



Genetic Divergence in Rice (*Oryza sativa* L.) Germplasms based on Agro-morphological Traits using Multivariate Analysis

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ABSTRACT

Background: A breeder's success in rice breeding depends on identifying and using genetic diversity at genotypic level. This involves evaluating different traits to select parent genotypes with high genetic variability. The purpose of this study is to analyze genetic diversity and assess yield and yield attributes in the collected diverse rice germplasm.

Methods: The study aimed to assess genetic diversity and perform multivariate analysis of 16 grain yield and yield attributes among 50 rice genotypes. The experiment was conducted using Randomized Block Design with three replications at P.G. Research Farm, Ranadevi, MSSoA, CUTM, Paralakhemundi, Odisha, during *Kharif* season of 2022-23.

Result: Cluster analysis categorized genotypes into six distinct clusters with Cluster I containing the most genotypes (40), followed by Cluster II (4) and Cluster V (3), while the rest had one genotype each. Cluster V exhibited the highest intra-cluster distance followed by Clusters II and I. The most significant inter-cluster distance was observed between Clusters V and II and then between Clusters VI and V, indicating substantial genetic divergence among these clusters. Grain yield plant⁻¹ contributed the most to total genetic divergence followed by biological yield plant⁻¹, 1000 grain weight and plant height. Five of 11 PCs analyzed had eigenvalues exceeding 1.0, accounting for 84.72% of the total cumulative variation. Based on *per se* performance, genotypes such as NLR 28523, NLR 9674, Khandagiri, Tulaipani and Barsha emerged as genetically diverse, offering promise for breeding programs.

Key words: Cluster analysis, Genetic divergence, Grain yield, Multivariate, Rice.

INTRODUCTION

Rice is the daily food for a significant portion of the world's population particularly in Asia and has played a key role in human nutrition for the past 10,000 years (Zhao *et al.*, 2020). It also comprises approximately 20 species with a fundamental chromosome number of 12. *Oryza sativa* L. is the primary variety cultivated, serving as the most crucial cereal food crop. Its cultivation is primarily concentrated in the wet season across a substantial geographic area. However, its productivity remains modest due to the scarcity of high-yield stable strains suitable for diverse seasons and agro-climatic zones throughout the nation. In 2022-23, global rice production reached 516.7 million metric tons, with China leading at 148.95 million, followed by India at 118.7 million and Indonesia at 34.6 million (FAO, 2023). Rice cultivation covered 165.25 million hectares globally with India having the largest area at 46.78 million hectares, followed by China at 30.50 million hectares. West Bengal was the top contributor in India, yielding 15.57 million tons followed by Uttar Pradesh (15.52) and Punjab (11.78) million tons (Zhang *et al.*, 2017). Other significant contributors included Andhra Pradesh, Tamil Nadu, Bihar, Chhattisgarh and Odisha. Rice comes in various forms, such as long-grain fragrant Indian basmati, Thai jasmine, short-grain sticky sushi and Italian arborio (Chang *et al.*, 2021). It also exists in various colors, including brown, black, red, white and golden rice enriched with vitamin A (Dubock, 2019). The conditions under which rice is cultivated vary, spanning from irrigated to rainfed and from lowland areas to higher altitudes.

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Even though it is well known that rice hybrids have a very high level of heterosis for grain yield, their grain quality is a major issue, mostly due to the parental line's highly varied grain quality (You *et al.*, 2022; Abd El-Aty *et al.*, 2022). Currently, there has been a growing emphasis on the breeding of quality traits, driven by increased consumer interest in rice quality. The preservation and exploitation of desirable

variations in breeding programs, along with the selection of suitable parental lines, are crucial aspects of successful genetic diversity (Salgotra and Chauhan, 2023). Also, this approach enhances the understanding of rice developmental patterns. Principal component analysis is a vital and widely utilized method, rooted in true eigen vector-based multivariate analysis for precisely assessing genetic diversity.

Understanding the genetic divergence among rice varieties is essential for breeding programs to develop high-yielding, climate-resilient cultivars to ensure food security because of fast-changing environmental conditions and growing global demands (Rezvi *et al.*, 2023). Several studies have aimed to investigate the extent of genetic variation among different rice lines, analyze the factors contributing to this diversity and elucidate its implications for rice breeding and crop improvement strategies (Swarup *et al.*, 2021; Qin *et al.*, 2021). Mahalanobis D² statistical method is a useful technique for quantifying the extent of genetic variation among genotypes and connecting the clustering patterns to their geographical origins. Genetic divergence plays a crucial role in selecting parent lines for hybridization programs (Nivedha *et al.*, 2024). Various researchers have explored genetic diversity, clustering patterns and the contribution of different traits to divergence and selection effectiveness (Tarunum *et al.*, 2023; Sujitha *et al.*, 2020). Multivariate analysis techniques offer a powerful toolset for dissecting complex datasets, providing insights into the intricate relationships between various agronomic traits, environmental factors and genetic markers within rice populations (Shrestha *et al.*, 2021). PCA emerges as data reduction technique, a true virtuoso in exploring intricate interdependencies. To elegantly simplify the intricate and multifaceted connections between sets of measured traits by revealing common threads and shared characteristics among independent variables. The objectives of this study are to assess the genetic divergence and perform multivariate analysis among 50 selected rice genotypes to identify suitable parental lines for hybridization programs and to enhance genetic parameters influencing yield attributes.

MATERIALS AND METHODS

Experimental location

The investigation was conducted at P.G. Research Farm, Ranadevi, MSSSoA, CUTM, Paralakhemundi, Odisha, during *Kharif* 2022-23 season. The experimental site is situated in the North Eastern Ghat zone of Odisha at 18.77027°N latitude and 84.10195°E longitude, at an elevation of 57 meters above sea level. The soil is well-drained, with a sandy loam texture and pH of around 7.4.

Genetic materials and planting details

The test entries consisted of 50 selected rice accessions from different regions of Andhra Pradesh, Odisha and West Bengal. Twenty-seven days old seedlings were transplanted in randomized block design (RBD) with three replicated plots using 20 × 15 cm spacing. Recommended practices and packages were followed during the crop period under transplanted conditions to ensure good crop growth.

Observations recorded

The observations were collected from randomly selected ten plants from each replication for 16 quantitative traits as per the objectives of the study. The data was recorded on plant height (PH), number of productive tillers plant⁻¹ (NPT),

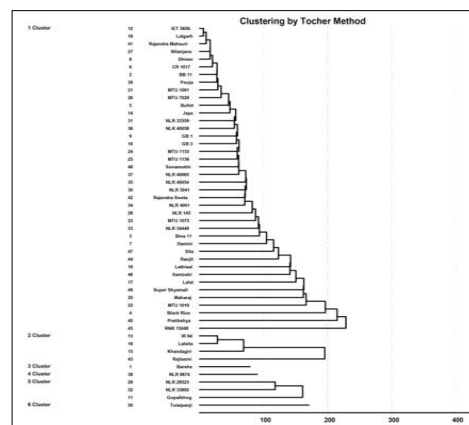


Fig 1: Dendrogram depicting the clustering of rice genotypes based on yield and its attributing traits.

Table 1: Clustering pattern of 50 rice genotypes for yield and its contributing traits using Tocher's method.

Cluster group	Number of genotypes	List of genotypes
I	40	IET 5656, Lalgarh, Rajendra Mahsuri, Nilanjana, Dhiren, CR 1017, BB 11, Pooja, MTU 1001, MTU 7029, Bullet, Jaya, NLR 33359, NLR 40058, GB 1, GB 3, MTU 1153, MTU 1156, Sonamukhi, NLR 40065, NLR 40054, NLR 3041, Rajendra Sweta, NLR 4001, NLR 145, MTU 1075, NLR 34449, Bina 11, Damini, Sita, Ranjit, Lathisal, Santoshi, Lalat, Super Shyamali, Maharaj, MTU 1010, Black Rice, Pratikshya, RNR 15048
II	4	IR 64, Lalsita, Khandagiri, Rajlaxmi
III	1	Barsha
IV	1	NLR 9674
V	3	NLR 28523, NLR 33892, Gopalbhog
VI	1	Tulaipanji

panicle length (PL), filled grains panicle⁻¹ (FGP), total grains panicle⁻¹ (TGP), spikelet fertility percentage (SF), 1000 grain weight (TW), straw yield plant⁻¹ (SYP), biological yield plant⁻¹ (BYP), grain yield plant⁻¹ (GYP) and harvest index (HI). The plants from the border were excluded to minimize the border effect. Days to 50% flowering (DFF) and days to maturity (DM) were recorded on plot basis. Randomly, 10 rice grains were selected to assess their dimensions, including grain length (GL), grain breadth (GB) and grain length to breadth ratio (LB), using a vernier calliper on a set of chosen grains.

Statistical analyses

The statistical analysis utilized the average data from each replication employing RBD design to determine the significance of genotypic variance across multiple genotypes as recommended by Rao (1952). The genetic divergence among the genotypes for 16 morphological characters was assessed using Mahalanobis D² statistics.

This analysis involved evaluating data collected for various traits following the methodologies established by Mahalanobis (1936) and the clustering pattern described by Ward (1963). Principal component analysis, a widely used method for analyzing multivariate data, was initially introduced by Pearson (1901) and developed by Hotelling (1933).

RESULTS AND DISCUSSION

An effective categorization of test entries based on distinct characteristics is essential for distinguishing genetically similar and dissimilar genotypes, a fundamental requirement for any genetic study. The analysis of the variance indicated distinctions among the different genetic types across all traits under investigation.

Grouping of genotypes into clusters

Using Tocher's method, 50 selected rice genotypes were divided into six distinct clusters as depicted in Table 1. The D² values were derived from the average of genotypes. The

Table 2: Average intra (diagonal) and inter (off-diagonal) cluster distances among six clusters of rice genotypes for yield and its contributing traits.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	119.68	292.46	286.74	318.54	370.36	240.57
Cluster II		169.37	402.50	427.44	833.28	346.69
Cluster III			0.00	207.03	361.90	371.20
Cluster IV				0.00	322.45	462.52
Cluster V					200.11	499.65
Cluster VI						0.00

Table 3: Description of genetically divergent clusters and D² distances among selected rice genotypes for yield and its contributing traits.

Cluster combination	Inter-cluster distance (D ² value)	Selected genotypes from the cluster	Distance between the selected genotypes (D ² value)
Cluster V and Cluster II	833.20	NLR 28523 and Khandagiri	1742.78
Cluster VI and Cluster V	499.65	Tulaipanji and NLR 28523	992.17
Cluster VI and Cluster IV	462.52	Tulaipanji and NLR 9674	627.20
Cluster IV and Cluster II	427.44	NLR 9674 and Khandagiri	803.85
Cluster III and Cluster II	402.50	Barsha and Khandagiri	691.12
Cluster VI and Cluster III	371.20	Tulaipanji and Barsha	387.07

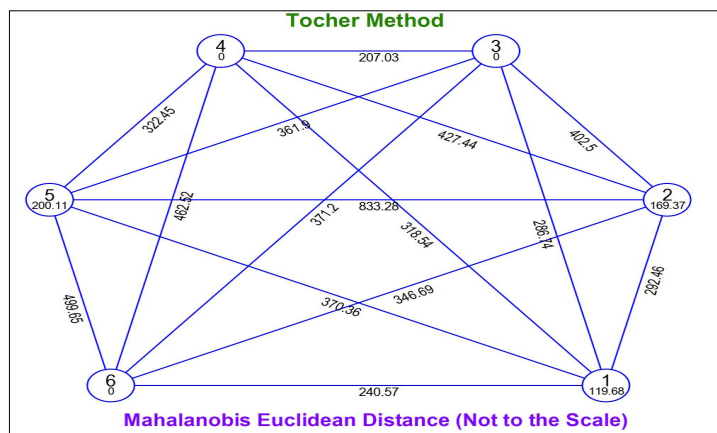


Fig 2: Diagram of intra- and inter-cluster distance (D² values) among six clusters using Tocher's method.

cluster dendrogram shows the variety of genotypes within the plant population studied (Fig 1). Among them, the total number of genotypes in Cluster I was highest (40), followed by Cluster II (4) and Cluster V (3). Clusters III (Barsha), V (NLR 9674) and VI (Tulaipanji) stood out as solitary clusters. Kushwaha *et al.* (2020) and Singh *et al.* (2022) found similar results about the non-association between geographical regions and genetic diversity. According to Rezk *et al.* (2024) and Sheeba *et al.* (2023), various traits contributed to genetic differences with no correlation observed between the origin of the genotypes and their genetic diversity.

Average intra and inter-cluster distances

The intra-cluster distances varied from zero (Clusters III, IV and VI) to 200.11 (Cluster V). The greatest inter-cluster distance (833.28) was observed between Cluster V and II

followed by Cluster VI and V (499.65), Cluster VI and IV (462.52), Cluster IV and II (427.44), Cluster III and II (402.5) and Cluster VI and III (371.2) indicating substantial genetic diversity among genotypes across these clusters (Table 2 and Fig 2). Crosses from genotypes in Cluster I, II and V exhibiting significant divergence are likely to yield more favorable offspring for achieving increased yield through genetic diversity (Chhodavadiya *et al.*, 2023; Thakur and Sarma, 2023).

The analysis of genetically divergent clusters and the computation of distances (D^2 values) among the studied genotypes of rice are given in Table 3. Upon careful examination of these distances, it was observed that NLR 28523 in Cluster V and Khandagiri in Cluster II showed the highest genotypic distance (1742.78). Similar patterns of significant genetic distances between rice lines were

Table 4: Cluster mean values for yield and its contributing traits of rice genotypes grouped by Tocher's method.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Days to 50% flowering	101.28	96.08	134.33	150.67	135.89	96.33
Days to maturity	131.60	126.33	162.67	180.67	166.22	127.67
Plant height (cm)	114.64	109.38	158.26	126.90	139.48	142.47
Number of productive tillers plant ⁻¹	10.63	11.63	9.60	10.93	10.31	20.33
Panicle length (cm)	23.11	22.49	22.49	24.00	24.79	27.00
Total grains panicle ⁻¹	169.83	96.28	162.40	149.03	240.60	166.93
Filled grains panicle ⁻¹	111.58	89.69	98.29	194.23	202.99	53.27
Spikelet fertility (%)	67.97	97.44	60.57	86.60	84.43	74.71
1000 grain weight (g)	21.29	24.43	26.97	17.11	16.26	18.50
Straw yield plant ⁻¹ (g)	23.55	22.28	29.29	48.55	43.39	26.07
Grain length (mm)	8.14	9.85	9.21	6.95	6.68	8.08
Grain breadth (mm)	2.36	2.22	2.69	2.51	2.38	1.92
Grain L/B ratio	3.50	4.45	3.42	2.78	2.82	4.21
Biological yield plant ⁻¹ (g)	48.53	47.16	54.70	84.86	77.64	46.10
Harvest index (%)	0.51	0.52	0.47	0.43	0.44	0.43
Grain yield plant ⁻¹ (g)	24.98	24.88	25.41	36.31	34.25	20.03

Table 5: Contribution of yield and its related traits towards total genetic divergence among rice genotypes.

Characters	Contribution to divergence (%)	No. of first rank
Grain yield plant ⁻¹ (g)	16.78	206
Biological yield plant ⁻¹ (g)	10.43	128
1000 grain weight (g)	8.90	109
Plant height (cm)	8.49	104
Harvest index (%)	7.43	91
Straw yield plant ⁻¹ (g)	7.33	90
Panicle length (cm)	6.42	79
Number of productive tillers plant ⁻¹	6.33	78
Spikelet fertility (%)	4.98	61
Grain length (mm)	4.32	53
Grain L/B ratio	3.50	43
Grain breadth (mm)	3.44	42
Filled grains panicle ⁻¹	3.43	42
Days to 50% flowering	3.21	39
Days to maturity	2.57	31
Total grains panicle ⁻¹	2.44	30

identified in other clusters, such as Tulaipanji in Cluster VI and NLR 28523 in Cluster V (992.17). Tulaipanji in Cluster VI and NLR 9674 in Cluster IV demonstrated a considerable genotypic distance (627.20). Also, NLR 9674 in Cluster IV and Khandagiri in Cluster II exhibited a considerable genotypic distance (803.85). Additionally, Barsha in Cluster III and Khandagiri in Cluster II showed D^2 distance (691.12) and Tulaipanji in Cluster VI and Barsha in Cluster III recorded a substantial genotypic distance (387.07). Identifying parental lines from these different groups has great potential for breeding programs. Crossing parents with greater genetic divergence can generate higher variability and strong beneficial traits in their offspring. These results were also in accordance with some previous studies (Mondal *et al.*, 2024; Roy *et al.*, 2023;2024).

Cluster means

Table 4 shows the cluster means for DFF and DM, which were highest in Cluster IV (150.67 and 180.67) and lowest in Cluster II (96.08 and 126.33). Similarly, the cluster mean for PH was highest in Cluster III (158.26) and lowest in Cluster II (109.38). The NPT exhibited highest mean value in Cluster VI (20.33) and lowest in Cluster III (9.60), while PL had its highest mean in Cluster VI (27.00) and lowest in Clusters II and III (22.49). For TW, Cluster III (26.97) recorded highest mean, while Cluster V (16.26) had lowest. The highest mean for TGP was in Cluster V (240.60), whereas lowest was in Cluster II (96.28). Cluster V (202.99) exhibited highest mean for FGP, whereas Cluster VI (53.27) had lowest. SF had the maximum mean in Cluster II (97.44) and Cluster III (60.57) had minimum value. GYP and BYP had highest mean in Cluster IV (36.31 and 84.86) and lowest in Cluster VI (20.03 and 46.10). HI and LB had highest mean in Cluster II (4.45 and 0.52) and lowest in Cluster IV (2.78 and 0.43). GL had highest mean in Cluster II (9.85) and lowest in Cluster V (6.68), while GB had highest mean in Cluster III (2.69) and lowest mean in Cluster VI

(1.92). Cluster IV (48.55) exhibited highest mean for SYP, whereas Cluster II (22.28) showed lowest mean. The findings demonstrated significant genetic divergence within the genotypes in these groups, suggesting their potential utility in targeted trait enhancement in plant breeding initiatives. Crossing genotypes within these clusters is also expected to produce substantial heterosis. This result aligns with earlier studies by Pavankumar *et al.* (2022) and Sudeepthi *et al.* (2020).

Contribution towards total divergence

The highest percentage contribution towards genetic divergence was observed in GYP (16.78%), followed by BYP (10.43%), TW (8.9%), PH (8.49%), HI (7.43%), SYP (7.33%), PL (6.42%), NPT (6.33%), SF (4.98%), GL (4.32%), LB (3.5%), GB (3.44%), FGP (3.43%), DFF (3.21%), DM (2.57%) and TGP (2.44%), as shown in Table 5 and Fig 3. All genotypes exhibited significant variability in economic traits especially in GYP suggesting the need for further study on allelic characterization. Previous studies also found similar results by different researchers (Bhargavi *et al.*, 2023; Bora *et al.*, 2023; Shrivastav *et al.*, 2022).

Principal component analysis

Principal component analysis serves to condense large datasets into more manageable principal components, preserving all crucial details by analyzing the correlations between variables. The eigenvector values, variation

Table 6: Eigenvalues, percent of variance and cumulative variance of yield and its contributing traits among rice genotypes.

	Eigenvalue	Variability (%)	Cumulative (%)
PC ₁	5.92	36.97	36.97
PC ₂	3.05	19.08	56.05
PC ₃	1.84	11.47	67.52
PC ₄	1.70	10.63	78.15
PC ₅	1.05	6.57	84.72

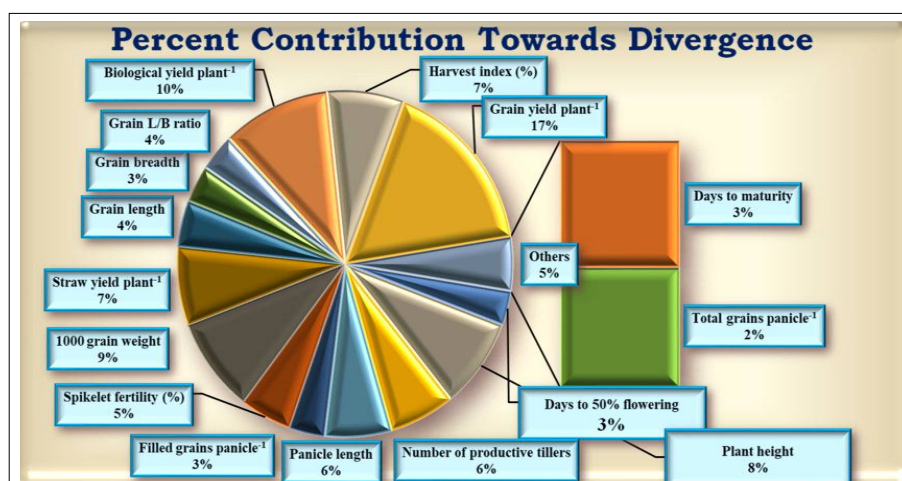


Fig 3: Per cent contribution of yield and its attributing traits towards total genetic divergence among genotypes.

percentages and cumulative percentages are presented in Table 6. Among the eleven components, five PCs observed eigenvalues surpassing 1.0, thus contributing significantly to the cumulative variability of 84.72% across the considered variables. PC₁, accounting for 36.97% of the total variation, along with the rest of PCs, contributed 56.05%, 67.52%, 78.15% and 84.72% to the overall variance. Components boasting multiple eigenvalues showcased heightened variability among rice genotypes, facilitating the identification of diverse parental selections. The scree plot provided insights into the percentage of variance explained by eigenvalues and principal components (Fig 4). In this study, PC₁ showed 36.97% variability, characterized by an eigenvalue of 5.92. The graph depicts PC₁ as exhibiting the highest degree of variability compared to other PCs. One earlier research reported a maximum variance of 38.72% in PC₁ across 49 rice lines (Christina *et al.*, 2021). Thus, selecting genotypes from PC₁ could prove advantageous for future breeding endeavors focused on improving traits (Tiwari *et al.*, 2022).

In this study, the biplot is constructed using PC₁ and PC₂ to analyze the associations between 50 rice accessions based on yield and its related attributes (Fig 5). Biplot based correlations among traits explained 56.05% of the total variation, providing a reliable estimate for evaluating their impact on yield and inter-similarities. All characters except TGP, SF, NPT and HI have higher vector lengths, indicating a more substantial influence on the variation in a particular dimension. The loading plot revealed substantial diversity among nearly all genotypes and variables. These findings are consistent with those reported by Sheela *et al.* (2020).

The contribution of 16 quantitative traits to the principal components is presented in Table 7. In PC₁, SYP (0.913) followed by BYP (0.908), DM (0.890), DFF (0.881), GYP (0.780), FGP (0.763), PH (0.556), TGP (0.500), GB (0.441), PL (0.300) and SF (0.184) showed favourable loadings, while the remaining traits exhibited negative loadings. PC₂ showed positive loadings for parameters such as TW (0.888), GL (0.754), NPT (0.484) and HI (0.434) with other factors such as FGP (-0.493) followed by SF (-0.416) and

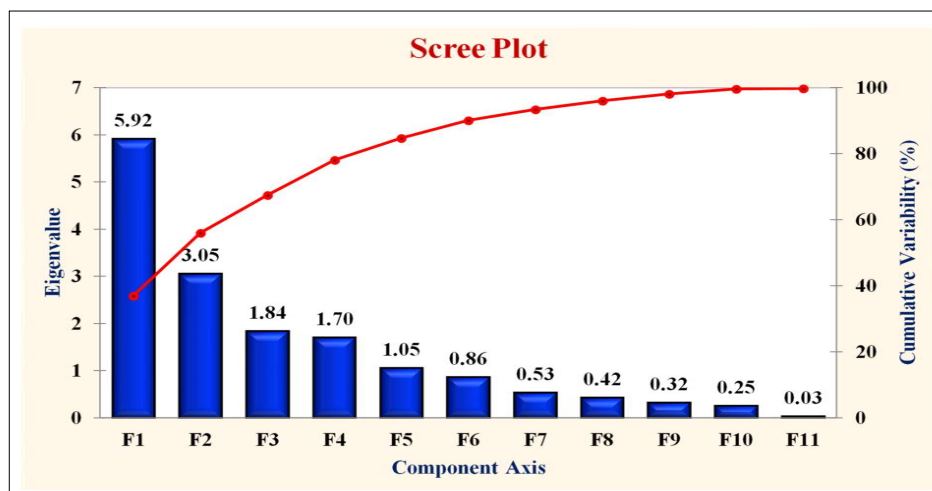


Fig 4: Scree plot depicting the clustering of genotypes based on yield and its attributing traits.

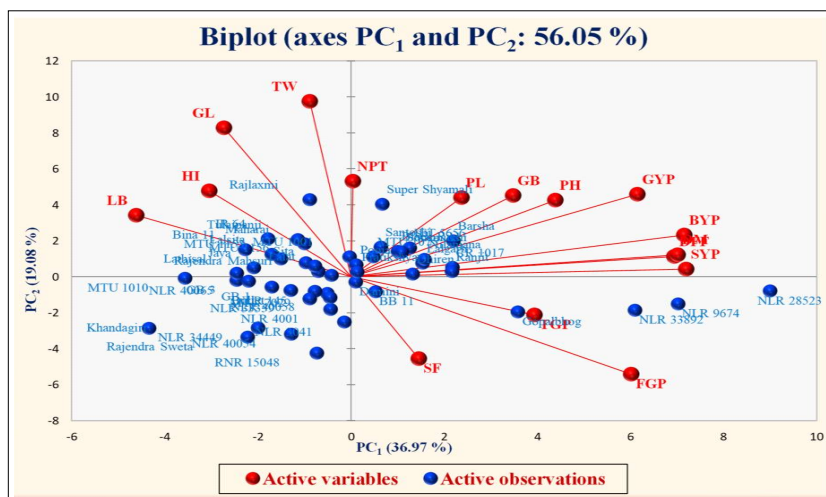


Fig 5: Biplot presenting the relationship between yield and its attributing traits among rice genotypes.

Table 7: Five PCs along with their factor loading values for yield and its contributing traits among rice genotypes.

Characters	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅
Days to 50% flowering	0.881	0.103	0.120	-0.096	0.001
Days to maturity	0.890	0.112	0.111	-0.097	-0.006
Plant height (cm)	0.556	0.388	-0.094	0.332	-0.418
Number of productive tillers plant ⁻¹	0.005	0.484	0.417	0.448	-0.148
Panicle length (cm)	0.300	0.400	-0.043	0.613	-0.102
Total grains panicle ⁻¹	0.500	-0.194	-0.511	0.415	0.502
Filled grains panicle ⁻¹	0.763	-0.493	-0.011	-0.129	0.287
Spikelet fertility (%)	0.184	-0.416	0.572	-0.549	-0.228
1000 grain weight (g)	-0.113	0.888	-0.119	-0.235	-0.021
Straw yield plant ⁻¹ (g)	0.913	0.038	0.301	0.026	0.074
Grain length (mm)	-0.347	0.754	0.292	-0.222	0.258
Grain breadth (mm)	0.441	0.411	-0.510	-0.429	-0.295
Grain L/B ratio	-0.585	0.311	0.579	0.126	0.398
Biological yield plant ⁻¹ (g)	0.908	0.211	0.203	-0.074	0.151
Harvest index (%)	-0.386	0.434	-0.455	-0.393	0.249
Grain yield plant ⁻¹ (g)	0.780	0.417	0.043	-0.200	0.235

TGP (-0.194) demonstrating negative loadings. Traits in PC₃ that recorded positive loadings were LB (0.579), SF (0.572), NPT (0.417), SYP (0.301), GL (0.292), BYP (0.203), DFF (0.120), DM (0.111) and GYP (0.043), while the remaining characters observed negative loadings. In PC₄, variables such as PL (0.613), NPT (0.448), TGP (0.415), PH (0.332), LB (0.126) and SYP (0.026) exhibited positive loading values and the remaining traits showed negative loadings. In PC₅, TGP (0.502) followed by LB (0.398), FGP (0.287) and GL (0.258) exhibited positive loadings, while PH (-0.418), followed by GB (-0.295), SF (-0.228) and NPT (-0.148) showed negative loadings. Among the studied traits, PC₁ and PC₂ contributed more to genetic divergence and accounted for a significant portion of the variability. Therefore, selecting traits with substantial variability will benefit future breeding endeavors.

CONCLUSION

The examination of variance indicated significant diversity among the 50 rice genotypes across all traits. Utilizing D² analysis, the genotypes were categorized into six clusters. Considering the inter-cluster distances, genotypes from Cluster V and II, followed by Cluster VI and V, Cluster VI and IV are suggested as potential parents for upcoming hybridization endeavors. GYP, BYP, TW and PH were identified as pivotal contributors to overall divergence, underscoring their importance for future crop improvements. The PCA of the selected genotypes indicated that the major five PCs, with eigenvalues larger than one explained 84.72% of the total cumulative variance. This found a strong connection between the traits evaluated. Based on analysis of cluster means and inter-cluster distance, the genotypes NLR 28523, NLR 9674, Khandagiri, Tulaipani and Barsha showed promise for their utilization in breeding programs aimed at producing high-yielding rice varieties with desirable quality traits.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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