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# Morpho-molecular Genetic Diversity Analysis of Basmati and Non-basmati Rice (*Oryza sativa* L.) Germplasm

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

This study evaluated genetic variability and relatedness among 34 rice genotypes, encompassing basmati and non-basmati varieties using morphological and molecular data. The findings revealed significant variability across all traits assessed. High phenotypic and genotypic coefficients of variation (PCV and GCV) were observed for traits such as days to 50% flowering, days to maturity, biological yield, grain yield, test weight, flag leaf length, and number of effective tillers. Notably, high heritability and genetic advance indicated that these traits are likely influenced by additive gene effects, making them suitable for selection in breeding programs. $D^2$ analysis confirmed substantial genetic diversity among the genotypes and five clusters were formed based on the morphological data. Molecular analysis using 13 SSR primers, out of 13 primers 11 primers are showed the banding pattern, with the highest polymorphism information content (PIC) value recorded at 0.79 for

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marker HvSSR04-46. Clustering based on Jaccard's dissimilarity coefficient revealed two distinct clusters. Based on the dendrogram pattern molecular data is divided into two clusters, cluster I and Cluster II, cluster I is subdivided into Cluster Ia and Cluster Ib. This research was conducted at Sardar Vallabhbhai Patel University of Agricultural & Technology, Modipuram, Meerut, Uttar Pradesh, highlights the potential for selecting diverse rice genotypes to enhance breeding programs.

*Keywords: Heritability; polymorphism; markers; genetic diversity.*

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the grass family (Poaceae) and is an edible starchy grain. it is the most important cereal crop which serves as a staple food for over 60% of the world's population. Asia produces and consumes roughly 90% of the world's rice (Singh *et al.,* 2015). India's slogan "Rice is life" is fitting because rice plays a significant role in Indian agriculture, contributing 15% to the annual GDP and meeting 43% of the country's calorie needs (Akter et al., 2001). According to the Food and Agricultural Organization (FAO) the United Nations if the population reaches 9.1 billion by 2050, the world's food production will need to rise by 70%. the use of semi-dwarf varieties and intensive input-based management techniques, rice production has increased significantly. Therefore, genetic enhancement for better yield seems the most important option. Rice is one of the most diverse cereal crop due to its adaptation to a wide range of geographical, ecological, and climatic regions. India has a remarkable diversity of rice cultivars, landraces, wild and weedy relatives. Rice germplasm is a rich reservoir of valuable genes that plant breeders can use for crop improvement. The two major rice species grown worldwide today are indica and japonica. The two cultivated rice species, *Oryza sativa* L. and *O. glaberrima* Steud., belong to a species group called the *Oryza sativa* complex together with the five wild taxa namely *O. rufipogon, O. longistaminat, O. barthii, O. glumaepatula* and *O. meridionalis.* Among these taxa only *O. rufipogon* produces fertile $F_1$ hybrids with *O. sativa* and therefore these two species are considered to belong to a single biological species. Together with all the circumstantial evidence, this suggests that *O. rufipogon* is the ancestor of *O. sativa*. Similarly, *O. barthii* is the ancestor of african rice i.e., *O. glaberrima*.

The success of a breeding programme is determined by the amount of genetic variability available for exploitation and the degree to which desirable characteristics are heritable (Dhanwani *et al.,* 2013). The term "variability" describes the tendency of individual genetic characteristics in a population to differ from one another. Variability is necessary for the development of genetic material when there is a significant amount of genetic variation among the breeding material's selection is effective (Banumathy *et al.,* 2010). Hybrid rice technology is one of such options to increase the rice yield beyond expectations which has been proven in China, now most of the rice growing area is under hybrids. It has been reported that hybrid rice yields 15-20% higher than the high-yielding varieties available in the same maturity group (Virmani 2003). In India, more than 70 rice hybrids have been released to date which cover around 6.0% of the total rice grown area out of these hybrids more than 50% are from the private sector (Katara *et al.,* 2017) and are preferred by the farmers of Central and North-Eastern states where the next green revolution is expected.

The $D^2$ analysis is a technique developed by Mahalanobis in 1936. It is used to evaluate the heritable diversity in rice breeding based on morphological, genetic, and physiological characteristics (Bhanu, 2010). There are several methods for estimating the diversity of germplasm including the analysis of phenotypic variation, biochemical analysis, and DNA polymorphisms. Phenotypic and biochemical characterizations are both unreliable due to their labor-intensive nature, environmental restrictions, and numerical & phenological limitations. On the other hand, DNA-based molecular markers are pervasive, repeatable, and extremely reliable (Ford-Lloyd *et al.,* 1997; Virk *et al.,* 2000; Song *et al.,* 2003).

The study aimed to evaluate genetic variability and relatedness among basmati and non-basmati rice varieties using morphological and molecular data. This will help identify diverse genotypes for future rice breeding programs.

## 2. MATERIALS AND METHODS

The field experiment titled "Genetic Diversity Analysis of Basmati and Non-Basmati Rice

(*Oryza sativa* L.) Germplasm" was conducted during the Kharif 2022 at the Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut–250110 (U.P.). Meerut is located at 29.01 degrees latitude in the north and 77.45 degrees longitude, at an altitude of 277m above mean sea level, representing the North-Western Plain Zone. The soil of the experimental field is fertile sandy loam. The experimental material consists of 34 rice genotypes for the present study. The names of 34 genotypes are mentioned below in Table 1. The seeds of all varieties were sown at the Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture & Technology (FAOSTAT, 2019) 34 genotypes were transplanted in a randomized complete block design in three replications with specific row and plant spacing (Cho, 2010). Necessary agronomical practices and plant protection measures were adopted for a good crop (Fazal et al., 2023). In this study, I have taken the field observations recorded on ten quantitative traits viz., 50% flowering, days to maturity, Plant height (cm), panicle length (cm), number of effective tillers per hill, inter-node length (cm), grain yield per plot (kg), biological yield per plot (kg), harvest index, and test weight (g), The calculated mean value for the recorded data was subjected to analysis of variance.

The genetic divergence in thirty-four genotypes of rice was estimated using the Mahalanobis $D^2$ statistic (1936) following Rao (1952). Inter and intra-cluster distances were calculated by Tocher's method as suggested by Rao (1952) to form the clusters (Garg *et al.,* 2011). This criterion was based on the concept that the degree of diversity can be measured by the distance between two clusters. The divergence increases with increasing distance between two clusters and *vice versa.*

## 2.1 DNA isolation

The genomic DNA was extracted from young leaves of 15–20 days-old rice seedlings and stored at -20°C for further processing. The DNA was extracted by using the CTAB extraction method as suggested by Doyle and Doyle in 1987, with a few modifications made to the DNA extraction buffer. CTAB is a cationic detergent that helps dissolve membranes and forms a complex with DNA. After disrupting the cells and incubating with hot CTAB isolation buffer, proteins are extracted using chloroform and isoamyl alcohol. The CTAB-DNA complex is then precipitated with isopropanol. The resulting DNA pellet, after centrifugation, is washed, dried, and redissolved. Treatment with RNase removes RNA and some polysaccharides.

### 2.1.1 Protocol for DNA extraction

- 300 mg frozen leaves of each rice genotype were crushed in liquid nitrogen using a mortar and pestle. Then, 1 ml of pre-warmed (65°C) extraction buffer was added, and the mixture was transferred into Eppendorf tubes. The samples were homogenized by gently inverting the tubes several times.

**Table 1. List of 34 Genotypes used in the study**

| S.no | Varieties | Type | S.no | Varieties | Type |
|---|---|---|---|---|---|
| 1 | Basmati -386 | Basmati | 18 | Jalmagna | Non-Basmati |
| 2 | Basmati-370 | Basmati | 19 | Anandi Dhan | Non-Basmati |
| 3 | Basmati-564 | Basmati | 20 | Tdk-1 | Non-Basmati |
| 4 | PB-1728 | Basmati | 21 | Pat Gc-203 | Non-Basmati |
| 5 | Punjab Basmati -3 | Basmati | 22 | Mirchia Dhan | Non-Basmati |
| 6 | Malviya Basmati | Basmati | 23 | Fr-13a | Non-Basmati |
| 7 | Pant Basmati-2 | Basmati | 24 | Bpt-5204 | Non-Basmati |
| 8 | PB-1637 | Basmati | 25 | S.Shree | Non-Basmati |
| 9 | VB-22 | Basmati | 26 | Tkm-6 | Non-Basmati |
| 10 | VB-23 | Basmati | 27 | Kbanku | Non-Basmati |
| 11 | VB-24 | Basmati | 28 | Canaceda | Non-Basmati |
| 12 | HR-1 | Basmati | 29 | Crassa Purple | Non-Basmati |
| 13 | HR-2 | Basmati | 30 | Pokkali | Non-Basmati |
| 14 | Type-3 | Basmati | 31 | Lal Sita | Non-Basmati |
| 15 | Pusa Basmati-1509 | Basmati | 32 | Kala B | Non-Basmati |
| 16 | Pusa Basmati-1 | Basmati | 33 | Kalanamak | Non-Basmati |
| 17 | Pusa Basmati-1121 | Basmati | 34 | Badshabahog | Non-Basmati |

- The samples in the Eppendorf tubes were incubated for 1 hour in a shaking water bath at 65°C
- Afterward, the tubes were allowed to cool at room temperature, and then 800µl of chloroform: iso-amyl alcohol (24:1) was added. The mixture was gently and properly mixed by inverting the centrifuge tubes for about 15-20 minutes
- Centrifuge the samples at 10,000 rpm for 10 minutes at 4°C.
- After centrifugation, the aqueous phase (supernatant) was transferred to fresh Eppendorf.
- The supernatant was mixed with 3-5 µl of (10 mg/ml) RNase and incubated for 30 minutes.
- After incubation, 600 µl of isopropanol was added and mixed gently for 3-5mins.
- Eppendorf tubes were centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was washed with 70% ethanol and dried.
- Finally, the pellet was re-suspended in 100 µl of TE buffer and stored at 4°C for immediate use.

## 2.2 Quantification of Genomic DNA by Spectrophotometer

The genomic DNA dissolved in TE buffer was used for quantification by ultraviolet (UV) absorbance at 260 nm. To measure the concentration, a BIORAD Smart Tech spectrophotometer was used. Reference was set against TE, and then, after thorough rinsing of the quartz cuvette, the absorbance of the sample was measured at 260 nm and 280 nm. The ratio of optical density (OD) 260/280 provides an estimate of nucleic acid purity. According to the standard, a properly purified DNA sample has a ratio between 1.8 and 2.0, and the concentration in g/ml was calculated as 1 OD at 260 nm is equivalent to 50 g/ml of double-stranded DNA (Sambrook et al., 1989). If a DNA sample is free of contamination from protein, phenol, or RNA, its concentration can be measured accurately by determining the amount of UV radiation that is absorbed by the bases present in an aliquot of the sample.

### 2.2.1 Protocol for DNA quantification by Spectrophotometer

- Make sure that the spectrophotometer's UV light source has been turned on and allow it to warm up for at least 10 minutes before use.
- Pipette 3000 µl of TE into a cuvette (path length: 10mm) to be used.
- Set a spectrophotometer for DNA measurement, then wavelength to be measured for 260 nm and 280 nm. Follow the instructions in the instrument manual. Insert cuvette.
- Zero spectrophotometer using cuvette containing TE as a blank, then set reference.
- Remove TE from the blank cuvette with a Pasteur pipette.
- Pipette 3ul of each sample into different 200µl PCR tube.
- Pipette 3 ml of TE into 4 ml cuvette and 3µl of the sample was added. Mix solution by pipetting up and down.
- The cuvette containing the sample was in the spectrophotometer, and $OD_{260}$ and $OD_{280}$ of each sample were recorded.
- The cuvette was rinsed several times using double distilled water or rinsed with a cuvette washing device. Replace the cuvette in the holder. The same process was repeated to record all samples' OD.

## 2.3 Semi - Quantification and Quality Analysis by Gel-Electrophoresis

The isolated genomic DNA was analyzed using agarose gel electrophoresis to assess its quality. In this technique, larger DNA molecules move more slowly through the gel due to greater frictional drag and less efficient passage through the pores. To visualize the large genomic DNA molecules, a 0.8% gel was used, capable of resolving DNA molecules in the range of 0.7 to 8.5 kb.

## 2.4 Polymerase Chain Reaction

DNA amplification of SSR was performed in a total volume of 25 µl. The following components were gently mixed in 0.2 ml thin-walled PCR tubes Taq Buffer 10x with $MgCl_2$ 2.5µl, 2.5mM each dNTP Mix 0.5µl, Primer (5 µm) Forward 1 µl, Reverse 1µl, Taq Polymerase (1U/µl) 1µl, DNA (25ng/µl) 1µl, Water (Milli Pore) 18 µl. Tubes were transferred to the DNA thermal cycler. Amplification reactions were performed in BIO-RAD MyCyclerTM Thermal cycler with the following thermal profile mentioned. Initial denaturation $95^0$C for 5 Min, denaturation $95^0$C for 1min, annealing

(based on the primer $T_m$ temperature) for 1 min, extension $72^0$C for 2 min, and total 35 PCR cycles.

## 2.5 Preparation of 2% (w/v) Agarose Gel for Amplified Products

Weighed 10 grams of agarose powder and suspended it in 500 ml of 1X TAE buffer. After heating the solution and adding 25 µl of ethidium bromide, I cast the gel in a casting tray, created wells, allowed it to solidify, and then placed it in the electrophoresis chamber filled with 1X TAE buffer. loaded the DNA samples mixed with 3X loading dye into the well and performed gel electrophoresis in 1x TAE buffer at 3-5 volts/cm for 2-3 hours.

## 2.6 Data Analysis

After completion of gel electrophoresis was photographed using a CCD camera attached to a gel documentation system with Quantity One software (Alpha Infotech). Scoring was done manually for each of the gel sections, and alleles were determined based on the positions of the bands (Gediya , 2019). Banding patterns for each of the microsatellite markers were recorded for each genotype by assigning a letter to each band (Jasim et al., 2018). Using various commercially synthesized DNA ladders (Bangalore Genei, Bangalore) respective allele sizes were estimated and alleles were numbered as ‘a1’, ‘a2," etc. In the data matrix, the presence of a band was represented by ‘1’ and ‘0’ for absence (Kaur *et al.,* 2021, Rezk *et al.,* 2024). This binary data matrix was then utilized to generate similarity data among genotypes. Only unambiguous bands were scored for the estimation of genetic similarity between the varieties using Jaccard’s similarity coefficient. Based on these data, UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering was carried out by applying the software NTSYS-pc (Rohlf, 1997).

## 2.7 Polymorphic Information Content (PIC)

PIC value is used to estimate the discriminatory power of the SSR marker. The formula for PIC calculation for SSR markers:

$$PIC = 1 - \Sigma pi^2$$

Where, pi stands for the frequency of $i^{th}$ allele and summation extends over n patterns.

## 3. RESULTS AND DISCUSSION

Analysis of variance (ANOVA) based on the mean values of ten quantitative traits in thirty-four rice genotypes recorded significant differences among the genotypes with respect to all the characters considered for the study (Table 2). This suggests that there is an inherent genetic difference among the genotypes for the traits studied.

The coefficient of variation is a crucial tool for comparing the variability found in various traits. For most of the traits in the genotypes examined a large range of variance was seen indicating that selection made with these characters is anticipated to be successful. The estimates of phenotypic and genotypic coefficient of variation for different quantitative characters of 34 genotypes are presented in Table 3. The high estimates of the genotypic coefficient of variation for quantitative traits related to yield were observed for days to 50% flowering (GCV % = 16.47) and the highest phenotypic coefficient of variation was observed in Flag leaf length (PCV % = 17.908).

Heritability estimates help in identifying genetic improvement and selection response by indicating how much genetic variation is transmissible relative to total variation. In the present investigation, heritability in the broad sense and genetic advance were calculated for all quantitative characters under study and is presented in Table 3. High estimates of heritability were found for most of the quantitative characters namely days to 50% flowering (99.7%), days to maturity (99.5%), plant height (84.4%), flag leaf length (81.2%), internode length (92%), biological yield (88.4%), Test weight (91.1%), Grain yield (88.9%), and panicle length (66.6%) recorded.

High heritability coupled with high GA indicates that most likely the heritability is due to the additive gene effect and selection may be effective for characters such as days to 50% flowering, days to maturity, plant height, flag leaf length, internode length, biological yield, 1000 grain weight, and grain yield.

**Table 2. Analysis of Variance For 11 Characters in Rice (O*ryza Sativa* L.)**

| Source of variation | Replication | Treatment | Error |
|---|---|---|---|
| Df | 2 | 33 | 66 |
| Days to 50% flowering | 4.971 | 941.70** | 0.809 |
| Days to maturity | 1.412 | 932.77** | 1.311 |
| Plant height (cm) | 96.436 | 726.57** | 42.025 |
| Flag leaf length (cm) | 205.28 | 49.25** | 3.515 |
| Internode length (cm) | 0.201 | 14.971** | 0.420 |
| Number of effective tillers /hill | 6.707 | 3.784** | 0.707 |
| Panicle length (cm) | 3.024 | 16.556** | 2.369 |
| Biological yield (kg) | 0.010 | 0.162** | 0.007 |
| Harvest Index | 27.769 | 34.494** | 15.792 |
| Test Weight | 0.393 | 22.315** | 0.653 |
| Grain Yield | 0.005 | 0.052** | 0.002 |

***Significant at 1% Level*

**Table 3. Mean, Range, GCV, PCV, heritability, and Genetic Advance for 11 characters in rice**

| | Min | Max | Mean | Heritability % | GA | GA% Mean | GCV | PCV |
|---|---|---|---|---|---|---|---|---|
| **DFF** | 80.3 | 139.0 | 107.5 | 99.743 | 36.435 | 33.893 | 16.474 | 16.495 |
| **DTM** | 112.3 | 171.0 | 138.9 | 99.58 | 36.222 | 26.087 | 12.69 | 12.717 |
| **PH** | 68.1 | 131.5 | 101.9 | 84.447 | 28.596 | 28.057 | 14.821 | 16.128 |
| **FL** | 19.5 | 35.0 | 24.6 | 81.265 | 7.251 | 29.979 | 16.144 | 17.908 |
| **IL** | 12.5 | 21.1 | 15.9 | 92.035 | 4.352 | 27.297 | 13.812 | 14.398 |
| **EF** | 6.7 | 11.9 | 8.5 | 59.212 | 1.606 | 18.823 | 11.875 | 15.432 |
| **PL** | 19.1 | 29.2 | 23.4 | 66.629 | 3.657 | 15.649 | 9.307 | 11.401 |
| **BY** | 1.2 | 2.1 | 1.6 | 88.406 | 0.441 | 27.54 | 14.219 | 15.122 |
| **HI** | 45.5 | 57.9 | 51.8 | 28.303 | 2.736 | 5.281 | 4.818 | 9.057 |
| **TW** | 16.5 | 27.4 | 22.2 | 91.701 | 5.301 | 23.876 | 12.103 | 12.639 |
| **GY** | 0.6 | 1.1 | 0.8 | 88.908 | 0.25 | 30.307 | 15.603 | 16.548 |

*DFF-Days to 50% flowering, DTM- Days to maturity, PH- Plant height, FL- Flag leaf length, IL- Inter node length, EF- Number of effective tillers per hill, PL- Panicle length, BY- Biological Yield, HI- Harvest index, TW- Test weight, GY- Grain Yield*

**Table 4 Clustering pattern of 34 genotypes of rice based on genetic divergence**

| Clusters | No of genotypes | Varieties |
|---|---|---|
| I | 11 | VB-22, VB-23, VB-24, Punjab Basmati -3, HR-2, Basmati -386, Pusa Basmati-1, Pusa Basmati-1121, PB-1637, Basmati-564, HR-1. |
| II | 6 | PB-1728, S.Shree, Basmati-370, Canaceda, Tkm-6, BPT-5204. |
| III | 5 | Malviya Basmati, Pant Basmati-2, Tdk-1, Type-3, Pat Gc-203. |
| IV | 10 | Kala B, Kalanamak, Jalmagna, Mirchia Dhan, Badshabahog, Kbanku, Fr-13a, Lal Sita, Anandi Dhan, Pokkali. |
| V | 2 | Pusa Basmati-1509, Crassa Purple. |

**Table 5. Intra and inter-cluster distance**

| | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V |
|---|---|---|---|---|---|
| Cluster I | **10.8** | | | | |
| Cluster II | 22.5 | **13.4** | | | |
| Cluster III | 17.7 | 32.8 | **13.6** | | |
| Cluster IV | 43.4 | 26.9 | 51.8 | **15.7** | |
| Cluster V | 20.7 | 32.7 | 20.8 | 51.1 | **31.2** |

**Table 6. Molecular characterization of 34 rice genotype by 11 SSR markers**

| Marker | Primer sequence | Total alleles | Polymorphic bands | Monomorphic bands | Polomorphic percentage | PIC |
|---|---|---|---|---|---|---|
| HvSSR01-41 | TGAGTGAGACTTGACAGTGC<br>AGTTAACACCAATGCTGACC | 2.00 | 2 | 0 | 100.00 | 0.39 |
| HvSSR01-53 | TGTCGTCCACGTAGTAGGAG<br>ACACTCCTCCTCTGTTCTCA | 1.00 | 1 | 0 | 100.00 | 0.58 |
| HvSSR02-33 | TAATGCACGCACAACTTTAC<br>TATAGAATGCTGACTGGGCT | 1.00 | 1 | 0 | 100.00 | 0.99 |
| HvSSR02-50 | TTTCAGGAATCTGATGCTTT<br>TTAATCAAAGCCCTAACAGC | 1.00 | 1 | 0 | 100.00 | 0.58 |
| HvSSR03-02 | TAGCGGAGTTGGAATAACAC<br>CTGCACTGCATACCTCATAA | 1.00 | 1 | 0 | 100.00 | 0.91 |
| HvSSR03-19 | AATTCAGTTCACGCATTCTT<br>AGCTGTTCGTCTGCATAGTT | 1.00 | 1 | 0 | 100.00 | 0.99 |
| HvSSR03-54 | GCCTATCAGGCTATCATCAC<br>GTGATCGACATTGAGGAGTT | 1.00 | 1 | 0 | 100.00 | 1.00 |
| HvSSR04-19 | TCGTGGAGTATCCTGTATCC<br>TTATAACTTGGAGCTCAGGC | 1.00 | 1 | 0 | 100.00 | 0.97 |
| HvSSR04-27 | ATGGATTTAGGCTTGTTTGA<br>ATACTGCGAAGGTGAAGAGA | 1.00 | 1 | 0 | 100.00 | 0.90 |
| HvSSR04-46 | GGCGCGCTTATATATGTACT<br>CGATTGCGTGGTGTAACTAT | 1.00 | 1 | 0 | 100.00 | 0.72 |
| HvSSR05-09 | CTCTCCATCTTGCAATCTTC<br>TGCATGACTCTATCAACCAG | 1.00 | 1 | 0 | 100.00 | 0.98 |

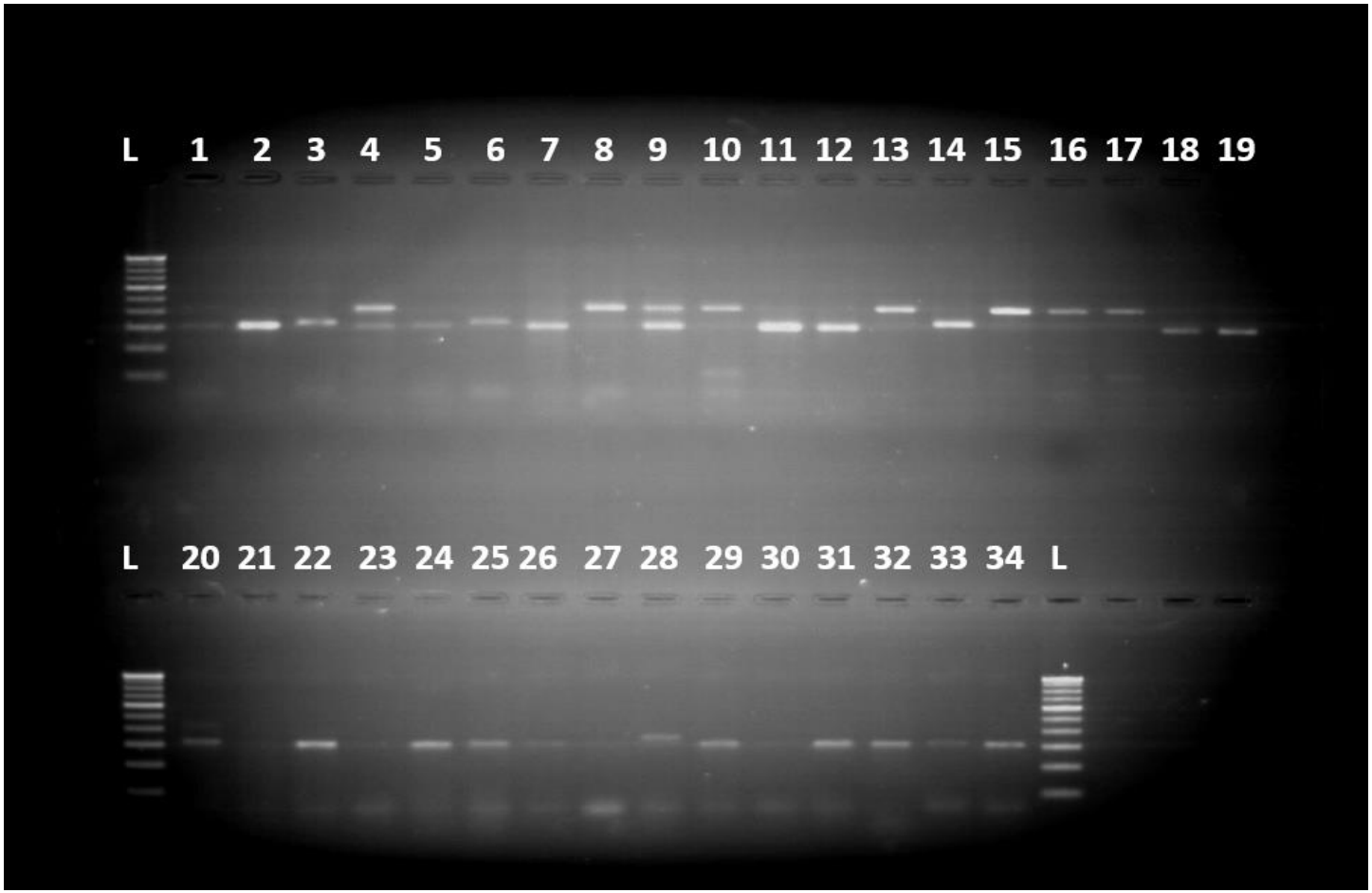


**Fig. 1. Banding profiles of the 34 rice genotypes with marker: HvSSR01-41**

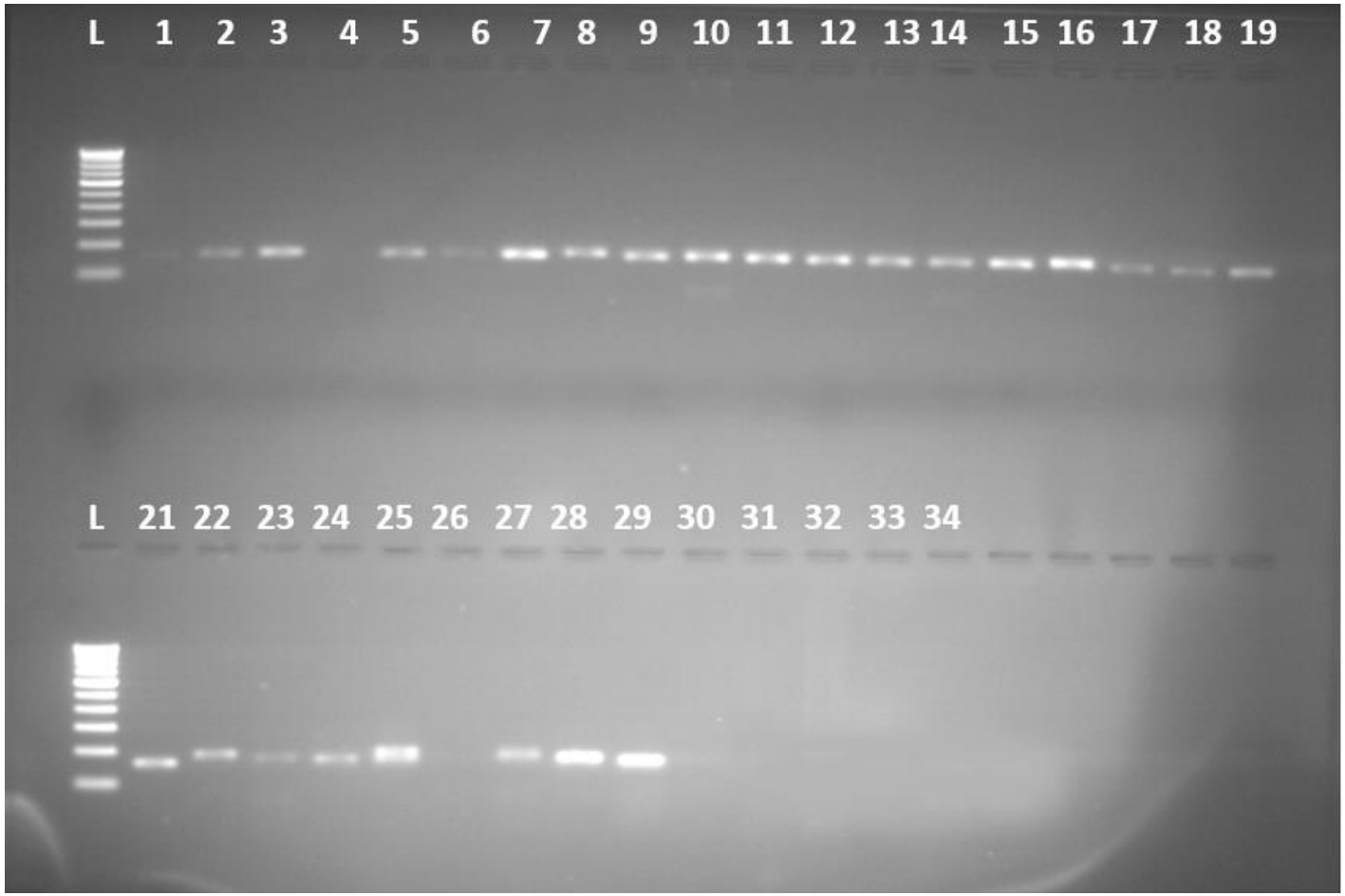


**Fig. 2. Banding Profiles of the 34 Rice Genotypes with Marker: Hvssr01-53**

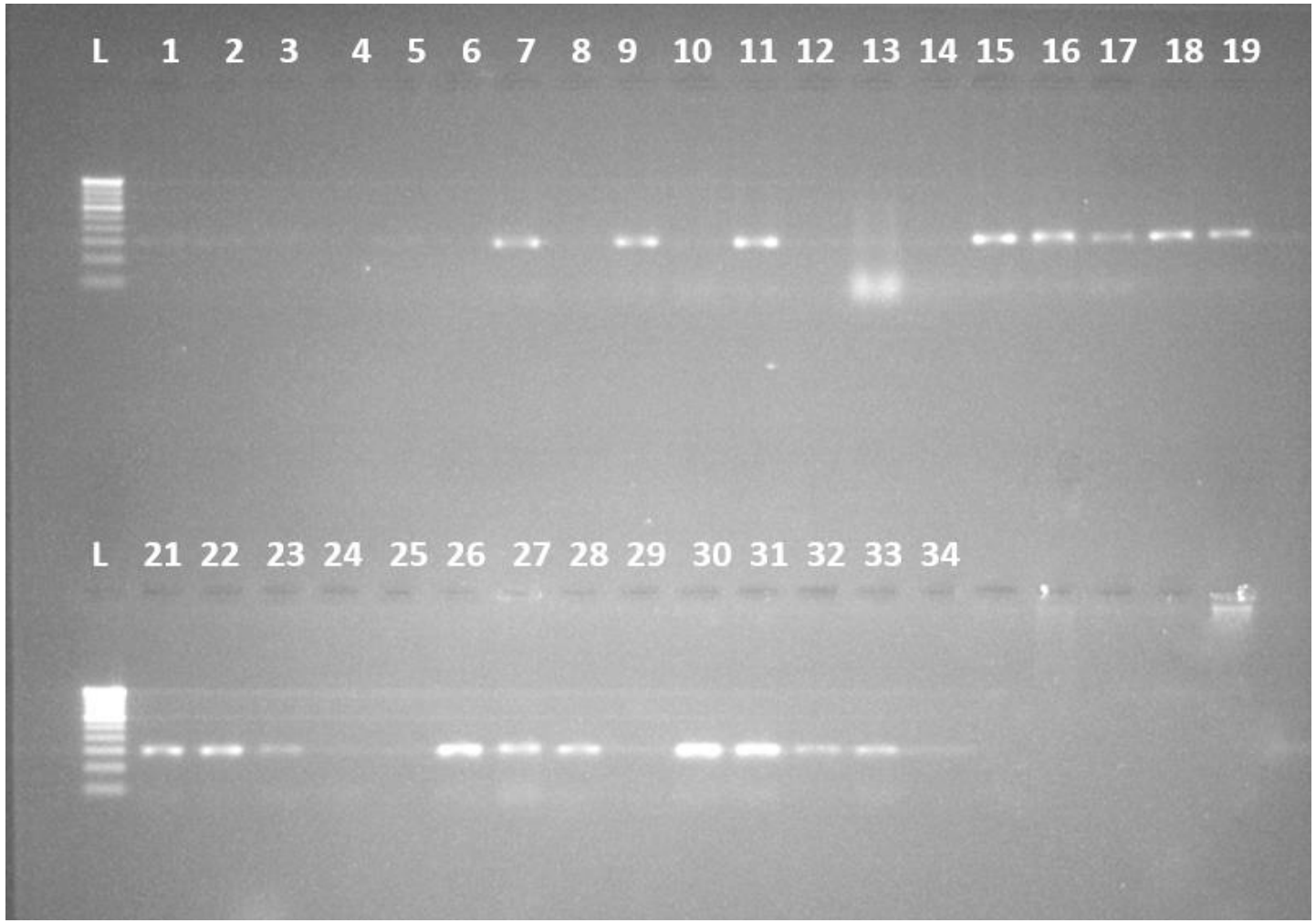


**Fig. 3. Banding Profiles of the 34 Rice Genotypes with Marker: Hvssr03-19**


**Fig. 4. Banding Profiles of the 34 Rice Genotypes with Marker: Hvssr05-09**

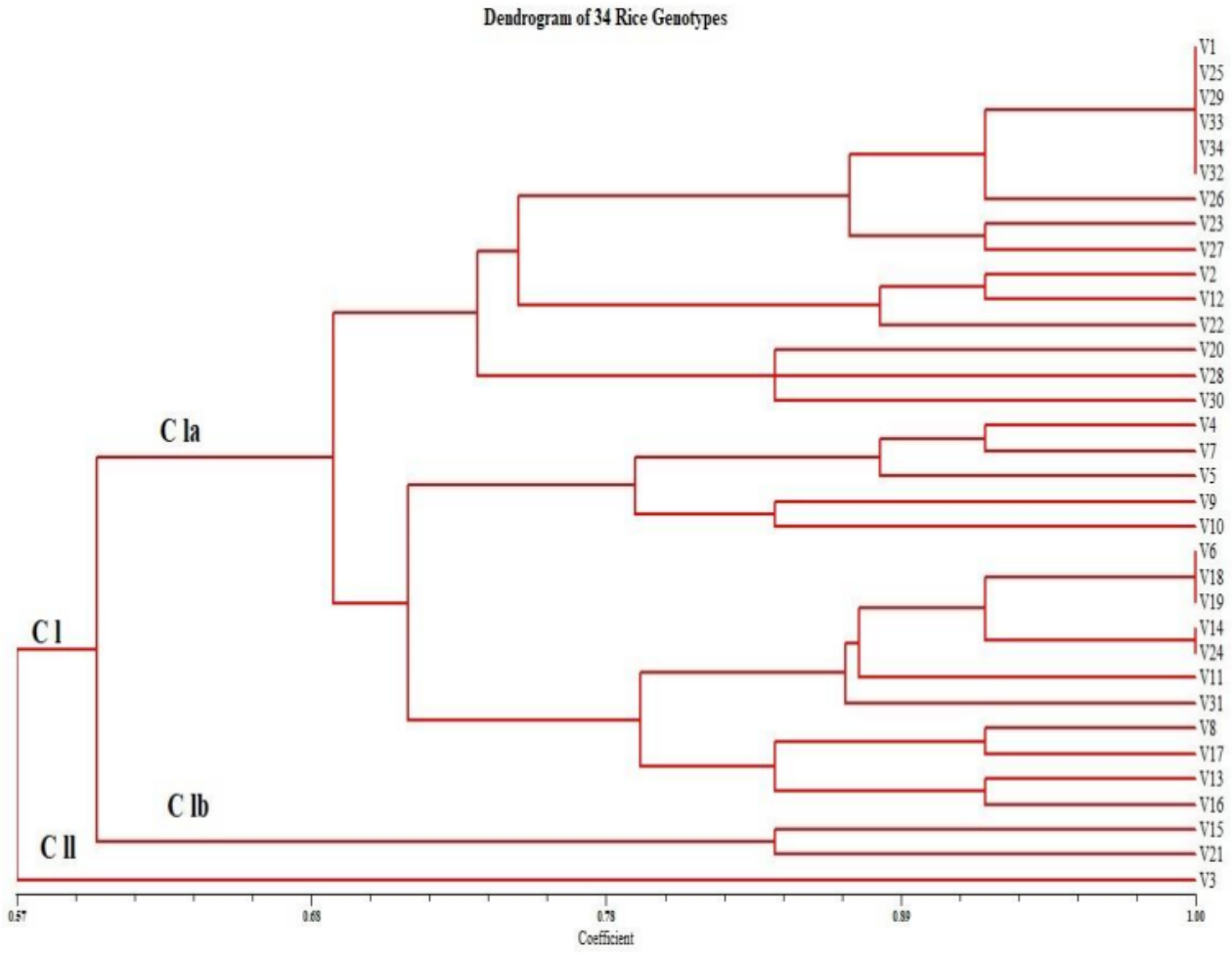


**Fig. 5. Dendrogram of 34 Rice Genotype**

The 34 entries were grouped into 5 clusters presented in Table 4. The highest number of genotypes appeared in cluster I which contains nine genotypes i.e VB-22, VB-23, VB-24, Punjab Basmati -3, HR-2, Basmati 386, PB-1637, Basmati-564, HR-1, pusa basmati -1, pusa basmati-1121. The second highest cluster is cluster lV having the ten genotypes it is comprising Kala B, Jalmagna, Mirchia Dhan, Kbanku, Fr-13a, Lal Sita, Anandi Dhan, Pokkali. kalanamk and badshabhog, The third highest cluster is cluster ll having the six genotypes it comprising PB-1728, S.Shree, Basmati-370, Canaceda, Tkm-6, Bpt-5204. The fourth highest cluster is cluster lll having the five genotypes It is comprising Malviya Basmati, Pant Basmati-2, Tdk-1, Type-3, Pat Gc-203 and last cluster is cluster V having the two genotypes it is comprising Pusa Basmati-1509, Crassa Purple.

The inter cluster distance was greater than intra cluster distance revealing considerable amount of genetic diversity among the rice material used for the study. The intra cluster distance is maximum in cluster ($D^2$ = 31.2) reveals the maximum genetic diversity followed by cluster lV ($D^2$ = 15.7) and cluster lll ($D^2$ = 13.6). The inter-cluster distance (D) is the main criterion for selection of genotypes. The inter-cluster $D^2$ values of the five clusters revealed that maximum inter-cluster generalized distance ($D^2$ = 51.8) was between cluster lV and cluster lll which exhibited maximum divergence followed by cluster IV and cluster I ($D^2$ = 43.4) while, the lowest ($D^2$ = 17.7) was between cluster III and cluster I indicate close relationship and similarity for most traits of all in this cluster.

Based on the clustering pattern it can be depicted that the genotypes belonging to Cluster III and cluster IV can be selected as parents showing high genetic diversity for further hybridization programme.

## 3.1 Molecular Characterization

13 SSR primers were custom synthesized from Genei laboratory and used for molecular diversity analysis of 34 rice genotypes. Out of 13 primers bands were showing only in 11 primers. The total number of bands, number of polymorphic bands, number of monomorphic bands, the average number of polymorphic bands per primer, percent polymorphism, and the polymorphic information content (PIC) were obtained (Table 6). Similarities of banding profiles using SSR primers are shown, based on the results obtained through the present study polymorphisms were observed. All bands are polymorphic in nature. The alleles produced by 11 SSR primers showed a high degree of polymorphism with all primers producing cent percent polymorphic bands. All 11 SSR primers showed hundred percent polymorphism (HvSSR01-41, HvSSR01-53, HvSSR02-33, HvSSR02-50, HvSSR03-02, HvSSR03-19, HvSSR03-54, HvSSR04-19, HvSSR04-27, HvSSR04-46, HvSSR05-09). Number of polymorphic alleles ranged from 1 to 2. Only one primer shown the two polymorphic bands (HvSSR01-41) and remaining primers shown the single polymorphic bands (Fig. 1 to Fig. 4).

The PIC values for each of the SSR loci studied varied depending on the allelic diversity and frequency among the genotypes. The average PIC value for SSR loci was 0.59, while the PIC values ranged from 0.34 to 0.79 (Table 6). The study revealed that primers have the highest PIC value is HvSSR04-46 (0.79) and followed by HvSSR01-41 (0.78). The analysis revealed that the primer had the lowest PIC value 0.39 observed for HvSSR04-19.

Jaccards similarity index showed a significant genetic variation among all rice genotypes were observed highly similarity 1.00 most abundant related to kbanku, Fr-13a, S.shree, etc. and lowest genetic similarity was found in Anandi dhan. A dendrogram was produced using the UPGMA algorithm based on the distance matrix expressed as the similarity coefficient. The UPGMA-based cluster demonstrates the increased degree of interlinkage and significant genetic similarity among all genotypes. The dendrogram showed one major cluster (CI) and one minor cluster (CII), each containing 33 genotypes and 1 genotype of rice genotypes respectively, were visible on the cluster dendrogram of the SSR marker. These clusters were separated by 0.61. Subcluster I (CIa) and subcluster II (CIb) were created from the cluster CI and comprise 31 and 2 genotypes, respectively. Cluster II (CII) contains one genotype (Fig. 5).

## 4. CONCLUSION

The potential parental combinations that are expected to generate maximum diversity based on $D^2$ statistics and SSR marker analysis were found to be Basmati 564 x Kalanamak, Basmati 564 x Kala B, Basmati 564 x Badshahbhog.

These might be used effectively in hybridization programmes for the improvement of aromatic rice.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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