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RESEARCH ARTICLE

Morphological and molecular characterization of advanced breeding lines of rice (*Oryza sativa* L.) under sub-tropical irrigated conditions of Jammu region

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Abstract

The present study aimed to characterize thirty advanced breeding lines of rice for yield and yield attributing traits using morphological and molecular markers. Analysis of variance revealed significant differences among the thirty rice germplasm lines. High heritability coupled with high genetic advance was observed for number of grains per panicle suggesting that this trait is likely governed by additive gene action and can be stabilized through appropriate selection. Number of grains per panicle exhibited the strongest direct positive effect and was significantly correlated with grain yield per plant. The D^2 statistics grouped the advanced lines of rice into five distinct clusters, with cluster II containing the highest number of lines (16), followed by cluster III (7) and clusters I and IV (3) while, cluster V had a single line. Grain yield per plant had highest contribution to genetic divergence (39.77 %) followed by number of grains per panicle (23.45 %). The maximum inter cluster distance was observed between cluster IV and cluster V (37.14) and cluster II and cluster IV (26.75), suggesting that the lines in these clusters can be used for future breeding programme to develop new cultivars. Out of 24 molecular markers 21 were found to be polymorphic and a total of 54 alleles were identified, ranging from 2-4 alleles per locus with an average of 2.52 alleles. Polymorphism information content (PIC) values ranged from 0.224 to 0.677. Among the molecular markers, RM408 amplified the maximum number of 4 alleles while the highest PIC value was observed in RM7102 (0.677). The study effectively differentiated the advanced lines and highlighted their diversification, with cluster analysis revealing similarities and differences among the lines for future breeding programs.

Keywords: advanced breeding lines; genetic parameters; heritability; molecular characterization

Introduction

Rice is popularly known as “Global Grain” due to its usage as a primary staple food in over hundred nations and being consumed by over half of the world’s population. Scientifically known as *Oryza sativa* L. (2n = 24) it is the primary cultivated species belonging to the genus *Oryza* within the *Poaceae* family. South Asia is often referred to as the “food basket” and “food bowl” of Asia because it is one of the primary centers for the domestication of rice (1). Globally, it is reported to provide 27 % of carbohydrates, 20 % of dietary protein and 3 % of dietary fat (2). The demand for rice is still increasing in Asia, where the consumption rate is at least 90 % and it is globally projected that demand for rice will rise to 650 million tonnes by 2050 (3). The world population is expected to rise 10 billion by 2050 and it requires 763 million tons of rice in 2020, while 852 million tons by 2035 (4). Globally, India ranks second with 46.38 million hectare area under rice cultivation with a production and productivity of 130.29 million tonnes and 28.09 quintals per hectare, respectively (5). While, in the Union Territory of Jammu and Kashmir it was cultivated over an area of 274.47 thousand hectare with production and productivity of 6047 thousand quintals and 22.03 quintals per hectare, respectively (6). In India, around 160 to 280 N latitude and 790 to 900 E longitudes or several agro-ecological zones where rice is cultivated under extremely diverse conditions (2). It is grown in areas where water depth reaches 2-3 m or more. Rice culture in Kuttanad district of Kerala is grown below the sea level, while in the Union Territory of Jammu and Kashmir; it is grown at an altitude of 2000 m above sea level with temperature range of 15-40 °C and average annual rainfall range from 30 mm in Rajasthan to more than 2800 mm in Assam. Characterization and proper documentation of distinguishing traits are important for scientific research. This process is essential for evaluating rice to improve its use as breeding material and to increase grain yields (7). Morphological characterization serves as a fundamental and conventional method for evaluating genetic variability and diversity. It plays a vital role in selecting and utilizing suitable parents for breeding programs. Mahalanobis D^2 statistical analysis is a reliable multivariate biometrical technique and was employed to examine the type and

extent of genetic diversity within genotypes based on yield and yield related traits (8). Tocher's method gives us estimations of the diversity of genotypes and displays clustering patterns using average D^2 values. Correlation studies are useful for assessing the relative importance of individual traits. Consequently, path coefficient analysis is utilized to understand both the direct and indirect contributions of a trait to yield, enabling the breeders to assess genetic traits based on their impact. Principal component analysis (PCA) is used to minimize redundancy and analyze correlations among a large set of variables by reducing them to a few principal components, preserving the essence of the information (9). The genetic diversity among rice subspecies has been widely identified using SSR markers, which have a high degree of allelic diversity detection capability, co dominant, highly reproducible and are short stretch tandem repeat motifs of 2-6 nucleotides distributed widely across the entire genome (10).

Materials and Methods

Experimental material

The experimental material used in the present study are thirty lines, out of which twenty-nine advanced breeding lines of rice received from International Rice Research Institute (IRRI) through Indian Institute of Rice Research, Hyderabad and one local check (SJR-5) maintained at the Division of Plant Breeding and Genetics SKUAST-Jammu. A brief description of experimental materials is given in Table 1.

Design and layout

During *Kharif* 2023, seeds of all the genotypes were sown in a nursery and thirty days old seedlings were transplanted in a Randomized Complete Block Design along with three replications having a plot size of 2 m^2 (2 rows each of 5 m length) with spacing of 20 × 15 cm. The genotypes were randomized in each replication

Data recording

Morphological data was collected by randomly selecting five plants from each genotype within each replication for evaluation of yield and yield contributing traits. This included observations on plant height (cm), total number of tillers per plant, number of effective tillers per plant and panicle length (cm) for each germplasm line present in each replication following specified guidelines. Days to 50 % flowering were determined based on visual assessment, while days to maturity were recorded when more than 80 % of the grains on the panicles were fully ripened. Postharvest traits such as 1000 grain weight (g), grain yield per plant, kernel length (mm), kernel breadth (mm) and L/B ratio were recorded following the harvesting, threshing and drying of the seeds.

Molecular diversity analysis

DNA extraction was done by Modified CTAB DNA extraction method described by Doyle and Doyle (1990) (11). The genotypes were grown offseason on pots in a glasshouse of Division of Plant Breeding and Genetics, SKUAST Jammu for 2-3 weeks and 15-20 days old seedlings were used for the extraction of genomic DNA. To evaluate genetic variation on a molecular level, a total of 24 genome-wide simple sequence markers were chosen based on polymorphism from previous literature. These markers were diluted in autoclaved double-distilled water to a concentration of 10 pmol. The details of the simple sequence repeat and their amplification temperatures are provided in Table 2 along with forward and reverse primers. The DNA quality and concentration were assessed using a spectrophotometer and verified on 0.8 % agarose gel electrophoresis. DNA genotyping was conducted using an Eppendorf Thermal cycler with a 15.0 µL reaction mixture comprising genomic DNA sample (1.0 µL), forward and reverse primers (0.5 µL each), PCR buffer (3.0 µL), 1.2 µL of $MgCl_2$, 0.38 µL of dNTPs, 0.15 µL of Taq polymerase and 8.27 µL of sterile water. Initially, a fresh master mix was prepared containing all

Table 1. Description of experimental materials utilized in the present study

S. No.	IRRI Nomenclature/check	Code allotted	Pedigree
1	ING-170	GPB-1	IR05N412/IRRI 168
2	ING-159	GPB-2	PR 37866-1B-1-4/IRRI 154//PSB RC 10 (IR 50404-57-2-2-3)
3	ING-181	GPB-3	TAICHUNG NATIVE 1/IR 24*5
4	ING-155	GPB-4	IR 10N108/IRRI 154
5	ING-150	GPB-5	IR 71700-247-1-1-2/ SAMBHA MAHSURI
6	ING-161	GPB-6	IR 111242/IR 105145
7	ING-203	GPB-7	IR 73718-1-2-1-3/PSB RC 10(IR 50404-57-2-2-3)
8	ING-162	GPB-8	IR 24*5/CHUGOKU 45
9	ING-169	GPB-9	PR35786-B-3-3-2-1-1/IRRI 154
10	ING-205	GPB-10	IR 69428-6-1-1-3-3/IRRI 123*2
11	ING-162	GPB-11	IR 10F365/IR09L342
12	ING-151	GPB-12	IRRI 154*2/IRRI 149
13	ING-165	GPB-13	PR 37139-3-1-3-1-2-1/IR05N412
14	ING-198	GPB-14	IR 47761-27-1-3-6/PSB RC 28 (IR 56381-139-2-2)
15	ING-157	GPB-15	PR 37866-1B-1-4/IRRI 156
16	ING-176	GPB-16	IR 24*5/DV 85
17	ING-163	GPB-17	IR08A176/IRRI 154//IR09A136
18	ING-171	GPB-18	IR10F336/IR 55179-3B-11-3
19	ING-204	GPB-19	IR 68077-82-2-2-2-3/IR00A117
20	ING-202	GPB-20	IR 73008-138-2-2-2/IR 68544-29-2-1-3-1-2//IR 72870-19-2-2-3
21	ING-205	GPB-21	IR 69428-6-1-1-3-3/2*IRRI 123
22	ING-159	GPB-22	PR 37866-1B-1-4/IRRI 154//PSB RC 10 (IR 50404-57-2-2-3)
23	ING-156	GPB-23	IRRI 156/IR09F436
24	ING-167	GPB-24	PR 37246-2-3-2-1-1-2-1/IRRI 154
25	ING-154	GPB-25	IR10F548/IR 55179-3B-11-3
26	ING-155	GPB-26	IR10N108/IRRI 154
27	ING-157	GPB-27	PR 37866-1B-1-4/IRRI 156
28	ING-152	GPB-28	IR09L272/IR09L337
29	ING-170	GPB-29	IR05N412/IRRI 168
30	SJR-5(Local Check)	GPB-30	IR25393-57/RD23//IR27316-96///SPRLR77205-3-2/SPRLR79134-51-2

Table 2. Details of the SSR markers and their annealing temperatures used for molecular profiling

S. No.	Markers	Chr No.	Forward sequence	Reverse sequence	T_a (°C)
1	RM583	1	AGATCCATCCCTGTGGAGAG	GCGAACTCGCGTTGTAATC	55 °C
2	RM220	1	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT	55 °C
3	RM110	2	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGGTCGAG	55 °C
4	RM263	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	55 °C
5	RM218	3	TGGTCAAACCAAGGTCCTTC	GACATACATTCTACCCCCGG	58 °C
6	RM439	3	TCATAACAGTCCACTCCCCC	TGGTACTCCATCATCCCATG	58 °C
7	RM471	4	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC	58 °C
8	RM317	4	GTTCAGTGT	TCAGTGCCACC	58 °C
9	RM430	5	AAACAACGACGTCCCTGATC	GTGCCTCCGTGGTTATGAAC	55 °C
10	RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	57 °C
11	RM162	6	GCCAGCAAAACCAGGGATCCCGG	CAAGGTCTTGTGCGGCTTGCGG	55 °C
12	RM225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	55 °C
13	RM11	7	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	55 °C
14	RM172	7	TGCAGCTGCGCCACAGCCATAG	CAACCACGACACCGCCGTGTTG	55 °C
15	RM408	8	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC	58 °C
16	RM80	8	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG	58 °C
17	RM245	9	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG	55 °C
18	RM242	9	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	55 °C
19	RM304	10	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGAG	55 °C
20	RM184	10	ATCCCATTCGCCAA	TGACACTTGGAGAG	55 °C
21	RM286	11	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	55 °C
22	RM287	11	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	55 °C
23	RM453	12	GGCCAACGTGTGTATGTCTC	TTAATGCCAAGACGGATGGG	58 °C
24	RM7102	12	TAGGAGTGTTTAGAGTGCCA	TCGGTTTGCTTATACATCAG	55 °C

components except the DNA sample. Subsequently, 1 µL of DNA was added to 0.5 mL Eppendorf tubes, followed by the addition of 14 µL of the prepared master mix. PCR products were resolved on 2.5 % agarose gels, stained with ethidium bromide and visualized under UV light. Allele sizes were estimated using a 100 bp ladder.

Results and Discussion

Variability and genetic parameters

The results of the analysis of variance revealed significant differences among the genotypes for most of the traits indicating their potential as superior parents for future hybridization programs, similar findings have been reported in the previous research (12-14). The estimate of genetic parameters represented in Table 3 showed that phenotypic variance was considerably higher than genotypic variance for most of the traits. This difference suggests that environmental factors have a minimal influence on the expression of these traits, indicating that the observed variation is primarily due to the inherent characteristics of the germplasm lines (15). The highest estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were found in grain yield per plant while, lower estimates of phenotypic and genotypic coefficients of variation were observed in days to maturity. Higher estimates of both GCV and PCV indicated that these traits have potential for selection (16). Heritability in the broad sense reflects the proportion of overall variation that can be passed from one generation to the next. Estimates of heritability help plant breeders to design effective and targeted breeding programs. The results of the current study demonstrated that the heritability varied from Kernel breadth (35.14 %) to grain yield (88.34 %). The highest genetic advance was observed in number of grains per panicle (32.97) and the least genetic advance was found in kernel breadth (0.28). High heritability paired with high genetic advance indicates additive gene interactions among the genes controlling the trait, making selection more effective (17). The results of the current study confirmed that trait such as the number of grains per panicle exhibited high genetic advance along with high heritability. The correlation coefficients (Table 4) for yield and its attributing traits were calculated at both genotypic and phenotypic levels.

Correlation analysis

The genotypic correlation coefficient was found to be higher than the phenotypic correlation coefficient. Grain yield per plant demonstrated a significant and high positive genotypic correlation with number of grain per panicles (0.471), followed by number of effective tillers per plant (0.384), total number of tillers per plant (0.367), 1000 grain weight (0.351) whereas it had a positive but non-significant correlation with days to 50 % flowering (0.276) panicle length (0.266), kernel breadth (0.222), kernel length (0.184), days to maturity (0.099) and plant height (0.043). Conversely, grain yield per plant had a negative

Table 3. Estimation of genetic parameters among yield and yield attributing traits

Characters	Genotypic variance(σ^2_g)	Phenotypic variance (σ^2_p)	GCV (%)	PCV (%)	Heritability (bs) (%)	Genetic advance	Genetic advance as percent of mean (%)
Days to 50 % flowering	13.88	27.39	3.55	4.99	52.70	7.46	5.21
Plant height (cm)	97.62	119.58	9.67	10.71	81.63	18.38	18.05
Total no. of tillers per plant	1.77	4.75	7.12	11.64	61.42	1.68	8.97
No. of effective tillers per plant	2.85	5.71	9.35	13.23	69.96	9.46	13.61
Panicle length (cm)	1.53	3.27	4.75	6.92	47.04	1.75	6.71
Days to maturity	5.37	16.08	1.59	2.76	50.40	5.75	1.90
1000 grain weight (g)	3.47	7.53	7.06	10.40	64.12	2.60	9.88
No. of grains per panicle	305.11	363.40	14.54	15.87	83.96	32.97	27.46
Kernel length (mm)	0.05	0.08	3.56	4.33	67.73	0.41	6.04
Kernel breadth (mm)	0.01	0.01	3.55	6.00	35.14	0.28	4.34
L/B ratio	0.03	0.05	4.71	6.19	57.82	0.70	7.37
Grain yield per plant (g)	49.30	55.81	22.75	24.20	88.34	19.59	44.04

Table 4. Genotypic and phenotypic correlation coefficient among yield and yield attributing characters

Characters	Days to 50 % flowering (no.)	Plant height (cm)	Total number of tillers per plant	Number of effective tillers per plant	Panicle length (cm)	Days to maturity (no.)	1000 grain weight (g)	Number of grains per panicle	Kernel length (mm)	Kernel breadth (mm)	L/B ratio	Grain yield per plant (g)
Days to 50 % flowering	1.00	0.508** 0.346**	0.575** 0.345**	0.431* 0.314**	0.631** 0.295**	0.782** 0.330**	0.080 0.021	0.478** 0.342**	-0.084 -0.06	-0.378* -0.288**	0.358* 0.199	0.276 0.191
Plant height (cm)		1.00	0.343* 0.262*	0.201 0.270**	0.664** 0.478**	0.419* 0.244*	0.067 0.034	-0.006 0.024	-0.017 -0.022	0.230 0.169	-0.100 -0.145	0.043 0.073
Total no. of tillers per plant			1.00	0.945** 0.799**	0.021 0.049	0.327* 0.221*	-0.173 0.015	-0.274 -0.084	-0.097 -0.089	-0.464** -0.084	-0.03 0.123	0.367* 0.328**
No. of effective tillers per plant				1.00	0.024 0.066	0.157 0.101	0.352* 0.069	0.256 -0.167	-0.150 -0.092	-0.447** -0.086	0.01 -0.061	0.384* 0.251*
Panicle length (cm)					1.00	0.297 0.130	0.372* 0.092	0.223 0.151	0.360* 0.146	0.220 0.028	0.244 0.204	0.266 0.130
Days to maturity						1.00	-0.321* -0.036	0.158 0.185	0.071 0.053	-0.456** -0.156	0.351* 0.196	0.099 0.061
1000 grain weight (g)							1.00	-0.272 -0.081	0.155 0.059	0.603** 0.185	-0.257 -0.171	0.351* 0.228*
No. of grains per panicle								1.00	0.160 -0.089	-0.422* -0.259*	0.370* 0.264*	0.471** 0.407**
Kernel length (mm)									1.00	0.568** 0.246*	0.341* 0.311*	0.184 0.159
Kernel breadth (mm)										1.00	0.732** 0.467**	0.222 0.182
L/B ratio											1.00	-0.008 -0.060
Grain yield per plant (g)												1.00

correlation with L/B ratio (-0.008). Path analysis was proposed by Wright (18) and further developed by Dewey and Lu (19) is employed to establish causal relationships by partitioning the genotypic and phenotypic correlation coefficients into direct and indirect effects.

Path analysis

The path coefficient analysis results (Table 5) indicated that other traits had a direct and indirect effect on grain yield per plant. The findings of the current study revealed that traits like days to 50 % flowering, plant height, 1000 grain weight, number of grains per panicle, kernel length, kernel breadth, total number of tillers per plant and number of effective tillers per plant were found to have a positive direct effect on the grain yield per plant whereas, direct but highest negative effects on grain yield per plant was exhibited by panicle length and days to maturity. Therefore, direct and positive effect of traits will help in producing high-yielding rice genotypes.

Diversity analysis using D^2 statistics

The genetic diversity among thirty advanced breeding lines of rice was assessed using the D^2 statistic based on various yield and yield attributing traits and clustering of thirty advanced lines using Tocher's method grouped into five distinct clusters. Among the five clusters, 4 (clusters I, II, III and IV) were major clusters, while cluster V was minor cluster. The clustering pattern (Table 6) revealed that 16 out of the 30 genotypes are in cluster II, showing that they are closely related between them compared to other genotypes. The diversity of genotypes within a cluster is lower than that of genotypes found distinct from it. Moreover, cluster III consists of seven and cluster I and IV have three lines, while cluster V have only one line (Fig. 1). The highest number of advanced breeding lines was observed in clusters II, III, IV and I, as many of these lines are related as sister lines according to their pedigree records. The advanced breeding lines emerged from a single source, were spread across multiple clusters, indicating a significant level of diversity within the genotypes. The intra and inter cluster distances for five different clusters expressed as average D2 values are shown in Table 7. The maximum intra-cluster distance was observed in cluster III (11.12) revealed significant genetic diversity among the germplasm lines within each cluster which can aid in selecting phenotypically superior lines. The maximum inter-cluster distance was observed between clusters IV and V (37.14) revealing that the genotype hybridization between these clusters would produce desirable segregates through the accumulation of advantageous genes over the segregating generations, followed by clusters II and IV (26.75), clusters I and IV (24.37), clusters I and V (22.90), clusters III and IV (21.69), clusters I and III (18.54) and clusters II and III (17.24). According to the cluster means (Table 8), most desirable mean for most of the traits found in cluster I followed by cluster IV and V. The number of grains per panicle, the grain yield per plant, plant height, kernel length and days to 50 % flowering, are larger contributors to the overall divergence revealing that genotypes selected based on these characteristics are desirable. The overall contribution of these traits to genetic divergence is 92.41 % showing that they are closely related between them.

Principal component analysis

Principal component analysis (PCA) was employed to assess the relative contribution of different traits to the overall variability. Among the 12 principal components (Table 9) obtained, only 5 had eigen values greater than 1 and contributing to approximately 83.60 % of the variability among the traits studied. Principal component 1 accounted for the highest variability 28.46 % with an eigen value 3.15, followed by principal component 2 contributed 21.72 % of variation with an eigen value 2.42, principal component 3 with an eigen value of 1.74 contributed 13.79 % of variation, principal component 4 contributed 11.24 % of variation with an eigen value 1.43 and principal component 5 contributed 8.39 % of variation had an eigen value 1.12. The results of loading component matrix studies indicate that PC 1 has a significant positive loading value association with characters such as days to 50 % flowering, length/breadth, plant height, total number of tillers per plant, number of effective tillers per plant, panicle length, number of grains per panicle, grain yield per plant and days to maturity. In PC 2 traits like days to 50 % flowering, plant height, days to maturity, panicle length, number of grains per panicle, kernel length, 1000 grain weight, kernel breadth and grain yield per plant contributed maximum to variability. The contribution of qualitative and yield-attributing traits to genetic variation within the germplasm has been revealed through the PCA. Biplot of PC1 versus PC2 demonstrated that the characters like 50 % flowering, number of grains per panicle, days to maturity, plant height, panicle length and grain yield per plant positively impacted the germplasm lines such as GPB-1,GPB-2, GPB-9, GPB-19 and GPB-24 (Fig. 2) and these lines are promising lines that can be used for further studies.

In the current study, the extent of genetic diversity among thirty advanced breeding lines of rice was assessed using 24 molecular (SSR) markers. The analysis (Table 10) included determining parameters such as the number of alleles per locus, polymorphism information content and percentage heterozygosity using 24 polymorphic SSR markers. The SSR banding profile of the advanced lines of rice obtained using the markers RM218 (Fig. 3), RM220 (Fig. 4), RM242, RM413 (Fig. 5), RM263 (Fig. 6) and RM11 were illustrated on plates 1 to 6. When thirty advanced breeding lines were analyzed using 24 SSR markers, 21 out of 24 were found to be polymorphic with a total of 54 alleles. The average number of alleles per locus was 2.52, ranging from 2 alleles (RM583) to 4 alleles (RM408). Among the 21 polymorphic markers, 11 markers were found heterozygous alleles. The observed heterozygosity percentage ranged from 3.33 % (RM218) to 68.00 % (RM110). The polymorphism information content (PIC) of the markers varied from 0.224 (RM172) to 0.677 (RM7102), with an average PIC value of 0.443. Out of 21 markers, 8 had a PIC value greater than 0.50, demonstrating that the markers are highly polymorphic. The data was analyzed using NTSYS pc version v2.10e software and a dendrogram (Fig. 7) was created using the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method based on Jaccard's similarity coefficient.

The dendrogram distinctly grouped the thirty advanced breeding lines of rice into six different clusters, organizing them based on genetic similarity with a coefficient ranged from 0.52 to 0.83. The distribution of advanced lines across the clusters is detailed in Table 11. The maximum number of lines were found in cluster II (11 lines) followed by cluster I (6 lines), cluster IV (5 lines), clusters III (4 lines), cluster VI (3 lines) and cluster V contained only one line. The clustering analysis effectively grouped the lines based on their phylogenetic relationships.

Table 5. Direct (diagonal) and indirect (off-diagonal) effects of different yield attributing characters in rice

Characters	Days to 50 % flowering (no.)	Plant height (cm)	Total number of tillers per plant	Number of effective tillers per plant	Panicle length (cm)	Days to maturity (no.)	1000 grain weight (g)	Number of grains per panicle	Kernel length (mm)	Kernel breadth (mm)	L/B ratio	Correlation with Grain yield per plant (g)
Days to 50 % flowering (no.)	0.906 (0.120)	0.113 (-0.029)	-0.110 (-0.044)	-0.034 (0.055)	-0.676 (0.014)	-0.193 (0.005)	0.025 (0.003)	0.213 (0.174)	-0.021 (-0.012)	-0.233 (-0.058)	0.086 (-0.037)	0.276 0.191
Plant height (cm)	0.562 (0.041)	0.223 (-0.086)	-0.042 (-0.033)	-0.016 (0.047)	-0.711 (0.024)	-0.103 (0.003)	0.021 (0.005)	-0.002 (0.012)	-0.004 (-0.004)	0.141 (0.034)	-0.024 (0.027)	0.043 0.073
Total no. of tillers per plant	0.636 0.041	0.049 -0.026	0.191 (-0.128)	0.076 (0.140)	0.002 (0.002)	-0.081 (0.003)	0.055 (0.002)	0.122 (-0.043)	-0.024 (-0.018)	-0.286 (-0.016)	-0.009 (0.023)	0.367* 0.151
No. of effective tillers per plant	0.477 (0.037)	0.044 (-0.023)	0.181 (-0.102)	0.080 (0.176)	-0.026 (0.003)	-0.038 (0.001)	0.048 (0.011)	0.114 (0.085)	-0.038 (-0.018)	-0.275 (-0.017)	0.004 (0.011)	0.384* 0.051
Panicle length (cm)	0.698 (0.035)	0.148 (0.041)	0.005 (-0.006)	-0.001 (0.011)	-0.107 (0.050)	-0.073 (0.002)	0.118 (0.015)	0.099 (0.077)	0.092 (0.029)	0.136 (-0.005)	0.058 (0.038)	0.266 0.130
Days to maturity (no.)	0.866 (0.039)	0.093 (-0.021)	-0.062 (-0.028)	-0.012 (0.017)	-0.318 (0.006)	-0.247 (0.016)	-0.102 (-0.005)	0.070 (0.094)	0.018 (0.011)	-0.281 (-0.031)	0.075 (-0.036)	0.099 0.161
1000 grain weight (g)	0.089 (0.002)	0.014 (-0.002)	0.033 (-0.001)	-0.012 (0.012)	-0.399 (0.004)	0.079 (-0.005)	0.317 (0.164)	-0.121 (-0.041)	0.039 (0.012)	0.372 (0.037)	-0.061 (0.032)	0.351* 0.228*
No. of grains per panicle	0.529 (0.041)	-0.001 (-0.002)	0.052 (0.010)	0.020 (0.029)	0.238 (0.007)	-0.039 (0.003)	-0.086 (-0.013)	0.446 (0.509)	-0.041 (-0.018)	-0.260 (-0.052)	0.089 (-0.049)	0.471** 0.407**
Kernel length (mm)	0.093 (-0.007)	-0.003 (0.001)	0.018 (0.011)	0.012 (0.016)	-0.386 (0.007)	-0.017 (0.008)	0.049 (0.009)	-0.071 (-0.045)	0.255 (0.202)	0.349 (0.052)	0.072 (-0.057)	0.184 0.159
Kernel breadth (mm)	-0.419 (-0.034	0.051 (0.014)	0.088 (0.010)	0.036 (0.015)	-0.236 (0.001)	0.112 (-0.002)	0.191 (0.030)	-0.188 (-0.132)	0.144 (0.052)	0.616 (0.201)	-0.176 (0.088)	0.222 0.182
L/B ratio	0.396 (0.024)	-0.022 (0.012)	0.007 (0.015)	-0.001 (-0.010)	-0.262 (0.010)	-0.077 (0.003)	-0.081 (-0.028)	0.165 (0.134)	0.077 (0.062)	-0.451 (-0.095)	-0.240 (0.189)	-0.008 -0.060

Table 6. Distribution of experimental material across distinct clusters

Cluster number	No. of germplasm lines	Name of germplasm lines
I	3	GPB-15, GPB-23, GPB-27
II	16	GPB-2, GPB-7, GPB-9, GPB-17, GPB-22, GPB-12, GPB-4, GPB-24, GPB-26, GPB-6, GPB-19, GPB-14, GPB-21, GPB-20, GPB-5, GPB-10
III	7	GPB-1, GPB-11, GPB-13, GPB-18, GPB-25, GPB-28, GPB-29
IV	3	GPB-3, GPB-8, GPB-16
V	1	GPB-30

Table 7. Inter and intra (diagonal) cluster distances among advanced breeding lines of rice

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	4.81	19.75	18.54	24.73	22.90
Cluster II		9.56	17.24	26.75	15.95
Cluster III			11.12	21.69	21.54
Cluster IV				7.88	37.14
Cluster V					0.00

Table 8. Cluster means for twelve characters in thirty advanced breeding lines of rice

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to 50 % flowering	107.38	108.76	98.69	96.33	112.67
Plant height (cm)	103.08	102.83	106.86	85.96	111.60
Total no. of tillers per plant	21.69	19.07	15.52	16.85	20.67
No. of effective tillers per plant	20.07	15.84	17.87	13.34	20.87
Panicle length (cm)	23.80	26.34	24.94	25.53	28.03
Days to maturity	145.54	146.30	141.95	143.32	149.67
1000 grain weight (g)	29.21	26.28	28.85	26.51	26.80
No. of grains per panicle	167.49	145.27	129.33	136.20	154.90
Kernel length (mm)	7.15	6.83	6.78	7.05	6.21
Kernel breadth (mm)	2.24	1.79	1.93	1.77	1.58
L/B ratio	3.19	3.81	3.52	2.98	3.99
Grain yield per plant (g)	33.38	37.24	25.68	46.96	29.14

Table 9. Eigen values and loading component matrix in different PCs for yield and its attributing traits

Components	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12
Eigen values	3.15	2.42	1.74	1.43	1.12	0.73	0.65	0.31	0.22	0.18	0.09	0.00
Variance (%)	28.46	21.72	13.79	11.24	8.39	5.13	4.42	2.61	1.84	1.50	0.76	0.04
Cumulative Proportion	28.46	50.18	63.97	75.21	83.60	88.73	93.15	95.76	97.60	99.10	99.86	100
Days to 50 % flowering	0.48	0.15	0.08	-0.14	0.05	-0.04	-0.05	0.57	-0.33	-0.46	0.19	-0.00
Plant height	0.26	0.38	-0.15	-0.17	-0.47	-0.10	0.19	-0.26	0.48	-0.37	-0.12	-0.00
Total number of tillers per plant	0.38	-0.09	-0.43	-0.01	0.18	0.24	0.21	0.06	-0.14	0.15	-0.68	0.02
Number of effective tillers per plant	0.34	-0.06	-0.47	-0.02	0.29	-0.01	0.18	-0.09	0.22	0.24	0.63	-0.01
Days to maturity	0.36	0.03	0.10	-0.07	-0.29	0.47	-0.65	-0.06	0.09	0.30	0.05	-0.00
Panicle length	0.25	0.44	0.11	0.20	-0.19	-0.43	0.09	-0.21	-0.46	0.44	0.01	-0.00
Number of grains per panicle	0.19	0.01	0.56	-0.20	0.14	-0.04	0.33	0.32	0.43	0.39	-0.10	-0.01
1000 grain weight	-0.08	0.38	-0.16	-0.04	0.48	-0.39	-0.53	0.10	0.29	0.01	-0.21	0.00
Kernel length	-0.05	0.32	0.02	0.67	0.05	0.32	0.10	0.17	0.14	-0.06	0.02	-0.51
Kernel breadth	-0.32	0.46	-0.18	-0.02	-0.09	0.32	0.13	0.31	-0.01	0.15	0.10	0.62
L/B ratio	0.29	-0.19	0.23	0.58	0.13	-0.07	-0.05	-0.17	0.15	-0.21	-0.03	0.59
Grain yield per plant	0.04	0.33	0.29	-0.26	0.48	0.38	0.11	-0.51	-0.18	-0.19	0.04	0.00

Table 10. Number of alleles per locus, heterozygosity, polymorphism information content and amplified product size

S.No.	Markers	No. of alleles	Heterozygosity (%)	Polymorphism information content (PIC)	Amplified product size (bp)
1	RM583	2	0.00	0.495	179-198
2	RM220	2	0.00	0.444	120-129
3	RM110	3	68.00	0.239	140-159
4	RM263	3	3.33	0.631	158-205
5	RM218	3	3.33	0.664	142-155
6	RM439	2	0.00	0.357	245-26
7	RM471	2	0.00	0.497	103-115
8	RM317	2	0.00	0.391	155-179
9	RM430	2	0.00	0.491	135-156
10	RM413	3	13.79	0.553	75-120
11	RM162	3	10.71	0.43	210-230
12	RM225	2	0.00	0.562	130-149
13	RM11	2	3.33	0.392	135-155
14	RM172	3	37.93	0.224	189-301
15	RM408	4	42.30	0.264	122-141
16	RM242	3	14.20	0.497	225-272
17	RM304	3	30.43	0.571	140-180
18	RM184	3	31.03	0.230	184-215
19	RM286	2	0.00	0.562	100-120
20	RM287	3	0.00	0.661	112-122
21	RM7102	2	0.00	0.677	109-118
	Average	2.52	12.82	0.443	-

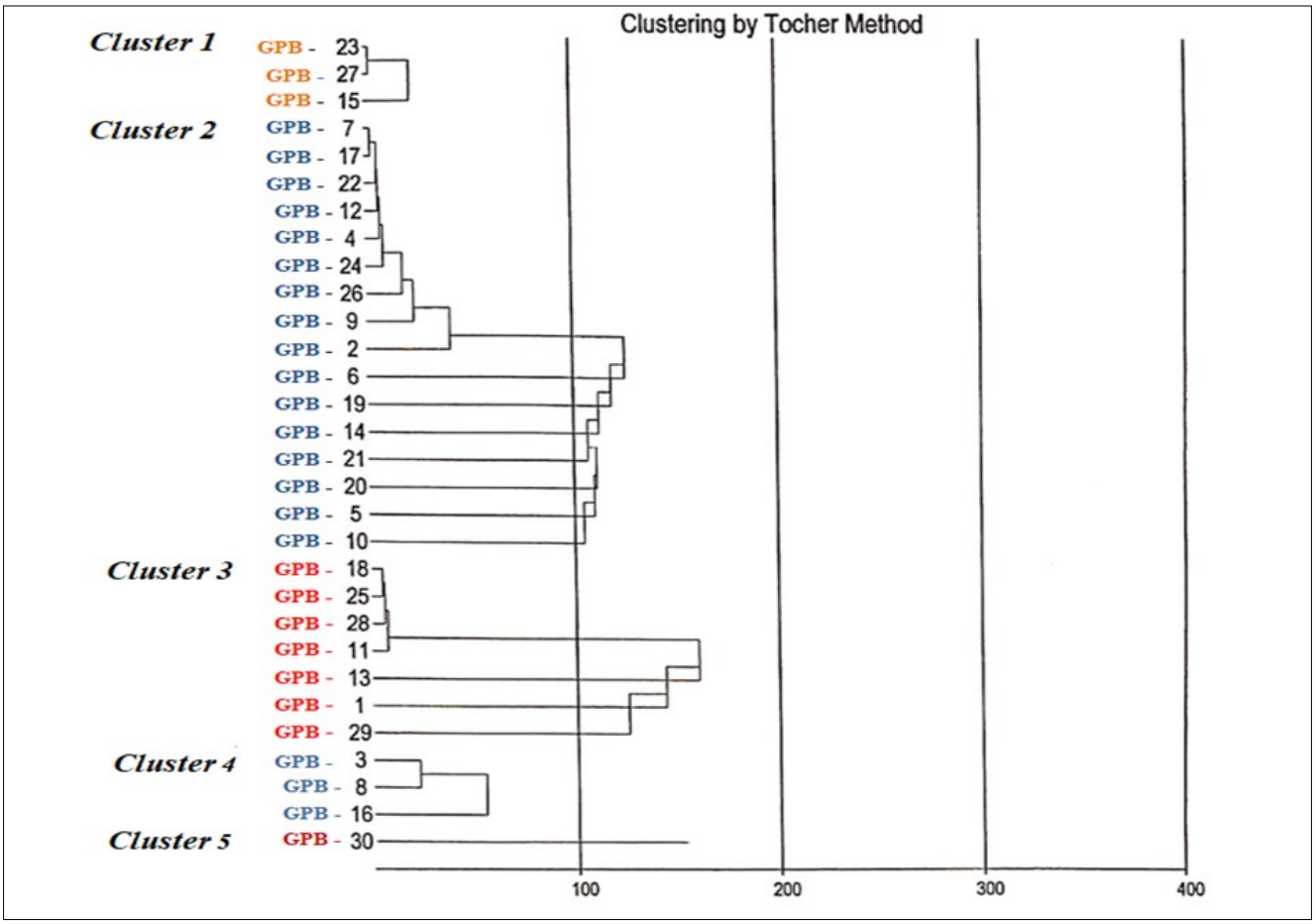

Fig. 1. Clustering patterns among advanced breeding lines based on Tocher's method.

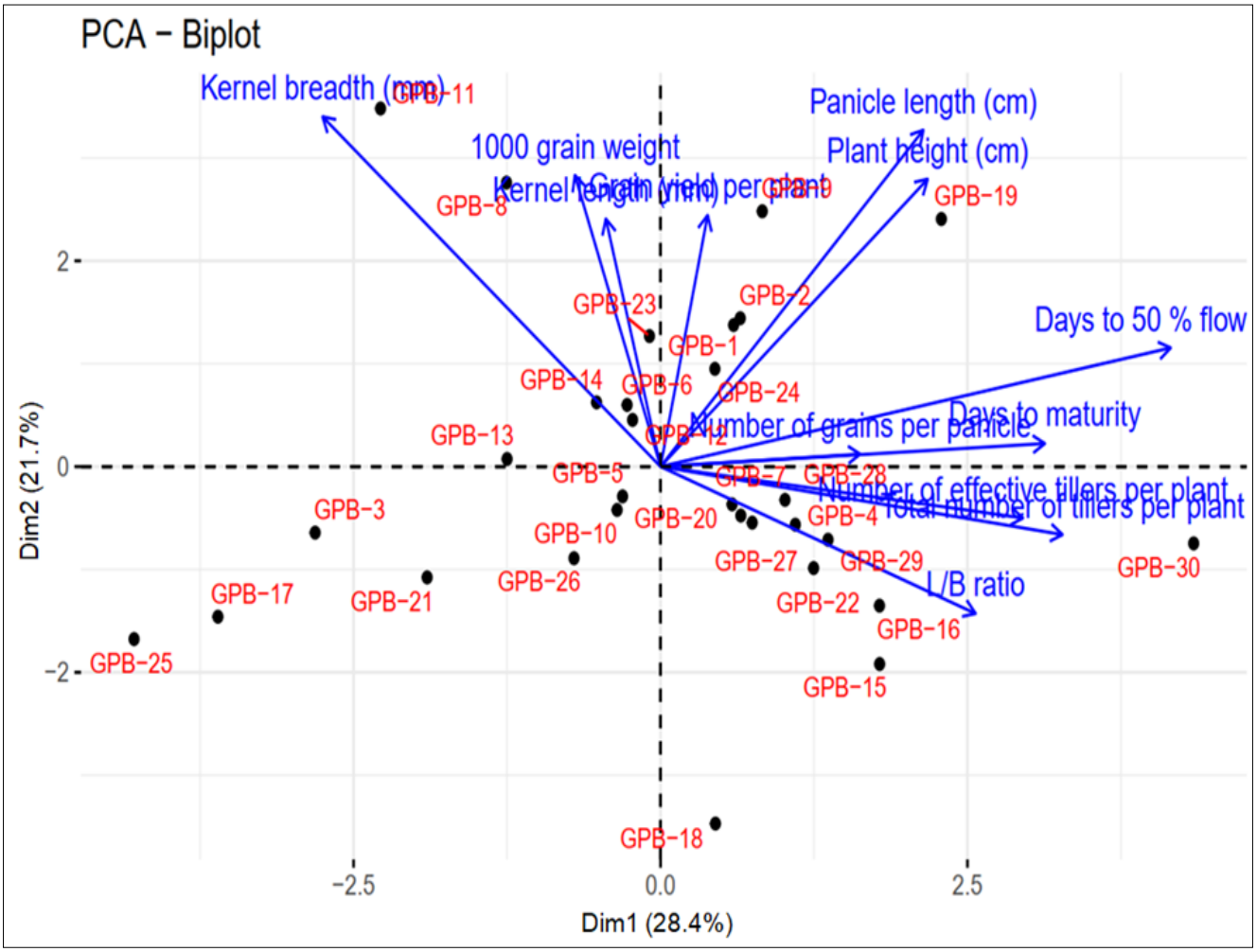

Fig. 2. Biplot of principal components analysis.

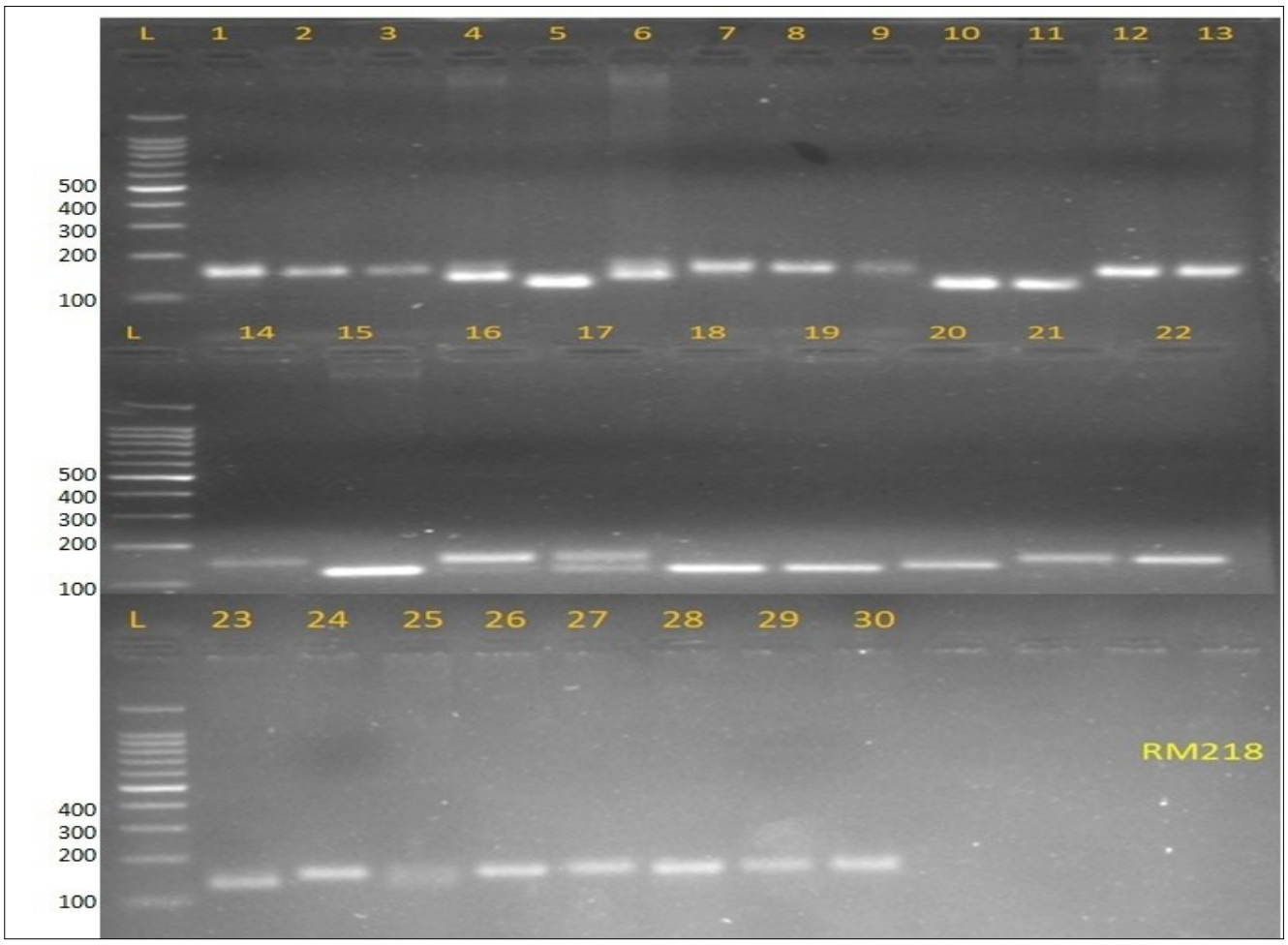

Fig. 3. SSR banding profile of the advanced breeding lines of rice with markers RM218.

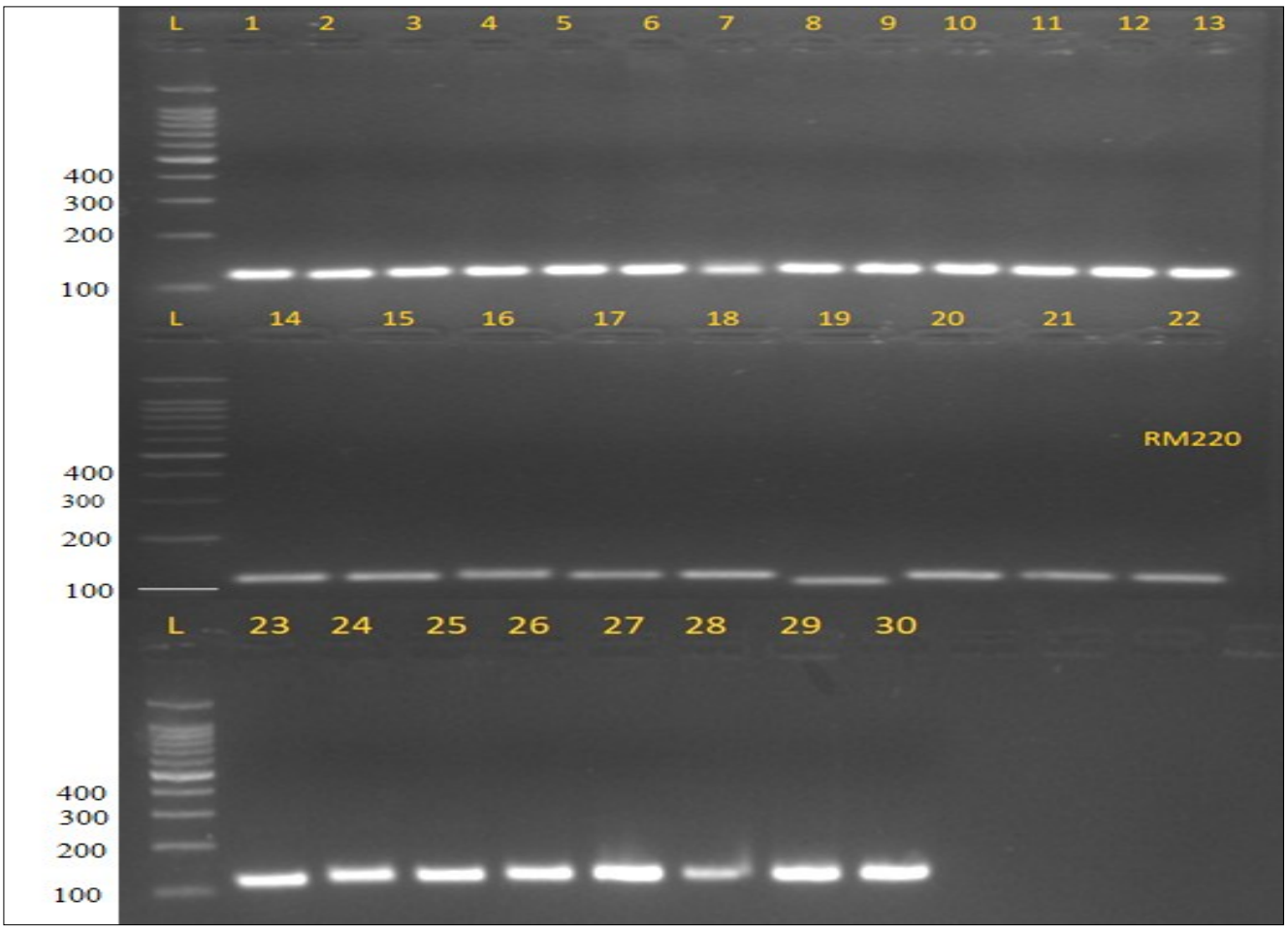

Fig. 4. SSR banding profile of the advanced breeding lines of rice with markers RM220.

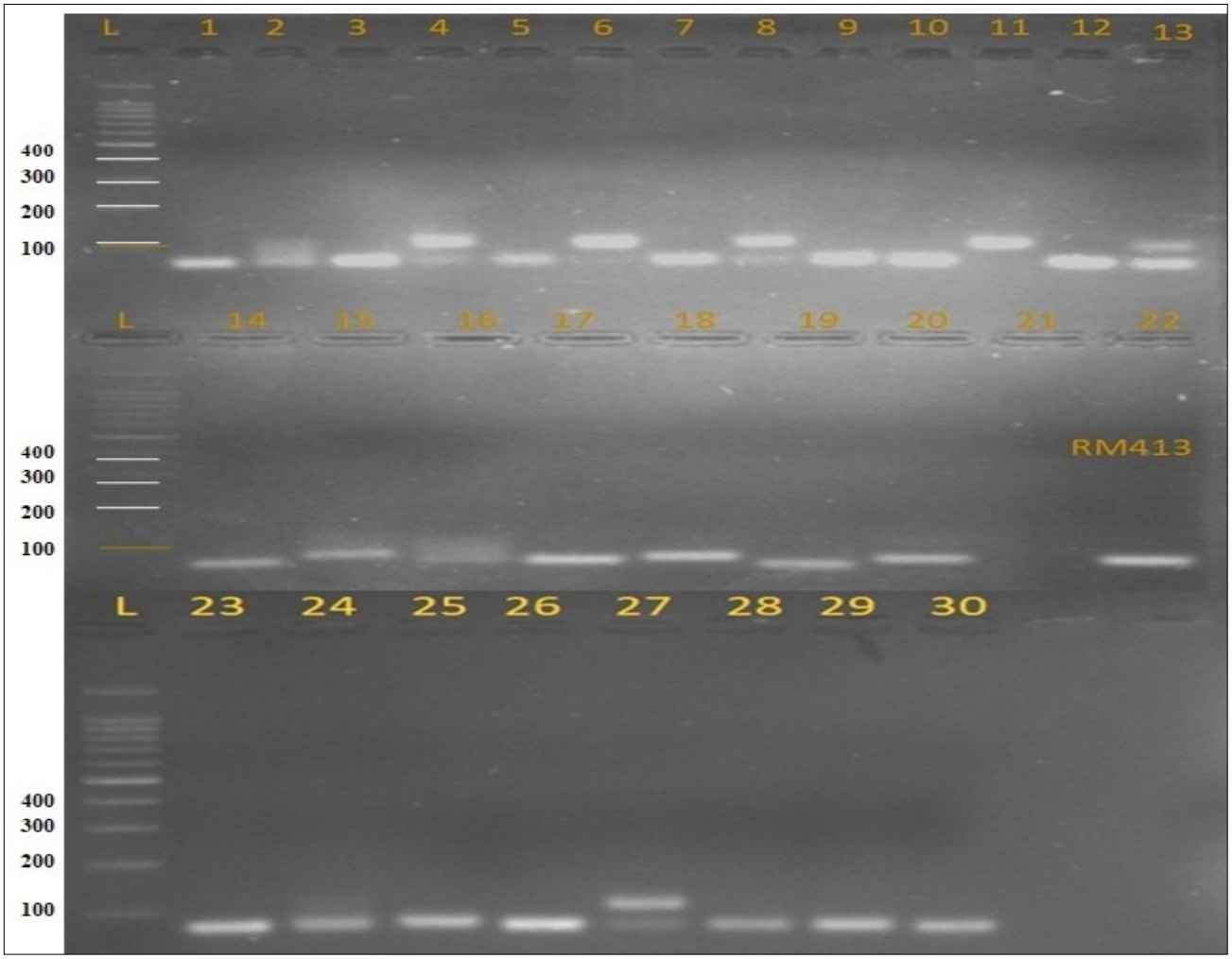

Fig. 5. SSR banding profile of the advanced breeding lines of rice with markers RM413.

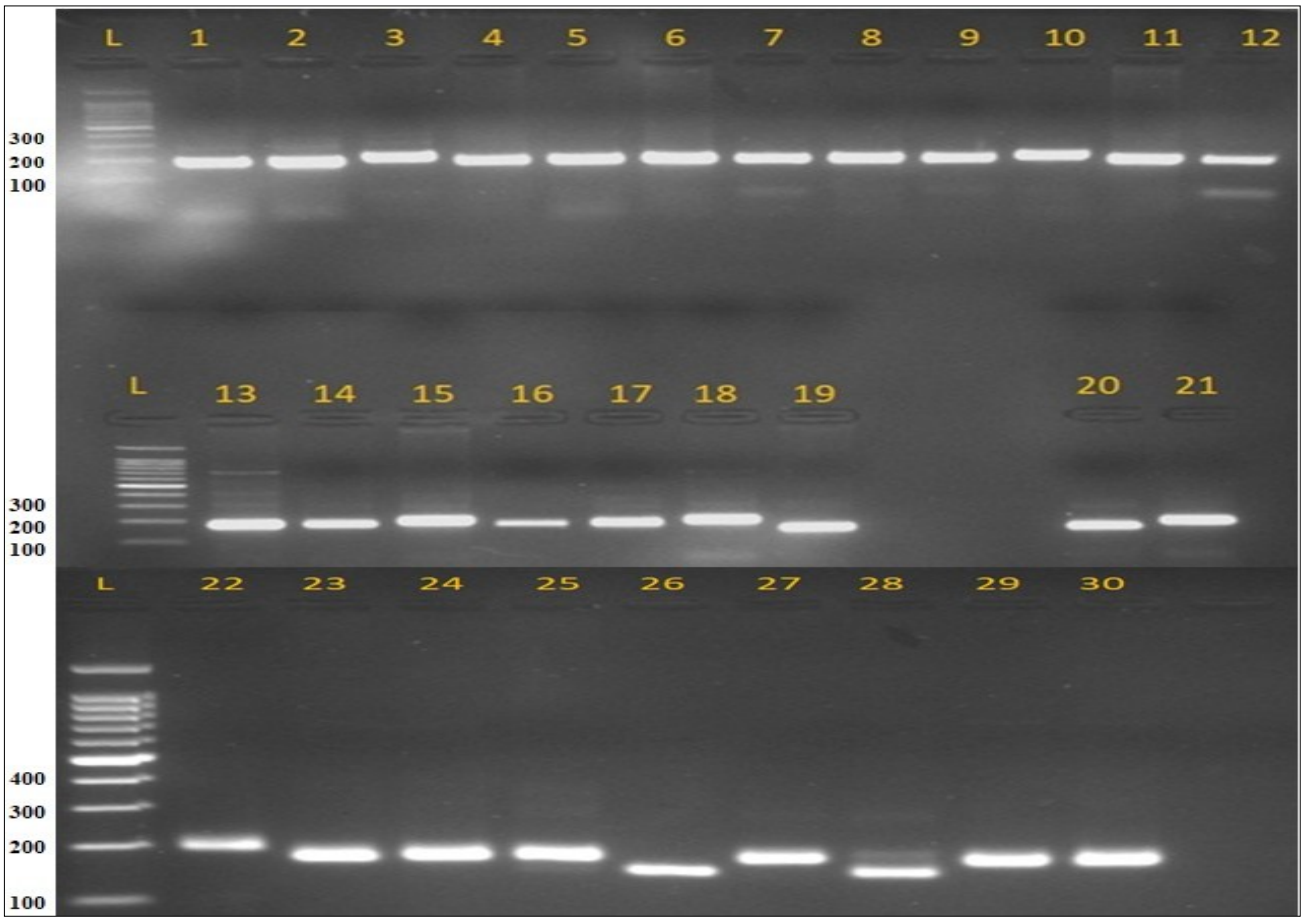

Fig. 6. SSR banding profile of the advanced breeding lines of rice with markers RM263.

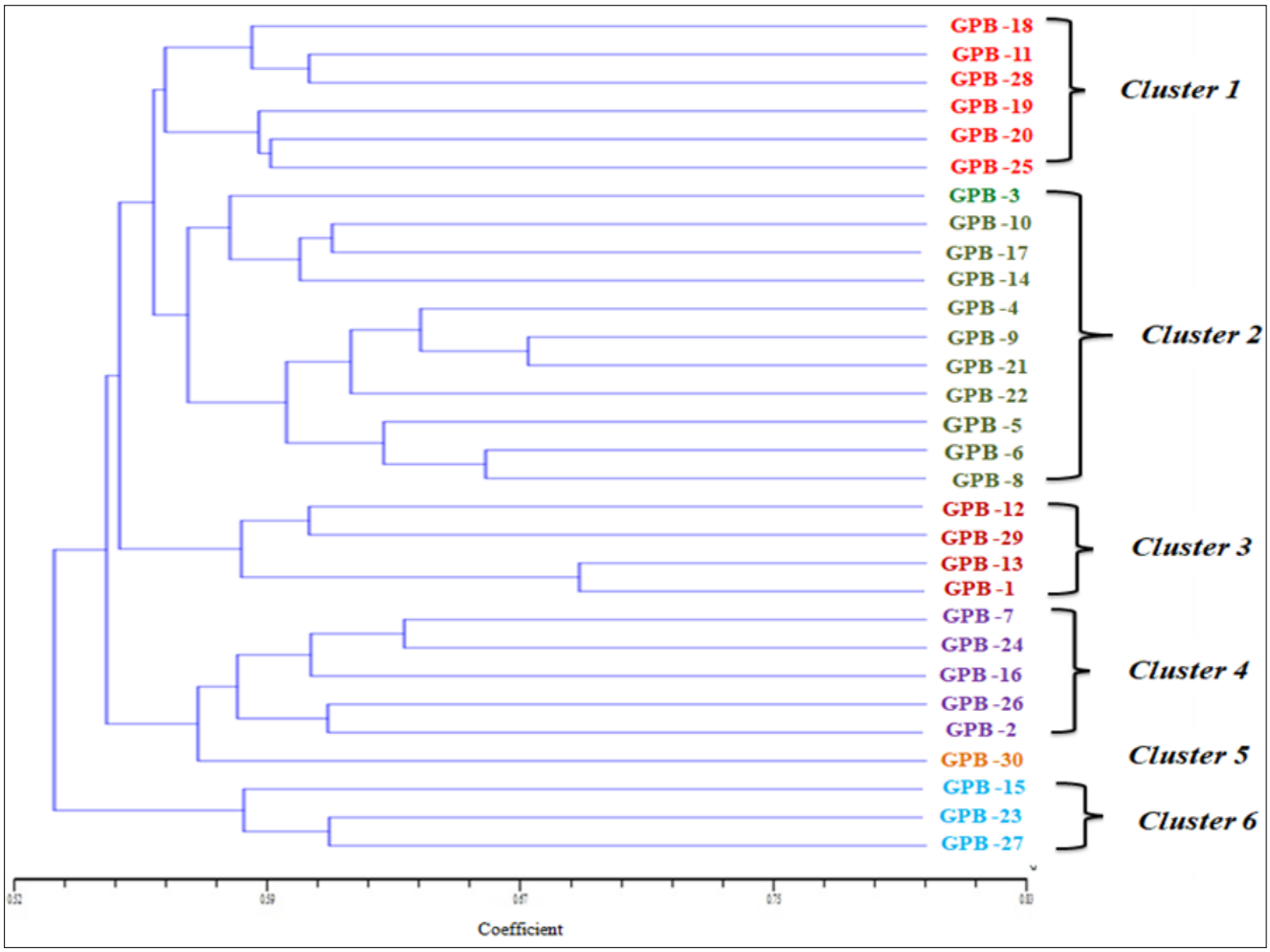

Fig. 7. Dendrogram exhibiting the clustering pattern among the 30 advanced breeding lines of rice based on Jaccard's similarity coefficient.

Table 11. SSR marker-based distributions of advanced breeding lines into different clusters

Cluster number	No. of lines	Name of lines
I	6	GPB-18, GPB-11, GPB-28, GPB-19, GPB-20, GPB-25
II	11	GPB-8, GPB-9, GPB-4, GPB-22, GPB-10, GPB-21, GPB-14, GPB-6, GPB-5, GPB-3, GPB-17
III	4	GPB-1, GPB-12, GPB-13, GPB-29
IV	5	GPB-2, GPB-7, GPB-26, GPB-16, GPB-24,
V	1	GPB-30
VI	3	GPB-15, GPB-27, GPB-23

Conclusion

In the present investigation, genetic variations in both morphological and molecular characteristics were observed among the thirty advanced breeding lines of rice assessed and differences in these genotypes were categorized into distinct clusters. Based on the genetic distance and similarity coefficient obtained from both morphological and molecular analysis a total of seven promising lines were found GPB-15, GPB-23, GPB-3, GPB-8, GPB-18, GPB-27 and GPB-30 based on diversity with respect to various traits recorded and can be used in further breeding programmes. Knowledge of genetic diversity within a population at both morphological and molecular level helps the breeder to formulate a successful hybridization programme and gain good results.

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Authors' contributions

RSS guided the overall research and participated in the design of the study. RK carried out the molecular genetic studies and field experiments drafted the manuscript. APS participated in conducting agronomical trials in the field. BK participated in the design of the study and performed the statistical analysis. MS conceived the study and participated in its design and coordination. SKR helped in designing the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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