



Morpho-biochemical and molecular diversity patterns of basmati rice (*Oryza sativa*) germplasm using protein and simple sequence repeat markers

NEELESH KAPOOR^{1,*}, ADITI TIWARI¹, ANKIT AGRAWAL¹, PRAFULLA KUMAR²,
MAHESH KUMAR BHARTI³, MUKESHKUMAR⁴, RAVINDRA KUMAR¹ and
RAKESH SINGH SENGAR¹

College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram,
Meerut, Uttar Pradesh 250 110, India

Received: 30 July 2024; Accepted: 04 June 2025

ABSTRACT

The present study was carried out during the rainy (*khari*) season of 2020–21 at Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh to analyse the agro-morphological characteristics of 20 different basmati rice (*Oryza sativa* L.) varieties which are indigenous to different states but are grown in the western Uttar Pradesh to check their survivability using qualitative agro-morphological, biochemical and molecular markers. For morphological characterization, four growth parameters, viz. plant height, panicle length, grains/plant, and tillers/plant were selected and from these parameters, the variety Basmati-386 was selected as tallest (160.33), maximum panicle length was evaluated for the variety CSR-30 (31.41). The variety Basmati-1612 exhibited the maximum number of grains/plant, with a count of 1055.55 and the maximum number of tillers/plant was observed for Punjab Basmati-4 (15.20). For biochemical characterization, total seed protein of the varieties was subjected to protein profiling through SDS-PAGE among which Vallabh Basmati-21 and Tarawadi Basmati were found highly polymorphic. Molecular characterization was performed using 10 SSR markers, of which seven primers produced results with eight varieties and a total of 52 reproducible band positions were amplified, of which 51 were polymorphic. The number of alleles produced by SSR primers ranged from two (RM-122 and RM-127) to four (RM-3), with RM-23 producing the maximum number of polymorphic bands.

Keywords: Basmati rice, Biochemical characterization, Morphological characterization, Polymorphism, SSR

Rice (*Oryza sativa* L.) is a member of the Poaceae family and major food crop cultivated worldwide because it overwhelms by half of the population for their everyday consumption. Additionally, due to its diploid nature, small genome size of 430 Mb, and a vast amount of well-conserved genetically diverse material approximately 100,000 accessions of rice germplasm worldwide rice serve as an important model for genetic studies (Chakravarthi and Naravaneri 2018). Among 10 rice producing countries of Asia, India ranks 2nd in global rice production. Rice can be

categorized into two types, aromatic rice and non-aromatic rice (Chakravarti *et al.* 2020). In contrast to non-aromatic rice types, which are often neutral in flavour and short-grained, aromatic rice cultivars have a sweet taste and aroma due to the chemical compound 2-acetyl-1-pyrroline (Hore 2019). The world's rice production has doubled over the past 25 years due to the application of enhanced technologies, including improved crop management techniques and high-yielding varieties and (Chakravarthi and Naravaneri 2018). Therefore, a genetic resource with significant genetic diversity is essential for accelerating crop genetic improvement, as it helps to distinguish genotypes into genetically distant and similar groups. Genetic diversity studies play a significant role in breeding and development programmes for closely related agricultural species because they ensure an efficient breeding system and appropriate use of germplasm resources (Ahn *et al.* 2020). Measuring physical features by itself could not be an effective criterion for evaluating the genetic diversity of plant germplasm. However, different biochemical studies enable the establishment of differences

¹College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh; ²UIET, Guru Nanak University, Hyderabad, Telangana; ³College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh; ⁴College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh. *Corresponding author email: drneeshbiotech@gmail.com

at various taxonomic levels, in taxonomic and genetic diversity assessment studies, one of the biochemical methods frequently used is sodium dodecyl-sulfate polyacrylamide gel electrophoresis (Shah *et al.* 2021). However, phenotypic and biochemical characterizations are not very reliable due to their high labour costs, environmental influences, and numerical and phenological limitations. Molecular characterization is a crucial step to determine whether any germplasm is part of the system's gene pool (Mishra *et al.* 2020). SSR technology is one of the best molecular marker technologies for identifying the genetic diversity of varieties (Sajib *et al.* 2021).

MATERIALS AND METHODS

Experimental material: The present study was carried out during the rainy (*kharif*) season of 2020–21 at Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh. Twenty basmati rice varieties (Table 1) were obtained from Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh and experiment was laid out in a randomized block design (RBD). For genomic DNA isolation and biochemical characterization, leaves and seed sample were obtained.

Evaluation of morphological traits: Yield and yield component data, including plant height (PLH), number of tillers/plant (NT), panicle length (PNL), and full grains/panicle (FGN), were collected from 15 plants/genotype, chosen from the first, last, and middle rows.

Biochemical characterization using protein profiling: The seeds of each rice genotype were dehusked, ground into a fine powder using a mortar and pestle, and 10 milligrams (0.01 g) of seed flour from each genotype were used for extraction. The seed flour was combined with a protein extraction buffer (0.05 M Tris-HCl, 5 M Urea, 0.2% SDS, and 1% mercaptoethanol) to facilitate protein extraction. Centrifuge was done at 15,000 rpm for 10 min at room temperature. The protein extracted was obtained as a clear supernatant, which was then preserved at -20°C. The Lowry Assay was then used to determine the protein concentration. SDS-PAGE was employed to analyze the protein profiles of the extracted samples, utilizing a 12.5% stacking gel and a 4% resolving gel, run at 50 V. The test samples were loaded as standard along with a protein marker (Himedia) of known molecular weight. Coomassie brilliant blue R250 (0.2% w/v) was then added, and shaking was continuously required. Following the gel preservation process, scanning and photographing were carried out.

Table 1 Basmati rice varieties used in the experiment, along with their pedigrees and sources

Basmati Varieties	Pedigree	Source
Basmati-370	Pusa Basmati 1121 and Pusa Basmati 1509	Indian Agricultural Research Institute, New Delhi
Basmati-386	Basmati-370 × Type-3	Indian Agricultural Research Institute, New Delhi
Basmati-1612	(Pusa 1121 × Pusa 1176) × Pusa 1509	Indian Agricultural Research Institute, New Delhi
CSR-30	Pak. Bas1' × 'BR4-10'	Central Soil Salinity Research Institute, Karnal, Haryana
Haryana Basmati-1	Pusa Basmati 1121 × IRBB60	Haryana Agricultural University, Hisar, Haryana
Malviya Basmati	HBR 92, Pusa Basmati, and Kasturi	Department of Genetics and Plant Breeding at Banaras Hindu University, Varanasi, Uttar Pradesh
Pant Basmati-2	Pusa Basmati-1 × Taraori Basmati	G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand
Pant Sugandh-3	(Basmati 370 × IR 8) × Pusa Basmati 1	G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand
Punjab Basmati-3	Basmati-370 × IR-8	Punjab Agricultural University, Ludhiana, Punjab
Punjab Basmati-4	Basmati-370 × IR-8	Punjab Agricultural University, Ludhiana, Punjab
Punjab Basmati-5	Basmati-370 × PB 1	Punjab Agricultural University, Ludhiana, Punjab
Pusa Basmati-1	Taj × PB-1	Indian Agricultural Research Institute, New Delhi
Pusa Basmati-1121	PB 1 × Taraori Basmati	Indian Agricultural Research Institute, New Delhi
Pusa Basmati-1609	Pusa Basmati 1 × PB 89	Indian Agricultural Research Institute, New Delhi
Pusa Basmati-1728	Pusa 1121 × Traditional Basmati variety	Indian Agricultural Research Institute, New Delhi
Pusa Sugandh-3	Pusa Basmati-1 × IR 50	Indian Agricultural Research Institute, New Delhi
Tarawadi Basmati	Traditional cultivars	-
Unnat Pusa Basmati	Pusa Basmati 1 (PB1) × IARI 5	Indian Agricultural Research Institute, New Delhi
Vallabh Basmati-21	PB 1 × Vikas	Indian Agricultural Research Institute in collaboration with the Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh
Vallabh Basmati-22	Basmati-370 × Pusa Basmati-1	Indian Agricultural Research Institute in collaboration with the Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh

The electropherogram of each variety was assessed for the presence or lack of bands in relation to variance in the protein banding pattern. Highly polymorphic variations were selected, and a dendrogram was constructed using NTSYS software with the

Molecular characterization: DNA from all 20 rice varieties was extracted using the CTAB method (Murray and Thompson 1980) with a few minor changes for molecular characterisation. A UV visible spectrophotometer operated at 260 and 280 nm wavelengths was used to quantify DNA, (Supplementary Table 1) and an agarose gel electrophoresis was employed to assess the quality of the samples. PCR amplification was conducted in a 15 µL reaction volume consisting of 6.25 µL of Master Mix (Genei), 0.25 µL of each forward and reverse primer, 5.25 µL of nuclease-free water, and 3 µL of 50 ng genomic DNA templates. Ten SSR primers covering the whole genome of rice were used for the diversity analysis (Table 2). The PCR procedure was conducted with the following conditions, initial denaturation at 95°C for 45 sec, followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at the marker-specific temperature for 45 sec, extension at 72°C for 45 sec, and a final extension at 72°C for 10 min. After staining with ethidium bromide, the PCR products were resolved on a 1.5% agarose gel and visualized under ultraviolet light. Using a 100 bp DNA ladder, the amplified fragment's size was identified through comparison. By comparing the migration distance of the amplified fragment with the molecular weight of the 100-base pair (bp) DNA ladder, the size of the most intensively amplified fragments is found for the molecular data analysis. After then, the major allele frequency and number of alleles per locus were numbered as "a1," "a2," etc. In a genotype, two bands of the same intensity were regarded as homozygous; in contrast, any band with varying intensities and overlaid conditions was regarded as an artifact, and diffused bands were categorized as "missing data". A 'null' allele for specific markers in a genotype was similarly defined as the absence of a band (designated as '0'), indicating the lack of binding sites for the SSR primer, after re-running the data matrix, genetic diversity and PIC values were calculated based on the presence ('1') or absence ('0') of SSR bands, and the data were exported in binary format. Percentage of Polymorphic Bands (PPB), Polymorphism Information Content (PIC) (Botstein *et al.* 1980), Effective Multiplex Ratio (EMR), Marker Index (MI) (Powell *et al.* 1996) and Resolving Power (RP) (Provost and Wilkinson 1999) of markers used were calculated. The pair-wise genetic similarity between the 30 wheat genotypes were generated using Jaccard's co-efficient. A dendrogram was generated using UPGMA clustering with binary data to visualize the genetic relationships among 20 rice varieties, using NTSYSpc version 2.02 (Rohlf 1998).

RESULTS AND DISCUSSION

Morphological characterization: Morphological characterization marks the first step in classifying and evaluating the germplasm. The analysis of variance was

estimated to examine the importance of variance among 20 different varieties of Basmati Rice. Supplementary Table 1 showed the significant variation among the all varieties which showed the scope of diversification of rice. The mean performance of different Basmati rice varieties for studied characters has been presented in the Table 2. Plant height showed significant variation among the varieties. It varied from 86.00–160.33 cm (Basmati-386) with general mean of 112.19. The range of variation for length of panicle was from 24.40–31.41 (CSR-30) with overall mean (27.95). The average number of grains/plant ranged from 302.93–1055.55, with Basmati-1612 recording the highest count. The number of tillers/plant ranged from 4.56–15.20, observed in Basmati-4. Among 20 varieties one variety showed significantly a greater number of tillers/plant. Among the four growth parameters, all parameters showed wide variations in tested varieties. Supplementary Table 2 compiles the results obtained from the analysis of 20 Basmati rice samples. On the basis of morphological characters, these 20 varieties of rice divided into 2 main cluster, Cluster 1 contains 15 varieties, viz. Malviya Basmati, Pant Sugandh-3, CSR-30, Pant Basmati-2, Vallabh Basmati-21, Pusa Basmati-1, Vallabh Basmati-22, Basmati-370, Tarawadi Basmati, Pusa Basmati-1728, Pusa Basmati-112, Haryana Basmati-1, Pusa Basmati-1609, Punjab Basmati-4 and UnnatPusa Basmati while Cluster 2 contain 5 varieties, viz. Punjab Basmati-3, Pusa Sugandh-3, Punjab Basmati-5, Basmati-1612 and Basmati-386 (Fig. 1). Plant height and number of grains varied from 86.00–160.33 and 302.93–1055.55 which is higher than the plant height and number of grains per panicle reported by the previous study of Tripathy *et al.* (2020) who reported plant height varied from 56–96 cm and number of grains/panicle from 42–115. Number of tillers reported by the study varied from 4.56 to 15.20 tillers/hill which is less in comparison to the previous study by Priyadarshini *et al.* (2022), who detected 5–22 tillers/hill across the genotypes. Maximum panicle length obtained by the study is 31.41 which is less comparable to the previous study of Fazal *et al.* (2022) who observed maximum panicle length of 40.1 cm.

Protein profiling by SDS-PAGE: Twenty different varieties of Basmati rice were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a 12.5% separating gel and 4% stacking gel (Supplementary Fig. 1). A total of 10 bands were observed during analysis. Bands 4 and 5 were assigned to the 40 kDa acidic (α) glutelin that is further fractionated into α_1 and α_2 subunits. 20 kDa basic (β) glutelin's β_1 and β_3 subunits were identified as bands 6 and 7, respectively. While, 13 kDa prolamin bands were given band numbers 8 and 9 which is likely similar to the previous study of Shah *et al.* (2021) who also reported presence of 40 kDa acidic protein, 20 kDa basic glutelin subunits and 13 kDa prolamin, while the total number of bands is less in comparison to the previous study of Khan *et al.* (2020) who observed a total 16 scorable bands using 12.5% separating gel. The Dice Similarity Coefficient for total seed protein varied between 0.30 and 1.0 across the

Table 2 Mean Performance of 20 Basmati rice varieties based on four quantitative characters

Genotypes	Plant height (cm)	Spike length	Numbers of grains	Number of tillers
Malviya Basmati	94.000	26.400	488.400	9.600
Pant Sugandh-3	108.000	27.593	821.533	6.667
CSR-30	113.000	31.417	694.350	12.000
Pant Basmati-2	115.333	26.520	470.550	4.567
Punjab Basmati-3	115.000	28.602	930.133	7.400
Vallabh Basmati-21	113.667	27.667	532.333	9.207
Pusa Basmati-1	114.333	28.000	302.933	7.200
Vallabh Basmati-22	104.667	28.480	496.333	10.067
Basmati-370	120.000	27.433	455.800	8.400
Pusa Sugandh-3	107.667	30.002	494.350	7.403
Tarawadi Basmati	142.333	25.600	380.556	7.400
Pusa Basmati-1728	91.667	29.807	495.556	8.800
Pusa Basmati-1121	108.333	26.167	589.444	7.407
Haryana Basmati-1	118.000	27.800	705.167	9.800
Punjab Basmati-5	109.467	26.800	895.167	7.000
Basmati-1612	112.333	27.607	1,055.556	8.600
Basmati-386	160.333	29.600	1,029.444	9.403
Pusa Basmati-1609	114.667	28.440	680.167	9.000
Punjab Basmati-4	86.000	24.407	725.167	15.200
Unnat Pusa Basmati	95.000	30.673	824.667	8.200
Mean	112.19	27.95	653.38	8.6605
Max	160.333	31.417	1,055.556	15.200
Min	86.000	24.407	302.933	4.567
CD	4.713	3.159	1.076	3.935
SEM	1.292	0.522	0.374	0.673
SE(d)	1.827	0.738	0.530	0.952
CV	6.986	8.212	3.099	9.153

tested varieties. Basmati-370 showed maximum co-efficient of 0.90 with Basmati-386 and Pusa basmati-1121. Vallabh Basmati-21 showed maximum similarity coefficient of 1.00 with Tarawadi Basmati (Fig. 2). The 20 rice genotypes were grouped into two main clusters; Cluster I comprised 7 subclusters and the largest number of varieties and Cluster II contains varieties VB-21 and Tarawadi Basmati which are highly polymorphic which is likely similar to the study of Khan *et al.* (2020) who also detected two clusters for the clustering of 87 breeding lines of Pakistan.

Molecular characterization: A set of 10 SSR primers were selected from literature covering whole rice genome and used for PCR amplification of simple sequence repeats. Among the 10 SSR markers, seven primers were found polymorphic and 3 primers are monomorphic (Supplementary Fig. 2 and 3), 7 polymorphic SSR loci amplified a total of 122 reproducible band locations. The average allelic richness per locus was 2.714, with variability ranging from 2–4 alleles. Among the polymorphic markers, 3 markers (RM-1, RM-122 and RM-127) produced two alleles each, 3 markers (RM-23,

RM-104 and RM-128) produced three alleles each and 1 marker (RM-1) generated four alleles. Marker RM-3 amplified the maximum number of alleles (Table 2). The average number of alleles detected (2.714) was higher than the average reported in previous diversity studies by Bordoloi *et al.* (2021), who found an average of 2.64 alleles/locus. Among the seven markers, the highest major allele frequency was 1 in one marker (RM-1), followed by 0.75 in one marker (RM-122) and 0.625 in one marker (RM-23). The highest PIC value, 0.479, was observed for RM-23, followed by 0.406 (RM-128), 0.375 (RM-104), 0.3437 (RM-127), 0.257 (RM-3), and 0.187 (RM-1), with an average PIC value of 2.40, indicating that these seven markers provided valuable information for distinguishing among the 20 Basmati varieties. The observed PIC values was less in comparison to previous studies by Sajib *et al.* (2021) who detected PIC values across all 9 loci ranged from 0.14 (RM-510) to 0.71 (RM-163), with an average PIC value of 0.48. SSR marker RM-23 exhibited a maximum resolving power (RP) of 2.5 with an average value of 1.178. From 100 bp (RM-1) to 250 bp (RM-127

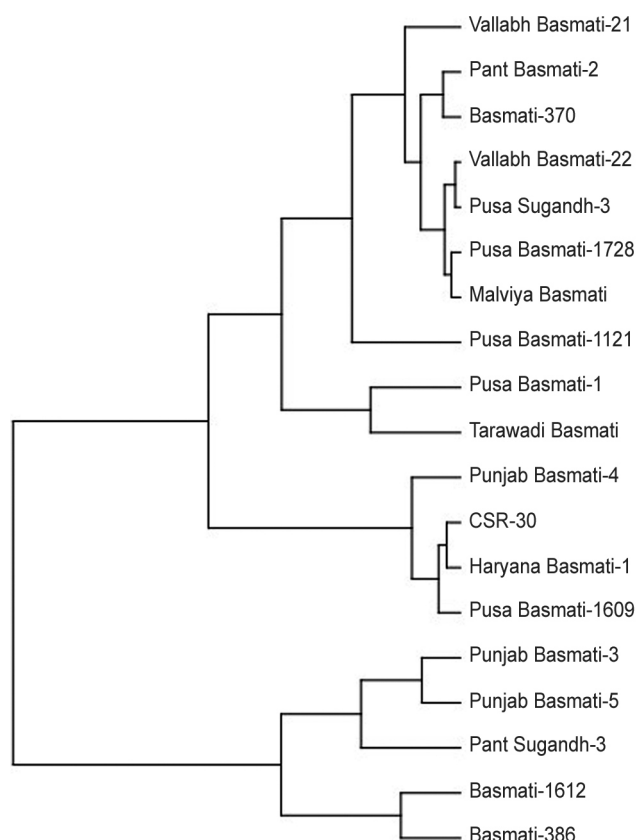


Fig. 1 Dendrogram showing clustering of 20 Basmati varieties on the basis of morphological characters.

and RM-128), the overall size of amplified products was provided (Table 3).

Cluster analysis indicated notable genetic variation among the 20 rice varieties. The similarity coefficients varied between 0.70 and 0.94, indicating diverse genetic

profiles among these genotypes. The hierarchical clustering method, implemented at a cutoff similarity coefficient of 0.7, resulted in the grouping of these genotypes into two major clusters, denoted as Cluster A and Cluster B. Cluster A was the larger cluster consisted of varieties, including Malviya Basmati, Pant Sugandh-3, CSR-30, Pant Basmati-2, Punjab Basmati-3, Pusa Basmati-1, Vallabh Basmati-22, Basmati-370, Pusa Sugandh-3, Tarawadi Basmati, Pusa Basmati-1728, Pusa Basmati-1121, Haryana Basmati-1, Punjab Basmati-5, Basmati-1612, Basmati-386, Pusa Basmati-1609, Punjab Basmati-4 and Unnat Pusa Basmati with the similarity coefficients ranged from 0.70 to 0.95. Cluster B encompassing only 2 genotypes, with similarity coefficients 0.72. This clustering pattern showed the genetic diversity within the rice varieties. The dendrogram generated from the cluster analysis visually represents the genetic relationships among these rice varieties, as shown in (Fig 3.). The UPGMA method was used to build the dendrogram using the similarity coefficient matrix. The dendrogram displayed a high degree of interconnectedness among the genotypes, indicating a considerable genetic similarity. This hierarchical clustering structure provides a comprehensive overview of the genetic relationships within the rice varieties, facilitating the identification of potential genetic resources for breeding purposes. The 20 basmati varieties were classified into 2 clusters by the dendrogram based on UPGMA that are less from previous studies of 53 rice accessions by Aljumali *et al.* (2018), who classified rice accessions in 10 clusters.

This study successfully characterized the agro-morphological, biochemical, and molecular traits of 20 Basmati rice varieties cultivated in the western region of Uttar Pradesh. Through morphological analysis, significant variability was observed among the varieties, demonstrating

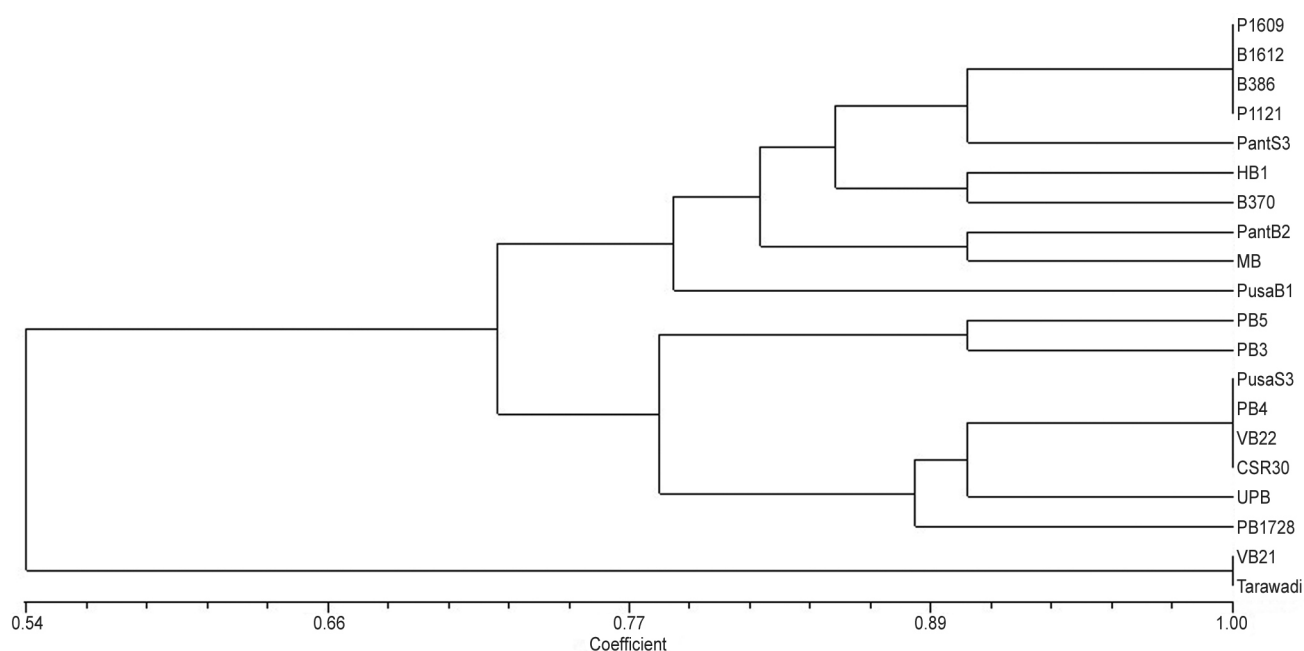


Fig. 2 A dendrogram illustrating the clustering of 20 Basmati varieties was constructed using UPGMA based on Jaccard's similarity coefficient derived from band numbers detected by SDS-PAGE.

Table 3 Molecular characterization of 20 rice varieties using 10 SSR marker

Primers	Primer sequence 5'- 3' end	Tm	Size of amplified product (bp)	T	P	M	PIC	EMR	RP	MI	PP
RM3	F- ACACTGTAGCGGCCACTG R- CCTCCACTGCTCCACATCTT	53	125-200	5	5	-	0.257	7.428	1.25	1.908	100
RM11	F- TCTCCTCTTCCCCGATC R- ATAGCGGGCGAGGCTTAG	53	100-150	10	2	8	0.187	1.485	1.75	0.277	20
RM19	F- CAAAAACAGAGCAGATGAC R- CTCAAGATGGACGCCAAGA	49	-	-	-	-	-	-	-	-	-
RM23	F- CATTGGAGTGGAGGCTGG R- GTCAGGCTTCTGCCATTCTC	53	125-200	12	12	-	0.479	7.428	2.5	3.558	100
RM24	F- GAAGTGTGATCACTGTAACC R- TACAGTGGACGGCGAAGTCG	53	-	-	-	-	-	-	-	-	-
RM104	F- GGAAGAGGAGAGAAAGATGTGTGTCG R- TCAACAGACACACCGCCACCGC	60	150-200	6	6	-	0.354	7.428	0.354	2.629	100
RM118	F- CCAATCGGAGCCACCGGAGAGC R- CACATCCTCCAGCGACGCCGAG	62	-	-	-	-	-	-	-	-	-
RM122	F- GAGTCGATGTAATGTCATCTGTGC R- GAAGGAAGGTATCGCTTTGTTGGAC	56	175-200	8	8	-	1.5	7.428	1.5	11.142	100
RM127	F- GTGGGATAGCTGCGTCGCGTCG R- AGGCCAAGGGTGTGGCATGCTG	61	125-250	4	4	-	0.343	7.428	0.25	2.547	100
RM128	F- AGCTTGGGTGATTTCTTGGAAGCG R- ACGACGAGGAGTCGCCGTGCAG	59	175-250	7	7	-	0.406	7.428	0.50	3.015	100

Tm, Annealing temperature; P, Number of polymorphic band; T, Total number of band; M, Number of monomorphic band; PIC, Polymorphism information content; EMR, Effective multiplex ratio; RP, Resolving power; MI, Marker index; PP, Polymorphism percentage.

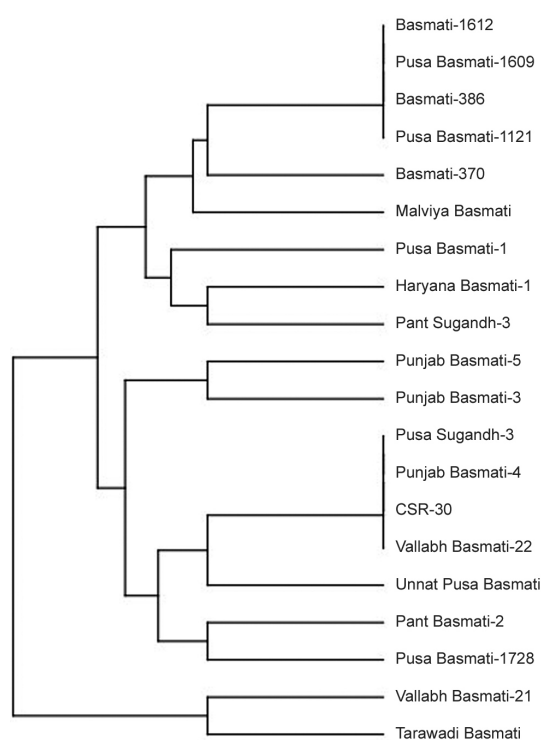


Fig. 3 A dendrogram illustrating the clustering of 20 Basmati varieties of rice was constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR marker analysis.

a broad scope for diversification. Basmati-386 stood out as the tallest variety, CSR-30 showed the longest panicle length, Basmati-1612 had the highest number of grains per plant, and Punjab Basmati-4 displayed the maximum number of tillers/plant. Biochemical characterization via SDS-PAGE revealed notable polymorphism, particularly in Vallabh Basmati-21 and Tarawadi Basmati. This protein profiling identified key bands corresponding to glutelin and prolamin subunits. Molecular characterization using SSR markers shows considerable genetic diversity among the varieties. Seven polymorphic SSR primers produced a total of 122 reproducible bands, averaging 2.714 alleles/locus. The marker RM-23 produced the most polymorphic bands and demonstrated the highest PIC value, highlighting its effectiveness in distinguishing between the varieties. Cluster analysis based on both biochemical and molecular data categorized the varieties into two main clusters, reflecting their genetic diversity and potential for breeding programs. The study's findings underline the importance of integrating morphological, biochemical, and molecular markers to comprehensively assess the genetic diversity and relationships among rice varieties. This integrated approach can facilitate targeted breeding strategies aimed at improving rice cultivars for better yield, adaptability, and quality. The identification of genetically diverse and high-yielding varieties such as Basmati-1612 and Punjab Basmati-4 provides valuable genetic resources for future

breeding efforts. Overall, this research contributes to the understanding of the genetic makeup and agronomic potential of Basmati rice, supporting efforts to enhance rice production and sustainability in the region.

REFERENCES

- Ahn S N, Bollich C N and Tanksley S D. 2020. RFLP tagging of a gene for aroma in rice. *Theoretical and Applied Genetics* **84**(7–8): 825–28.
- Aljumali S J, Rafii M Y, Latif M A, Sakimin S Z, Aroli I W and Miah G. 2018. Genetic diversity of aromatic rice germplasm revealed by SSR markers. *Biomed Research International*. <https://doi.org/10.1155/2018/7658032>
- Bordoloi D, Sarma D, Barua N S and Das B K. 2021. Morphological, biochemical and molecular characterization of indigenous aromatic rice of Assam. *Research Square*. <https://doi.org/10.21203/rs.3.rs-455040/v1>
- Botstein D, White R L, Skolnick M and Davis R W. 1980. Construction of a genetic linkage map in human using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**: 314–31.
- Chakravarthi B K and Naravaneni R. 2018. SSR marker-based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). *African Journal of Biotechnology* **5**(9): 684–88.
- Chakravarthi S K, Kumar H, Lal J P and Vishwakarma M K. 2020. Induced mutation in traditional aromatic rice frequency and spectrum of viable mutations and characterizations of economic values. *The Bioscan* **7**(4): 739–42.
- Fazal U, Khan A M, Khan F U, Iqbal N, Ibrahim M and Bangash S A K. 2022. Evaluation of the agro-morphological traits, seed characterization and the genetic diversity of local Rice (*Oryza sativa* L.) varieties of Pakistan. *Research Square*. <https://doi.org/10.21203/rs.3.rs-1736575/v1>
- Hore D K. 2019. Rice diversity collection, conservation and management in north-eastern India. *Genetic Resources and Crop Evolution* **52**(8): 1129–40.
- Khan S A, Shinwari Z K and Rabbani M A. 2020. Study of total seed protein pattern of rice (*Oryza sativa* L.) breeding lines of Pakistan through SDS-PAGE. *Pakistan Journal of Botany* **45**(3): 871–76.
- Mishra A, Kumar P, Sengar S R, Kumar P, Singh R, Chaudhary R, Kumar A and Fatima P. 2020. Assessment of diversity by using morphological, biochemical and molecular approaches of selected basmati rice (*Oryza sativa* L.). *Journal of Applied and Natural Science* **8**(1): 69–76.
- Murray M G and Thompson W. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**(19): 4321–26.
- Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**: 225–38.
- Priyadarshini P, Lal M K and Pal M. 2022. Variability in photosynthetic traits is associated with biomass accumulation and grain yield in Basmati rice germplasm. *Plant Physiology Reports* **27**: 618–24.
- Provost A and Wilkinson M J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical Applied Genetics* **98**: 107–12.
- Rohlf F J. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. *Systematic Biology* **47**(1): 147–58.
- Sajib A M, Hossain M M, Mosnaz A T M J, Hossain H, Islam M M, Ali M S and Prodhan S H. 2021. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). *Journal of Biosciences and Biotechnology* **1**(2): 107–16.
- Shah F, Huang L J, Cui H K, Nie M L, Shah T, Chen C and Wang K. 2021. Impact of high temperature stress on rice plant and its traits related to tolerance. *Journal of Agricultural Science* **149**: 545–56.
- Shah S M A, Rahman H U, Abbasi M, Rabbani M A, Khan I A, Shinwari Z K and Shah Z. 2021. Interspecific variation of total seed protein in wild rice germplasm using SDS-PAGE. *Pakistan Journal of Botany* **43**(4): 2147–52.
- Tripathy K S, Mohapatra R B, Parida K A, Pradhan K P, Senapati N, Dash B G, Lenka D, Nayak K P and Mishra R D. 2020. Revealing genetic variation and clustering pattern in upland rice using morpho-economic traits. *Electronic Journal of Plant Breeding* **6**(3): 801–12.