Genomic (Microsatellite Marker), Physiological, and Morphological (DUS) Profiling of Traditional Rice (Oryza sativa L.) Varieties

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Abstract

Rice (*Oryza sativa* L.) is a critical staple crop, providing food security for over one-third of the global population. The preservation and characterization of traditional landraces are essential for sustaining biodiversity and improving crop resilience. This study aimed to assess the genetic diversity of 23 rice landraces from Bihar using agro-morphological traits and molecular markers. A comprehensive Distinctiveness, Uniformity, and Stability (DUS) test was conducted, evaluating 33 phenotypic characteristics. The results revealed significant variability, with 6 traits exhibiting monomorphism, 1 partially monomorphic, 6 dimorphic, and 20 polymorphic, indicating high genetic diversity among genotypes. Notably, landraces such as *Sathi-1* and *Parwa Pankhi* displayed rare traits, enhancing their potential for breeding programs. Molecular characterization, performed using 15 simple sequence repeat (SSR) markers, identified 81 alleles, including 7 unique alleles, reinforcing the genetic distinctiveness of the landraces. The Polymorphism Information Content (PIC) values ranged from 0.032 to 0.87, demonstrating high marker efficiency in genotype differentiation. The integration of morphological and molecular markers provided a robust framework for rice landrace conservation, aiding in the identification of valuable genetic resources for future breeding initiatives. These findings underscore the significance of conserving traditional landraces to enhance rice genetic improvement and sustainability.

1. Introduction

Rice (Oryza sativa L.) is the world's most important food crop, serving as the primary staple for over one-third of the global population. It is classified into three subspecies: indica, japonica, and javanica [1] and is cultivated across diverse agro-ecological conditions in tropical and subtropical regions. By 2050, rice demand is expected to reach around 943.60 million tons, requiring an annual increase by 5.80 million tons from current production levels [2]. India produced a record 135. 75 million tonnes of rice in the 2022–2023 crop year, according to the Ministry of Agriculture and Farmers Welfare. This is a significant increase over the 129. 47 million tons of the prior year. India's rice output covers 47. 83 million hectares of land, according to estimates. Rice contributes 75% of caloric intake and 55% of protein content in the average daily human diet [3]. Due to its significance in food security, Asian farmers have preserved a vast array of rice landraces, which play a crucial role in local food security, sustainable agriculture, and genetic improvement [4]. These landraces and their wild relatives harbour valuable genes for yield improvement, quality enhancement, and resistance to biotic and abiotic stresses [5]. Their adaptability to specific environments makes them a critical genetic resource for future breeding programs.

To conserve and utilize traditional landraces effectively, systematic collection, characterization, and conservation are essential. Understanding genetic variability among landraces is fundamental for their classification, breeding potential, and conservation [6]. Molecular markers, such as SSR markers, have proven to be precise and reliable tools for distinguishing rice genotypes at the DNA level, unaffected by environmental factors [7]. SSR markers have been widely used for genetic diversity analysis, germplasm conservation, and trait mapping in various crops [8,9,10,11]. A precise assessment of genetic diversity is crucial for effective breeding strategies and conservation programs. Therefore, the present study aims to characterize and evaluate the genetic diversity of 23 rice landraces from Bihar using agro-morphological traits and molecular markers.

2. Material and Methods

The present study aimed to characterize the Distinctiveness, Uniformity, and Stability (DUS) of 23 rice landraces (Table 1.) from Bihar using morphological descriptors and molecular markers. The field experiment was conducted during Kharif 2018 in a Randomized Block Design (RBD) with three replications. A total of thirty-one (Table 2.) agro-morphological traits *viz.* Coleoptile colour, Basal leaf sheath colour, Leaf: Pubescence of blade surface- Culm: attitude, Flag leaf: attitude of blade(early observation) Spikelet : density of pubescence of lemma, Lemma: anthocyanin colouration of keel, Lemma: anthocyanin colouration of area below apex Spikelet : colour of stigma, Stem: anthocyanin colouration of internode Flag leaf: attitude of blade(late observation)- Spikelet :colour of tip of lemma, lemma and palea colour, Purple **-**Panicle Colour of awns(late observation), Panicle's length of awn, Panicle : distribution of awns length, Grain: 1000 fully developed grains Leaf: senescence Panicle: exertion, Decorticated grain: aroma-1, Leaf : intensity of green, Leaf: auricles, Leaf anthocyanin colouration of auricles, Leaf collar, Leaf ligule, Leaf : shape of lingule, Leaf length, Leaf collar colour, Stem: intensity of anthocyanin colouration of nodes, Panicle awns, Panicle: presence of second branching, Decorticated grain colour evaluated for characterization.

A total of twenty-two rice (*Oryza sativa* L.) genotypes, comprising traditional landraces and improved aromatic cultivars, were collected from diverse sources to capture wide genetic variability. These included Pusa Sugandha-2 and Tarori Basmati (IARI, Pusa), Rajbhog, Karahani, Kariywa, Dihawan, Moti, Sugapankhi, Jadhan, Lalka Dhan, Dudha Ladu, Kankirbi, Sugandha, Sathi-1, Sathi-2, Marcha-1, Marcha-2 (RAU, Pusa), Jaswa-3 and Parwa Pankhi (IGKV, Raipur), CSR-30 (CSIR, Karnal), Kasturi (BAU, Sabour), Lalmati (BAU, Ranchi), and Basmati-570 (PAU, Ludhiana).

Table 1
descriptors List of the DUS descriptors and frequency of the genotypes in the different state of the trait.

Sl. No.	Characteristics	States	Note	Frequency	Percentage
1	Coleoptile colour	Colourless	1	23	100
2	Basal leaf sheath colour	Green; Light purple	1; 2	16; 7	69.56; 30.43
3	Leaf pubescence of blade surface	Absent; Weak; Medium; Strong	1; 3; 5; 7	1; 9; 4; 9	4.3; 39.13; 17.39; 39.13
4	Culm attitude	Erect; Semi-erect; Open	1; 3; 5	14; 5; 4	60.86; 21.73; 17.39
5	Flag leaf attitude (early)	Erect; Semi-erect	1; 3	13; 10	56.52; 43.47
6	Spikelet pubescence (lemma)	Absent; Weak; Medium; Strong; Very strong	1; 3; 5; 7; 9	2; 1; 3; 12; 5	8.6; 4.3; 13.04; 52.17; 21.73
7	Lemma anthocyanin colouration of keel	Absent/very weak; Weak; Very strong	1; 3; 9	20; 1; 2	86.59; 4.3; 8.6
8	Lemma anthocyanin colouration below apex	Absent; Very strong	1; 9	16; 7	69.56; 30.43
9	Stigma colour	White; Yellow; Purple	1; 3; 5	11; 5; 7	47.82; 21.73; 30.43
10	Stem anthocyanin of internodes	Absent; Present	1; 9	16; 7	69.56; 30.43
11	Flag leaf attitude (late)	Erect; Semi-erect; Horizontal	1; 3; 5	14; 6; 3	60.86; 26.09; 13.09
12	Spikelet colour of lemma tip	White; Yellowish; Brown; Red; Black	1; 2; 3; 4; 6	3; 10; 7; 2; 1	13.09; 43.47; 30.43; 8.6; 4.3
13	Lemma & palea colour	Straw; Brown spots; Brown; Black	1; 3; 5; 9	11; 3; 5; 4	47.82; 13.09; 21.73; 17.39
14	Presence of awns	Absent; Present	1; 9	9; 14	39.13; 60.86
15	Panicle awn colour (late)	Yellowish white; Yellowish brown; Brown; Red; Black	1; 2; 3; 6; 9	10; 1; 1; 1; 1	43.47; 4.3; 4.3; 4.3; 4.3
16	Panicle length of awns	Very short; Short; Medium; Long; Very long	1; 3; 5; 7; 9	3; 2; 3; 4; 2	13.09; 8.6; 13.09; 17.39; 8.6
17	Panicle distribution of awns	Tip only; Upper half only	1; 3	5; 9	21.73; 39.13
18	Leaf senescence	Early; Medium; Late	3; 5; 7	7; 15; 1	30.43; 65.21; 4.3
19	Sterile lemma colour	Straw; Gold; Red	1; 2; 3	15; 6; 2	65.21; 26.02; 8.6
20	Panicle exertion	Partly exerted; Mostly exerted	3; 5	3; 5	13.09; 21.73
21	Decorticated grain aroma	Absent; Present	1; 9	12; 11	52.17; 47.82
22	Leaf green intensity	Light; Medium; Dark	3; 5; 7	13; 8; 2	56.52; 34.70; 8.6
23	Leaf auricles	Present	9	23	100
24	Anthocyanin of auricles	Colourless	1	23	100
25	Leaf collar	Present	9	23	100
26	Leaf ligule	Present	9	23	100
27	Leaf ligule shape	Split	3	23	100
28	Leaf collar colour	Absent	1	23	100
29	Node anthocyanin intensity	Weak; Medium; Strong	3; 5; 7	9; 12; 2	39.13; 52.17; 8.6
30	Panicle secondary branching	Absent; Present	1; 9	21; 2	91.30; 8.6
31	Decorticated grain colour	White; Light brown; Variegated brown; Red	1; 2; 3; 6	18; 2; 1; 2	78.86; 8.6; 4.3; 8.6

2.1. Morphological Characterization of genotype

Rice genotypes was done in six rows of 6 meters each, with a spacing of 15 cm × 20 cm. Observations on 31 agro-morphological descriptors were recorded at different growth stages following the DUS test guidelines for rice issued by the Indian Institute of Rice Research (IIRR), Hyderabad. Among the 31 morphological traits, visually assessed characteristics were directly used for distinctiveness evaluation without statistical analysis. In contrast, 17 measurable traits were analysed statistically to assess variability estimates. Additionally, agro-economic metric traits were recorded from five randomly selected plants from the middle row of each plot, except for days to maturity, days to flowering, and grain yield, which were recorded on a plot basis. 1000-grain weight was measured from a random seed sample collected from each plot.

Genetic diversity analysis was performed on 23 rice genotypes (Fig. 1) using 33 qualitative morphological traits. The dataset, stored in CSV format, was imported and processed in R (version 4.4.3). All traits were treated as categorical and converted to factors. Multiple Correspondence Analysis (MCA) was employed using the Facto-MineR and facto-extra packages to visualize trait and genotype associations in reduced dimensions. Two key plots were generated: MCA variable plot and genotype plot, coloured by contribution and cos² values, respectively. To assess genetic relationships, pairwise dissimilarities were calculated using Gower's distance, suitable for qualitative data, through the cluster package. Hierarchical clustering was performed using Ward's method (hclust), and a dendrogram was visualized with branch colours representing four major clusters using the den extend package. A dissimilarity heatmap was generated using plots: heatmap.2, applying the "viridis" colour palette to highlight trait-based differentiation among genotypes. Cluster validation was conducted using silhouette analysis to evaluate intra-cluster homogeneity and inter-cluster separation.

2.2. Molecular Characterization of marker

Five grams of seeds from each entry in earthen pots and 14-day seedlings were used for subsequent DNA extraction. Genomic DNA was extracted using the CTAB method [12] with necessary modifications and quantified using gel electrophoresis's markers (Table 1.3) were used for amplification through PCR, and the products were visualized under UV light and documented for further evaluation. The presence or absence of amplified bands was recorded using binary scoring (1 = presence, 0 = absence) as per 13]. The molecular size (bp) of DNA fragments was determined using a DNA ladder marker. The polymorphism information content (PIC) of SSR markers was calculated following) [14]. The UPGMA dendrogram based on Gower's distance provides a visual representation of genetic relatedness among the different rice genotypes. It clusters genotypes based on overall similarity, accounting for both qualitative and quantitative traits.

Table 2
List of SSR markers used in experimentation

S.NO.	PRIMER	FORWARD SEQUENCE	REVERSE SEQUENCE	ANNEALING TEMPERATURE	PRODUCT SIZE (bp)	REPEAT MOTIFS
1.	RM566	CCCAACTACGATCAGCTCG	CTCCAGGAACACGCTCTTTC	55ºC	239	(AG)15
2.	RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	55ºC	247	(AG)10
3.	RM555	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC	55ºC	223	(AG)11
4.	RM324	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC	55ºC	175	(CAT)21
5.	RM521	TTCCCTTATTCCTGCTCTCC	GGGATTTGCAGTGAGCTAGC	55ºC	260	(TC)14
6.	RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	55ºC	251	(AG)16
7.	RM28166	TGCTTGCAAACATTGCTTCTGG	ACTGATGTACTGAACACGGGAAGG	55ºC	194	(CT)12
8.	RM5791	GAAGCAGAATACGCTTCGC	ACGTCACAAGGGTTCTTGC	55ºC	103	(AGC)8
9.	RM319	ATCAAGGTACCTAGACCACCAC	TCCTGGTGCAGCTATGTCTG	55ºC	134	(GT)10
10.	RM416	GGGAGTTAGGGTTTTGGAGC	TCCAGTTTCACACTGCTTCG	55ºC	114	(GA)9
11.	MRG 2894 IRRI 2894	TATGCTCTCTCCTTCAGGCC	CTTACCAACTCCGCACTTGC	55ºC	211	-
12.	MRG2805.HAU 2805	AGAGGAAGAAGCCAAGGAGG	CATCAACGTACCAACCATGG	55ºC	110	-
13.	RM321	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG	55ºC	200	(CAT)5
14.	RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	55ºC	110	(GA)16
15.	RM70	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC	55ºC	130	(ATT)33

3. Results

3.1. Morphological diversity based on DUS descriptors

Morphological characterization of 23 rice landraces using 31 DUS descriptors revealed substantial variability across traits. Six descriptors coleoptile colour, presence of auricles, auricle anthocyanin, collar, ligule shape, and collar colour were monomorphic, indicating a high level of conservation among the studied genotypes. Panicle secondary branching was also largely uniform, with only minor variation observed in Tarori Basmati and Basmati-370.Six traits exhibited dimorphism, including basal sheath colour, internode anthocyanin pigmentation, lemma apex colouration, grain aroma, awn presence, and awn distribution. In contrast, the remaining 18 descriptors were polymorphic, showing considerable variation in key agronomic and diagnostic traits such as lemma and palea colouration, panicle exertion, flag leaf attitude, stigma colour, and grain characteristics. These polymorphic traits contributed significantly to varietal differentiation and discrimination among genotypes.

3.2. Cluster analysis based on morphological traits

Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) grouped the genotypes into three major clusters (Fig. 2). Cluster I comprised elite aromatic fine-grain genotypes such as Pusa Sugandha-2, Tarori Basmati, and Basmati-570, indicating a relatively narrow genetic base.

Cluster II included intermediate genotypes such as Sathi-1, Sathi-2, and Lalmati. Cluster III consisted of highly diverse traditional landraces, including Dudha Ladu, Moti, Dihawan, and Parwa Pankhi, reflecting broader variability.The clustering pattern clearly distinguished improved aromatic cultivars from traditional landraces. The heat map based on pairwise dissimilarity further supported these groupings, illustrating the extent of morphological divergence among genotypes (Fig. 2).

3.3. Genetic diversity and population structure using SSR markers

The molecular characterization aimed to assess genetic diversity and relatedness among the 23 rice genotypes and establish their distinctiveness at the DNA level. A total of 15 SSR primers (RM 555, RM 5791, RM 566, RM 28166, RM 70, RM 286, RM 319, RM 321, RM 324, RM 416, RM 431, RM 520, RM 521, MRG 2805, and MRG 2894) generated 81 alleles, with allele numbers ranging from 3 to 7 per locus and an average of 3.52 alleles per primer.The polymorphism information content (PIC) values ranged from 0.32 (RM 286) to 0.87 (RM 520), with a mean value of 0.725 (Table 3), confirming that the primers were highly informative for detecting genetic variability. Nine primers (RM 520, RM 521, RM 431, RM 5791, RM 321, RM 566, RM 70, RM 319, and RM 555) exhibited high PIC values, demonstrating strong discriminatory power.

Table 3
Analysis of primer pairs used for the amplification of genomic DNA extracted from twenty-three genotypes of Rice

Primers	Size of alleles	No. of alleles	unique alleles	% Of unique alleles	PIC	null alleles
RM566	225–279	6	1	16.6	0.81	1
RM520	198–272	7	1	14.3	0.87	0
RM555	218–249	4	0	0	0.76	2
RM324	153–203	6	1	16.6	0.70	0
RM521	237–295	6	0	0	0.87	4
RM431	227–274	5	0	0	0.83	3
RM28166	186–220	4	0	0	0.7	3
RM5791	355–386	5	0	0	0.82	2
RM319	115–140	4	0	0	0.77	0
RM416	119–128	3	0	0	0.6	0
MRG 2894 IRRI 2894	190–231	6	1	16.6	0.54	0
MRG2805.HAU 2805	91–132	6	0	0	0.67	0
RM321	210–253	7	1	14.3	0.82	1
RM286	108–118	5	0	0	0.32	0
RM70	138–230	7	2	28.5	0.8	0

A total of 74 shared alleles and 7 unique alleles were identified. Unique alleles were amplified by primers RM 520, RM 70, RM 321, RM 324, RM 566, and MRG 2894, with RM 70 producing two unique alleles. Null alleles were detected in several genotypes, particularly with primers RM 28166, RM 5791, RM 566, RM 555, RM 521, RM 431, and RM 321, possibly due to locus-specific mutations or amplification failure. Genetic similarity coefficients, based on the presence/absence of SSR bands, ranged from 0.00 to 0.7333 (Table 4). The highest similarity (0.7333) was observed between Dihawan–Moti and Sugapankhi–Lalka Dhan, while complete dissimilarity (0.00) was recorded in several genotype pairs, including Pusa Sugandha-2–Kasturi, Pusa Sugandha-2–Sathi-1, and Rajbhog–Tarori Basmati, indicating substantial genetic divergence.

UPGMA dendrogram analysis grouped the genotypes into four major clusters (Fig. 3). Cluster I, the largest, comprised 13 landraces including Sugapankhi, Lalka Dhan, Rajbhog, Karahani, Moti, and Dihawan, reflecting moderate diversity within traditional types. Cluster II contained Kankirbi and CSR-30, while Cluster III included six genotypes such as Lalmati, Basmati-370, Marcha-1, Marcha-2, and Tarori Basmati. Cluster IV consisted solely of Sathi-2, indicating its distinct genetic identity. At a higher stringency level (50% similarity), these clusters further subdivided into sub-clusters, separating aromatic derivatives from traditional landraces and highlighting the relatively narrow genetic base of Basmati types compared to the broader variability observed among indigenous landraces. The SSR profiles, including representative gel images, confirmed a high degree of polymorphism among the studied genotypes (Fig. 3).

Overall, the SSR markers (Table 3) proved highly effective in detecting intra- and inter-genotypic variability. The clustering pattern demonstrated that elite aromatic Basmati cultivars exhibited close genetic relatedness due to repeated selection for grain quality traits, whereas traditional landraces such as Moti, Dihawan, and Parwa Pankhi represented genetically divergent pools. These findings underscore the potential of landraces as valuable genetic resources for broadening the genetic base, enhancing adaptive traits, and supporting germplasm conservation and utilization in rice breeding programmes.

Table 4
Estimates of fifteen SSR primer pairs-based Dice similarity coefficients among twenty-three rice landraces used in the

G02	G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12	G13	G14	G15	G16
	0.533															
G03	0.400	0.467														
G04	0.333	0.600	0.533													
G05	0.400	0.667	0.667	0.600												
G06	0.267	0.467	0.533	0533	0.733											
G07	0.333	0.267	0.400	0.133	0.467	0.400										
G08	0.400	0.333	0.333	0.333	0.533	0.466	0.600									
G09	0.267	0.400	0.333	0.333	0.600	0.666	0.733	0.73								
G10	0.267	0.267	0.267	0.267	0.333	0.400	0.467	0.467	0.600							
G11	0.200	0.133	0.200	0.067	0.266	0.333	0.667	0.533	0.533	0.533						
G12	0.200	0.133	0.133	0.067	0.200	0.267	0.467	0.467	0.400	0.467	0.600					
G13	0.214	0.214	0.143	0.143	0.214	0.143	0.286	0.357	0.286	0.357	0.429	0.500				
G14	0.067	0.200	0.067	0.200	0.133	0.133	0.133	0.267	0.200	0.267	0.133	0.200	0.357			
G15	0.231	0.154	0.308	0.231	0.308	0.308	0.308	0.461	0.385	0.385	0.385	0.308	0.083	0.231		
G16	0.000	0.000	0.064	0.129	0.064	0.064	0.064	0.064	0.064	0.129	0.193	0.129	0.207	0.323	0.293	
G17	0.000	0.000	0.154	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.077	0.083	0.385	0.273	0.148
G18	0.000	0.074	0.148	0.074	0.074	0.074	0.074	0.000	0.074	0.074	0.000	0.000	0.000	0.148	0.261	0.429
G19	0.062	0.000	0.062	0.125	0.062	0.062	0.125	0.062	0.062	0.125	0.187	0.187	0.067	0.125	0.214	0.485
G20	0.064	0.064	0.000	0.129	0.064	0.064	0.064	0.064	0.064	0.129	0.064	0.064	0.000	0.193	0.148	0.187
G21	0.062	0.000	0.000	0.000	0.000	0.000	0.062	0.062	0.000	0.062	0.187	0.062	0.133	0.125	0.286	0.364
G22	0.000	0.071	0.071	0.071	0.143	0.143	0.071	0.143	0.143	0.000	0.071	0.071	0.000	0.214	0.167	0.138
G23	0.148	0.074	0.222	0.222	0.148	0.074	0.074	0.074	0.148	0.074	0.000	0.000	0.000	0.074	0.174	0.214

4. Discussion

The present study demonstrated substantial morphological variation among rice landraces using DUS descriptors, highlighting their importance in varietal identification and germplasm characterization. The occurrence of monomorphic traits suggests the conservation of essential diagnostic features, likely maintained through selection pressure and genetic stability. In contrast, polymorphic traits such as lemma and palea colouration, panicle exertion, stigma colour, and grain characteristics exhibited considerable variability, making them reliable markers for distinguishing genotypes. Such morphological diversity is particularly valuable in the context of DUS testing and varietal protection, where distinctiveness is a key requirement. Similar patterns of morphological variation in rice germplasm have been reported previously, emphasizing the utility of qualitative descriptors in diversity analysis and classification [15–18].

The clustering pattern based on morphological traits revealed a clear distinction between elite aromatic cultivars and traditional landraces. The grouping of Basmati genotypes into a single cluster reflects their narrow genetic base, which can be attributed to prolonged selection for specific grain quality traits. In contrast, the wide dispersion of traditional landraces across clusters indicates a broader genetic base and greater heterogeneity. The molecular clustering further corroborated these observations, with SSR-based dendrograms clearly separating aromatic and non-aromatic genotypes at higher similarity thresholds. The distinct clustering of certain genotypes, such as Sathi-2, indicates unique genetic backgrounds that may be valuable for breeding programs. Comparable clustering patterns distinguishing improved cultivars from indigenous landraces have also been documented in earlier studies [19–22].

SSR markers proved to be highly effective in detecting genetic variability among the studied genotypes. The high PIC values observed for most primers indicate their strong discriminatory ability and informativeness. The identification of unique alleles further highlights their potential utility in genotype identification, fingerprinting, and marker-assisted selection. The presence of null alleles in certain genotypes may be attributed to mutations in primer binding regions or amplification inefficiencies, as reported in previous studies. Compared to morphological markers, SSR markers offer several advantages, including co-dominant inheritance, high reproducibility, and independence from environmental influences. These characteristics make them particularly suitable for precise genetic characterization and diversity assessment. Similar findings regarding the robustness and effectiveness of SSR markers in rice have been widely reported [23–30].

The findings of this study underscore the critical role of traditional rice landraces as reservoirs of genetic diversity. The high level of divergence observed among these genotypes indicates their potential as valuable donors for broadening the genetic base of cultivated varieties. Incorporating such diverse

germplasm into breeding programs can enhance adaptability to environmental stresses, improve yield stability, and introduce novel traits. The narrow genetic base observed in elite aromatic cultivars highlights the need for genetic diversification to reduce vulnerability to biotic and abiotic stresses. The combined use of morphological and molecular approaches in the present study provides a comprehensive framework for germplasm evaluation and utilization. The congruence between these approaches strengthens the reliability of the results and supports their application in conservation strategies, varietal improvement, and marker-assisted breeding. Overall, the study emphasizes the importance of systematic characterization and conservation of indigenous rice germplasm to ensure sustainable crop improvement and food security.

5. Conclusion

Among the 31 morphological characteristics evaluated, 6 were monomorphic, 1 was partially monomorphic, 6 were dimorphic, and 20 exhibited polymorphisms. The dimorphic and polymorphic traits demonstrated their efficacy in distinguishing genotypes, highlighting their potential for precise characterization. Notably, the landrace Sathi-1 emerged as the most distinct genotype, exhibiting rare morphological features such as no panicle exertion, black lemma and palea colouration, early flowering, and early maturity. Similarly, the landrace Parwa Pankhi was characterized by a unique trait its exceptionally long sterile lemma. The most discriminatory descriptors for genotype differentiation included lemma and palea colouration, as well as the flag leaf attitude at both early and late growth stages. The dendrogram provides clear evidence of the genetic structure and diversity among rice genotypes, aligning with earlier studies emphasizing the utility of traditional landraces for introducing novel alleles into breeding programs. The use of Gower's distance with UPGMA proves effective in integrating diverse trait types for comprehensive genotype classification. Molecular characterization using microsatellite markers further reinforced genotype distinctiveness, revealing unique or variety-specific alleles that can serve as DNA fingerprints for the precise identification and conservation of rice genotypes. The application of fifteen SSR markers demonstrated a high degree of genetic polymorphism, enabling the unique genotyping of all twenty-three rice entries analysed. These findings underscore the effectiveness of combining morphological and molecular markers for comprehensive characterization, facilitating genetic conservation and informed breeding strategies.

Abbreviations

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Declarations

Ethical approval and consent to participate

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Competing Interest

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Author Contribution

N.K. was responsible for data collection, conducting experiments, literature review, and manuscript drafting. P.C.G. and D.S. contributed to experimental design and assisted in data interpretation. T.A.M. and K.K.G. supported molecular analysis and laboratory work. N. and R.K. contributed to data analysis and validation of results. D.R.* conceptualized the research, supervised the overall study, guided the experimental work, and critically revised the manuscript. All authors read and approved the final manuscript.

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Data availability

All data generated or analysed during this study are included in this article and its supplementary information files. and its supplementary information files.

References

1. Zhang G. 2022. The next generation of rice: inter-subspecific indica-japonica hybrid rice. *Front. Plant Sci.* 13:8578.
2. Mohanty T.A., Kumaresan D., Manonmani S., Ramalingam S., Boopathi N.M., Natarajan S. 2025. Enhancing rice breeding through two-line hybrids: integrative analysis of combining ability, heterosis, MGIDI, and grain quality traits. *Euphytica* 221(3):29.
3. Zafar S., Jianlong X. 2023. Recent advances to enhance nutritional quality of rice. *Rice Sci.* 30(6):523–536.
4. Pradhan S.K., Das S.R., Patra B.C. 2021. Advances in Rice Breeding: Stress Tolerance, Climate Resilience, Quality & High Yield. *ICAR-National Rice Research Institute*, Cuttack, India. p. x + 426.
5. Broccanello C., Bellin D., DalCorso G., Furini A., Taranto F. 2023. Genetic approaches to exploit landraces for improvement of *Triticum turgidum* ssp. *durum* in the age of climate change. *Front. Plant Sci.* 14:1101271.
6. Lazaridi E., Kapazoglou A., Gerakari M., Kleftogianni K., Passa K., Sarri E., Bebeli P.J. 2024. Crop landraces and indigenous varieties: a valuable source of genes for plant breeding. *Plants* 13(6):758.
7. Bidyananda N., Jamir I., Nowakowska K., Varte V., Vendrame W.A., Devi R.S., Nongdam P. 2024. Plant genetic diversity studies: insights from DNA marker analyses. *Int. J. Plant Biol.* 15(3):607–640.
8. 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 Rep.* 37:102051.
9. Saxena P., Malik L., Pattanaik S., Gudipalli P. 2024. Genetic diversity analysis of a germplasm collection of red sanders (*Pterocarpus santalinus* L.f.) using cross-species SSR, ISSR and RAPD markers. *S. Afr. J. Bot.* 174:856–869.
10. Mallikarjuna B.P., Alam B.K., Suresha P.G., Saini M., Rajendran A., Patil B.S., Bhat J.S. 2025. Genomic approaches for achieving higher nutrient use efficiency in oilseeds. In: *Breeding Climate Resilient and Future Ready Oilseed Crops*. Springer, Singapore. p.163–186.
11. Su Y., Fu J., Xie H., Huang Z., Li Y., Luo Y., Liu Y. 2025. SSR markers development and their application in genetic diversity of burdock (*Arctium lappa* L.) germplasm. *BMC Plant Biol.* 25(1):196.
12. Doyle J.J., Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13–15.
13. Hartigan J.A. 1975. *Clustering Algorithms*. John Wiley & Sons, Hoboken.
14. Anderson J.A., Churchil G.A., Autrique J.E., Tanksley S.O., Sorrels M.E. 1993. Optimizing parent selection for genetic linkage maps. *Genome* 36:181–186.
15. Gayathri N.K., Srujana Y., Venkateswarlu N.C. 2023. DUS characterization of rice (*Oryza sativa* L.) germplasm. *Electron. J. Plant Breed.* 14(1):314–322.
16. Lavanya K., Chiranjeevi M., Surender R., Yadav P.A.S., Fiyaz A.R., Sudhakar, Rao L.S. 2021. Characterization of rice traditional varieties (*Oryza sativa* L.) based on DUS descriptors. *Pharma Innov. J.* 10(3):760–771.
17. Kalyan B., Radha Krishna K.V., Subba Rao L.V. 2017. DUS characterization for germplasm of rice. *Int. J. Curr. Microbiol. Appl. Sci.* 6(10):3480–3487.
18. Prasanna G.S., Joshi J.L., Muraleedharan A. 2024. Distinctiveness, Uniformity and Stability (DUS) characterization in twenty-five landraces of rice (*Oryza sativa* L.). *J. Adv. Biol. Biotechnol.* 27(3):77–84.
19. Ansari A., Pranesti A., Telaumbanua M., Alam T., Wulandari R.A., Nugroho B.D.A. 2023. Evaluating the effect of climate change on rice production in Indonesia using multimodelling approach. *Heliyon* 9(9):e.
20. Gaikwad K.B., Singh N., Kaur P., Rani S., Babu H.P., Singh K. 2021. Deployment of wild relatives for genetic improvement in rice (*Oryza sativa*). *Plant Breed.* 140(1):23–52.
21. Pourabed E., Noushabadi M.R.J., Jamali S.H., Alipour N.M., Zareyan A., Sadeghi L. 2015. Identification and DUS testing of rice varieties through microsatellite markers. *Int. J. Plant Genomics* 2015:965073.
22. Mondal B., Singh S.P., Joshi D.C. 2014. DUS characterization of rice (*Oryza sativa* L.) using morphological descriptors and quality parameters. *Outlook Agric.* 43(2):131–137.
23. Nivedha R., Manonmani S., Kalaimagal T., Raveendran M., Kavitha S. 2024. Assessing the genetic diversity of parents for developing hybrids through morphological and molecular markers in rice (*Oryza sativa* L.). *Rice* 17(1):17.
24. Al-Musawi B.H., Al-Badeiry N.A.H., Al-Anbari M.A. 2019. Efficiency of SSR markers system in accessing genetic variation for rice (*Oryza sativa* L.) genotypes. *Plant Arch.* 19(Suppl. 1):318–325.
25. Kiranmayee B., Kemparaju K.B., Sundaram R.M., Raju C.D., Balram M., Prasad A.H., Manasa Y. 2022. Genetic diversity analysis of maintainer lines using SSR markers in rice (*Oryza sativa* L.). *Oryza* :5(9), 393–399.
26. Singh N., Choudhury D.R., Tiwari G., Singh A.K., Kumar S., Srinivasan K., Singh R. 2016. Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC Genet.* 17:1–13.
27. 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 J. Biol. Sci.* 31(3):103944.
28. Pathaichindachote W., Panyawut N., Sikaewtung K., Patarapuwadol S., Muangprom A. 2019. Genetic diversity and allelic frequency of selected Thai and exotic rice germplasm using SSR markers. *Rice Sci.* 26(6):393–403.
29. Jasim Aljumaili S., Rafii M.Y., Latif M.A., Sakimin S.Z., Arolu I.W., Miah G. 2018. Genetic diversity of aromatic rice germplasm revealed by SSR markers. *Biomed. Res. Int.* 2018(1):7658032.
30. Kumar S.J., Susmita C., Agarwal D.K., Pal G., Rai A.K., Simal-Gandara J. 2021. Assessment of genetic purity in rice using polymorphic SSR markers and its economic analysis with grow-out-test. *Food Anal. Methods* 14:856–864.

Figures

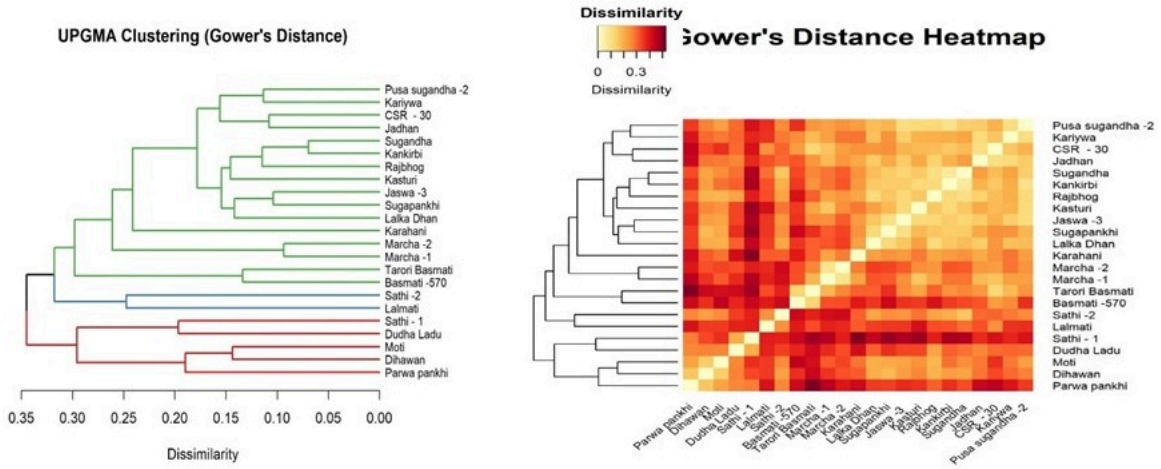

Figure 1

The different genotypes used for the DUS testing studies.

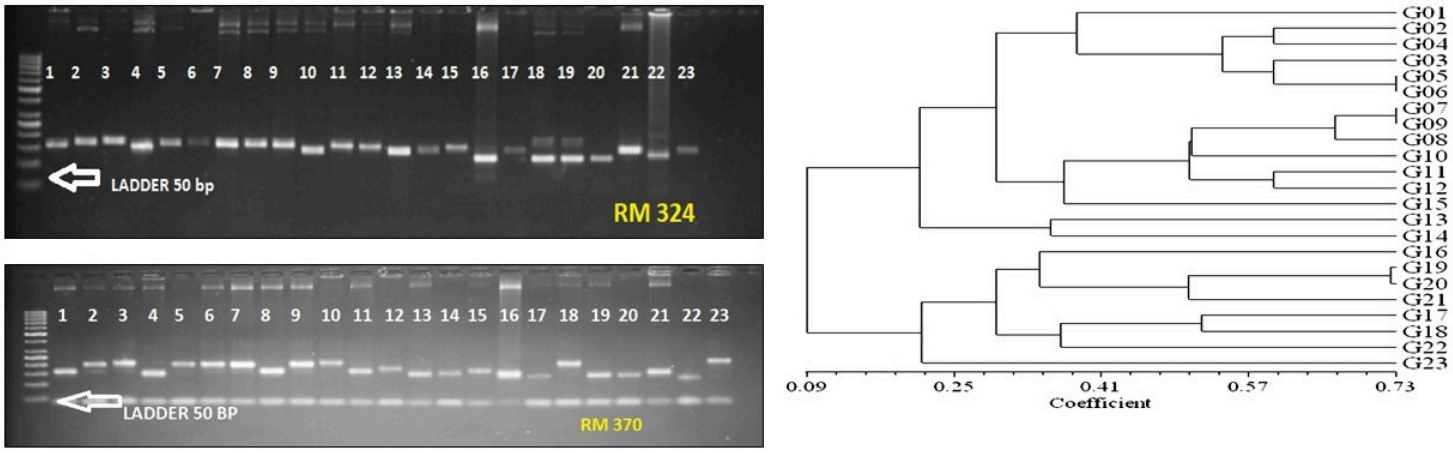

Figure 2

Dendrogram showing Clustering pattern of 23 genotypes based on morphological DUD traits and Heat map based on pair-wise dissimilarity matrix of morphological traits.

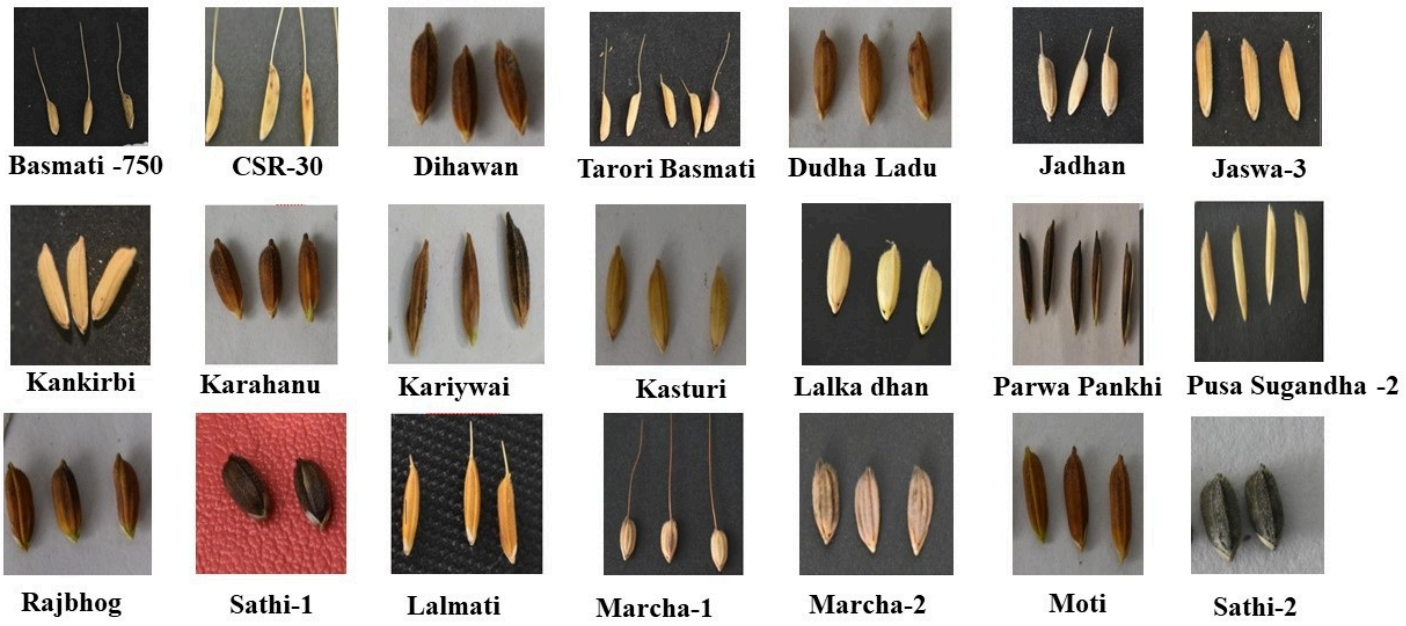

Figure 3

Representative gel image obtained molecular profiling of landraces and dendrogram depicting the clustering pattern based SSR markers