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DUS TRAIT EVALUATION AND MICRO-SATELLITE MARKERS BASED MOLECULAR PROFILING OF PROMINENT RICE (ORYZA SATIVA L.) VARIETIES GROWN IN BIHAR

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ABSTRACT
This research was carried out at the Rice Breeding Section, Pusa Farm, Dr. Rajendra Prasad Central Agricultural University (RPCAU) in Samastipur, Bihar. Twenty-two rice cultivars cultivated in Bihar and enhanced varieties were sown using a randomized block design (RBD) with three replications and a standard spacing of 20 × 15 cm to analyse agro-morphologically and molecularly using SSR markers. The evaluation was conducted by the DUS descriptors provided by the Directorate of Rice Research in Hyderabad. The analysis of DUS characteristics revealed that the decorticated grain colour was observed as white in all twenty-two rice varieties. The aroma of decorticated grain was found to be absent in all twenty-two varieties except Rajendra Bhagwati. The polymorphic information content (PIC) values, which indicate allelic diversity and frequency based on simple sequence length polymorphism, ranged from 0.710 to 0.917, with an average value of 0.820 per locus. Among the primer pairs, higher PIC values were observed in RM 566, RM 520, RM 555, RM 324, RM 521, and RM 431, which are referred to be highly informative markers. Clustering analysis, based on a similarity threshold of 75 units, resulted in the subdivision of the entries into multiple sub-clusters: IA, IIA, IIIA, B, IC, IIC, ID, IID, IIID, IE, IIE, IF, and IIF.

KEYWORDS: DUS, decorticated, PIC, Clustering, Markers, R.B.D.

1. INTRODUCTION

Rice is one of the most crucial food crops in the world, providing the primary source of nutrition for majority of people (Mohidem et al., 2022). It has been named the "Global Grain" (Debsharma et al., 2022) because of its widespread importance. According to projections, rice demand is expected to reach approximately 943.60 million tons by 2050, necessitating a yearly increase of 5.80 million tons from current output levels (FAO, 2017). However, fulfilling this demand is difficult because industrial, residential, and infrastructural development has decreased cultivable land by more than 1% (Mohanty et al., 2025). More than 800 million individuals worldwide are currently hungry and food insecure. Given the restricted fertile land for cultivation and the growing global population, enhancing rice production has become unavoidable (Mohanty et al., 2025). India produced 137.82 million tonnes of rice in the 2023–24 crop year, grown over 47.6 million hectares (Chaturvedi et al., 2023). With an increasing demand, ongoing attempts are being made to improve productivity per hectare.

One of the factors that allows rice to flourish in a variety of environmental circumstances is its incredible genetic variety. This extensive intra-species diversity is essential for genetic enhancement, sustainable agriculture, and global food security (Kimwemwe et al., 2023). Rice germplasm is an essential resource for breeding programs focused on improving desirable characteristics because it contains a large collection of useful genes and gene combinations (Gaikwad et al., 2021). The agro-morphological characterization of rice accessions is a crucial step in effectively conserving and exploiting this diversity, given its importance.

The success of any breeding program is fundamentally reliant on the extent of genetic variability within a crop, which is crucial for developing high-yielding varieties (Padulosi, 1993). The initial step in improving rice productivity involves evaluating and characterizing existing rice genotypes or varieties at both morphological and molecular levels, as phenotypic and genotypic diversity provide valuable insights for plant breeders (Singh, 1989). In India, the only legally recognized method for varietal identification and genetic purity assessment remains seed certification, which relies on grow-out tests conducted in field plots based solely on morphological characteristics. While these methods have proven to be effective, intensive plant breeding programs have led to the development

of new varieties with reduced phenotypic distinctiveness due to the utilization of closely related genetic populations. Additionally, the expression of morphological traits is often influenced by environmental factors, making these characteristics unreliable for assessing genetic purity (Cooke, 1999). Morphological traits are largely quantitative, influenced by multiple genes, and require extensive replication, trained personnel, and significant resources for evaluation. Furthermore, the limited number of genes governing morphological differences may not accurately reflect genetic divergence across the entire genome (Singh et al., 1999). Consequently, morphological traits alone may be insufficient for distinguishing and identifying existing and newly developed rice varieties, necessitating more precise techniques.

Given that the existing UPOV (International Union for the Protection of New Varieties of Plants) models were not fully suited to India's agricultural requirements, the Government of India enacted the Protection of Plant Varieties and Farmers' Rights Act (PPV&FRA) in 2001. This legislation facilitates plant variety protection based on Distinctness, Uniformity, and Stability (DUS) testing, along with novelty criteria. Notably, the Act recognizes both farmers and breeders as equal contributors to food security, acknowledging their role in varietal development (Patra, 2000).

The principles of distinctness, uniformity, and stability serve as fundamental criteria for classifying a variety as a unique genetic entity. The registration process under PPV&FRA encompasses three categories of plant varieties: newly bred varieties developed by breeders, extant varieties, and farmers' varieties. For breeder-developed varieties, registration is granted only if they satisfy the conditions of novelty, distinctness, uniformity, and stability, which are evaluated through the DUS test.

India's structured rice improvement initiatives have led to the development and release of nearly 700 improved rice varieties under the Seeds Act of 1966. Even before this formal framework, several agricultural research institutions had been actively engaged in breeding improved rice varieties, which have now become widely cultivated (Nagarajan et al., 2008). These rice varieties exhibit significant variability concerning their morphological, agronomic, grain, and cooking characteristics.

The effectiveness of biochemical markers, such as electrophoresis of proteins and isoenzymes, in distinguishing crop varieties has been widely reported. Although these markers are relatively stable and less influenced by environmental factors, they exhibit limited polymorphism, which restricts their ability to differentiate closely related genotypes (Bostein et al., 1980; Ainsworth & Sharp, 1989; Tanksley et al., 1989; Aldrich et al., 1992). Consequently, a more precise descriptor capable of directly scanning the genome is essential for accurate crop variety identification.

DNA markers provide a significant advantage over morphological and biochemical markers, as they allow for the development of genotype-specific profiles. These markers cover the entire genome, exhibit higher polymorphism, and are unaffected by environmental factors or developmental stages. The application of DNA fingerprinting for rice variety identification was first demonstrated by Dallas (1988). Various DNA fingerprinting techniques, including Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Simple Sequence Repeats (SSRs), Inter Simple Sequence Repeats (ISSRs), and Amplified Fragment Length Polymorphisms (AFLPs), have been instrumental in distinguishing rice varieties (Powell et al., 1996; Joshi et al., 2000). Each of these methods presents specific advantages and limitations.

Among molecular markers, microsatellites, also known as Simple Sequence Repeats (SSRs), are recognized for their high information content, versatility, and reproducibility in genetic studies (Salimah et al., 1995; Rafalski et al., 1996; McCouch et al., 1997; Nagaoka & Ogihara, 1997). Over the past few decades, the application of molecular markers in rice genetics and breeding has expanded significantly. SSR markers have gained prominence due to their ease of amplification via PCR and the substantial allelic variation they exhibit at each locus. Typically composed of one to six nucleotide repeats, SSRs are abundant, highly polymorphic, and distributed throughout the genome. Their species specificity and co-dominant nature make them highly effective genetic markers in rice breeding programs. To date, thousands of SSR markers have been developed for rice research, with their chromosomal locations and polymorphism levels well-documented. Therefore, evaluating and characterizing available rice genotypes at both morphological and molecular levels is crucial for broadening the genetic base of improved rice varieties (Ogunbayo et al., 2005).

2. MATERIALS AND METHODS

2.1 Materials

In this research, twenty-two rice cultivars cultivated in Bihar and enhanced varieties **(Table 1)** were analyzed agromorphologically and molecularly using SSR markers.

2.2 Location

This research was carried out at the Rice Breeding Section, Pusa Farm, affiliated with Dr. Rajendra Prasad Central Agricultural University (RPCAU) in Samastipur, Bihar, during the kharif season of 2018. Geographically situated at 25.98°N latitude and 85.67°E longitude, with an elevation of 51.8 meters above mean sea level, this area is part of the Middle Gangetic Plains agro-climatic zone, which is ideal for rice farming and agricultural research. The kharif season, which runs from June to October, corresponds with the monsoon season and provides ideal circumstances for rice cultivation and field testing.

Bihar, located in eastern India, lies between 24°20'10" N and 27°31'15" N latitude and between 83°19'50" E and 88°17'40" E longitude. Its subtropical climate experiences significant seasonal shifts, with summer temperatures reaching 45 to 47 degrees Celsius and winter temperatures dropping as low as 2 to 3 degrees Celsius in December

and January. The state has an average yearly temperature of 25.5°C and an annual precipitation of about 1,097 mm, which is mostly due to the southwest monsoon.

2.3 DUS Characterization

The genotypes were sown using a randomized block design (RBD) with three replications and a standard spacing of 20 × 15 cm for agro-morphological characterization. The evaluation was conducted in accordance with the DUS descriptors provided by the Directorate of Rice Research in Hyderabad (**Table 2**). To guarantee ideal growth and precise evaluations throughout the experiment, standard crop management practices were meticulously followed.

2.4 Molecular Characterization

DNA Amplification

For molecular characterization, DNA was extracted from 200 mg of rice leaf tissue collected during the seedling stage using the standard CTAB method. PCR amplification was carried out employing fifteen SSR primer pairs (**Table 3**) obtained from the Gramene database. The final PCR reaction was prepared in a 25. 0 µl volume, which included: 1. 0 µl (20 ng/µl) DNA template, 2. 0 µl dNTPs (2. 5 mM each), 0. 5 µl Taq polymerase (3U/µl), 2. 5 µl reaction buffer (10X), 1. 0 µl forward primer (20 µM), 1. 0 µl reverse primer (20 µM), and 17. 0 µl nuclease-free water. The thermal cycling conditions comprised an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension was conducted at 72°C for 7 minutes, and the PCR products were stored at 4°C until they were subject to further analysis.

Gel Electrophoresis and Band Visualization

After PCR amplification, the DNA samples underwent agarose gel electrophoresis using a 2% agarose gel prepared in 1X TBE buffer. Ethidium bromide (10 µl) was added to 200 µl of TBE buffer for staining purposes. The electrophoresis was performed at 110 volts for 1 hour and 10 minutes. Once separation was achieved, the gel was observed under a UV transilluminator within a gel documentation system, and images were taken utilizing an integrated camera. The banding patterns created by each primer set were analyzed individually, and fragment sizes were established by comparing the migration distances with a 50-base pair (bp) DNA ladder. The detection of a band at a specific position was recorded as '1,' whereas its absence was noted as '0' for further data analysis.

Statistical analysis.

From the marker data acquired from fifteen SSR markers across 22 rice genotypes, a phylogenetic tree was developed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The analysis was conducted using the NTSYSpc software to evaluate genetic relationships among the genotypes. The resulting dendrogram depicted the distance-based interrelationships between the tested genotypes. Furthermore, the polymorphism information content (PIC) was computed according to the formula suggested by Anderson et al. (1997) to assess the discriminatory capability of the markers utilized.

$$\mathbf{PICi} = 1 - \sum_{j=1}^{k} P^2 ij$$

Where,

k is the total number of alleles detected for a locus of a marker,

Pij is the frequency of the jth allele for the ith marker, and the summation extends over k alleles.

3. RESULTS AND DISCUSSION

DUS characterization

The basal leaf sheath color for all nineteen varieties was green except for three varieties via., Rajendra Nilam, Gautam, and Dular, which exhibited light purple basal leaf sheath color. Similarly, the coleoptile color of nineteen rice varieties was colorless, whereas green color was observed in three varieties, namely Pooja, Kalanamak, and Dular. Pubescence of blade surface was found to be weak in Rajendra Bhagwati, Rajendra Nilam, Rajendra Saraswati, Rajendra Shweta, Dhanlaxmi, Saroj, Gautam, BPT-5204, Dinesh and Dular whereas, medium pubescence was observed in Rajendra Kasturi, Rajendra Suwashini, Rajendra Mahsuri, Prabhat, Richharia, Sita, Pooja, Swarna sub-1 and kalanamak (**Table 4**).

Long length of leaf blade was observed in seven varieties via Rajendra Bhagwati, Rajendra Kasturi, Dhanlaxmi, Gautam, Sahbhagi, Pooja and Dular whereas in Rajendra Shweta and Dinesh it was found short. In rest of the rice varieties showed medium length of leaf blade. The leaf senescence was early in Prabhat, Saroj, and Sahbhagi, while, late in Dhanlaxmi, Swarna sub-1, and BPT-5204, and found medium in the remaining varieties of rice.

The color of the sterile lemma was observed to be straw colored in all the rice varieties except the Kalanamak variety, in which it was purple colored. The color of lemma and palea was brown (tawny) in Richharia and Dular, brown spots were prominent on straw in Saroj and Gautam, and black in Kalanamak. In the rest of the varieties straw color of the lemma and palea was found.

The pubescence was found to be strong for Sabour Surbhi while absent in Turanta and Sahbhagi varieties of rice. Leaf auricles, ligules, and leaf collars were present in all varieties under study. The shape of the ligule and color of the ligule also did not show any variation and were found to be split and absent, respectively, for all the varieties. Culm is attributed to be semi-erect for Sahbhagi and Kalanamak. The rest of the varieties showed an erect culm. The character leaf anthocyanin coloration of auricles was found to be colorless for all the rice varieties that were taken for the study.

The early observation on the flag leaf was found to be erect in fifteen varieties, whereas semi-erect was found in three varieties, Rajendra Bhagwati, Sahbhagi, and Sabour Surbhi. Horizontal flag leaf attitude (early observation) was found in Prabhat, Richharia, Pooja, and Kalanamak. The density of pubescence of lemma in the spikelet was weak in twelve varieties, medium was found in nine varieties, namely Rajendra Nilam, Rajendra Shweta, Turanta, Dhanlaxmi, Sita, Swarna sub-1, Dinesh, Dular and Sabour Surbhi where as the Kalanamak variety of rice recorded strong density of lemma pubescence.

All the varieties were grouped in weak and absent or very weak for anthocyanin coloration of keel whereas the anthocyanin coloration of area below apex was absent in fifteen rice varieties, weak in six varieties namely Rajendra Mahsuri, Saroj, Gautam, Sita, Sahbhagi and Swarna sub-1 whereas strong anthocyanin coloration of area below apex was found show variation among the varieties. The stigma color was found to be white in the fifteen varieties, light green was two varieties, namely BPT-5204 and Dinesh, purple was found in Dular, Gautam, Rajendra Nilam, light purple was found in Kalanamak, and yellow in Rajendra Bhagwati. None of the varieties showed the presence of anthocyanin coloration on internodes except Rajendra Nilam and Dular, whereas anthocyanin coloration on nodes of the stem was found weak for all the varieties. The late observation on flag leaf attitude was found to be semi-erect for eleven rice varieties, horizontal for Richharia, and erect for five varieties, namely Dhanlaxmi, Saroj, Gautam, Kalanamak, and Dinesh.

The curvature of the panicle main axis at the ripening stage was semi-straight in twelve varieties. The remaining ten varieties via Rajendra Nilam, Rajendra Shweta, Prabhat, Richharia, Turanta, Dhanlaxmi, Saroj, Swarna sub-1, BPT-5204, and Dinesh showed straight curvature of panicle. The color of the tip of the lemma of the varieties was classified into White (Rajendra Saraswati, Rajendra Kasturi, and Rajendra Suwashini), Brown (Turanta, Gautam, and Dular), and Black (Kalanamak). Rice variety Rajendra Nilam had a purple lemma tip, whereas the remaining varieties had a yellowish tip of the lemma.

The panicle awns were absent in eighteen rice varieties, whereas present in Rajendra Bhagwati, Rajendra Saraswati, Sita, and Pooja. However, the length of the awn was short (Rajendra Saraswati, Sita and Pooja) and very short (Rajendra Bhagwati). The presence of secondary branching of the panicle was observed in all the varieties. The exertion of panicles was recorded as well-exerted in eighteen varieties, while partially exerted in the remaining four varieties, namely Rajendra Shweta, Rajendra Mahsuri, BPT-5204, and Dinesh. Awn color was observed to be yellowish white, and awns were distributed on the tip only for Rajendra Bhagwati, Rajendra Saraswati, Sita, and Pooja.

The decorticated grain color was observed as white in all twenty-two rice varieties. The aroma of decorticated grain was found to be absent in all twenty-two varieties except Rajendra Bhagwati. Decorticated grain width was found to be narrow in thirteen varieties, medium in eight rice varieties, namely Rajendra Bhagwati, Rajendra Nilam, Richharia, Sita, Sahbhagi, Pooja, Dinesh, and Dular, while broad decorticated grain width was observed in Turanta. Similar results were observed by Umarani E. et al. (2017), Anjali K. et al. (2018), Manjunathan G.A et al. (2018), Pragnya K. et al. (2018), Parikh M. et al. (2012), Sarawgi et al. (2012), Binse and Sarawgi (2008).

Molecular characterization:

The polymorphic information content (PIC) values, which indicate allelic diversity and frequency based on simple sequence length polymorphism, ranged from 0.710 for RM 5791 to 0.917 for MRG 2805 HAU 2805, with an average PIC value of 0.820 per SSR primer pair (**Table 5**). A notably higher magnitude of PIC values was recorded for primer pairs RM 566, RM 520, RM 555, RM 324, RM 431, MRG 2894 IRRI 2894, MRG 2805 HAU 2805, and RM 70. Similar findings were previously reported by Pal et al. (2004), Jain et al. (2004), Tang et al. (2009), and Das et al. (2012).

Among the primer pairs, higher PIC values were observed in RM 566, RM 520, RM 555, RM 324, RM 521, and RM 431 generated a substantial number of allelic variants per primer (**Table 5**). This was attributed to variations in the length of amplified simple sequence repeats flanked by these primer pairs. Furthermore, RM 521, RM 324, RM 555, and RM 520 exhibited a higher percentage of unique alleles, demonstrating high polymorphic information content and a greater number of alleles. Similar trends were also observed in the studies conducted by Ram B. J. et al. (2018), Rahman M. et al. (2012), Prathayusha A. et al. (2009), and Kshirsagar et al. (2012).

The genetic similarity coefficient was assessed among 22 rice entries by analyzing the presence and absence of amplified products generated by 15 primer pairs targeting unique microsatellite flanking sequences distributed across chromosomes. The highest similarity coefficient values were recorded between Turanta and Dhanlaxmi (0.484), as well as Swarna sub-1 and BPT-5204 (0.484), among the pairwise combinations. Additionally, a relatively high similarity coefficient was observed between Rajendra Shweta and Rajendra Kasturi (0.473), Rajendra Kasturi and Rajendra Saraswati (0.412), Rajendra Kasturi and Rajendra Suwashini (0.375), Prabhat and Richharia (0.375), Saroj and Gautam (0.312), Gautam and Sita (0.303), Pooja and Sita (0.242), Saroj and Rajendra Kasturi (0.242), and Saroj and Rajendra Shweta (0.228). Several pairwise comparisons, including those between Rajendra Bhagwati and Richharia, Rajendra Bhagwati and Kalanamak, Rajendra Nilam and Saroj, Rajendra Nilam and Pooja, Rajendra Nilam and Kalanamak, and others, yielded a similarity coefficient of zero, suggesting substantial molecular diversity among the rice entries studied. These findings align with the observations of Behera et al. (2012), Pachauri et al. (2013), Saheewala et al. (2014), and Priyadarshini et al. (2018).

Clustering analysis, based on a similarity threshold of 75 units, resulted in the subdivision of the entries into multiple sub-clusters: IA, IIA, IIIA, B, IC, IIC, ID, IID, IIID, IE, IIE, IF, and IIF (**Fig. 2**). Sub-cluster IA was further divided into the mono-genotypic sub-cluster IAa, containing Rajendra Mahsuri, and sub-cluster IAb, which

comprised Saroj. Similarly, sub-cluster IIA was split into a di-genotypic group consisting of Turanta and Dhanlaxmi, along with a mono-genotypic sub-cluster containing Gautam. Sub-cluster IIIA comprised only Sita, while Cluster B was further classified into IB (Prabhat) and IIB (Richharia). Sub-cluster IC was separated into ICa, which contained Rajendra Bhagwati, and ICb, which included Rajendra Nilam. Likewise, IIC was subdivided into IICa (Rajendra Saraswati) and IICb (Rajendra Kasturi, Rajendra Suwashini, and Rajendra Shweta). Additional sub-clusters included ID (Swarna sub-1 and BPT-5204), IID (Dular), IIID (Dinesh), IE (Sahbhagi), IIE (Kalanamak), IF (Pooja), and IIF (Sabour Surbhi). These clustering patterns are consistent with the studies conducted by Sarkar et al. (2013), Rani et al. (2017), and Saheewala et al. (2014).

CONCLUSION

Microsatellite marker-based analysis revealed unique or variety-specific alleles which could be useful as DNA fingerprints of rice genotypes. The use of fifteen microsatellite markers in the analysis exhibited a remarkably higher level of genetic polymorphism, which allowed unique genotyping of twenty- two entries included in the analysis. A notably higher magnitude of PIC values was recorded for primer pairs RM 566, RM 520, RM 555, RM 324, RM 431, MRG 2894 IRRI 2894, MRG 2805 HAU 2805, and RM 70. The highest similarity coefficient values were recorded between Turanta and Dhanlaxmi (0.484), as well as Swarna sub-1 and BPT-5204 (0.484), among the pairwise combinations. Additionally, a relatively high similarity coefficient was observed between Rajendra Shweta and Rajendra Kasturi (0.473), Rajendra Kasturi and Rajendra Saraswati (0.412), Rajendra Kasturi and Rajendra Suwashini (0.375), Prabhat and Richharia (0.375) suggesting substantial molecular diversity among the rice entries studied

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Author Contribution
RC, NK, N and RK were involved in planning and designing of experiment, RC and NK carried out the research work and RC, NK, DS and TAM were involved in preparation and review of manuscript.

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

Ethical Approval and Consent to Participate
Not applicable.

Consent for Publication
Not applicable.

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

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Table 1: List of the twenty-two rice cultivars used in the investigation

Sl. No.	**Name of Varieties**	**Sl. No.**	**Name of Varieties**
1.	Rajendra Bhagwati	12.	Saroj
2.	Rajendra Nilam	13.	Gautam
3.	Rajendra Saraswati	14.	Sita
4.	Rajendra Kasturi	15.	Sahbhagi Dhan
5.	Rajendra Suwasini	16.	Pooja
6.	Rajendra Sweta	17.	Swarna sub-1
7.	Rajendra Mahsuri 1	18.	BPT5204
8.	Prabhat	19.	Kalanamak
9.	Richharia	20.	Dinesh

10.	Turanta	21.	Dular
11.	Dhanlaxmi	22.	Sabour Surbhit

Table 2: DUS Guidelines based on DRR, Hyderabad, India.

Sl. No.	Characteristics	States
1.	Coleoptile color	Colorless Green Purple
2.	Basal leaf Sheath color	Green Light purple Uniform purple
3.	Leaf Pubescence of the blade surface	Absent Weak Medium Strong
4.	Culm attitude	Erect Semi–erect Open Spreading
5.	Flag leaf attitude of the blade (early observation)	Erect Semi-erect Horizontal Drooping
6.	Spikelet density of pubescence of lemma	Absent Weak Medium Strong Very strong
7.	Lemma anthocyanin coloration of keel	Absent or very weak Weak Medium Strong Very strong
8.	Lemma anthocyanin coloration of area below apex	Absent Weak Medium Strong Very strong
9.	Spikelet color of stigma	White Light green Yellow Light purple Purple
10.	Stem anthocyanin coloration of internodes	Absent Present
11.	Flag leaf attitude of blade (late observation)	Erect Semi-erect Horizontal Deflexed
12.	Panicle curvature of main axis	Straight Semi-straight Deflexed Drooping
13.	Spikelet color of tip of lemma	White Yellowish Brown Red Purple Black
14.	Lemma and palea color	Straw Gold and gold furrows on straw background Brown spots on straw Brown (tawny) Reddish to light purple Purple spots /furrows on straw Purple Black
15.	Panicle color of awns (late observation)	Yellowish white Yellowish brown Brown Reddish brown Light red Red Light purple Purple Black
16.	Panicle length of awns	Very short Short Medium Long Very long
17.	Panicle distribution of awns	Tip only Upper half only Whole length
18.	Leaf senescence	Early Medium Late
19.	Sterile lemma color	Straw Gold Red Purple

20.	Panicle exertion	Partly exerted Mostly exerted Well exerted
21.	Decorticated grain aroma	Absent Present
22.	Leaf intensity of green color	Light Medium Dark
23.	Leaf auricles	Absent Present
24.	Leaf anthocyanin coloration of auricles	Colorless `Light purple Purple
25.	Leaf collar	Absent Present
26.	Leaf ligule	Absent Present
27.	Leaf shape of ligule	Truncate Acute Split
28.	Leaf length of blade	Short (<30 cm) Medium (30-45cm) Long (>45cm)
29.	Leaf collar color	Absent Present
30.	Stem intensity of anthocyanin coloration of nodes	Weak Medium Strong
31.	Decorticated grain color	Absent Present
32.	Panicle awns	Absent Present
33.	Panicle presence of secondary branching	Absent Present
34.	Decorticated grain length	Narrow Medium Broad

Table 3: List of the 15 SSR markers used for DNA amplifications

S.No	PRIMER	Forward Sequence	Reverse Sequence	Annealing temp.	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	NA	NA
12.	MRG2805.HAU 2805	AGAGGAAGAAGCCAAGGAGG	CATCAACGTACCAACCATGG	55ºC	NA	NA
13.	RM321	CCAACACTGCCACTCTGTTC	GAGGATGGACACCTTGATCG	55ºC	200	(CAT)5
14.	RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	55ºC	110	(GA)16
15.	RM70	GTGGACTTCATTTCAACTCG	GATGTATAAGATAGTCCC	55ºC	130	(ATT)33

Character / Varieties	Coleoptile color	Basal leaf sheath color	Leaf pubescence of blade surface	Culm attitude	Flage leaf attitude of blade (early observ.)	Spikelet density of pubescence of lemma	Lemma anthocyanin coloration of keel	Lemma anthocyanin coloration of area below apex	Skiplet color of stigma	Stem anthocyanin coloration of internodes	Flag leaf attitude of blade (late observation)	Panicle curvature of main axis
Rajendra Bhagwati	colorless	green	weak	erect	Semi-erect	weak	weak	absent	yellow	absent	Semi-erect	Semi-straight
Rajendra Nilam	colorless	Light purple	weak	erect	erect	medium	Absent or very weak	absent	purple	present	Semi-erect	straight
Rajendra Saraswati	colorless	green	weak	erect	erect	weak	Absent or very weak	absent	white	absent	Semi-erect	Semi-straight
Rajendra Kasturi	colorless	green	medium	erect	erect	weak	weak	absent	white	absent	Semi - erect	Semi-straight
Rajendra Suwashini	colorless	green	medium	erect	erect	weak	Absent or very weak	absent	white	absent	Semi-erect	Semi-straight
Rajendra Shweta	colorless	green	weak	erect	erect	medium	Absent or very weak	absent	white	absent	Semi-erect	straight
Rajendra Mahsuri	colorless	green	medium	erect	erect	weak	Absent or very weak	weak	white	absent	Semi-erect	Semi-straight
Prabhat	colorless	green	medium	erect	horizontal	weak	Absent or very weak	Absent	white	absent	Semi-erect	straight
Richharia	colorless	green	medium	erect	horizontal	weak	Absent or very weak	absent	white	absent	horizontal	straight
Turanta	colorless	green	absent	erect	erect	medium	weak	absent	white	absent	Semi-erect	straight
Dhanlaxmi	colorless	green	weak	erect	erect	medium	Absent or very weak	absent	white	absent	erect	straight
Saroj	colorless	green	weak	erect	erect	weak	Absent or very weak	weak	white	absent	erect	Straight
Gautam	colorless	Light purple	weak	erect	erect	weak	Absent or very weak	weak	purple	absent	erect	Semi-straight
Sita	colorless	green	medium	erect	erect	medium	Absent or very weak	weak	white	absent	Semi-erect	Semi-straight
sahbhagi	colorless	green	absent	Semi-erect	Semi-erect	weak	medium	weak	white	absent	Semi-erect	Semi-straight
Pooja	green	green	medium	erect	horizontal	weak	Absent or very weak	absent	white	absent	Semi-erect	Semi-straight
Swarna sub-1	colorless	green	medium	erect	erect	medium	Absent or very weak	weak	white	absent	Semi-erect	Straight
BPT-5204	colorless	green	weak	erect	erect	weak	Absent or very	absent	Light green	absent	Semi-erect	Straight

							weak					
Kalanamak	green	green	medium	Semi-erect	horizontal	strong	strong	strong	Light purple	absent	erect	Semi-straight
Dinesh	colorless	green	weak	erect	erect	medium	Absent or very weak	absent	Light green	absent	erect	Straight
Dular	green	Light purple	weak	erect	erect	medium	weak	absent	purple	present	Semi-erect	Semi-straight
S.Surbhi	colorless	green	strong	erect	Semi-erect	medium	weak	absent	white	absent	Semi-erect	Semi-straight

Table 4: Qualitative morphological trait variation among 22 rice varieties

Character / Varieties	**Spikelet color of tip of lemma**	**Lemma and palea color**	**Panicle color of awns(late observ.)**	**Panicle length of awns**	**Decorticated grain width**	**Panicle distribution of awns**	**Leaf senescence**	**Sterile lemma color**	**Panicle exertion**
Rajendra Bhagwati	yellowish	straw	Yellowish white	Very short	medium	Tip only	medium	straw	Well exerted
Rajendra Nilam	Purple	straw	-	-	medium	-	medium	straw	Well exerted
Rajendra Saraswati	White	straw	Yellowish white	short	narrow	-	medium	straw	Well exerted
Rajendra Kasturi	White	straw	-	-	narrow	-	medium	straw	Well exerted
Rajendra Suwashini	White	straw	-	-	narrow	-	medium	straw	Well exerted
Rajendra Shweta	yellowish	straw	-	-	narrow	-	medium	straw	Mostly exerted
Rajendra Mahsuri	yellowish	straw	-	-	narrow	-	medium	straw	Mostly exerted
Prabhat	yellowish	straw	-	-	narrow	-	early	straw	Well exerted
Richharia	yellowish	Brown (tawny)	-	-	medium	-	medium	straw	Well exerted
Turanta	Brown	straw	-	-	broad	-	medium	straw	Well exerted
Dhanlaxmi	yellowish	straw	-	-	narrow	-	late	straw	Well exerted
Saroj	yellowish	Brown spot on straw	-	-	narrow	-	early	straw	Well exerted
Gautam	Brown	Brown spots on straw	-	-	narrow	-	medium	straw	Well exerted
Sita	yellowish	straw	Yellowish white	short	medium	Tip only	medium	straw	Well exerted
sahbhagi	yellowish	straw	-	-	medium	-	early	straw	Well exerted
Pooja	yellowish	straw	Yellowish white	short	medium	Tip only	medium	straw	Well exerted
Swarna sub-1	yellowish	straw	-	-	narrow	-	late	straw	Well exerted
BPT-5204	yellowish	straw	-	-	narrow	-	late	straw	Mostly exerted
Kalanama	Black	black	-	-	narrow	-	medium	purple	Well

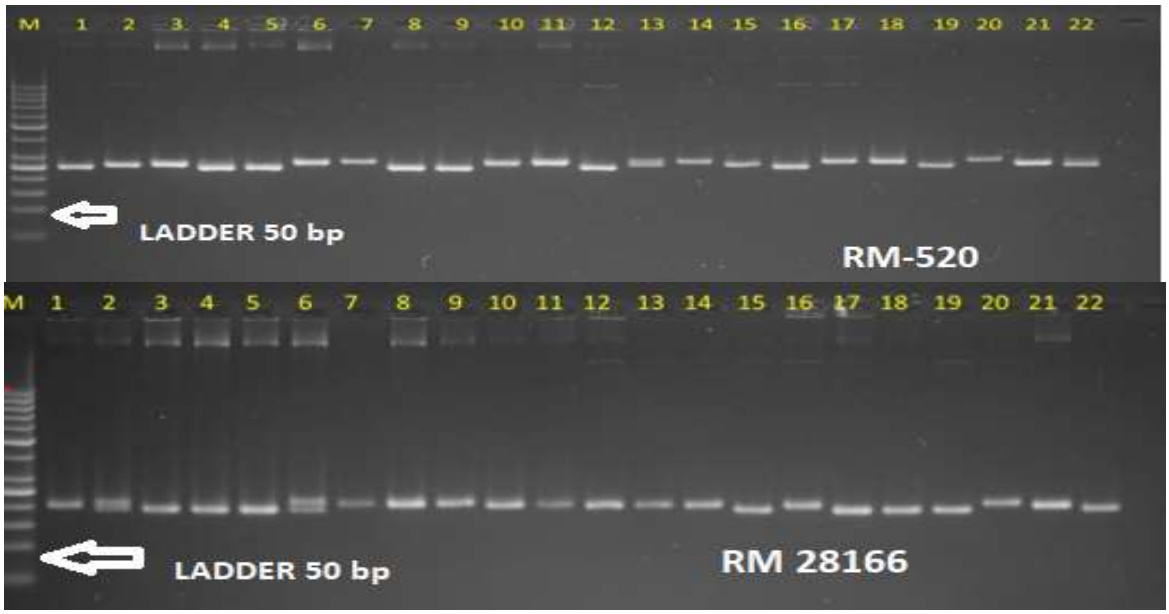
k									exerted
Dinesh	yellowish	straw	-	-	medium	-	medium	straw	Mostly exerted
Dular	Brown	Brown (tawny)	-	-	medium	-	medium	straw	Well exerted
S.Surbhi	yellowish	straw	-	-	narrow	-	medium	straw	Well exerted

Character / Varieties	**Leaf auricles**	**Leaf anthocyanin coloration of auricles**	**Leaf collar**	**Leaf ligule**	**Decorticated grain color**	**Leaf shape of ligule**	**Leaf length of blade**	**Decorticated grain aroma**	**Leaf intensity of green color**	**Leaf collar color**	**Stem anthocyanin coloration of node**	**Panicle awns**	**Panicle presence of secondary branching**
Rajendra Bhagwati	present	colorless	present	present	white	split	long	present	light	absent	weak	present	present
Rajendra Nilam	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
Rajendra Saraswati	present	colorless	present	present	white	split	long	absent	medium	absent	weak	present	present
Rajendra Kasturi	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
Rajendra Suwashini	present	colorless	present	present	white	split	medium	absent	light	absent	weak	absent	present
Rajendra Sweta	present	colorless	present	present	white	split	short	absent	medium	absent	weak	absent	present
Rajendra Mahsuri 1	present	colorless	present	present	white	split	medium	absent	light	absent	weak	absent	present
Prabhat	present	colorless	present	present	white	split	medium	absent	dark	absent	weak	absent	present
Richharia	present	colorless	present	present	white	split	medium	absent	dark	absent	weak	absent	present
Turanta	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
Dhanlaxmi	present	colorless	present	present	white	split	long	absent	medium	absent	weak	absent	present
Saroj	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
Gautam	present	colorless	present	present	white	split	long	absent	medium	absent	weak	absent	present
Sita	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	present	present
Sahbhagi	present	colorless	present	present	white	split	long	absent	medium	absent	weak	absent	present
Pooja	present	colorless	present	present	white	split	long	absent	medium	absent	weak	present	present
Swarna sub-1	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
BPT 5204	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present
Kalanamak	present	colorless	present	present	white	split	medium	present	medium	absent	weak	absent	present
Dinesh	present	colorless	present	present	white	split	short	absent	medium	absent	weak	absent	present
Dular	present	colorless	present	present	white	split	long	absent	medium	absent	weak	absent	present
S.Surbhi	present	colorless	present	present	white	split	medium	absent	medium	absent	weak	absent	present

Table 5: SSR markers with their number of alleles, no. of unique alleles, percentage of unique alleles, polymorphism information content (PIC), and no. of entries having null alleles in the 22 rice cultivars.

Primers	Size of alleles (bp)	No. of alleles	No. of unique alleles	Percentage of unique alleles	PIC	No. of entries having null alleles
RM566	228-277	8	0	0	0.828	0
RM520	248-291	11	4	36.37	0.896	0
RM555	232-288	11	6	54.54	0.853	0
RM324	108-184	11	6	54.54	0.878	1
RM521	257-356	16	10	62.50	0.816	0
RM431	269-338	10	2	20.00	0.879	0
RM28166	191-218	8	3	37.50	0.771	0
RM5791	89-439	19	8	42.10	0.710	1
RM319	123-136	5	1	20.00	0.739	0
RM416	101-114	5	0	0	0.789	1
MRG 2894 IRRI 2894	167-189	7	1	14.28	0.843	0
MRG2805.HAU 2805	60-131	7	3	42.85	0.917	1
RM321	200-216	5	0	0	0.775	1
RM286	96-125	7	0	0	0.791	1
RM70	129-214	10	4	40.00	0.819	0

Fig 1: PCR amplification of 22 rice varieties with SSR markers RM520 & RM28166



1.	Rajendra Bhagwati	9.	Richharia	17.	Swarna Sub-1
2.	Rajendra Nilam	10.	Turanta	18.	BPT-5204
3.	Rajendra Saraswati	11.	Dhanlaxmi	19.	Kalanamak

4.	Rajendra Kasturi	12.	Saroj	20.	Dinesh
5.	Rajendra Suwashini	13.	Gautam	21.	Dular
6.	Rajendra Shweta	14.	Sita	22.	Sabour Surabhi
7.	Rajendra Mahsuri	15.	Sahbhagi		
8.	Prabhat	16.	Pooja		

Fig 2: Dendogram depicting the classification of 22 rice varieties constructed through UPGMA method and based on SSR markers

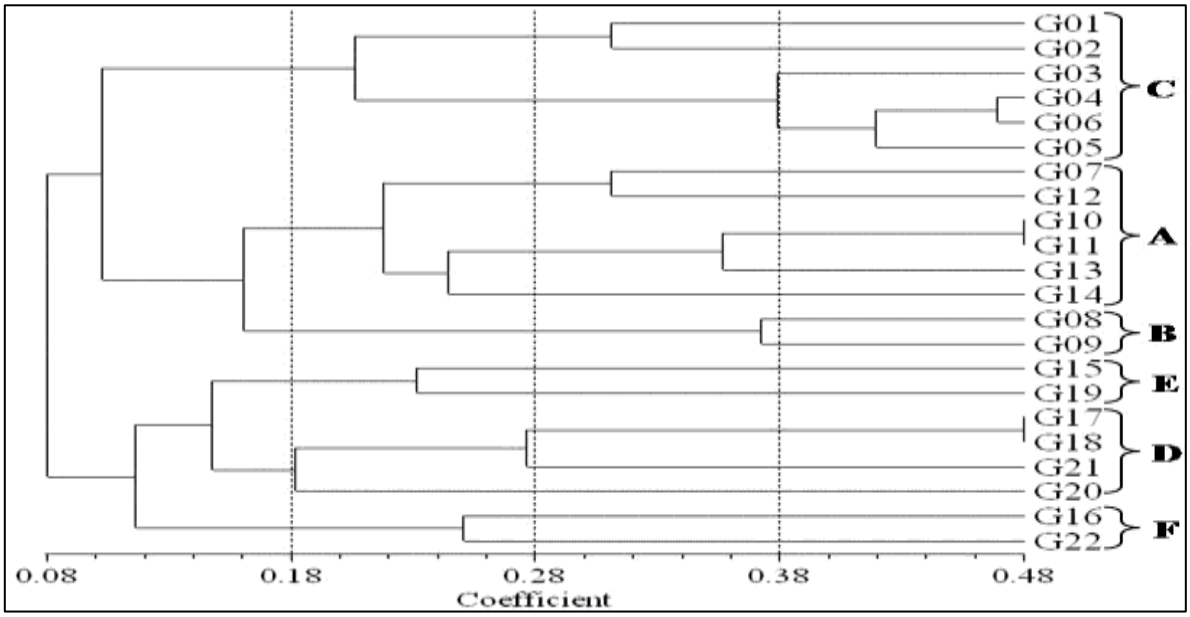


Table 6: Estimates of fifteen SSR primer pairs based similarity coefficients among twenty-two rice varieties used in the present study

Entries	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.313	0.258	0.182	0.276	0.343	0.250	0.194	0.000	0.133	0.061	0.067	0.063	0.129	0.125	0.125	0.063	0.065	0.000	0.000	0.258	0.000
2		0.121	0.171	0.129	0.162	0.059	0.182	0.061	0.125	0.057	0.000	0.059	0.061	0.059	0.000	0.118	0.182	0.000	0.059	0.061	0.063
3			0.412	0.400	0.333	0.121	0.125	0.000	0.065	0.000	0.000	0.061	0.125	0.121	0.000	0.061	0.063	0.080	0.061	0.063	0.129
4				0.375	0.474	0.171	0.177	0.118	0.121	0.056	0.242	0.114	0.059	0.114	0.000	0.057	0.059	0.074	0.057	0.059	0.000
5					0.471	0.129	0.333	0.067	0.000	0.000	0.000	0.065	0.067	0.065	0.000	0.000	0.000	0.087	0.065	0.000	0.000
6						0.216	0.167	0.111	0.114	0.053	0.229	0.054	0.111	0.108	0.000	0.108	0.056	0.069	0.108	0.167	0.000
7							0.182	0.182	0.250	0.114	0.313	0.177	0.182	0.059	0.118	0.118	0.061	0.077	0.118	0.121	0.063
8								0.375	0.129	0.235	0.065	0.121	0.063	0.000	0.061	0.061	0.063	0.080	0.000	0.063	0.000
9									0.129	0.353	0.258	0.182	0.000	0.000	0.061	0.121	0.125	0.080	0.061	0.188	0.000
10										0.485	0.267	0.375	0.194	0.188	0.063	0.063	0.000	0.000	0.063	0.129	0.000
11											0.242	0.343	0.235	0.229	0.057	0.057	0.059	0.000	0.114	0.177	0.000
12												0.313	0.194	0.188	0.125	0.063	0.000	0.000	0.125	0.194	0.000
13													0.303	0.235	0.177	0.118	0.121	0.154	0.059	0.061	0.125

14														0.242	0.242	0.121	0.063	0.000	0.182	0.063	0.000
15															0.177	0.177	0.061	0.231	0.177	0.121	0.000
16																0.118	0.182	0.154	0.118	0.121	0.250
17																	0.485	0.231	0.177	0.303	0.063
18																			0.121	0.250	0.129
19																			0.077	0.080	0.167
20																				0.242	0.000
21																					0.129