et al.*, 2012). In this study, allele counts and PIC values for each of the fourteen SSR markers were calculated in order to examine the polymorphism levels among the various genotypes. each marker pairs differed significantly in their ability to determine variability among the genotypes. The PIC values varied widely among loci and ranged from 0.076 (RM408) to 0.80 (RM212) with an average of 0.57 per marker, it indicates that greater magnitude of diversity among the plant materials (Table 3). The SSR markers had high PIC values, which show genotype-to-genotype allele diversity and frequency Ashraf *et al.*, 2016; Li *et al.*, (2023). Similar results were found with Neeraja *et al.*, 2005; Saini *et al.*, 2004 Siwach *et al.*, (2004). The number of alleles detected per primer pair ranged from 1 to 3 with an average of 2.074.

### Cluster Analysis and Principle Component Analysis

Cluster analysis was employed to evaluate the genetic diversity among the studied rice genotypes. By analysing genetic data that highlights differences between individuals (polymorphic data), calculated the similarity index. The similarity matrix was computed using SSR marker based on Jaccard's coefficient following the UPGMA method using SHAN programme of NTSYS-pc 2.02, this score, ranged from 0.30 to 0.96. A maximum genetic similarity 96% between the genotypes GNV-GP-11-4017 & GGV-05-01. The lowest similarity 30% was observed between AG-7 and BR-6926-3-1-4-5-4. The genotypes from clusters with high genetic divergence (i.e., low similarity index values) have a better scope for inclusion in a breeding programme (Rezk *et al.*, 2024; Sarif *et al.*, 2020).

A UPGMA based clustering by using all the 29 alleles (amplification products) generated by 13 polymorphic SSR markers grouped the genotypes into 10 clusters at 85% similarity level and the results are presented in Figure 4. The list of all the ten clusters along with genotypes included is presented in Table 4. Analysis of SSR marker data grouped the genotypes into 10 clusters, indicating significant genetic diversity at the molecular level. Among the different clusters, the cluster size varied from 10 (Cluster IV) to 1 (Clusters VI, XI and X). Similar results were reported by (Oladoso *et al.*, 2015; Ravi *et al.*, 2003). The SSR marker data were able to differentiate the accessions into separate clusters; this elucidated the potentiality of SSR markers for the characterization of germplasm accessions. Among the ten clusters the maximum number of genotypes were grouped in cluster III, IV, V followed by cluster I. The genotypes within this cluster possess a related genetic background. Nevertheless, hybridizing the most divergent members within the cluster is a promising strategy for developing adaptive genotypes for direct use in breeding. This method may be more practical than wide crosses between distinct clusters (I to X), which are typically foundational, pre-breeding activities requiring extensive subsequent selection.

Principle components analysis of twenty-four rice genotypes was carried out according to the similarity coefficient. Among all the rice genotypes selected for the present investigation, BPT5204, GNV-GP-11-4048, PSBRC-18, BELINELLU, PK-8573-4-1, AG-7 were more diverse from other genotypes as, it formed separate sub group (Figure 5).

### Conclusion

This study successfully employed SSR markers to quantify the high degree of genetic diversity within the rice germplasm of the Tungabhadra command area. The effectiveness of these markers in identifying genetically distant genotypes, which were then organized into distinct clusters, provides direct molecular evidence that local farming and seed management practices are successfully maintaining rice biodiversity. This molecular data offers a reliable framework for breeders to strategically select diverse parental lines. By facilitating the intercrossing of these parents, this marker-based approach accelerates the development of superior varieties and ultimately contributes to food security.

**Table 1 :** Details of the genotypes used in the study.

S. No.	Accession Name	Category
1	Beli nellu	Landrace
2	GNV-GP-11-4082	Germplasm accessions
3	GNV-GP-11-4029	Germplasm accessions

4	GNV-GP-11-4017	Germplasm accessions
5	GNV-GP-11-4048	Germplasm accessions
6	GNV-GP-11-4083	Germplasm accessions
7	GNV-GP-11-4087	Germplasm accessions
8	GNV-GP-11-4005	Germplasm accessions
9	GNV-GP-11-4050	Germplasm accessions
10	GNV-GP-11-4027	Germplasm accessions
11	GNV-GP-11-4047	Germplasm accessions
12	BPT-5204	Pure line
13	GGV-05-01	Pure line
14	IET-19251	Pure line
15	P27PO4	Hybrid
16	GNV-GP-11-4014	Pure line
17	AG-7	Breeding line
18	PSBRC-18	Breeding line
19	OM6162	Breeding line
20	A-69-1	Breeding line
21	BR-6926-3-1-4-5-4	Breeding line
22	PK-8573-4-1	Breeding line
23	CB-01-001	Breeding line
24	CB-01-508	Breeding line

**Table 2 :** Details of SSR primers employed for molecular diversity analysis, including primer sequences and their respective chromosome number

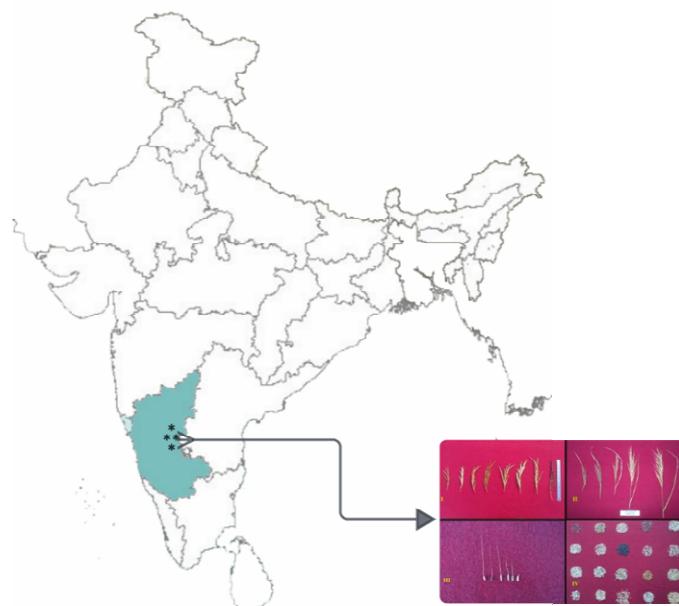
Sl. No	Locus name	Trait	Chromosome No	Sequence	Annealing temperature	Product size
1	RM 212	Grain length	1	F 5' CCACTTCAAGCTACTACCAG 3' R5'CACCCATTGCTCTCATTATG3'	55	136
2	RM 315	Panicle length	1	F 5' GAGGTACTCCTCCGTTTCAC3' R5'AGTCAGCTCACTGCAAGTG3'	55	133
3	RM 242	Days to heading	9	F 5' GAGCCAAATAAGATCGCTGA3' R5'TGCAAGCAGCAGATTAGTG3'	55	225
4	RM 1	Grain yield per plant	1	F 5' GCGAAAACACAATGCAAAAA3' R5'GCGTTGGTTGGACCTGAC3'	55	113
5	RM 11	Days to maturity	7	F 5' TCTCCTCTCCCCGATC3' R5'ATAGCGGGCGAGGCTTAG3'	55	140
6	RM 152	No. of tillers	8	F 5' GAAACCACCAACACCTCACCG3' R5'CCGTAGACCTTCTGAAGTAG3'	55	151
7	RM 408	Days to maturity	8	F 5' CAACGAGCTAACTCCGTCC3' R5'ACTGCTACTGGTAGCTGACC3'	55	128
8	RM 5 36	Panicle number	11	F 5' TCTCTCTCTTGTGCTC3' R5ACACACCAACACGACCACAC3'	55	243
9	RM 7	Spikelet number	3	F 5' TTCGCCATGAAGTCTCTCG3' R5CCTCCCATCTTCGTT3'	55	180
10	RM 224	Panicle length,	11	F 5' ATCGATCGATCTCACGAGG3' R5TGCTATAAAAGGCATTGGG3'	55	157
11	RM 263	harvesting index	2	F 5' CCCAGGCTAGCTCATGAACC3' R5GCTACGTTGAGCTACCACG3'	55	199
12	RM 190	Panicle length	6	F 5' GCTACAAATAGCCACCCACACC3' R5CAACACAAGCAGAGAAGTGAAGC3'	55	2
13	RM 13	Grain yield,	5	F 5' TCCAACATGGCAAGAGAGAG3' R5GGTGGCATTGATTCAG3'	55	141
14	RM 201	Seed set percent,	9	F 5' CTCGTTTATTACCTACAGTACCC3' R5CTACCTCCTTCTAGACCGATA3'	55	158

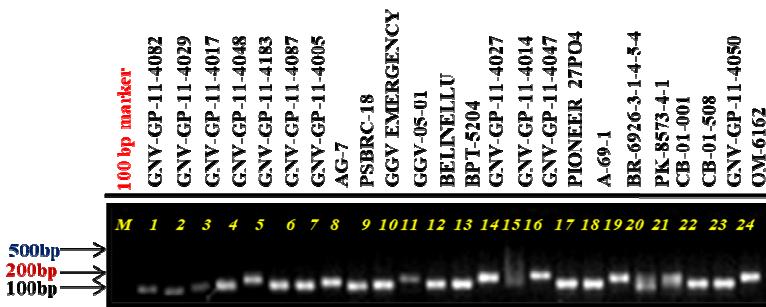
**Table 3 :** Details of markers, allelic frequency, PIC value and genetic diversity of the SSR markers across twenty-four cultivars.

Marker	Number of alleles	Number of p polymorphic alleles	Polymorphism (%)	PIC
<b>RM212</b>	2	2	100	0.8074
<b>RM315</b>	2	2	100	0.7663
<b>RM242</b>	2	2	100	0.7481
<b>RM1</b>	2	2	100	0.3536
<b>RM11</b>	3	1	80.00	0.2212
<b>RM152</b>	2	2	100	0.5609
<b>RM408</b>	2	2	100	0.0767
<b>RM536</b>	2	2	100	0.4881
<b>RM7</b>	2	2	100	0.6923
<b>RM224</b>	2	2	100	0.5968
<b>RM263</b>	2	2	100	0.6371
<b>RM190</b>	2	2	100	0.7649
<b>RM13</b>	2	2	100	0.3694
<b>RM201</b>	2	2	100	0.5331

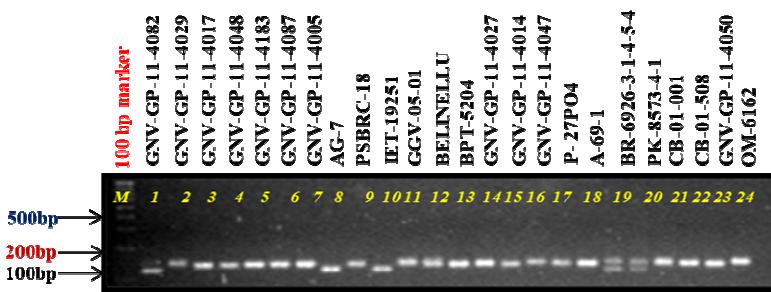
**Table 4 :** Grouping of twenty-four genotypes of rice into ten clusters on the basis of dendrogram analysis.

Cluster no	No of genotypes	List of genotypes
<b>I</b>	2	GNV-GP-11-4082, GNV-GP-11-4087
<b>II</b>	1	GNV-GP-11-4005
<b>III</b>	5	GNV-GP-11-4017, GGV-05-01, CB-01-001, GNV-GP-11-4047, BR-6926-3-1-4-5
<b>IV</b>	5	GNV-GP-11-4048, GNV-GP-11-4014, GNV-GP-11-4027, OM 6162, GNV-GP-11-4083, P-27PO4
<b>V</b>	5	BELINELLU, PK-8573-4-1 A-69-1, CB-01-508, GNV-GP-11-4050,
<b>VI</b>	2	GNV-GP-11-4029, G-EMERGENCY(IET19251)
<b>VII</b>	1	GNV-GP-11-4027
<b>VIII</b>	1	PSBRC-18
<b>IX</b>	1	BPT-5204
<b>X</b>	1	AG-7

**Fig. 1 :** Map showing the distribution of rice genotypes used in the present study across the Tungabhadra Command Area of Karnataka.

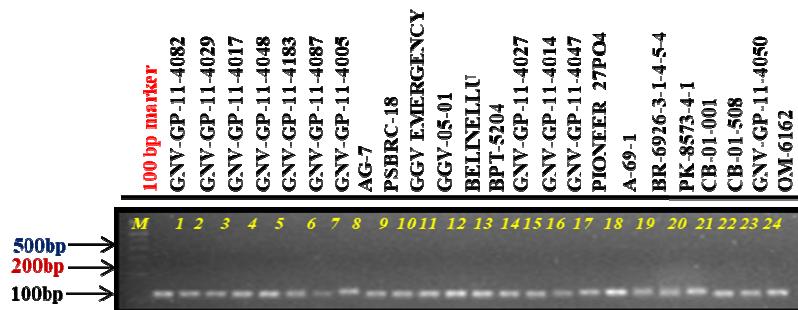


(A) RM212 on Chromosome 1 is associated with grain length



(B) RM315 on Chromosome 1 is associated with panicle length

Fig. 2 : SSR marker profile of indica rice genotypes generated by the primer RM212 (A) & RM315 (B)

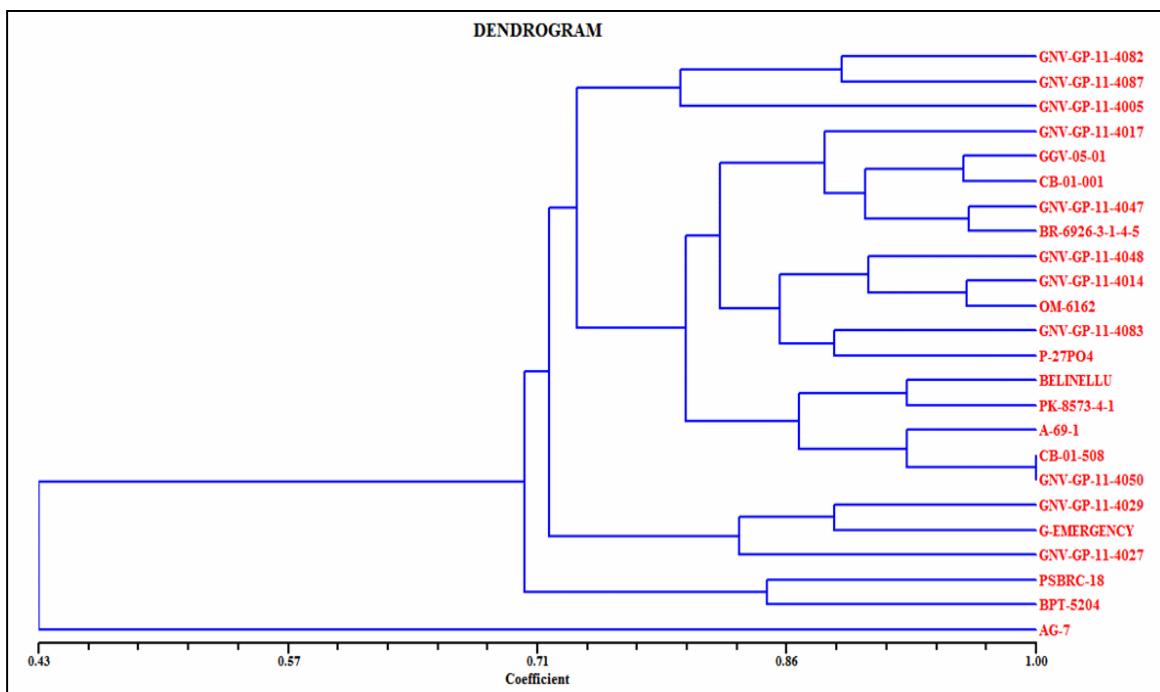


(C) RM408 on chromosome 8 is associated with the total root length

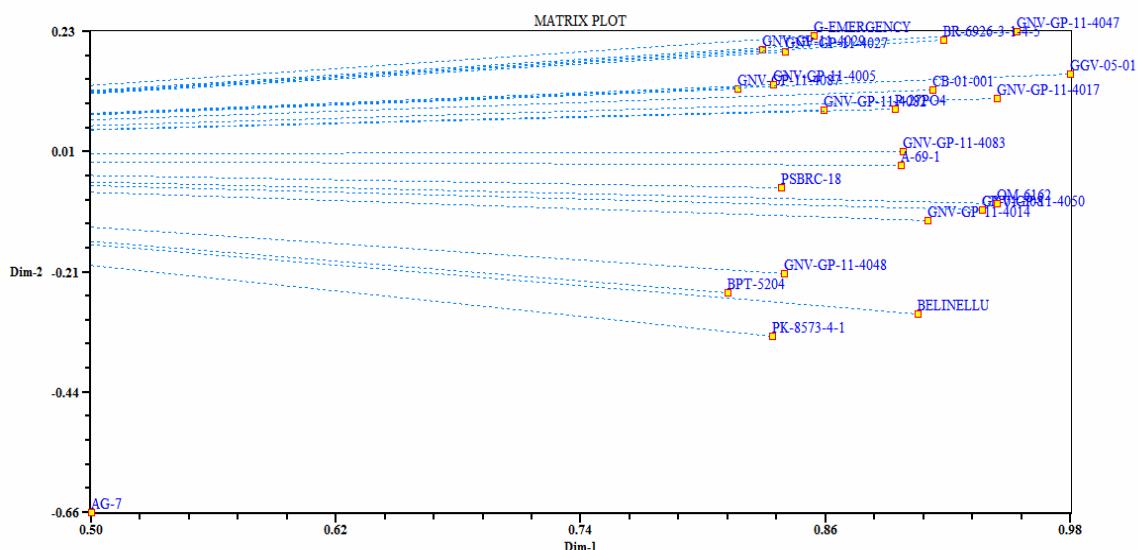


(D) RM11 on chromosome 7 is associated with the amylose content

Fig. 3 : SSR marker profile of indica rice genotypes generated by the primer RM408 (C) & RM11 (D)



**Fig. 4 :** Dendrogram resulting from UPGMA cluster analysis of 24 rice genotypes, based on data derived from 14 SSR markers. The values at the forks show the percentage contribution of the group bracketed by the fork by bootstrapping.



**Fig. 5 :** Two-dimensional scaling of rice genotypes based on Principal Component Analysis (PCA).

## Acknowledgements

We thank the Department of Genetics and Plant Breeding, Marker Assisted Laboratory, University of Agricultural Sciences, Bengaluru, India, for providing the facilities and resources for this research.

## Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

## Author Contributions

Raghavendra N R wrote the manuscript, conducted the laboratory analysis, and interpreted the data. Mohammed Ibrahim was responsible for data collection and sample preparation. Lokesha, R and Shailaja Hittalamani supervised the research, provided critical feedback, and revised the final manuscript. Authors have reviewed and approved for publication.

## References

Akagi, H., Yokozeki, Y., Inagaki, A., & Fujimura, T. (1996). Microsatellite DNA markers for rice chromosomes. *Theoretical and Applied Genetics*, **93**(7), 1071–1077. <https://doi.org/10.1007/BF00230127>

Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D., & Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage maps. *Genome*, **36**(1), 181–186. <https://doi.org/10.1139/g93-024>

Ashraf, H., Husaini, A. M., Ashraf Bhat, M., Parray, G., Khan, S., & Ganai, N. A. (2016). SSR based genetic diversity of pigmented and aromatic rice (*Oryza sativa* L.) genotypes of the western Himalayan region of India. *Physiology and Molecular Biology of Plants*, **22**(4), 547–555. <https://doi.org/10.1007/s12298-016-0377-8>

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter*, **1**(4), 19–21. <https://doi.org/10.1007/BF02712670>

Joshi, R. K., & Behera, L. (2007). Identification and differentiation of indigenous non-Basmati aromatic rice genotypes of India using microsatellite markers. *African Journal of Biotechnology*, **6**(4). <https://www.ajol.info/index.php/ajb/article/view/56210>

Karakousis, A., Barr, A. R., Chalmers, K. J., Ablett, G. A., Holton, T. A., Henry, R. J., Lim, P., & Langridge, P. (2003). Potential of SSR markers for plant breeding and variety identification in Australian barley germplasm. *Australian Journal of Agricultural Research*, **54**(12), 1197–1210. <https://doi.org/10.1071/AR02178>

Kostova, A., Todorovska, E., Christov, N., Hristov, K., & Atanassov, A. (2006). Assessment of Genetic Variability Induced by Chemical Mutagenesis in Elite Maize Germplasm via SSR Markers. *Journal of Crop Improvement*, **16**(1–2), 37–48. [https://doi.org/10.1300/J411v16n01\\_03](https://doi.org/10.1300/J411v16n01_03)

Li, X., Wang, X., Cui, Z., Shi, W., Huang, J., & Wang, J. (2023). Development of Polymorphic Microsatellite Markers and Identification of Applications for Wild Walnut (*Juglans regia* L.) in Middle Asia. *Diversity*, **15**(10), 1073. <https://doi.org/10.3390/d15101073>

Liu, K., & Muse, S. V. (2005). PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics (Oxford, England)*, **21**(9), 2128–2129. <https://doi.org/10.1093/bioinformatics/bti282>

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., Huang, N., Ishii, T., & Blair, M. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology*, **35**(1–2), 89–99.

Mukta, S., Bappy, M. N. I., Bhuiyan, J., Zohora, F. T., & Afrin, D. (2024). Assessment of genetic diversity in Bangladeshi rice (*Oryza sativa* L.) varieties utilizing SSR markers. *Gene Reports*, **37**, 102051. <https://doi.org/10.1016/j.genrep.2024.102051>

Nachimuthu, V. V., Muthurajan, R., Duraialaguraja, S., Sivakami, R., Pandian, B. A., Ponniah, G., Gunasekaran, K., Swaminathan, M., K K, S., & Sabariappan, R. (2015). Analysis of Population Structure and Genetic Diversity in Rice Germplasm Using SSR Markers: An Initiative Towards Association Mapping of Agronomic Traits in *Oryza Sativa*. *Rice*, **8**(1), 30. <https://doi.org/10.1186/s12284-015-0062-5>

Nagy, S., Poczai, P., Cernák, I., Gorji, A. M., Hegedüs, G., & Taller, J. (2012). PICcalc: An Online Program to Calculate Polymorphic Information Content for Molecular Genetic Studies. *Biochemical Genetics*, **50**(9), 670–672. <https://doi.org/10.1007/s10528-012-9509-1>

Neeraja, C. N., Hariprasad, A. S., Malathi, S., & Siddiq, E. A. (2005). Characterization of tall landraces of rice (*Oryza sativa* L.) using gene-derived simple sequence repeats. *Current Science*, **88**(1), 149–152. <https://www.jstor.org/stable/24110106>

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, **76**(10), 5269–5273. <https://doi.org/10.1073/pnas.76.10.5269>

Oladosu, Y., Rafii, M. Y., Abdullah, N., Malek, M. A., Rahim, H. A., Hussin, G., Ismail, M. R., Latif, M. A., & Kareem, I. (2015). Genetic variability and diversity of mutant rice revealed by quantitative traits and molecular markers. *Agrociencia*, **49**(3), 249. <https://openurl.ebsco.com/contentitem/gcd:102623354?sid=ebsco:plink:crawler&id=ebsco:gcd:102623354>

Panaud, O., Chen, X., & McCouch, S. R. (1996). Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Molecular and General Genetics MGG*, **252**(5), 597–607. <https://doi.org/10.1007/BF02172406>

Ram, S. G., Thiruvengadam, V., & Vinod, K. K. (2007). Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. *Journal of Applied Genetics*, **48**(4), 337–345. <https://doi.org/10.1007/BF03195230>

Rashmi, D., Bisen, P., Saha, S., Loitongbam, B., Singh, S., Pallavi, & Singh, P. K. (2017). Genetic Diversity Analysis in Rice (*Oryza sativa* L.) Accessions using SSR Markers. *International Journal of Agriculture, Environment and Biotechnology*, **10**(4), 457. <https://doi.org/10.5958/2230-732X.2017.00057.2>

Ravi, M., Geethanjali, S., Sameeyafarheen, F., & Maheswaran, M. (2003). Molecular Marker based Genetic Diversity Analysis in Rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*, **133**(2), 243–252. <https://doi.org/10.1023/A:1025513111279>

Rezk, A. A., Mohamed, H. I., & El-Beltagi, H. S. (2024). Genetic variability and diversity analysis in *Oryza sativa* L. genotypes using quantitative traits and SSR markers. *Saudi Journal of Biological Sciences*, **31**(3), 103944. <https://doi.org/10.1016/j.sjbs.2024.103944>

Rohlf, F. J. (1988). *NTSYS-pc: Numerical taxonomy and multivariate analysis system*. Exeter Publishing.

Saini, N., Jain, N., Jain, S., & Jain, R. K. (2004). Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica*, **140**(3), 133–146. <https://doi.org/10.1007/s10681-004-2510-y>

Sarif, H. M., Rafii, M. Y., Ramli, A., Oladosu, Y., Musa, H. M., Rahim, H. A., Zuki, Z. M., & Chukwu, S. C. (2020). Genetic diversity and variability among pigmented rice germplasm using molecular marker and morphological traits. *Biotechnology & Biotechnological Equipment*,

34(1), 747–762. <https://doi.org/10.1080/13102818.2020.1804451>

Singh, R. K., Sharma, R. K., Singh, A. K., Singh, V. P., Singh, N. K., Tiwari, S. P., & Mohapatra, T. (2004). Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. *Euphytica*, **135**(2), 135–143. <https://doi.org/10.1023/B:EUPH.0000014905.10397.08>

Siwach, P., Jain, S., Saini, N., Chowdhury, V. K., & Jain, R. K. (2004). Allelic Diversity Among Basmati and Non-Basmati Long-grain Indica Rice Varieties using Microsatellite Markers. *Journal of Plant Biochemistry and Biotechnology*, **13**(1), 25–32. <https://doi.org/10.1007/BF03263186>

Sneath, P. H. A., Sneath, P. H. A., Sokal, R. R., & Sokal, U. R. R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. W. H. Freeman.

Swarup, S., Cargill, E. J., Crosby, K., Flagel, L., Kniskern, J., & Glenn, K. C. (2021). Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science*, **61**(2), 839–852. <https://doi.org/10.1002/csc2.20377>

Temnykh, S., Park, W. D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y. G., Ishii, T., & McCouch, S. R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **100**(5), 697–712. <https://doi.org/10.1007/s001220051342>