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Research Note

Studies on morphological and molecular divergence in rice

Ayanabha Kole* and Indra Deo

Department of Genetics & Plant Breeding, G. B. Pant University of Agriculture & Technology, Udham Singh Nagar- 263145, Uttarakhand, India
***E-Mail:** ayankole1999@gmail.com

Abstract
Morphological and molecular diversity was studied in a population of 20 rice genotypes, comprising mainly traditional aromatic non-basmati rice. Morphological diversity based on 13 yield traits using D^2 statistics and molecular diversity from 17 polymorphic SSR markers resulted in six and five clusters, respectively with minor changes in cluster composition. The D^2 values ranged from 17.32 to 2521.56. The highest average intra- and inter-cluster distance ($\sqrt{D^2}$) was 12.3 in Cluster I and 46 between Cluster III and IV. Cluster - I and VI played critical role in inter-cluster distance. Relative contribution of days to flowering to the divergence was highest, followed by secondary panicle branches, panicle exertion and test weight. Molecular analysis revealed 50 alleles with a range of 2 - 4 alleles and PIC values ranging from 0.095 to 0.685 with mean of 0.402. RM390 appeared as the potential marker for polymorphism investigations. Considering genetic divergence, crosses have been suggested for exploitation of heterosis in the F_1 generation and isolation of transgressive segregates in advanced segregating generations.

Keywords: Diversity, Morphological, SSR markers, Aromatic rice.

Rice (*Oryza sativa* L., 2n=24), the staple food of more than half the world's population, has played an essential role in human civilization for millennia (Manjunatha *et al.*, 2020). With its origins in ancient Asia, rice has been cultivated and cherished for its versatility, nutritional value and abundant yield (Rathna *et al.*, 2019). Aromatic rice, also known as scented rice, possesses a unique characteristic that sets it apart from the conventional white rice varieties. Unlike the subtle taste of regular rice, aromatic rice entices the senses with its alluring aroma, making it a delightful culinary experience. Cultivated and cherished in various regions around the world, these aromatic grains have earned a reputation for their exceptional quality and ability to transform ordinary meals into extraordinary feasts. In year 2022–2023, India has exported 4.56 million MT of Basmati rice worth Rs. 38524.11crores / 47877.50 US $ Mill. to mainly Iran, Saudi Arab, Iraq, UAE, Yemen Republic (APEDA, 2023).

It is thought that aromatic rice first appeared in the Indian subcontinent, notably in the present-day nations of Pakistan, Bangladesh, and India (Behera and Panda, 2023). Aroma in rice is a complex character controlled by a number of volatile chemicals, 2-acetyl-1-pyrroline being the main one (Kole, 2005). Aromatic rice varieties in the international market are characterized by long grains with high kernel elongation ratio (Verma and Srivastav, 2020). But grain size of many Indian land races and farmers' varieties with pleasing as well as appealing aroma are short to medium. Almost every state in India is endowed with such group of aromatic rice, which are specifically cultivated in the prevailing agro-climatic conditions. In comparison to long grain aromatic varieties, some indigenous aromatic non-basmati rice varieties are well acclaimed for their aroma, superior taste and other quality attributes (Pachauri *et al.,* 2010).

Diversity analysis plays a critical role in rice breeding; as it provides valuable insights into the genetic variability present within rice germplasm. This analysis is essential for ensuring the sustainable development

and improvement of rice varieties. It also helps in selection of parents for cross-breeding programme (Govindraj *et al*., 2015).

The experimental material used in the present study comprised of 20 different rice genotypes procured from the ICAR- National Institute of Plant Biotechnology, New Delhi, India. Among them, Pusa 44 and Sambha Mashuri were non-aromatic, PB1121 and Taraori Basmati were aromatic basmati type, while the other 16 genotypes were aromatic non-basmati types from various parts of India.

The field experiment was conducted in a randomized complete block design (RCBD) with three replications during the *kharif* (warm wet) seasons (July-December month) of 2022 at the N. E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar. It is situated in the Tarai belt of Uttarakhand, 30 km southern end of foot hills of Shivalik range of the Himalayas at 29.5°N latitude, 79.30°E longitude and an altitude of 243.84 m above mean sea level. The characteristic feature of the soil is clay loam and calcareous in nature having a pH of ~8.06. The seeds of each genotype were sown in the nursery bed with the onset of monsoon. Thirty days old seedlings were transplanted under puddled field condition. Single seedling was transplanted in each hill. Each plot consisted of three rows of three meter long with inter- and intra-row spacing of 20 cm and 15 cm, respectively. Fertilizer dose was @ N: P: K = 80:40:40 kg ha^{-1}. Standard crop management practices were followed for raising a healthy crop (DRR, 2005). Data were recorded on 13 quantitative traits viz. plant height, days to 50 % flowering, flag leaf area and angle, panicle number per hill, panicle exertion, panicle length, primary and secondary branches per panicle, number of filled grain per panicle, test weight, straw yield per hill and grain yield per hill from five plants taken randomly from the middle row in each replication and the mean of the same were used for D^2 analysis (Mahalanobis, 1936) and grouping of genotypes were done following Tocher's method (Rao, 1952). For the analysis of filed data SPAR1C software of Indian Agricultural Statistical Research Institute, New Delhi, India was used. Along with this molecular diversity analysis was also performed using 17 polymorphic markers out of 19 SSR markers. Grouping of rice genotypes into different clusters was done following UPGMA hierarchical classification method with DARwin 6.0 software.

Genetic Diversity analysis using Mahalanobis' D^2 statistic Variance analysis exhibited significant differences due to genotypes for all the 13 traits, suggesting that there was enough differences among the genotypes. Wilk's Lambda criterion ($\lambda = 0.225 \times 10^{-7}$, and V= 1086.23 with 247 df) revealed highly significant differences among the genotypes for the aggregate effect of all the thirteen characters. The D^2 values ranged from 17.32 (between Nuakalajeera and Govindbhog) to 2521.56 (between Jeerakashal and Mushkabudgi). Based on relative magnitude of D^2 values, the genotypes were distributed into six clusters (**Table 1**). The majority (12, all aromatic) of rice genotypes were contained in Cluster-I, followed by three (2 basmati and one non-aromatic) in Cluster II, two (aromatic) in Cluster-III and rest three (2 aromatic and one non-aromatic) were solitary.

The clustering patterns shows that genetic diversity was not associated with geographical distribution. Many landraces of different geographic locations fell into the Cluster I. Shanmugam *et al*. (2023) reported no relation between geographical distribution and genetic divergence. The tendency to form such type of distribution overcoming the geographical barriers indicates that geo-spatial isolation could not be the singular reason for divergence in natural population.

The study revealed that the average intra-cluster distance ($\sqrt{D^2}$) varied from 6 in cluster III to 12.3 in cluster I (**Table 2**). Cluster III showed a maximum inter-cluster distance ($\sqrt{D^2}$) of 46 with Cluster IV and a minimum of 18 with Cluster VI. This indicated a considerable amount of divergence within and between the clusters. A critical role was played by Cluster - I and VI for between clusters distance. Crossing, therefore, could be logically effected between diverse genotypes, based on D^2 values.

Table 1. Distribution of genotypes into various clusters

Cluster	Number of Genotype	Name of genotypes
I	12	Seeragasambha, Govindbhog, Nuadhusura, Ketakijoha, Nuakalajeera, Burmabhusi, Bishnubhog, Katarni, Marcha, Jeerakasal, Tulsiphula, NKSLR80
II	3	Pusa 44 (non-aromatic), Taraori Basmati, PB1121
III	2	Jasmine, Mushkibhog
IV	1	Chandanchur
V	1	Samba Masuri (non-aromatic)
VI	1	Sonasal

Table 2. Average intra-and inter-cluster D^2 values

Cluster	I	II	III	IV	V	VI
I	**151.74(12.3)**	783(27.98)	1914(43.76)	388.9(19.72)	343.3(18.28)	1027.25(32.05)
II		**139.67(11.8)**	490.67(22.15)	835.33(28.9)	566.67(22.73)	388.67(19.71)
III			**36(6)**	2116.5(46)	1266(35.58)	306.5(17.5)
IV				**0**	287(16.94)	1491(38.6)
V					**0**	798(28.2)
VI						**0**

Bold figures indicate intra-cluster values; Values in parenthesis indicate $\sqrt{D^2}$ values

Cluster I (**Table 3**) was characterized by tall plant height, late flowering, little flag leaf with moderate angle of spreading, high filled grain number, low test weight and low yielding types. Cluster-II had highest mean value for panicle length, test weight and grain yield and the lowest value for secondary branches. The rest of the characters showed moderate mean values. Cluster- III had the earliest days to flowering, wide flag leaf angle, highest panicle exertion and test weight, while yield was lowest. Cluster- IV was mono-genotypic and characterized by the shortest plant height, very late (last) flowering, erect flag leaf with the highest flag leaf area, lowest length of panicle, panicle just exerted with low seed yield. Cluster-V also was solitary and the genotype was characterised by erect flag leaf, highest mean values for primary and secondary branches of panicle with incomplete panicle exertion, moderately late to flowering and high seed yield. In cluster VI number of panicles was highest, while flag leaf area, primary branches and test weight were lowest.

The relative contribution of days to flowering to the total divergence was highest (12.2), followed by secondary branches (9.7), panicle exertion (9.6), test weight (9.2), straw weight (8.7), flag leaf area (8.2) and filled grain number (8.1). Other characters had less contributions. Contributions towards divergence have been reported for days to flowering (Kole, 2000; Chandra *et al*., 2007, Mishra *et al*., 2020, Sar and Kole, 2022), for length of panicle (Chand *et al*., 2005; Medhabati *et al*., 2013); and for test weight (Chand *et al*., 2005; Reddy *et al*., 2006, Chamundeswari, 2016**;** Mishra *et al*., 2020, Sar and Kole, 2022).

Molecular diversity analysis using SSR markers: Microsatellite markers were utilized to characterize the field evaluated genotypes. Out of 19 SSR markers, two (RG136 and RM122) were observed to be monomorphic. A total of 50 alleles were detected from 17 polymorphic microsatellite markers, which explained a broad spectrum of polymorphism in the studied population (**Table 4**). The mean number of alleles per marker was 2.94 and the number varied from two (RM 13, NBB197, RM130 etc.) to four (RM 390, RM 264, RM 206 and RM 164). The overall size of the amplified product varied from 90 bp (RM 556) to 290 bp (RM 164). Most of the amplified products of the markers were between 100 bp to 200

Table 3. Cluster means of 13 quantitative characters in rice

Cluster	Plant Height (cm.)	Days to Flowering	Flag Leaf Area (cm2)	Flag Leaf Angle	Panicle No.	Panicle Exertion (cm)	Panicle Length	Primary Branch	Secondary Branch	Filled Grain No.	Test Weight (g)	Straw Weight (g)	Grain Yield (g)
I	136.18	138.53	23.23	33.71	13.54	3.02	24.16	11.86	40.61	127.81	10.90	68.71	9.91
II	120.07	107.67	33.38	23.44	15.18	6.67	27.51	9.80	15.31	84.17	21.40	43.52	16.49
III	117.97	75.00	30.05	38.33	15.40	7.35	22.77	9.30	23.10	76.94	22.23	23.27	9.27
IV	86.13	146	40.63	10.33	11.53	0	16.53	12.8	27	56.57	15.87	53.66	9.54
V	91.4	125.33	34.26	8.67	11.27	-1.93	21.07	12.67	50.93	157	13.43	44.13	15.87
VI	132.8	90.67	22.87	29.67	16.53	6.1	23	8.8	26.6	90.53	10.84	29.67	11.73
Relative contribution (%)	5.7	12.20	4.6	8.2	6.83	9.6	5.8	5.4	9.7	8.1	9.2	8.7	7.2

Table 4. Number of alleles and PIC value among 17 SSR markers.

S. NO.	MARKERS	Number of Alleles	PIC
1	RM 224	3	0.445
2	RM 390	4	0.685
3	RM31	3	0.605
4	RM611	3	0.490
5	RM 13	2	0.268
6	NPB197	2	0.338
7	RM 130	2	0.095
8	RM 39	3	0.598
9	RM 264	4	0.683
10	RM 400	3	0.460
11	RM 153	2	0.095
12	MP 1	3	0.295
13	RM 206	4	0.355
14	RM 164	4	0.573
15	RM 556	2	0.180
16	RM 21	3	0.495
17	RM 167	3	0.178
	AVERAGE	2.94	0.402

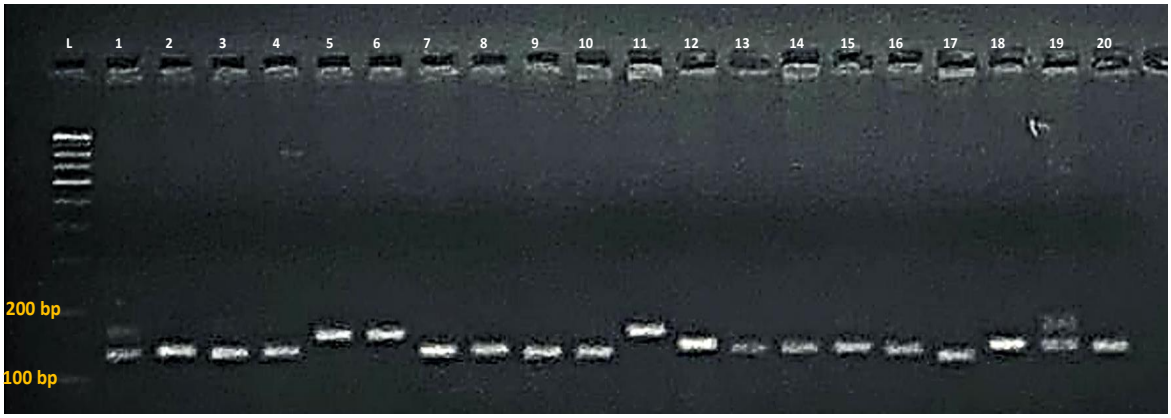

Fig 1. SSR banding pattern of the 20 genotypes (L1-L20) with marker MP1

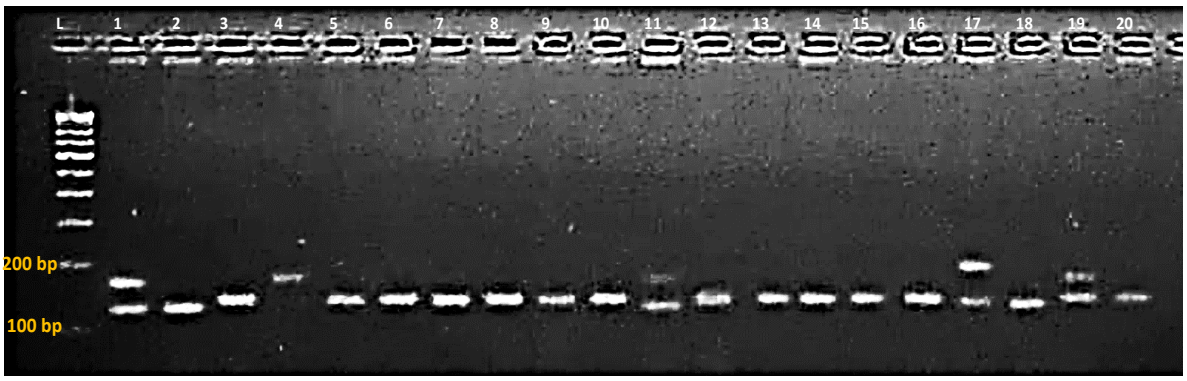

Fig 2. SSR banding pattern of the 20 genotypes (L1-L20) with marker RM206

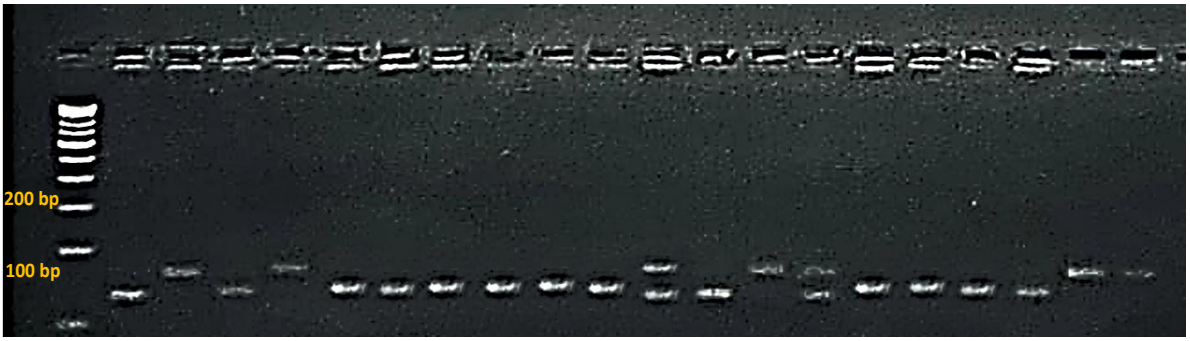

Fig 3. SSR banding pattern of the 20 genotypes (L1-L20) with marker RM21

bp (**Fig.1-3**). This broad range explained the significant variation in repeat counts across various alleles. The amount of diversity was assessed by determining the PIC value for each of the 17 microsatellite markers. The value of polymorphism information content (PIC) reflects allelic variation. Polymorphism information content of the markers ranged from 0.095 for RM 153 to 0.685 for RM 390 with average PIC value of 0.402. The mean PIC value indicates an occurrence of an average amount of polymorphism (Botstein *et al.,* 1980).

The 20 genotypes were divided into five primary groups by utilizing the UPGMA-based clustering with DARwin 6.0 software (**Table 5 and Fig. 4**). Comparison of cluster composition based on molecular diversity showed the near similarity in cluster I with major number of 13 genotypes (merging cluster V and VI), followed by clusters III with 3 genotypes (added Seeragasambha, Katarni from cluster I, to Chandanchoor of monogenotypic cluster-VI) and cluster IV with 2 genotypes (retaining one basmati and one nonaromatic genotypes of Cluster II, throwing out PB1121 to a solitary Cluster V). Cluster II retained only aromatic variety Jasmine from Thailand, while Mushkbudgi of Cluster III was clubbed to Cluster I. Therefore, redistribution of genotypes from morphological diversity to molecular diversity is minor.

Considering morphological and molecular divergence, relative contribution of traits towards genotypic diversity and *per se* performance of the genotypes, crossing between the intra-cluster genotypes Nuakalajeera × PB1121 (Cluster I having highest D^2 value) and inter-cluster genotypes PB 1121 × NKSLR 80 (Cluster

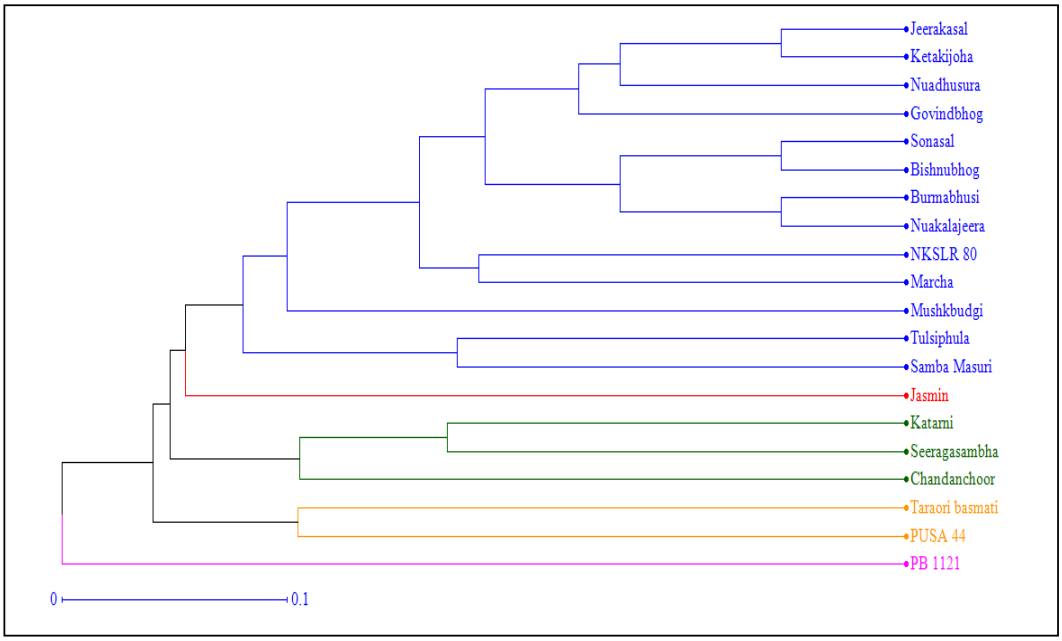

Fig. 4. Hierarchical cluster analysis based on molecular marker genotyping

Table 5. Grouping of genotypes into various clusters based on SSR marker analysis.

Cluster	Number of genotypes	Name of genotypes
I	13	Samba Masuri (non-aromatic), Jeerakasal, Ketakijoha, Nuadhusura, Govindbhog, Sonasal, Bishnubhog, Burmabhusi, Nuakalajeera, NKSLR80, Marcha, Muskbudgi, Tulsiphula,
II	1	Jasmine
III	3	Seeragasambha, Katarni, Chandanchoor
IV	2	Pusa44 (non-aromatic), Taraori Basmati
V	1	PB 1121

II × I), Nuakalajeera × Jasmine and Jeerakashal × Mushkbudgi (Cluster I × III), Govindbhog × Samba Masuri (Cluster I × V) and Katarni × Sonasal (Cluster I × VI) could be made for exploitation of heterosis in the F_1 generation and isolation of transgressive segregates from an array of recombinants in advanced segregating generations.

In conclusion, both morphological and molecular analyses revealed considerable amount of divergence among 20 genotypes in the population. The clustering patterns showed near-similarity with minor changes in grouping of the genotypes between the two methods of cluster formation. Therefore, selection of parents based on commonness in grouping of the genotypes emanating out of morphological and molecular diversity analyses is likely to be rewarding in cross-breeding programmes.

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