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Genetic Diversity of Agro-morphological Traits and Blast Disease Reaction in Elite Rice Genotypes for Hybrid Breeding

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ABSTRACT

Global rice production faces enormous challenges from climate-induced abiotic and biotic stresses, with rice blast disease caused by the fungus *Pyricularia oryzae* posing a major threat to yield and food security. Developing and deploying rice cultivars with resistance to blast disease remains the most effective and sustainable approach to mitigate this constraint. To identify potential sources of resistance, a study was conducted during 2022–2023 focusing on the development of hybrid rice parental lines. Out of 43 genotypes evaluated, 18 exhibited resistance to a virulent blast isolate, while the remaining 25 showed susceptible reactions. Agro-morphological traits such as plant height, panicle length, days to maturity, number of effective tillers, total spikelets per panicle, filled grains per panicle, spikelet fertility percentage, grain yield per hill, and notably the blast disease severity score, were key contributors to phenotypic variability among these genotypes. Based on these traits, the genotypes were categorized into five distinct clusters. Principal component analysis revealed that the first three components explained 67.45% of the total variation. Genotypes G22 (IR126055-46-3-2-B), G13 (BHR362- 8-6-5-26-9), G11 (BHR360- 86-41-7-26-1), and G10 (BHR359-11-30-7-2) from cluster III exhibited high yield potential, whereas genotypes G42 (IR127278-114-3-3-2), G38 (BHR383-53-8-13-1-1), and G43 (BR9390-6-2-2-1) from cluster IV showed notable blast resistance, underscoring their value for hybrid rice development programs. To employ these traits, genotypes from the aforementioned clusters could be made testcross with promising CMS (Cytoplasmic Male Sterile) lines that would offer a good scope to explore the source of high yielding and blast resistance elite restorer or maintainer lines. These findings offer valuable insights for rice breeders aiming to develop high yielding and blast-resistant hybrid varieties tailored to the agro-ecological conditions of Bangladesh.

Keywords: Genetic diversity, cluster, PCA, blast disease

INTRODUCTION

Rice serves as the primary food source for over half of the world's population, contributing significantly to daily energy and protein intake; hence, ensuring its sustainable production is vital for global food security (Saha *et al.*, 2021). To address the challenges posed by population growth, climate change, and limited arable land, hybrid rice has emerged as a key technological innovation. Hybrid rice consistently demonstrates a yield advantage of 15-20% over conventional varieties (Virmani *et al.*, 2001; Yuan, 2017). However, rice production faces

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significant challenges due to climate-related disruptions, which often trigger a combination of abiotic and biotic stresses. Among the biotic stresses, rice blast disease caused by the fungus *Pyricularia oryzae* is one of the most devastating diseases, leading to major yield reductions and threatening global rice production (Salleh *et al.*, 2022; Yadav *et al.*, 2017). This disease severely affects aromatic and hybrid rice varieties, resulting in substantial crop losses (Ye *et al.*, 2025; Nalley *et al.*, 2016). The development of rice hybrids is a key strategy for overcoming the yield plateau, as hybrid rice often demonstrates superior performance due to heterosis (hybrid vigor) (Nivedha *et al.*, 2024). For an effective hybrid breeding program, it is essential to first identify and select genetically distinct parental genotypes. Crossing these lines must be aimed at maximizing desirable traits in the offspring (Nivedha *et al.*, 2024). Consequently, a thorough evaluation of these elite genotypes is crucial, particularly by assessing their disease resistance alongside numerous yield and yield-related characteristics. Assessing rice germplasm for desirable traits is a complex endeavor, as key agronomic features like grain yield, plant height, and tiller number are polygenic and strongly influenced by environmental interactions (Wang *et al.*, 2024; Deepika *et al.*, 2021). Similarly, blast resistance is governed by multiple genes whose effectiveness may vary in durability and expression (Debnath *et al.*, 2018; Yadav *et al.*, 2017).

Multivariate statistical tools, including Principal Component Analysis (PCA) and cluster analysis, play an important role in breeding programs. These methods help in evaluating trait variability, identifying key contributors to genetic divergence, and grouping genotypes based on overall similarity (Deepika *et al.*, 2021; Nivedha *et al.*, 2024). Integrating these approaches enables breeders to efficiently select elite genotypes that are both high-yielding and blast-resistant, thereby improving the precision of parental selection (Salleh *et al.*, 2022).

Although chemical treatments and antibiotics are available to manage blast disease, they pose risks to human health and the environment. In contrast, the use of resistance (R) genes offers a safer and more sustainable alternative, providing broad-spectrum and long-lasting protection. Identifying resistant sources through systematic screening is a critical step in breeding programs aimed at developing durable blast-resistant varieties. A genetically diverse gene pool is essential for initiating such programs (Sivaranjani *et al.*, 2010; Nihad *et al.*, 2020). Genetic diversity analysis evaluates the genetic distance among the selected genotypes and shows the relative contribution of evaluated traits towards the total divergence (Falconer, 1960). Several researchers have used agro-morphological traits for diversity analysis and characterization of Bangladesh rice germplasm accessions (Siddique *et al.*, 2011; Banik *et al.*, 2012; Khalequzzaman *et al.*, 2012; Baktiar *et al.*, 2013; Siddique *et al.*, 2013; Islam *et al.*, 2014; Ahmed *et al.*, 2015a, 2015b; Kulsum *et al.*, 2015; Akter *et al.*, 2016; Biswash *et al.*, 2016; Siddique *et al.*, 2016a; Akter *et al.*, 2017; Islam *et al.*, 2017; Akter *et al.*, 2018; Islam *et al.*, 2018; Siddique *et al.*, 2018; Islam *et al.*, 2019; Muti *et al.*, 2020; Khalequzzaman *et al.*, 2022a; Khalequzzaman *et al.*, 2023). Hence, this study aims to evaluate elite rice genotypes to identify promising sources of blast resistance and to initiate testcross program for the identification of restorer and maintainer lines for developing blast resistant heterotic hybrid rice varieties.

MATERIALS AND METHODS

The experimental materials consisted of 43 elite rice (*Oryza sativa* L.) genotypes. Nine advanced breeding lines- IR126055-46-3-2-B, IR126066-85-5-2-1, IR126069-48-3-2-2, IR126076-67-3-2-3-4, IR126037-59-3-2-3-4, IR126072-83-3-3-1, IR126076-122-1-1-2, IR127278-152-1-3-1 and IR127278-114-3-3-2 originated from the Hybrid Rice Development Consortium (HRDC), International Rice Research Institute (IRRI), Philippines. All advanced materials were obtained from the Hybrid Rice Division, Bangladesh Rice Research Institute (BRRI), Gazipur. The genotypes code, genotypes/ advanced lines name and blast disease reactions are provided in Table 1.

Evaluation of leaf blast disease resistance

To assess blast disease reaction, the genotypes were grown from October to December 2022 in a randomized complete block design (RCBD) with three replications at the Blast Nursery of Plant Pathology Division, BRRI, Gazipur. A sowing frame was used to place 5–7 seeds per genotype per grid. The highly susceptible check variety US2 was planted along the borders of each bed to serve as a disease severity benchmark. Artificial inoculation was performed using a spore suspension of the virulent isolate U23-i7-k177-z06-ta423 of *Pyricularia oryzae* (Khan *et al.*, 2016). At the 4–5 leaf stage (21 days after sowing), seedlings were sprayed with a spore solution adjusted to $3–8 \times 10^4$ spores/mL. Meteorological data for the experimental period (October to December, 2022) were obtained from the meteorological station of the Bangladesh Rice Research Institute (BRRI), Gazipur. Monthly average air temperatures for October, November, and December were 29.2°C, 25.3°C, and 21.9°C, respectively. Relative humidity ranged from 62.1% to 74.0%. Rainfall was recorded only in October (9 mm), with no precipitation in November and December. To promote infection, nursery beds were covered with polythene sheets and misted using a sprinkler system to maintain humidity levels above 80% for six consecutive days. Disease scoring was initiated once the susceptible check (US2) showed severe blast symptoms. Leaf blast disease severity was recorded from three seedlings per genotype using the 0–5 JIRCAS rating scale (Hayashi and Fukuta, 2009). A score of 0 indicated no visible symptoms, while 5 represented spindle-shaped lesions exceeding 3 mm in diameter with lesion coalescence. scores between 0 and 2 were considered as resistant, whereas scores from 3 to 5 were categorized as susceptible.

Evaluation of agro-morphological traits

The 43 genotypes were transplanted for agro-morphological assessment during the Boro 2023–24 season at the research farm of Hybrid Rice Division, Gazipur. The field experiment followed an RCBD layout with three replications. Thirty-day-old seedlings were transplanted at a spacing of 20 × 20 cm between rows and 15 × 15 cm between plants, using one seedling per hill. Fertilization included Urea, TSP, MP, Gypsum, and $ZnSO_4$ were applied at the rates of 270:130:120:70:10 kg/ha, respectively. All fertilizers except urea were applied during final land preparation. Urea was top-dressed into three equal splits: at 15 days after transplanting (DAT), 30 DAT (tillering stage), and 45 DAT (just before panicle initiation). Standard agronomic practices recommended by BRRI for irrigated rice were strictly followed. Eight agro-morphological traits were recorded during field evaluation, based on observations from ten randomly selected plants for each genotype. The data were recorded of the following characters: Days to maturity (DM), Plant height (PH), Effective tillers per hill (ETPH), Panicle length (PL), Filled grains per panicle (FGPP), Total spikelets per panicle (TSPP), Spikelet fertility (SF %) and Grain yield per hill (GYPH).

Statistical analysis

Correlations, principal component and cluster analyses were done through several programme packages using R studio. The clustering was performed using the Ward's method, and correlation analysis was conducted through Pearson's test using R programming language. For data visualization and multivariate analysis, the R packages ggplot2, FactoMineR, Factoextra, and ggrepel were employed, while clustering procedures were further supported by Factoextra and cluster packages. To illustrate phenotypic correlation matrices, a heatmap was generated using the metan package in R Studio. Descriptive statistics for the nine evaluated traits were generated using the Statistical Tool for Agricultural Research (STAR, version 2.0.1; IRRI, Philippines).

Table 1. **List of the materials used in the study with leaf blast reactions.**

Code	Genotype	DR	Code	Genotype	DR
G1	BHR350-4-12-3-1-2	S	G23	IR126066-85-5-2-1	S
G2	BHR351-50-22-3-2-2	S	G24	IR126069-48-3-2-2	S
G3	BHR352-22-4-7-2	R	G25	IR126076-67-3-2-3-4	R
G4	BHR353-39-21-4-3	S	G26	IR126037-59-3-2 -3-4	S
G5	BHR354-55-27-9-2	R	G27	BHR372-8-11-1-1-1	S
G6	BHR355-5-26-7-3	R	G28	BHR373-8-65-22-29-10	S
G7	BHR356-13-20-3-4	S	G29	BHR374-90-9-2-8-15	S
G8	BHR357-19-29-4-3	S	G30	BHR375-6-5-26-21-2	S
G9	BHR358-12-11-7-2	S	G31	BHR376-B-6-52-6-10	S
G10	BHR359-11-30-7-2	S	G32	BHR377-60-81-9-34-2	S
G11	BHR360- 86-41-7-26-1	S	G33	BHR378-12-13-1-1-1	R
G12	BHR361-8-65-2-6-4	R	G34	BHR379-6-15-10-1-1	R
G13	BHR362- 8-6-5-26-9	R	G35	BHR380-26-1-8-1-1-2	R
G14	BHR363-86-52-6-4-7	S	G36	BHR381-66-11-9-1-1	S
G15	BHR364-86-5-26-14	S	G37	BHR382-50-3-3-1-1	S
G16	BHR365-76-90-2-11	R	G38	BHR383-53-8-13-1-1	R
G17	BHR366-126-4-2-2	R	G39	IR126072-83-3-3-1	R
G18	BHR367-18-2-1-14-1	S	G40	IR126076-122-1-1-2	S
G19	BHR368-5-B-16-2	S	G41	IR127278-152-1-3-1	R
G20	BHR369-6-55-16-1	S	G42	IR127278-114-3-3-2	R
G21	BHR370-51-8-1-3	R	G43	BR9390-6-2-2-1	R
G22	IR126055-46-3-2-B	R			

Legend: HRD= Hybrid Rice Division, BRRI= Bangladesh Rice Research Institute, HRDC= Hybrid Rice Development Consortium, IRRI= International Rice Research Institute, DR= Disease Reaction, R=Resistance, and S=Susceptible.

RESULTS

Reaction of genotypes to blast isolates

Following screening, 18 genotypes showed resistant reactions and 25 genotypes showed susceptible reactions (score 3-5) to the blast isolate (Table 1).

Pearson's correlation coefficient

Correlation analysis revealed the relationships among the studied traits to take decision in designing an effective breeding strategy. As shown in Fig. 1, grain yield per hill (GYPH) was significantly and positively correlated with spikelet fertility (SF%) (0.52***) and effective tillers per hill (ETPH) (0.31*). Total spikelets per panicle (TSPP) displayed a highly significant and positive correlation with filled grains per panicle (FGPP) (0.90***). Panicle length (PL) showed a strong positive correlation with and plant height (PH) (0.84***) and days to maturity (DM) (0.33*). FGPP had non-significant positive correlation with GYPH (0.14). PH had significant but negative correlation with SF (-0.34*).

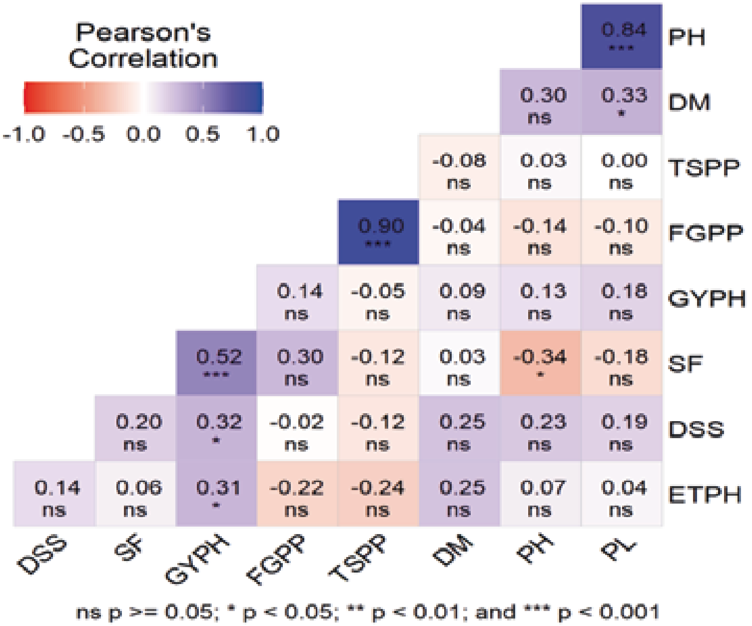

Fig. 1. Pearson's correlation analysis of the studied traits.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a widely used multivariate statistical method for distinguishing genotypes based on trait variation. Fig. 2 and 3 illustrate the eigenvalues and the proportion of total phenotypic variance accounted for by each principal component among elite rice genotypes. Out of nine components, only three exhibited eigenvalues greater than 1, collectively explaining approximately 67.45% of the total trait variability (Fig. 2). The first principal component (PC1) accounted for the largest share of variance at 26.59%, followed by PC2 with 20.90%, and PC3 with 19.96% (Fig. 3).

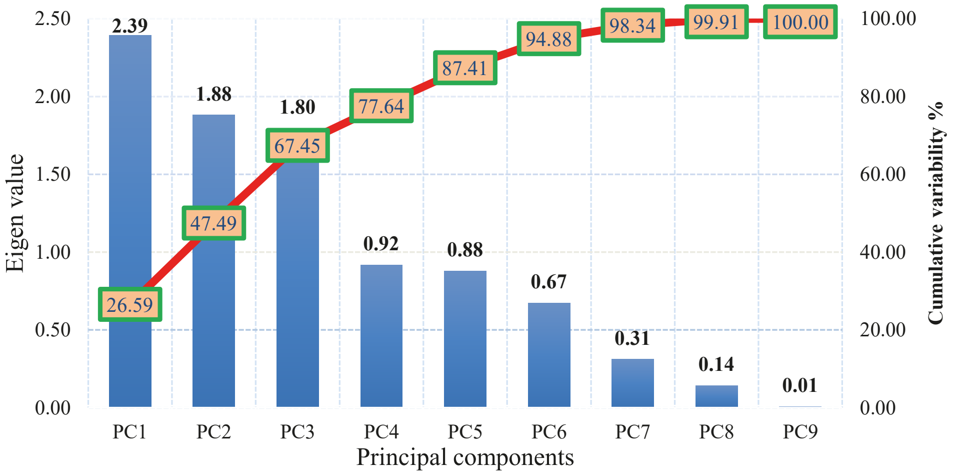

Fig. 2. Principal component analysis of elite rice genotypes showing eigenvalues and their contribution.

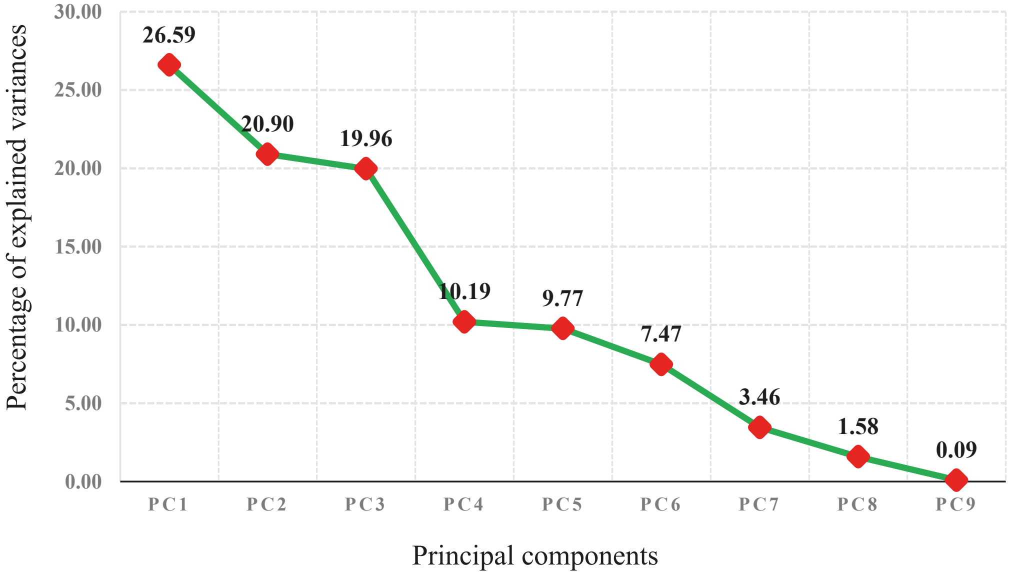

Fig. 3. Contribution of principal components to total explained variance in phenotypic diversity of elite rice genotypes to total variance.

Analysis of principal components and their corresponding vector loadings (Table 2) revealed that PC1 was strongly influenced by panicle length (0.75), plant height (0.74), days to maturity (0.54), effective tillers per hill (0.40), and grain yield per hill (0.23), all contributing positively. PC2 showed positive associations with total spikelets per panicle (0.82), filled grains per panicle (0.77), panicle length (0.50), plant height (0.49), days to maturity (0.22), and grain yield per hill (0.20). PC3 was primarily influenced by spikelet fertility percentage (0.85), grain yield per hill (0.78), effective tillers per hill (0.40), and filled grains per panicle (0.24).

Table 2. Contribution of variability of the first three principal components.

Variable	PC1	PC2	PC3
Days to maturity (days)	0.54	0.22	0.18
Plant height (cm)	0.74	0.49	-0.27
Effective tillers per hill (count)	0.40	-0.21	0.40
Panicle length (cm)	0.75	0.50	-0.15
Filled grains per panicle (count)	-0.57	0.77	0.24
Total spikelets per panicle (count)	-0.49	0.82	-0.12
Spikelet fertility percentage (percent %)	-0.21	-0.01	0.85
Grain yield per hill (g)	0.23	0.20	0.78
Disease severity score (ordinal values)	0.42	0.06	0.31
% of explained variances	26.59	20.90	19.96
Cumulative % of variances	26.59	47.49	67.45

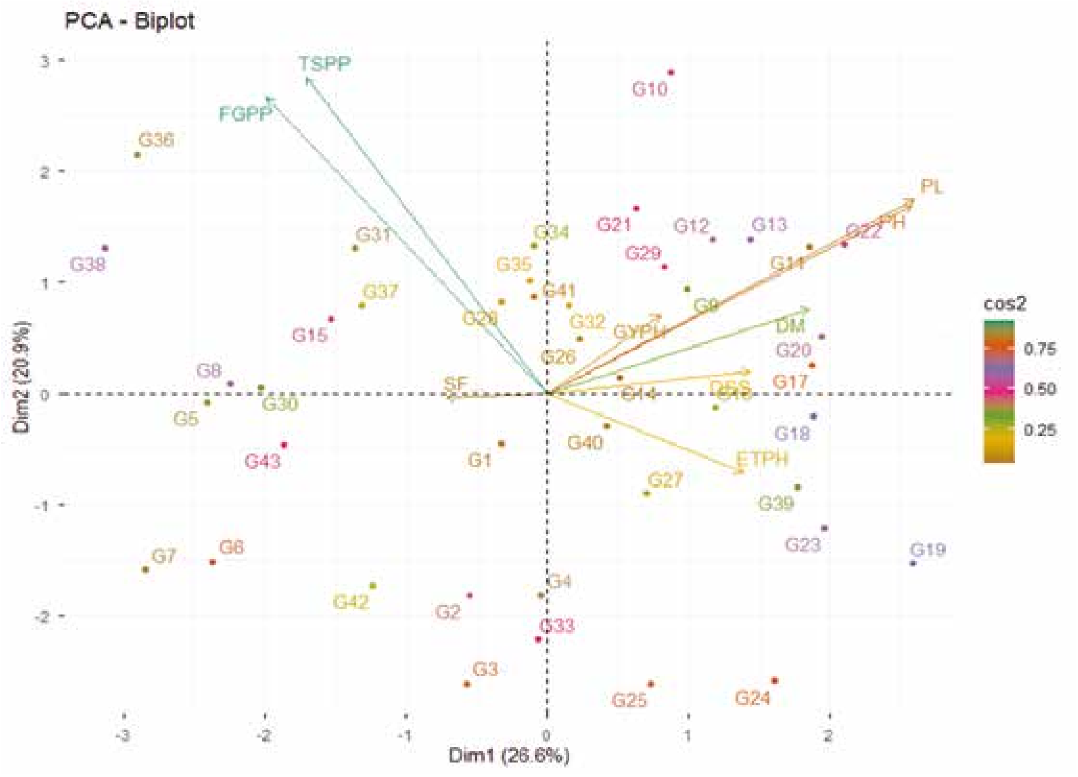

Fig. 4. The Biplot of 43 elite genotypes. The arrows show the contribution of the traits. Cos2 is the quality representation of both individuals and variables. (DM: days to maturity, PH: plant height (cm), ETPH: effective tillers per hill, PL: panicle length, FGPP: filled grain per panicle, TSPP: total spikelets per panicle, SF%: spikelet fertility percentage, GYPH: grain yield per hill (g) and DSS: Disease Severity Score).

A two-dimensional PCA biplot (Figure 4) was generated using principal component scores to visualize the extent and direction of trait contributions across components. The first dimension (Dim1) accounted for 26.6% of the total variation, while the second dimension (Dim2) explained 20.9%. Traits were grouped into two major components: Dim1 was primarily linked to GYPH, PL, and PH. These traits had long vectors and high cos² values, indicating strong influence. The Dim2 captured variation associated with TSPP and FGPP, which also showed strong contributions. Traits like SF, ETPH, and disease severity score (DSS) had shorter vectors and lower squared cosine values, indicating minimal representation in these two dimensions. Genotypes G22, G13, G11, G8, G12, G29, G20, G17, and G18 were positioned close to the grain yield vector, suggesting potential for yield-related traits. Genotypes G36, G37, G38, and G15 showed strong associations with TSPP and FGPP. The vector for DSS points towards the positive Dim1 and slightly positive Dim2 direction. Genotypes located opposite to the DSS vector (arrow) in the PCA biplot especially those farthest from it are likely to have low disease severity scores and are ideal candidates for blast resistance. In relation to the DSS vector, the genotypes G7, G6, G42, G2, G3, G38, G43 and G5 are the promising candidates for blast resistance and could be prioritized for breeding programs. Notably, genotypes G22, G13, G11, and G10 emerged as top performers for overall yield, while G10 showed strong expression of traits aligned with Dim2.

Trait Characterization

Descriptive statistics outlining the genetic variation among traits are presented in Table 3. Days to maturity among the genotypes ranged from 132 days (G38) to 162 days (G37). Plant height varied between 84 cm (G6) and 127 cm (G22). The number of effective tillers ranged from 5 (G38) to 10 (G23, G24, G33, G40). Panicle length extended from 19 cm (G7) to 27 cm (G10–G13, G17). Filled grains per panicle ranged between 95 (G19) and 255 (G36), while total spikelet's per panicle varied from 115 (G19) to 294 (G36). Spikelet fertility percentages spanned 66% (G42) to 97% (G7). Grain yield per hill ranged from 15 g (G30) to 41 g (G5). Disease severity scores varied from 0 (G5, G7, G10, G19, G26, G30, G36, G38, G42) to 5 (G14, G16).

Table 3. Descriptive statistics for nine agro-morphological traits of 43 rice genotypes.

SL No.	Variable	Mean	Min	Max	SD	SE
1	Days to maturity (days)	149.51	132	162	7.07	0.82
2	Plant height (cm)	104.88	84	127	10.29	1.49
3	Effective tillers per hill (count)	7.79	5	10	1.25	0.18
4	Panicle length (cm)	23.28	19	27	2.5	0.35
5	Filled grains per panicle (count)	169.77	95	255	35.04	5.47
6	Total spikelets per panicle (count)	200.98	115	294	41.18	6.96
7	Spikelet fertility percentage (percent %)	84.88	66	97	7.59	1.23
8	Grain yield per hill (g)	27.91	15	41	6.47	0.94
9	Disease severity score (ordinal values)	1.81	0	5	1.5	0.24

Cluster analysis

Based on multivariate analysis of nine studied traits, the 43 genotypes were classified into five separate clusters (Table 4 and Fig. 5). Cluster III encompassed the largest number of genotypes (11). Clusters I and IV each comprised 10 genotypes. Clusters II and V were the smallest, with six genotypes in each.

Table 4. Arrangement of 43 genotypes in different clusters.

Name of the cluster	Number of genotypes	Name of the genotypes code
I	10	G1, G4, G14, G17, G18, G27, G29, G32, G35, G40
II	6	G2, G3, G5, G6, G7, G8
III	11	G9, G10, G11, G12, G13, G17, G20, G21, G22, G26, G34
IV	10	G15, G28, G30, G31, G36, G37, G38, G41, G42, G43
V	6	G19, G23, G24, G25, G33, G39

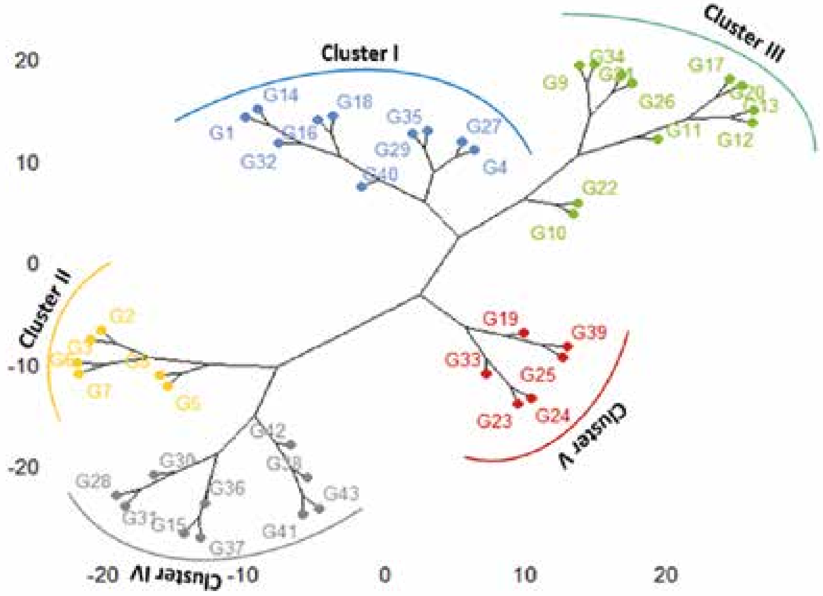

Fig. 5. Illustration of an un-rooted phylogenic tree among 43 rice genotypes based on Euclidean distance.

The mean performance of nine characters across five distinct clusters is presented in table 5. Notable differences were observed among clusters for most traits. Cluster V recorded the highest averages for days to maturity (154.17 days) and effective tillers per hill (9.00). Cluster IV showed the greatest mean values for filled grains per panicle (196.3) and total spikelets per panicle (241.2), along with the lowest disease severity (0.7), indicating strong resistant to blast. Cluster III exhibited the highest averages for panicle length (26.27 cm) and grain yield per hill (33.36). Spikelet fertility percentage peaked in Cluster II (94.50%). Meanwhile, Cluster I had the lowest average plant height (105.6 cm).

Table 5. Cluster means value of nine characters in the studied materials.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to maturity (days)	150.20	142.50	152.73	146.70	154.17
Plant height (cm)	105.6	89.83	115	101.9	105.17
Effective tillers per hill (count)	7.7	7.83	7.91	7	9
Panicle length (cm)	23.2	19.83	26.27	22.1	23.33
Filled grains per panicle (count)	164	175	178.27	196.3	114.33
Total spikelets per panicle (count)	195.6	185.5	208.64	241.2	144.33
Spikelet fertility (%)	83.7	94.5	87.18	81.2	79.17
Grain yield per hill (g)	28	32.83	33.36	20.6	25
Disease severity score (ordinal values)	3.8	1.17	1.82	0.7	1

Table 6 presents both intra- and inter-cluster distances among the five identified clusters. The intra-cluster distances varied from 2.69 to 3.77, with Cluster III showing the lowest internal variation and Cluster V exhibiting the highest variation. Regarding inter-cluster relationships, the highest distance was observed between Cluster I and Cluster V (D^2 = 5.09), indicating substantial genetic divergence. This was followed by distances between Cluster I and IV ($D^2 = 4.90$), Cluster III and IV ($D^2 = 4.77$), and Cluster IV and V ($D^2 = 4.65$). The smallest inter-cluster distance was recorded between Cluster II and Cluster III ($D^2 = 3.55$). These results highlight considerable diversity among the clusters, which is beneficial for selecting genetically distinct genotypes in hybrid rice breeding programs.

Table 6. Intra- (Italic) and Inter- cluster divergence of 43 rice genotypes.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	*3.01*	4.13	4.4	4.90	5.09
Cluster II		*2.96*	3.55	4.27	4.38
Cluster III			*2.69*	4.77	4.64
Cluster IV				*2.72*	4.65
Cluster V					*3.77*

DISCUSSION

Grain yield in rice is a complex quantitative trait influenced by numerous factors. These include indirect contributors such as plant height, growth duration, effective tiller number, panicle length, and seed setting percentage as well as direct factors such as panicle number, grains per panicle, filled grains and thousand-grain weight (Sakamoto & Matsuoka, 2008; Huang *et al.*, 2011). Due to this complexity, direct selection for yield traits can be challenging and time-intensive. In contrast, selecting traits that are strongly correlated with yield offers a more efficient and practical approach (Ahmadikhah *et al.*, 2008). The variation among the evaluated genotypes reflected a distinct interaction between blast resistance and genotypes. Some genotypes demonstrated strong resistance, while others showed susceptible reactions. These contrasting reactions emphasize that blast resistance varies across genotypes and is governed by both resistance genes and responsiveness to pathogen pressure. Such findings are valuable for breeding programs, as they help identify genotypes with more consistent resistance under pathogen pressure, thereby supporting the development of durable blast resistant hybrid cultivars.

Principal Component Analysis (PCA) revealed three components with eigenvalues of 2.39, 1.88, and 1.80, accounting for 26.59%, 20.90%, and 19.96% of the total variance, respectively. These components are essential in identifying key traits for selecting superior genotypes from diverse breeding populations, as supported by findings from Majid *et al.* (2013) and Akhtar *et al.* (2022). Understanding the relationships among yield components is essential for effective genotype selection in breeding programs. Correlation analysis plays a vital role in pinpointing traits that significantly impact yield, guiding breeders in prioritizing traits for improvement (Eriksson *et al.*, 2018). Correlation analysis revealed a significant positive association between SF (%) and GYPH implies that the higher the fertility rate, the higher the grain yield. Bist *et al.* (2025) and Bhadru *et al.* (2012) stated that the lower the sterility rate, the higher the grain yield. A significant positive correlation between grain yield and number of effective tillers per hill was stated by Islam *et al.* (2025) and Gupta *et al.* (2023), Debsharma *et al.* (2020) and Kohnaki *et al.,* (2013). FGPP was positively correlated with GYPH. The positive connection of GY with FGPP was reported by Perween *et al.,* (2020) and Kohnaki *et al.,* (2013). These results indicate that selection for these traits could effectively enhance grain yield in the evaluated rice genotypes. Integrating these traits into breeding will speed up the development of high-yielding rice cultivars to meet national food demand. The number of FGPP had a highly significant and positive correlation with TSPP was reported by Ara *et al.,* (2023). A highly significant and positive correlation between PL and PH also stated by Galib *et al.* (2025). Similarly, the findings of positive correlation between PL and DM corroborate with Bist *et al.,* (2025). The FGPP was not significantly correlated with GYPH; similar to the results of Li *et al.,* (2019).

PCA and cluster analysis are effective tools for assessing variability in quantitative traits and identifying high-performing genotypes (Sawarkar *et al.*, 2025). PCA helps reduce data dimensionality while retaining maximum variability, allowing genotypes to be ranked based on component scores (Shoba *et al.*, 2019). Similar patterns of variation were reported by Kumar *et al.* (2021), who found 31.848% and 19.553% variation in PC1 and PC2, respectively, using 119 rice breeding lines. PCA-based clustering revealed substantial genetic diversity and trait associations, which are crucial for targeted breeding efforts. The PCA biplot (Fig. 4) highlighted key traits driving variability and genotype differentiation, consistent with studies by Chiquet *et al.* (2018) and Saha *et al.,* (2022). Traits near the origin contributed little to variance, and clustered genotypes indicated strong phenotypic associations (Kose *et al.*, 2018). Notably, our analysis indicated a robust association among GYPH and a cluster of agronomic traits, including PL, PH, ETPH, DM and DSS as evidenced by the acute angles by their respective dimension vectors. The association between GYPH and PH suggests a compact plant architecture with shorter stature and more tillers or panicles, which can reduce lodging risk and improve yield stability (Hairmansis *et al.*, 2010; Avakyan & Dzhamirze, 2018). Similarly, the angle between GYPH and DM implies that longer growth periods may enhance yield through increased biomass and grain filling (Al-Karaki, 2012). Genotypes G22, G13, G11, G8, G12, G29, G20, G17, and G18 were closely aligned with the GYPH vector, indicating their potential as high-yielding candidates for direct selection or use in breeding programs. Rao *et al.* (2021) and Prakriti *et al.* (2017) documented variability in rice germplasm, with panicle length ranging from 16–33.9 cm and 21.7–29.6 cm, respectively. In a more recent study, Sultana *et al.* (2025) reported trait ranges in T. Aman rice, including effective tillers per hill (10.03–13.05), panicle length (21.82–23.0 cm), sterile spikelets per panicle (12.65–13.05), and grains per panicle (123.53–141.33).

Cluster analysis grouped the 43 genotypes into five distinct clusters. This classification revealed substantial genetic diversity among the genotypes, particularly in traits related to yield and disease resistance. The genotypes demonstrated a wide range of variability, with several unique entries showing potential for use in breeding programs. Different authors reported that genetic diversity among different rice genotypes has been classified into distinct cluster groups. For instance, Girma *et al.* (2018) grouped 64 genotypes into six clusters using seven yield-related traits. Bekis *et al.* (2021) classified 30 lowland rice genotypes into five clusters based on 17 morphological and yield traits, while Worede *et al.* (2014) divided 24 upland rice genotypes into two clusters using 17 morpho-agronomic traits. The phylogenetic tree further illustrated the genetic relationships and diversity among the cultivars. Such clustering is instrumental for breeders in selecting genetically distant parents for developing hybrids with enhanced yield potential and resistance to blast disease. The insights gained from this study contribute to breeding strategies aimed at meeting the evolving demands of agriculture and consumers.

The cluster mean value indicated that neither any cluster contained the genotypes with all desirable traits which could be selected and utilized directly in the crossing program of hybrid rice breeding. Thus, hybridization between different clusters is needed to develop desirable genotypes. The analysis of cluster distance revealed that the inter-cluster distances were higher than the intra-cluster distance indicating wider genetic diversity among the genotypes of different groups. The above result indicated that the genotypes included in the cluster I and cluster V were more diverse than the genotypes of others cluster. Therefore, genotype selection for hybridization from the cluster I and V may give the desirable heterosis for heterotic rice hybrids. The genotypes under cluster IV having lower DSS values. So, the genotypes of this cluster could be used as a male parent of testcross program for identifying blast resistant maintainer or restorer lines. These results are in agreement with those of Khan *et al.* (2014) and Latif *et al.,* (2011). Genotypes of distantly located clusters were suggested to use in hybridization programs for obtaining a wide spectrum of variation among the segregates as suggested by Yadav *et al.* (2011) and Latif *et al.,* (2011). The present study provided important information for blast resistance at the seedling stage, but it may not fully represent responses at reproductive stages. Similarly, the morphological traits are influenced by environmental factors; therefore, assessment of morphological traits in a single environment potentially confounds only genetic effects. So, phenotypic screening with molecular markers at both the seedling and reproductive stages alongside multi-location or multi-season trials would facilitate the identification of stable and broad-spectrum resistance.

CONCLUSION

Multivariate analyses of nine quantitative traits were performed to determine the genetic diversity of 43 genotypes, which were grouped into five different clusters. The 67.45% variation was explained by the first three primary axes. Cluster III and IV represent high yielding and disease resistant cluster, respectively. So, testcrosses could be made of the aforementioned clusters with promising CMS line that would offer a good scope to identify blast-resistant and high yielding elite restorer or maintainer lines. The study revealed that blast resistance varied among genotypes, reflecting genetic differences in resistance genes and pathogen responsiveness. Future research should focus on validating these findings across diverse environments and pathogen populations, as well as integrating molecular markers (SSR or SNP) to identify genes for blast resistance (*R*-genes), fertility restoration (*Rf*), wide compatibility (WC), identifying yield QTLs and heterotic loci for greater heterosis and introducing them into the background of hybrid parental genotypes to accelerate the rate of heterosis for hybrid rice breeding program.

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AUTHOR’S CONTRIBUTIONS

M. Akhlasur Rahman (M.A.R) and Umakanta Sarker (US): Conceptualization, designing the experiment, validation, resources, monitoring of the experiments, writing-manuscript draft, review and editing. Laila Ferdousi Lipi (LFL): Methodology, validation, experimentation, data curation, data analysis, data interpretation, writing first manuscript draft, review and editing. Mohammad Mehfuz Hasan Saikat (MMHS) and Md. Motaher Hossain (MMH): review and editing.

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