



Integrated multivariate analysis reveals genetic diversity for yield, grain quality, and stress tolerance in rice (*Oryza sativa* L.) genotypes

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Abstract

Rice (*Oryza sativa* L.) is a major staple crop, and simultaneous improvement of yield, grain quality, and stress resistance is essential under changing climatic conditions. However, comprehensive diversity analyses that integrate agronomic, quality, and stress related traits remain limited in rice improvement programs. Therefore, the present study was conducted to evaluate 36 rice varieties during the *Kharif* season of 2025 at Nuthankal Farm, Hyderabad, using a Randomized Complete Block Design (RCBD) with two replications. Data on 22 traits, including six agronomic, 12 grain quality, and four biotic and abiotic stress-related parameters, were analyzed using analysis of variance (ANOVA), cluster analysis, and principal component analysis (PCA). The results revealed significant differences among the genotypes for all the measured parameters, indicating the presence of high genetic variability. The cluster analysis grouped the genotypes into four distinct clusters, with the highest number of genotypes in Cluster II and only one genotype in Cluster IV, which was highly divergent. The genotypes in Clusters II and III were found to be superior for yield, milling quality, and moderate stress resistance, whereas Cluster IV genotypes had unique characteristics such as early flowering and herbicide resistance. PCA revealed that the first six components explained 81.58 % of the total variation, with major contributions from yield, milling percentage, head rice recovery, amylose content, and stress scores. NP 10325-3-14, NP 7373, NP 9156, and NP 9558 exhibited resistance to bacterial leaf blight. Based on the identified trait-specific donor genotypes, NP 9156, NP 9153 and NP-9558 can be crossed with the genetically divergent and herbicide-tolerant HTM-N22 to combine high yield, superior grain quality, and herbicide tolerance. Likewise, NP 10325-3-11 or NP 10325-3-8 × NP 9156/NP 9153 are promising combinations for improving cooking quality with high yield, while NP 7373 or NP 10325-3-5 × NP 9156/NP 9153 may facilitate the development of high-yielding cultivars with improved head rice recovery.

Keywords: Rice, Cluster analysis, Euclidean distance, eigenvalue, grain quality, herbicide tolerance

INTRODUCTION

Rice (*Oryza sativa* L.) serves as an important staple food that nourishes more than half of the world's population (UNFPA, 2025). It also plays an important role in supplying the necessary amount of energy and protein for humans. The increasing pressure of the population and changing climatic conditions require the breeding of new rice varieties that would provide increased yields and better stress resistance along with improved quality characteristics. Unfortunately, conventional genetic diversity analysis mostly relies on yield and morphological features, and rarely pays attention to grain quality and stress resistance (Zandalinas and Mittler, 2022).

The use of such traits in the assessment of genetic diversity gives a realistic idea about the true extent of variability among rice genotypes (Shrestha *et al.*, 2022). Nonetheless, the collection of such diverse data does not permit the use of traditional analysis techniques. In this regard, multivariate methods such as principal component analysis and clustering allow efficient evaluation of variability and grouping of genotypes based on their contribution (Dinesh *et al.*, 2023). Hence, the goal

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of this study is to estimate genetic diversity in rice using integrated trait assessment, determine main sources of variability, and categorize genotypes for further cross-breeding and production of superior varieties.

MATERIALS AND METHODS

The experiment was carried out during the *kharif* 2025 season at Nuthankal Farm, Hyderabad. The environment in which this experiment was conducted may be regarded as typical for irrigated rice cultivation. A total of 36 rice varieties were employed for conducting this study, 15 advanced breeding lines (G1 to G15) and 20 commercial varieties (G16 to G35) from Nuziveedu Seeds, Hyderabad and one genotype mutant HTM-N22 (G36) was derived from upland rice variety Nagina 22 (N22) through ethyl methane sulfonate (EMS) an induced mutagenesis (Shoba *et al.*, 2017). The experiments were conducted using the randomized block design, with two replications and with 10 sqm plot size. The spacing for transplanting each variety was maintained at 20 x 15 cm. The experiment was conducted using standard agronomic practices, i.e., typical for rice cultivation in the region. Proper plant protection measures were also taken.

Screening of rice genotypes for major biotic stresses and herbicide tolerance

Thirty-six rice genotypes were evaluated for resistance to major biotic stresses and herbicide tolerance under controlled and field conditions. Screening for bacterial leaf blight (BLB) was carried out under greenhouse conditions using TN-1 and PR 129 as susceptible and resistant checks, respectively. The genotypes were planted in two rows of 4 m length and inoculated at the tillering stage (40 days after transplanting) with a local isolate of *Xanthomonas oryzae* pv. *oryzae* (Xoo) maintained at Nuziveedu Seeds Pvt. Ltd., Hyderabad. Disease reaction was recorded 14 days after inoculation and scored according to the Standard Evaluation System (SES) (IRRI, 2014). Similarly, Blast resistance was evaluated under uniform blast nursery (UBN) conditions with HR12 and IR 64 as susceptible and resistant checks, respectively. Artificial inoculation was performed using a local isolate (SP-28) of *Magnaporthe oryzae*, and disease scoring was done 15 days after inoculation following SES (IRRI, 2014).

Screening for brown planthopper (BPH) resistance was done using the seed box screening method with three replications. TN-1 and PTB-33 were used as susceptible and resistant checks, respectively. Germinated seeds were raised up to the two-leaf stage, and second to third instar nymphs were released at a density of 8–10 nymphs per seedling. Damage scoring was carried out when the susceptible check showed complete wilting (10–12 days) based on SES (IRRI, 2014; Ishwarya Lakshmi *et al.*, 2021).

Screening for imazethapyr tolerance was performed in a randomized complete block design (RCBD) with

three replications using all 36 genotypes. The herbicide imazethapyr (Pursuit™) was applied at 2.5 ml L⁻¹ on 15-day-old seedlings. Herbicide injury was visually assessed 15 days after application following the procedure described by Shoba *et al.* (2017).

Evaluation of agro-Morphological, Grain and Cooking Quality Parameters

Data were recorded on 22 parameters viz., (six) agro-Morphological, (12) quality, (Three) biotic and (One) abiotic stress. Five plants were randomly selected from each genotype in each replication to collect data on agronomic and grain yield parameters. Characterization of the genotypes was done according to the descriptions and guidelines specified by applicable standard protocols of Standard Evaluation System (SES) for Rice developed by International Rice Research Institute (IRRI, 2014). Agronomic and yield-related traits such as days to 50% flowering, days to maturity, plant height (cm), panicle length (cm), ear bearing tillers, and plot yield (kg) were recorded following guidelines issued by IRRI, (2014). Maturity traits were recorded on a plot basis. Quality parameters like hulling percentage, milling percentage, head rice recovery (%), 1000 grain weight (g), kernel length (mm), kernel breadth (mm), length/breadth ratio (mm), grain aroma, grain chalkiness, volume expansion ratio, water uptake, kernel length after cooking (mm), elongation ratio, alkali spreading value (%), and amylose (%) content were estimated following standard laboratory protocols (Aravind Kumar *et al.*, 2021). The amylose content was determined by following Juliano (1971), while the alkali spreading value was assessed following the procedure described by Little *et al.* (1958). Biotic stress observations such as bacterial leaf blight, blast, and brown planthopper incidence along with herbicide tolerance response were assessed using standard score scales recommended by IRRI (2014) and established under crop evaluation protocols.

Data Analysis

The analysis of variance, clustering and PCA was conducted using the GRAPES open-source software. The clustering dendrogram and principal component analysis (PCA) plot was generated using RAISINS software.

RESULTS AND DISCUSSION

Analysis of variance

The variance analysis showed that there was significant variation ($P < 0.05$) among rice genotypes for all traits. This clearly showed that there is a considerable amount of genetic variation in the varieties that were under evaluation. The same observation was found by Soundharya *et al.* (2024), Pallavi *et al.* (2025) and Umadevi *et al.* (2019) in rice (Table 1).

Cluster analysis

The cluster analysis was done using the UPGMA method, based on Euclidean distance. The optimal number of clusters is revealed by using the K-means algorithm,

Table 1. ANOVA conducted for 18 yield and grain quality traits among 36 rice genotypes

S.No.	Character	Mean Squares		
		Replication Df (1)	Genotypes df (35)	Error df (35)
1	Grain yield	0.645	5.150**	0.907
2	Days to flowering	1.125	101.423**	2.125
3	Days to maturity	1.125	107.614**	2.125
4	Plant height	1.62	114.636**	13.079
5	Panicle length	7.867	11.597**	1.241
6	Ear bearing tillers	0.142	19.364**	0.749
7	Hulling per cent	0.087	4.483**	0.037
8	Milling per cent	0.451	2.876**	0.049
9	Head rice recovery	0.05	74.592**	0.138
10	Test weight	0.763	28.450**	0.313
11	Kernel length	0.028	0.366**	0.009
12	Kernel breadth	0.007	0.150**	0.001
13	Kernel length/breadth ratio	0.001	0.338**	0.003
14	Volume expansion ratio	0.001	0.056**	0.002
15	Water uptake	7.605	8850.796**	5.474
16	Kernel length after cooking	0	0.726**	0.009
17	Alkali spreading value	0.001	1.508**	0.008
18	Amylose content	0.04	4.940**	0.034

** Significant at 1 % level

as depicted in **Fig. 1**. The analysis of 36 rice genotypes showed four distinct clusters based on 18 grain quality, agronomic, and yield, related traits, as depicted in **Fig. 2 and Table 2**. Ahmed *et al.* (2019) reported a similar study in rice, 30 genotypes grouping into four clusters using yield and grain quality traits. Out of the four clusters formed, Cluster IV (Blue) contained a solitary genotype viz., HTM-N22, HTM-N22 combined the earliest flowering time (84 days), the tallest plant height, the shortest panicles, and the lowest head rice recovery and amylose content among all evaluated genotypes. Cluster I (Pink) consisted of 9 rice genotypes, followed by cluster II (Black) accommodated 21 of the 36 genotypes, making it the largest and most heterogeneous group. Its size

showed that a significant proportion of the germplasm shares a similar genetic background, likely coming from a common breeding lineage or repeated use of related parent material in the NP 10325-3 and related selection series. Large, inclusive clusters like this often occur when panels mainly consist of advanced breeding lines derived from a small number of founder crosses. Selection for farming functionality usually leads to similar phenotypes even if the pedigrees vary (Umadevi *et al.*, 2019). Cluster III (yellow) consisted of 5 rice genotypes. Laghari *et al.* (2025), in their study on rice genetic diversity, reported that cluster analysis classified genotypes into four major groups based on genetic similarity and trait variation, with certain clusters comprising a greater number of

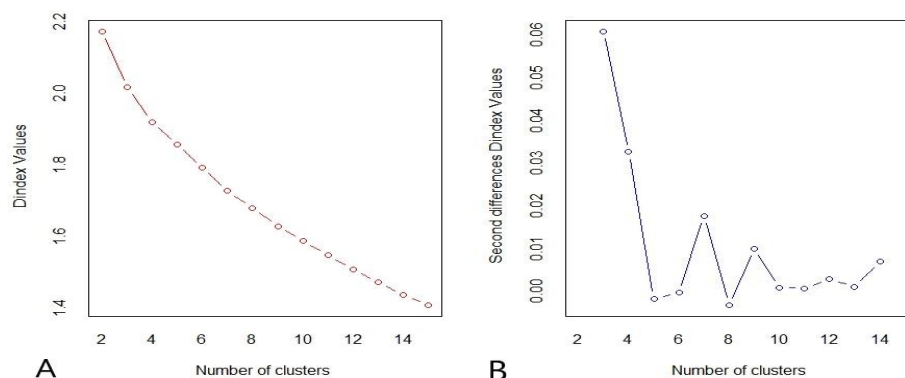


Fig. 1. Graphical depiction of the D-index used to determine the optimal number of clusters among 36 rice genotypes: (A) D-index values and (B) second-order differences of the D-index values

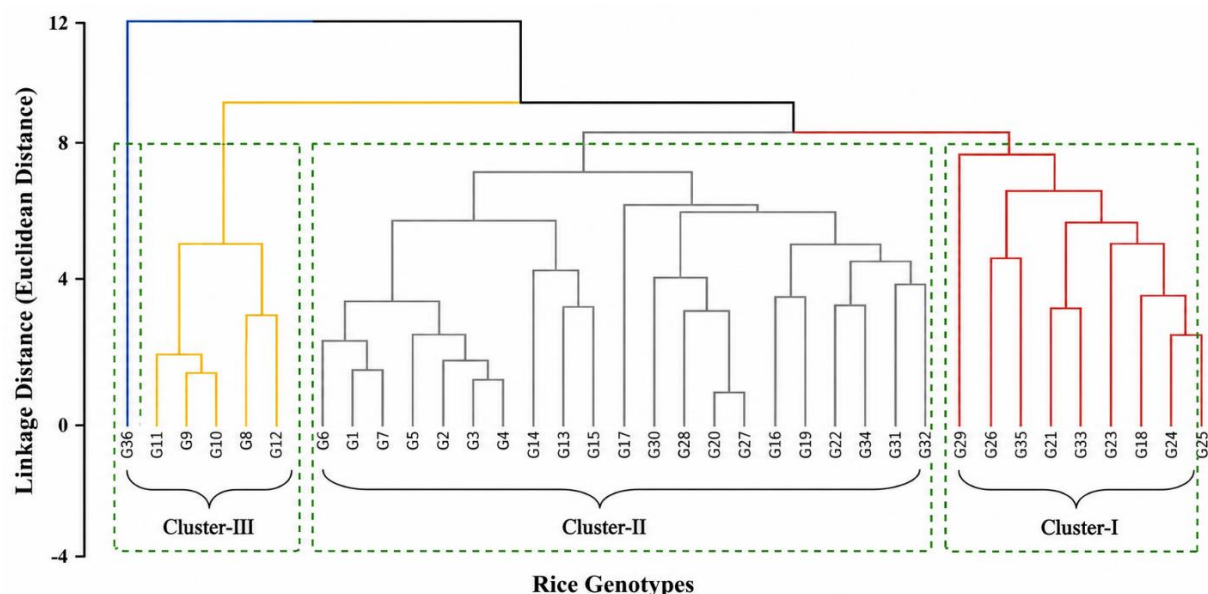


Fig. 2. Dendrogram showing the genetic relationships among 36 rice genotypes based on UPGMA cluster analysis using grain yield, quality, and stress-related traits

Note: For genotypes name refer Table 7

Table 2. Clustering of 36 rice genotypes

Cluster Number	Number of genotypes	Name of genotypes
Cluster I (Pink)	9	NP 9121, NP 950, NP 9308, NP 9156, NP 9153, NP 9183, NP 9024, NP 11092, NP 9558
Cluster II (Black)	21	NP 10325-3-11, NP 10325-3-14, NP 10325-3-26, NP 10325-3-28, NP 10325-3-5, NP 10325-3-6, NP 10325-3-8, NP 196-42-2-4-1, NP 196-42-2-4-3, NP 196-42-2-4-5, NP 9311, NP 7373, NP 7814-1, NP 3114, NP 459, NP 9624, NP 9954, NP 9359-9, NP 1818, NP 8912, NP 102
Cluster III (Yellow)	5	NP 10358-15-3, NP 10358-15-4, NP 10358-15-6, NP 10358-15-8,
Cluster IV (Blue)	1	HTM-N22

genotypes due to shared genetic backgrounds. Cluster I showed late flowering and late maturity. It had a moderate plant height and relatively high productive tillers. The grain yield was also relatively high. The genotypes in Cluster I showed good milling quality and grain appearance, making its genotypes valuable for improving processing quality (Table 3). Cluster II had the longest panicle length, highest hulling percentage, the highest milling percentage, highest test weight, and the highest alkali spreading value. It also produced the highest grain yield per plot. The combination of high milling recovery, excellent grain yield, and good cooking quality showed that Cluster II could be used as the best source of elite breeding material for improving both productivity and grain quality. Cluster III had the most productive tillers, with an average of 10.0. It also achieved the highest grain yield per plot at among all clusters, highest test weight, longest kernel length, highest kernel elongation after cooking, and intermediate amylose content. Additionally, these genotypes showed lower water uptake at 228.6 %

and had intermediate milling quality. The combination of superior yield traits, long and slender grains, and good cooking quality suggests that Cluster III contains valuable genotypes for developing high-quality, high-yielding rice varieties. Since this cluster included only five genetically different genotypes, these accessions may serve as unique genetic resources that should be preserved and used in breeding programs.

Further confirmation about the substantial genetic divergence among clusters was obtained by analyzing intra and inter-cluster distance. The highest inter-cluster distance was observed between Cluster-II and Cluster-IV that was 10.20 followed by Cluster-III and Cluster-IV as 9.17 (Table 4). It infers that genotypes from these clusters are genetically most divergent and may be used in hybridization programs to obtain maximum heterosis and transgressive segregants. The lowest inter-cluster distance was recorded between Cluster-I and Cluster-II as 6.13, reflecting a relatively closer genetic similarity

Table 3. Cluster mean values for 18 grain yield, quality traits in 36 rice genotypes

Cluster	Days to flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Ear bearing tillers	Grain yield in plot (kg)	Hulling %	Milling %	Head rice recovery %
1	102.1	128.7	107.3	23.8	7.6	7.7	80.0	72.9	61.7
2	100.0	135.6	114.0	25.7	4.4	8.8	82.4	74.7	54.6
3	103.9	128.6	107.3	24.3	10.0	9.8	80.0	73.0	59.6
4	84.0	125.5	116.5	17.0	8.0	4.2	79.0	71.5	43.0
Cluster	Test weight (g)	Kernel length (mm)	Kernel breadth (mm)	Kernel length/breadth ratio	Volume expansion ratio	Water uptake (ml)	Kernel length after cooking (mm)	Alkali spreading value (mm)	Amylose content (%)
1	15.2	5.5	1.8	3.1	5.0	267.1	9.2	5.6	21.3
2	19.1	5.5	2.2	2.5	5.0	377.0	9.0	7.0	22.7
3	22.2	6.0	2.2	2.8	4.9	228.6	10.0	4.9	23.0
4	18.7	5.0	2.3	2.2	4.5	90.0	11.0	5.0	17.7

Table 4. Intra and Inter-Cluster Distances (Average Euclidean Distance between Clusters)

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	4.74	6.13	6.14	9.01
Cluster 2		3.31	6.70	10.20
Cluster 3			5.30	9.17
Cluster 4				0.00

between the groups. With respect to intra-cluster distances, Cluster-III (5.30) and Cluster-I (4.74) indicated moderate internal variability indicating diversity within the cluster. Cluster-IV showed zero intra-cluster distance as it contained only one genotype. Similar findings reported by Islam *et al.* (2024) and Laghari *et al.* (2025) in rice for grain quality and yield traits. The genotypes present in clusters II and IV hold promise for improving rice productivity and quality, while genotypes present in cluster IV may be considered to be genetically distinct. Intercrosses between genotypes representing clusters showing the maximum inter-cluster distance, i.e., cluster II × cluster IV or cluster III × cluster IV, may produce better recombinants.

Principal Component Analysis

Principal Component Analysis (PCA) is an efficient multivariate technique that selects those characteristics making the greatest contribution to variability in a rice population. In the present study, 18 principal components were generated, which was equal to the number of traits analyzed. Principal components with eigenvalues <1 were considered to possess less information than the original variables and were not considered further in this work for interpretation (Cardoso *et al.*, 2026). Of these, the first six principal components jointly explained 81.58 % of the variation, reflecting its major contribution to the total diversity of the characteristics studied (Table 5). These results follow in the footsteps of the earlier findings

on rice reported by Chandraker *et al.* (2024), showing that six principal components explained 81.36 % of the total variation among rice genotypes.

The first principal component (PC1) had an eigenvalue of 4.15, which explains 23.06 % of the total variation. This component was mainly influenced by hulling percentage, milling percentage, and water uptake. According to Tesfa *et al.* (2024), traits with loading values greater than ± 0.30 play a significant role in forming the component. Thus, PC1 mainly reflects variation related to grain processing quality, especially hulling and milling recovery, as well as water absorption characteristics. The strong influence of these quality traits indicates that processing efficiency is a key source of genetic diversity among the rice genotypes studied. These results were similar to those reported by Zhong *et al.* (2021) in rice.

The second principal component (PC2) accounted for 19.31 % of the total variation with an eigenvalue of 3.48. This led to a cumulative variance of 42.37 %. PC2 showed positive loadings for kernel breadth, test weight, kernel length after cooking, and ear-bearing tillers. On the other hand, it had significant negative loadings for the kernel length/breadth ratio and alkali spreading value. As a result, PC2 mainly distinguished genotypes based on grain physical traits and cooking quality. This indicates that differences in grain size, grain weight, and cooking behavior played a major role in genetic variation.

Table 5. Eigen values of 18 principal components for grain yield and quality traits among 36 rice genotypes

Principal components	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	4.15	23.06	23.06
PC2	3.48	19.31	42.37
PC3	2.48	13.76	56.13
PC4	2.24	12.44	68.57
PC5	1.25	6.94	75.51
PC6	1.09	6.08	81.58
PC7	0.81	4.49	86.07
PC8	0.67	3.74	89.81
PC9	0.59	3.27	93.07
PC10	0.36	2.02	95.09
PC11	0.31	1.75	96.83
PC12	0.17	0.93	97.76
PC13	0.16	0.87	98.63
PC14	0.11	0.63	99.26
PC15	0.09	0.48	99.75
PC16	0.03	0.17	99.92
PC17	0.01	0.06	99.98
PC18	0.00	0.02	100.00

This is supported by similar observations reported by Pushpa *et al.* (2022) grain size in rice.

The third principal component (PC3) explained 13.76 % of the total variation with an eigenvalue of 2.48. This raised the cumulative explained variance to 56.13 %. PC3 was mainly influenced by head rice recovery, grain yield per plot, days to flowering, and volume expansion ratio. PC3 represents a mix of yield performance, milling quality, and cooking quality. This shows that these important traits work together to create genetic variability. The positive link between grain yield and head rice recovery is especially beneficial since both traits are key targets in rice improvement programs. The fourth principal component (PC4) explained 12.44 % of the total variation and had an eigenvalue of 2.24, leading to a cumulative variance of 68.57 %. This component was mainly influenced by kernel length, kernel length after cooking, and the kernel length/breadth ratio. In contrast, amylose content had a strong negative effect. These results suggest that PC4 mainly represents variation in grain appearance and cooking quality. The differing relationship between kernel dimensions and amylose content indicates that genotypes with longer kernels often varied in starch composition. This may affect consumer preferences and cooking properties. The fifth principal component (PC5) accounted for 6.94 % of the total variation, with an eigenvalue of 1.25. This component showed a strong positive loading for days to maturity. In contrast, plant height and panicle length had negative contributions. Therefore, PC5 mainly reflected variation linked to maturity duration and plant structure. It suggests that differences in growth habit

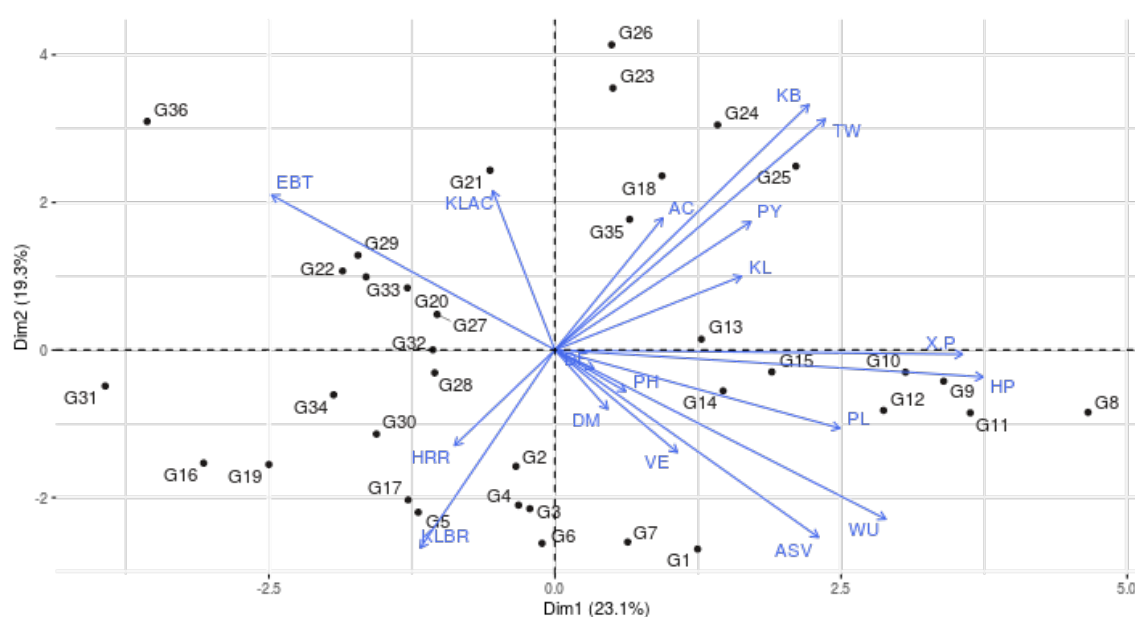
provided an additional, but smaller, source of genetic diversity. The sixth principal component (PC6) contributed 6.08% of the total variation with an eigenvalue of 1.09. This increased the cumulative explained variance to 81.58 %. Plant height, days to maturity and amylose content mainly influenced this component. While PC6 accounted for a small portion of the variation, it provided extra diversity related to plant stature, crop duration, and grain quality.

PCA biplot developed using various quantitative and qualitative traits of rice, with a clear view of the interrelation between the traits (**Table 6 & Fig. 3**). This distinguishes between yield and grain quality, considering the characteristics of the rice plants, with the yield and grain quality traits being closely associated with the PC1 and PC2 axes, respectively. With the characteristics of the rice plants, the cosine of the angle between the two vectors provides information on the traits' correlation: If the angle is acute, the correlation between the two traits is said to be positive, and if it is a right angle, the correlation between them would be zero, while if the angle is obtuse, indicating a smaller angle, the correlation would be negative (Cardoso *et al.*, 2026).

The PCA biplot (**Fig. 3**) explained 42.37 % of the total variation (PC1 = 23.06 %; PC2 = 19.31 %). The way the genotypes are spread across all four quadrants shows significant genetic diversity among the tested germplasm. The direction and length of the trait vectors indicate how much they contribute to the observed variation and how they relate to each other.

Table 6. Loadings (Eigenvectors) of Correlation Matrix among 18 grain yield and quality traits over six principal components

S.No.	Variables	PC1	PC2	PC3	PC4	PC5	PC6
1	Days to flowering	-0.04	-0.03	0.37	-0.28	0.13	-0.20
2	Days to maturity	-0.05	-0.10	-0.19	-0.14	0.60	-0.47
3	Plant height	-0.07	-0.07	-0.29	0.11	-0.52	-0.50
4	Panicle length	-0.29	-0.14	0.17	0.23	-0.34	-0.21
5	Ear bearing tillers	0.29	0.27	0.23	-0.22	0.11	-0.12
6	Grain yield in plot	-0.20	0.22	0.41	-0.02	-0.04	0.06
7	Hulling %	-0.44	-0.05	-0.09	0.01	0.12	0.24
8	Milling %	-0.41	-0.01	-0.03	-0.08	0.10	0.28
9	Head rice recovery %	0.10	-0.16	0.43	0.08	-0.19	0.00
10	Test weight	-0.28	0.40	0.05	0.19	0.10	-0.07
11	Kernel length	-0.19	0.13	0.24	0.48	0.25	-0.12
12	Kernel breadth	-0.26	0.42	-0.13	-0.09	-0.10	0.00
13	Kernel length/breadth ratio	0.14	-0.34	0.24	0.35	0.21	-0.12
14	Volume expansion ratio	-0.12	-0.18	0.30	-0.06	-0.13	0.11
15	Water uptake	-0.34	-0.29	0.07	-0.10	0.11	-0.18
16	Kernel length after cooking	0.06	0.27	-0.08	0.43	0.08	-0.23
17	Alkali spreading value	-0.27	-0.32	-0.13	-0.14	0.01	-0.13
18	Amylose content	-0.11	0.23	0.21	-0.40	-0.13	-0.40

**Fig. 3. Biplot graph representing illustrating rice traits loadings, rice genotype distribution patterns, and genotype-trait relationships**

Note: For genotypes name refer Table 7

The traits represented by long vectors played a bigger role in distinguishing genotypes than those with shorter vectors. The acute angles ($<90^\circ$) seen among kernel breadth, test weight, kernel length, amylose content, and grain yield per plot showed strong positive relationships

among these traits. This means that genotypes with larger grains and higher test weight usually had better grain yield and quality. Water uptake and alkali spreading value were also closely linked, highlighting their joint effect on cooking quality. Likewise, kernel length after cooking was

positively associated with plant height, indicating these traits tended to increase together in the tested germplasm. In contrast, obtuse angles greater than 90° indicate negative correlations. Ear-bearing tillers were positioned opposite to plant height, panicle length, water uptake, and alkali spreading value. This suggests that genotypes with more productive tillers generally had shorter plants and lower values for these grain quality traits. Similarly, head rice recovery showed an opposite relationship to kernel breadth, test weight, and grain yield. This indicates a negative relationship between milling recovery and grain size-related traits in this germplasm. Such conflicting relationships should be carefully considered during simultaneous selection in breeding programs.

The location of the genotypes in relation to the trait vectors offers important information for identifying better breeding materials. Genotypes that fall in the direction of kernel breadth, test weight, kernel length, amylose content, and grain yield per plot like NP-9156, NP-9153, NP-9121 and NP-9558, are likely to have better grain size, weight, yield, and quality. Similarly, NP-950 is positioned kernel length after cooking, pointing to relatively higher value for these traits. In contrast, genotypes in the lower-right quadrant, such as NP-10325-3-11 and NP-10325-3-8, are closely linked to water uptake and alkali spreading value, suggesting better cooking quality. Genotypes in the upper-left quadrant, such as HTM-N55 relates to ear-bearing tillers. Meanwhile, genotypes in the lower-left quadrant, like NP-7373 and NP-10325-3-5, connect to head rice recovery and kernel length/breadth ratio, showing differing performance for these traits. NP-10358-3, NP-1818 and HTM-N22 are most divergent lines, which are located at the edge of the biplot.

Bacterial leaf blight (BLB) screening

Screening 36 rice genotypes for bacterial leaf blight (BLB) using the IRRI Standard Evaluation System (SES) showed significant differences in disease reaction among the tested materials (**Table 7**). None of the genotypes had complete immunity (score 0). Four genotypes, NP 10325-3-14, NP 7373, NP 9156, and NP 9558, were marked as resistant (scores 1–3), indicating that they could be valuable sources for breeding BLB resistance. Nine genotypes (NP 10325-3-6, NP 950, NP 9308, NP 9153, NP 9183, NP 9359-9, NP 1818, NP 8912, and NP 11092) displayed moderate resistance (scores 3.1–5), suggesting they have some partial resistance that might help with long-term disease management. Eight genotypes fell into the susceptible category (scores 5.1–7), while the remaining 15 genotypes scored above 7 and were considered highly susceptible, showing a poor reaction to BLB infection. The variability observed points to valuable genetic diversity for resistance to bacterial leaf blight. The resistant genotypes are promising resources for creating rice cultivars that can withstand the disease.

Blast disease screening

The evaluation of 36 rice genotypes for blast disease

showed a generally good level of resistance. None of the genotypes were classified as immune based on the IRRI SES scale. However, 24 genotypes displayed resistance, with scores between (1–3) and NP 102, were moderately resistant (**Table 7**). Only three genotypes, NP 7373, NP 9624, and HTM-N22, were susceptible. Additionally, three genotypes, NP 3114, NP 459, and NP 9558, were highly susceptible. The presence of resistant genotypes showed that blast resistance is commonly found among the evaluated germplasm. This makes these accessions valuable for breeding programs that aim to improve resistance to *Magnaporthe oryzae*.

Brown plant hopper (BPH) screening

The response of the rice varieties to brown planthopper (BPH) infestation varied greatly, showing significant genetic diversity for insect resistance. None of the tested varieties displayed complete immunity. Thirteen varieties were considered resistant (scores 1 to 3) (**Table 7**). Four varieties (NP 7814-1, NP 9024, NP 1818, and NP 8912) showed moderate resistance, while only NP 102 was found to be susceptible. In contrast, 18 varieties had scores above 7 and were classified as highly susceptible, making up the largest response group. The fairly high number of susceptible varieties suggests that resistance to brown planthopper is less common than blast resistance in this germplasm. However, the resistant varieties identified in this study are valuable genetic resources for breeding BPH-resistant rice types.

Herbicide tolerance screening

Herbicide tolerance assessment following the IRRI evaluation protocol showed a stark difference among the 36 rice genotypes. Thirty-five genotypes were found to be susceptible (score 9) (**Table 7**) indicating severe injury or full sensitivity to the herbicide treatment. In contrast, only one genotype, HTM-N22, showed tolerance (score 1), showing it can withstand herbicide application with little injury. The very low number of herbicide-tolerant genotypes indicates that herbicide tolerance is rare in the evaluated germplasm. Therefore, the genotype HTM-N22 is a valuable genetic resource for developing herbicide-tolerant rice cultivars and may act as a potential donor parent in breeding programs aimed at better weed management strategies.

The current study showed significant genetic diversity among the 36 rice varieties regarding agronomic performance, grain quality, and resistance to major biotic and abiotic stresses. The notable variation in all traits confirmed the existence of usable genetic resources for rice improvement. Hierarchical cluster analysis divided the varieties into four distinct groups. Clusters II and III included top-performing varieties for grain yield, processing efficiency, and cooking quality. In contrast, the EMS-derived mutant HTM-N22 formed a separate group and was the only herbicide-tolerant variety. The large distances between clusters, especially between Clusters II and IV, suggest the potential for creating broad genetic

Table 7. Evaluation of rice genotypes against bacterial leaf blight, blast, brown planthopper, and herbicide tolerance

S.No.	Genotypes	Code	Bacterial leaf blight	Blast	Brown planthopper	Herbicide tolerance
1	NP 10325-3-11	G1	7.00	1.33	1.65	9
2	NP 10325-3-14	G2	2.33	1.00	8.33	9
3	NP 10325-3-26	G3	9.00	1.00	9.00	9
4	NP 10325-3-28	G4	7.67	1.00	2.53	9
5	NP 10325-3-5	G5	9.00	2.00	9.00	9
6	NP 10325-3-6	G6	5.00	1.00	1.83	9
7	NP 10325-3-8	G7	7.00	1.00	1.08	9
8	NP 10358-15-3	G8	9.00	1.00	2.40	9
9	NP 10358-15-4	G9	9.00	1.00	8.85	9
10	NP 10358-15-6	G10	9.00	1.00	2.48	9
11	NP 10358-15-8	G11	7.67	1.00	8.18	9
12	NP 10358-15-9	G12	9.00	1.00	9.00	9
13	NP 196-42-2-4-1	G13	7.00	1.00	9.00	9
14	NP 196-42-2-4-3	G14	7.00	1.00	9.00	9
15	NP 196-42-2-4-5	G15	9.00	1.00	9.00	9
16	NP 9311	G16	7.67	1.00	9.00	9
17	NP 7373	G17	2.33	6.00	9.00	9
18	NP 9121	G18	7.67	1.67	1.67	9
19	NP 7814-1	G19	8.33	1.77	5.00	9
20	NP 3114	G20	7.00	9.00	7.67	9
21	NP 950	G21	4.33	1.33	7.67	9
22	NP 459	G22	9.00	8.73	9.00	9
23	NP 9308	G23	5.00	5.00	1.67	9
24	NP 9156	G24	1.67	1.00	2.33	9
25	NP 9153	G25	5.00	1.00	1.00	9
26	NP 9183	G26	5.00	1.00	1.00	9
27	NP 9624	G27	7.00	7.00	7.67	9
28	NP 9954	G28	9.00	5.00	9.00	9
29	NP 9024	G29	5.67	5.00	5.00	9
30	NP 9359-9	G30	5.00	2.33	7.67	9
31	NP 1818	G31	5.00	5.00	4.33	9
32	NP 8912	G32	5.00	1.00	5.00	9
33	NP 11092	G33	5.00	5.00	1.67	9
34	NP 102	G34	7.00	5.00	7.00	9
35	NP 9558	G35	1.00	7.93	1.00	9
36	HTM-N22	G36	8.33	7.00	9.00	1

combinations, hybrid vigor, and new variations through careful hybridization. Principal component analysis further showed that factors like grain processing quality, grain size, yield characteristics, and cooking quality were the main contributors to overall genetic variation, making up over 81.58 % of the total diversity. Based on the PCA biplot results, the genotype NP 9308, NP 9153 and NP 9156 were identified as candidate genotypes and highly commercial variants for higher grain yield, high grain quality, moderate to highly resistance for bacterial

blight, plant hoppers and rice blast, hence these lines are suitable as parents for hybridization with HTM-N22 for introgression of novel trait herbicide tolerance. In conclusion, the combination of cluster analysis and PCA was an effective approach to analyze diversity and identify potential genotypes. Crosses between varieties in highly divergent clusters, such as Cluster II and Cluster IV; Cluster I and Cluster IV, are recommended for breeding programs to improve yield, grain quality, and stress resistance in rice.

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