



RESEARCH ARTICLE

Unveiling genetic variability and cause-effect relationships of morphological traits of rice (*Oryza sativa* L.) genotypes in the Terai agro-climatic zone of West Bengal

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Abstract

Rice (*Oryza sativa* L.) is an essential staple food crop for billions of people worldwide. Understanding genetic variability is essential for breeding programs targeting yield and resilience. This study assessed 52 rice genotypes across two kharif seasons (2021-23) in West Bengal's Terai zone. Key traits were evaluated using a randomized block design (RBD) with three replications. Key characteristics such as plant height (PH), days to 50 % flowering (DFF), number of effective tillers per plant (NETP), panicle length (PL), number of grains per panicle (NGPP), 1000-grain weight (TGW), grain yield per plant (GYP) and harvest index (HI) were assessed. Statistical analysis revealed significant variability among genotypes. Among the traits, GYP exhibited the highest genotypic (GCV) and phenotypic coefficients of variation (PCV). Additionally, PH, NETP, NGPP, TGW, HI and GYP exhibited high heritability and genetic advancement, highlighting their potential for selection. Path coefficient analysis identified HI and NGPP as the most influential traits that directly and positively affected GYP. Principal Component Analysis (PCA) attributed 78.61 % of the total variation to four principal components, highlighting HI, GYP, NGPP and PL as major contributors to genetic diversity. Genotypes such as Piolee, Motia Saru and Dhan Sali exhibited superior performance for specific traits, making them potential candidates for future breeding programs. These findings emphasize the potential of exploiting genetic variability in rice to develop high-yielding, regionally adapted varieties, contributing to sustainable agricultural practices in the Terai region.

Keywords

genetic variability; grain yield; heritability; *Oryza sativa*; path analysis; principal component analysis

Introduction

Rice (*Oryza sativa* L.) is the second most cultivated cereal crop globally. As a staple crop, it sustains more than 3.5 billion people worldwide and provides up to half the daily caloric intake in Asian countries (1). With the global population projected to reach 9.8 billion by 2050, rice production must expand significantly to meet the growing demand for food (2, 3). Rice is

cultivated in over 100 countries, with Asia contributing 90 % of global production and consumption. It shapes diets, economies and lifestyles, earning its title as the Global Grain (4).

The effectiveness of any plant breeding program is primarily determined by the genetic variability present within the crop species. Understanding genetic variability in germplasm is crucial, as it helps to identify suitable parents for hybridization under different conditions. More significant variability among parent plants increases the chances of producing heterotic combinations and expands the range of variability in the segregating generations (5). Rice breeding programs characterize germplasm based on agro-agro-morphological traits (6). Rice yield is influenced by traits like plant height (PH), days to 50 % flowering (DFF), number of effective tillers per plant (NETP), number of grains per panicle (NGPP), panicle length (PL), 1000-grain weight (TGW), harvest index (%) (HI) and grain yield per plant (g) (GYP) (7, 8). These traits are often correlated. Genetic variability, measured through PCV, GCV, heritability and genetic gain (GA), underpins crop improvement. This variability underpins the breeding strategies developed for crop improvement. The degree of variability is assessed using GCV and PCV and analyzing trait association helps identify the relationships among the various traits (9). Heritability is determined by calculating the genetic variance (VG) proportion to the overall phenotypic variance (VP). Breeders utilize this metric to estimate the expected response for selection and determine the efficiency of desired traits that are inherited across generations. However, relying solely on heritability estimates is insufficient; these estimates do not reflect the likelihood of a trait being passed on but quantify the proportion of a trait's expression influenced by genetic factors rather than environmental influences. Consequently, combining

heritability with genetic advancements offers a more accurate prediction of genetic gains under selection. Correlation and path analyses examine trait relationships, with path analysis distinguishing direct and indirect effects on dependent variables (10, 11, 12).

Many researchers have highlighted the importance of genetic variability in rice and its roles in selecting suitable parent plants for hybrid vigour or obtaining desirable segregants (13, 14). This study seeks to explore genetic variability and identify desirable *Oryza sativa* (rice) genotypes tailored explicitly for the terai agro-climatic zone.

Principal Component Analysis (PCA) is a dimensionality reduction method for multivariate data, widely used to assess the importance and influence of each factor on overall variance. It also provides insights into how individual traits influence total variance (5). PCA is essential in rice breeding, as it assesses genetic diversity, highlights significant components, enhances variability and ranks genotypes effectively (15). The study focuses on analyzing morphological traits and measuring genetic variation to identify superior genotypes with high yield potential and desirable yield-attributing traits for inclusion in future breeding programs aimed at rice variety development.

Materials and Methods

Experimental site

The study on agro-morphological traits was conducted at the Agricultural Instruction Farm of Uttar Banga Krishi Vishwavidyalaya, located in Pundibari, Cooch Behar, over two consecutive kharif seasons (2021-22 and 2022-23). The farm was positioned at a latitude of 26°19'86" N and a longitude of 89°23'53" E, with an altitude of 43 meters above mean sea level (Fig. 1).

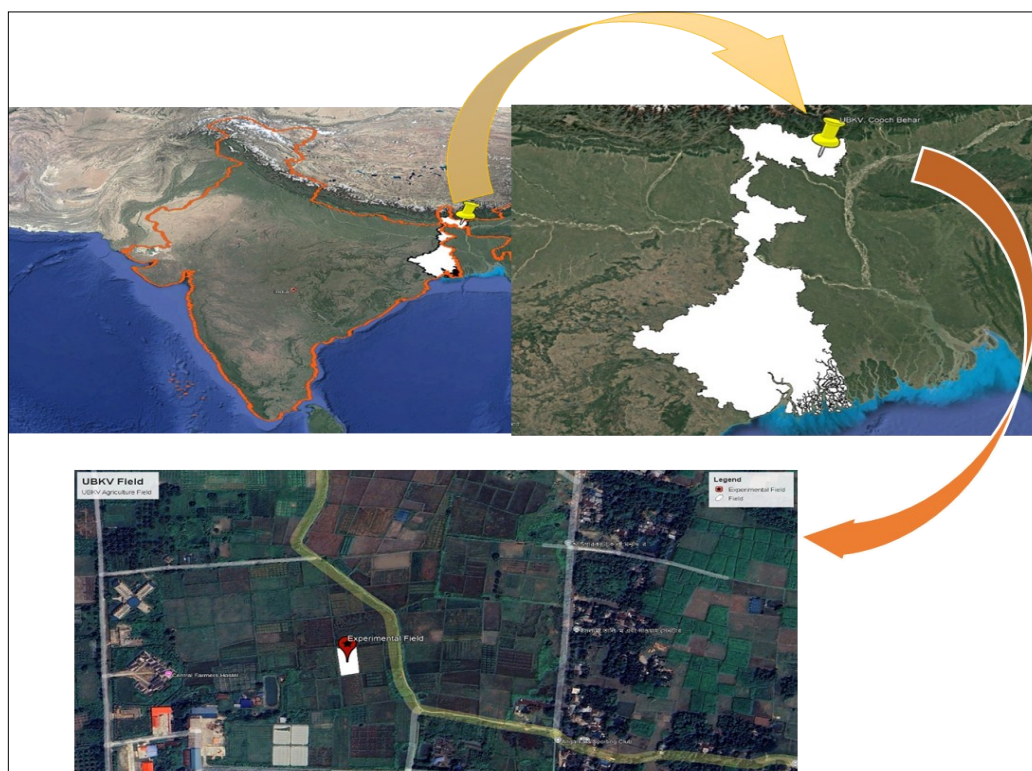


Fig. 1. Geographical Representation of the Experimental Field Location at Uttar Banga Krishi Vishwavidyalaya (UBKV), West Bengal, India.

Experimental materials

The experimental material for this study included 52 rice genotypes, which were collected from the National Rice Research Institute (NRRI) in Cuttack, Odisha; Bihar Agricultural University (BAU) in Sabour; and the Regional Agricultural Research Station (RARS) in Titabor, Assam. Table 1 lists the rice genotypes cultivated during the kharif season and their origins. The soil at the research site was sandy loam with a soil pH of 5.73, characteristic of the West Bengal terai region.

Experimental layout

In both the kharif seasons, 52 rice genotypes (Table 1) were cultivated using a randomized block design (RBD) with three replications to ensure reliable comparisons. Each plot measured 2 m × 1.5 m and spacing of 50 cm was maintained among plots, while plant-to-plant spacing was 15 cm apart and rows were separated by 20 cm. Standard procedures were followed for fertilizer application, top-dressing and crop management to ensure crop health and productivity throughout the study (16) and the details of these have been provided in Table S1.

Recorded observations

Observations were recorded for eight traits viz., plant height (PH), days to 50 % flowering (DFF), number of effective tillers per plant (NETP), panicle length (PL), number of grains per panicle (NGPP), 1000-grain weight (TGW), grain yield per plant (GYP) and harvest index (HI). Data were collected from 5 randomly selected and tagged plants in each replication and genotype.

PH was recorded in cm, measured from ground level to the tip of the main panicle just before harvest from each of the five selected plants in each replication and genotype. The

measurements were then averaged to represent each replication's plant height (PH). DFF were determined by noting when 50 % of the plants in each plot showed initial flowering emergence. PL was measured in cm from the base node portion of selected plants up to the topmost spikelet. NETP was counted at maturity in each plot and the average was calculated. NGPP was determined by counting grains from randomly selected panicles and calculating the mean. TGW was obtained by weighing randomly selected seeds by electrical balance and taking the average in grams (g). GYP was determined by harvesting five plants per plot, weighing the total grain yield and averaging in g (16, 17). Finally, HI was measured following the standard guidelines in percentage (18).

Statistical analysis

Data were collected from each of the five selected plants in the replication and the mean values across the plants chosen for each replication were used for statistical analysis. The data were analyzed using a randomized block design to evaluate the variance among genotypes (treatments). The analysis of variance (ANOVA) for assessing the randomized complete block design was performed using the package 'agricolae' in R software (19). Genotypic (GCV) and phenotypic (PCV) coefficients of variation were computed following standardized methods, with GCV and PCV categorized into high (>20 %), moderate (10-20 %) and low (<10 %), according to recommendations (20). Heritability in the broad sense, represented as $h^2(bs)$, was calculated by dividing genotypic variance by total phenotypic variance and classified into low (<30 %), medium (30-60 %) and high (>60 %) ranges as per referenced guidelines (21). The genetic advances under selection were determined (22), with the variability package in R used to calculate genetic parameters for traits across years

Table 1. List and origin of rice genotypes cultivated during kharif (2021-22 and 2022-23) in the present study

Sl No.	Name of Genotypes	Origin	Biological Status	Sl No.	Name of Genotypes	Origin	Biological Status
1	BR-11-Sub-1	Bihar	Elite Line	27.	Haripuwa Sali	Assam	Traditional cultivar
2	Bangaladeshi (Banga Bandhu White)	Assam	Elite Line	28.	Bhogali bora	Assam	Released Variety
3	Bangaladeshi (Banga Bandhu Red)	Assam	Elite Line	29.	Teti Sali	Odisha	Landraces
4	Ranjit	Assam	Released Variety	30.	Jayamati	Assam	Released Variety
5	Naveen	Odisha	Unknown	31.	Aviant	Assam	Traditional cultivar
6	Dhan Sali	Assam	Unknown	32.	Betguti Sali	Assam	Traditional cultivar
7	Aki Sali	Assam	Unknown	33.	Chakhao sempak	Manipur	Elite Line
8	Paro Chakua Sali	Assam	Traditional cultivar	34.	Jal kuwari	Assam	Released Variety
9	Moirangphou Khonkngangbi	Manipur	Elite Line	35.	Bahadur	Assam	Released Variety
10	Padum Sali	Assam	Traditional cultivar	36.	Mahsuri	Assam	Released Variety
11	Piolee	Unknown	Released Variety	37.	Motia saru	Assam	Landraces
12	Mulagabhoru	Assam	Released Variety	38.	Diphalu	Assam	Released Variety
13	Borsalpona	Assam	Landrace	39.	Hatisali	Assam	Traditional cultivar
14	Kati Sali	Assam	Land Race	40.	Sial Sali	Assam	Traditional cultivar
15	Luit	Assam	Released variety	41.	Aghani bora	Assam	Released variety
16	Satyanjan	Assam	Released variety	42.	Nefabi Julsi	Assam	Traditional cultivar
17	Dhalamukh Sali	Others	Unknown	43.	Badsa bhog	West Bengal	Elite Line
18	Disha	Manipur	Elite Line	44.	Sita bhog	West Bengal	Landrace
19	Keteki joha	Assam	Released variety	45.	Shanti bhog	Assam	Elite Line
20	Kalamalani Sali	Odisha	Traditional Cultivar/Folk Variety	46.	Ratna	Odisha	Released Variety
21	Pual Sali	Assam	Traditional cultivar	47.	Gudmatia	Unknown	Unknown
22	Kunchenyfoe	Assam	Traditional	48.	AC-35014(Nal Dhan)	Odisha	Landrace
23	Kushal	Assam	Released Variety	49.	Manipur black rice	Manipur	Landrace
24	Kanaklata	Odisha	Released variety	50.	AC-35038 (Godhi Akhi)	Odisha	Landraces
25	Gajep Sali	Assam	Traditional cultivar	51.	Lalat	Odisha	Released variety
26	Maniram	Others	Released variety	52.	Swarna	Odisha	Released variety

(23). Trait associations were assessed through Pearson's correlation coefficients with the metan package in R (24), while the corrplot package provided a visual correlation matrix (25). Path-coefficient analysis was computed using the variability package (23). Principal component analysis (PCA), focusing on components with eigenvalues exceeding one, was conducted using the Past 4.03 software package, transforming the original variable set into uncorrelated principal components (26). Biplots of these traits were created to illustrate individual and collective trait distributions (26).

Results and Discussion

Agro-morphological analysis is a valuable tool for plant breeders in selecting promising genotypes or parents for future breeding programs. Improving grain yield is the primary objective of rice breeding programs. According to this study, the analysis of variance (ANOVA) (Table 2) showed significant differences among the rice genotypes across eight key agro-morphological traits, namely PH, DFF, NETP, PL, NGPP, TGW, HI and GYP, indicating the presence of substantial variations among the genotypes. Therefore, it highlighted the potential for enhancing these traits through selection (17, 27, 28).

Genetic variability of rice genotypes

Table 3 summarises the overall mean, range and genetic parameters for the eight traits, while Fig. 2 presents a violin plot that visualizes the distribution of these eight yield-related traits. The PH varied from 93.40 cm at its lowest to 187.80 cm at its highest, with an overall mean value of 140.05 cm; DFF spanned from 74.00 to 121.00 days, with an average of 106.95 days. NETP fluctuated between 7.00 to 16.80, with a mean of 11.36; PL ranged between 20.20 cm to 33.20 cm, with a mean value of 26.64 cm; NGPP ranged between 73.60 to 244.40, with a grand mean of 161.46; TGW varied between 10.90 g and 31.60 g, with an average of 22.02 g; HI spanned 17.68 % to 49.13 %, with a grand mean of 33.25 %; and GYP ranged from 7.00 g to 32.90 g, with a mean yield of 16.06 g.

The importance of genotypic variance highlights whether rice genotypes exhibit sufficient variability for specific traits. Consequently, selecting high-performing parental types could significantly enhance GYP and related traits. DFF and PL showed low PCV and GCV values (8.43 % and 8.36 % for DFF; 7.73 and 7.55 for PL), below 10 %.

Table 2. Analysis of variance (ANOVA) for eight traits in 52 rice genotypes

Source of Variation	d.f.	PH	DFF	NETP	PL	NGPP	TGW	HI	GY_P
Year	1	632.12**	48.33**	1.75**	7.69**	4384.63**	0.54**	1019.48**	876.77**
Replication	4	2.85	3.63	13.28	0.59	31.73	0.72	54.62	10.94
Genotype	51	413.29**	160.78**	6.81**	8.22**	2078.89**	32.24**	57.02**	28.89**
Error	204	2.95	2.71	0.51	0.37	17.80	0.17	4.95	1.79

Table 3. Genetic parameters for eight traits of 52 rice genotypes over both seasons

Sl No.	Traits	Mean	Range		PCV (%)	GCV (%)	Heritability (broad sense) (%)	Genetic advance as % of the mean
			Min	Max				
1	PH	140.05	93.40	187.80	10.29	10.25	99.27	21.04
2	DFF	106.95	74.00	121.00	8.43	8.36	98.32	17.07
3	NETP	11.36	7.00	16.80	16.69	16.02	92.10	31.67
4	PL	26.64	20.20	33.20	7.73	7.55	95.54	15.21
5	NGPP	161.46	73.60	244.40	20.04	19.93	98.94	40.84
6	TGW	22.02	10.90	31.60	18.27	18.22	99.46	37.42
7	HI	33.25	17.68	49.13	16.52	15.82	91.70	31.21
8	GY_P	16.06	7.00	32.90	24.17	23.41	93.86	46.73

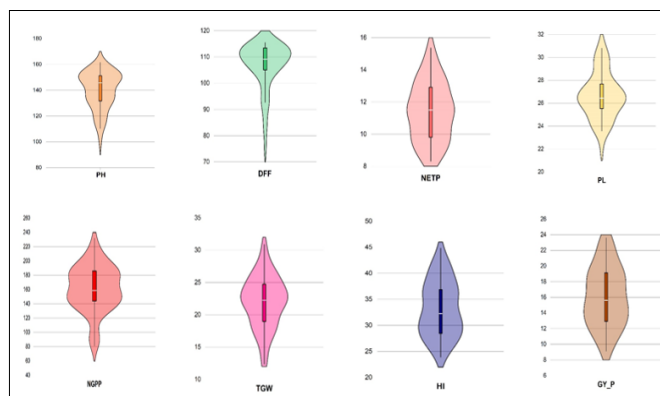


Fig. 2. Violin plot for yield and its attributing traits in rice.

Moderate PCV and GCV (between 10-20 %) were observed for PH (10.29 % and 10.25 %), NETP (16.69 % and 16.02 %), NGPP (20.04 % and 19.93 %), TGW (18.27 % and 18.22 %) and HI (16.52 % and 15.82 %). High PCV and GCV values above 20 % were noted for GYP (24.17 % and 23.41 %). The genetic factor assessed for the eight parameters showed that GYP exhibited the highest PCV and GCV values, exceeding 20 %, aligning with findings (29, 30) for GYP. The observed PCV and GCV values across these traits showed minimal differences, indicating that genetic influences dominate the expression in determining these traits over environmental effects. The broad-sense heritability values are presented in Table 3 and found all the traits such as PH (99.27 %), DFF (98.32 %), NETP (92.10 %), PL (95.54 %), NGPP (98.94 %), TGW (99.46 %), HI (91.70 %) and GYP (93.86 %) demonstrated high heritability. It indicated minimal environmental influence on trait expression and suggested a strong correlation between genotypic and phenotypic variation. Thus enhancing their potential for effective selection. A study like this evaluated 107 elite rice genotypes and identified high heritability across all traits, including DFF, PH, NETP, PL, NGPP, TW and GYP (27).

Genetic advance estimates were categorized into three levels: high genetic advance at over 20 %, moderate genetic advance from 10-20 % and low genetic advance below 10 % (21). DFF (17.07 %) and PL (15.21 %) displayed moderate genetic advancement as a percentage of the mean (27). Based on this study, PH, NETP, NGPP, TGW, HI and GYP showed high heritability with significant genetic advance as % of the mean. This indicated PH, NETP, NGPP, TGW, HI and GYP traits were governed by additive genes, making selection

effective due to their positive response to selection. A similar finding was reported for PH, NETP, NGPP, TGW, HI and GYP (27). This study found that GYP exhibited high GCV and PCV values, high heritability and considerable genetic advance, indicating the influence of additive gene action and highlighting the strong potential for improving the trait through selection.

Correlation studies between yield and its attributing traits

The genotypic correlation for the eight yield-related traits in rice is summarized in Table 4 and illustrated in Fig. 3. The analysis revealed that PH had a positive significant correlation with DFF (0.384). PL demonstrated a significant positive correlation with NGPP (0.348). NGPP exhibited substantial positive correlations with HI (0.334) and GYP (0.327). The genetic correlation analysis identified that GYP was significantly and positively associated with NGPP (0.327) and HI (0.733). A similar study observed a positive significant relationship between GYP and NGPP and HI (17, 31, 32, 33). It suggested that enhancing these traits would result in a higher GYP in this study. However, NGPP displayed a significant negative correlation with TGW (-0.455) and PH showed a highly significant negative correlation with HI (-0.438). These negative associations may result from competition for shared resources, such as nutrient availability, among the genotypes.

Analysis of path coefficient for yield and its contributing traits

This study performed a path analysis to evaluate the interrelationships among eight traits. Table 5 illustrates GYP as the resultant variable; the remaining traits were considered contributing factors. The coefficients derived from the correlation analysis were partitioned into direct and indirect effects of yield-related traits on GYP. The residual effect was 0.1699, indicating that the selected traits sufficiently explained GYP variability in rice.

The path analysis revealed that all traits exerted positive direct effects. Among these, HI (0.925) had the highest direct influence, followed by DFF (0.334), NETP (0.321), PH (0.162), NGPP (0.096), TGW (0.087) and PL (0.016). Most Notably, HI and NGPP demonstrated strong positive associations and positive direct effects with GYP (34, 35). HI and NGPP showed positive correlations with GYP and positive direct effects. These traits could, therefore, be strategically targeted to enhance rice GYP efficiently.

Principal component analysis

This study firmly states that PCA was applied to eight yield-contributing traits on 52 rice genotypes. PCs with eigenvalues exceeding one and accounting for at least 5 % of variation were considered significant (36). Eigenvalues represent the contribution of each PC to total variation, while factor loadings, or component loadings, indicate correlations between original variables and PCs.

The analysis (Table 6) revealed that four PCs explained 78.61 % of the total variation. PC1 explained 28.55 % of total variability, whereas PC2, PC3 and PC4 contributed 19.03 %, 18.16 % and 12.87 %, respectively. Similar findings were reported on 24 rice genotypes across 13 traits, where the initial four PCs explained 72.24 % of the overall variability in rice genotypes (37).

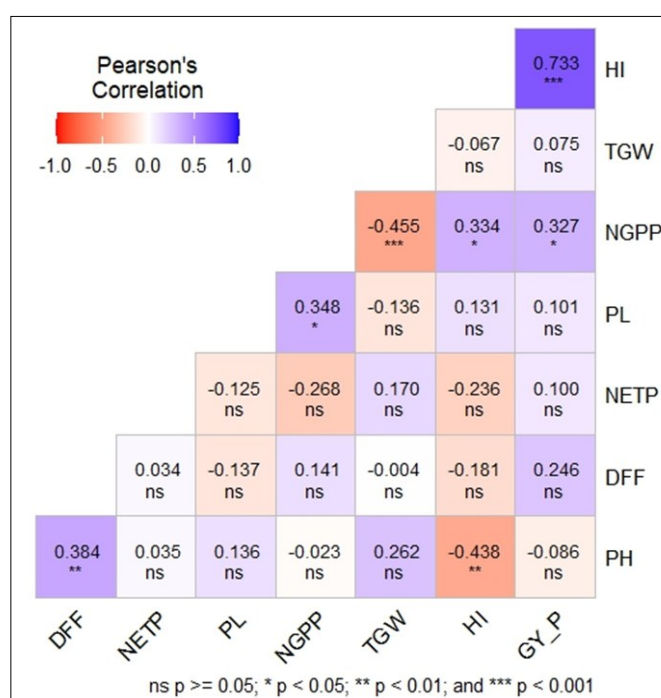


Fig. 3. Genotypic correlation matrix showing relationships among eight yield-attributing traits in rice, with significant positive and negative correlations.

Table 4. Genotypic correlation among eight yield attributing traits in rice

Traits	DFF	NETP	PL	NGPP	TGW	HI	GY_P
PH	0.384**	0.035	0.136	-0.023	0.262	-0.438**	-0.086
DFF		0.034	-0.137	0.141	-0.004	-0.181	0.246
NETP			-0.125	-0.268	0.170	-0.236	0.100
PL				0.348*	-0.136	0.131	0.101
NGPP					-0.455**	0.334*	0.327*
TGW						-0.067	0.075
HI							0.733**

Table 5. Path coefficient analysis reflecting the direct (diagonal) and indirect (off-diagonal) impacts of eight yield-contributing traits on GY_P in rice

Traits	PH	DFF	NETP	PL	NGPP	TGW	HI	Correlation with GY_P
PH	0.162	0.129	0.011	0.002	-0.002	0.023	-0.411	-0.086
DFF	0.063	0.334	0.011	-0.002	0.014	-0.001	-0.169	0.246
NETP	0.006	0.011	0.321	-0.002	-0.026	0.015	-0.222	0.100
PL	0.022	-0.047	-0.039	0.016	0.034	-0.012	0.125	0.101
NGPP	-0.004	0.047	-0.087	0.006	0.096	-0.039	0.314	0.327*
TGW	0.042	-0.001	0.056	-0.002	-0.044	0.087	-0.062	0.075
HI	-0.072	-0.061	-0.077	0.002	0.032	-0.006	0.925	0.733**

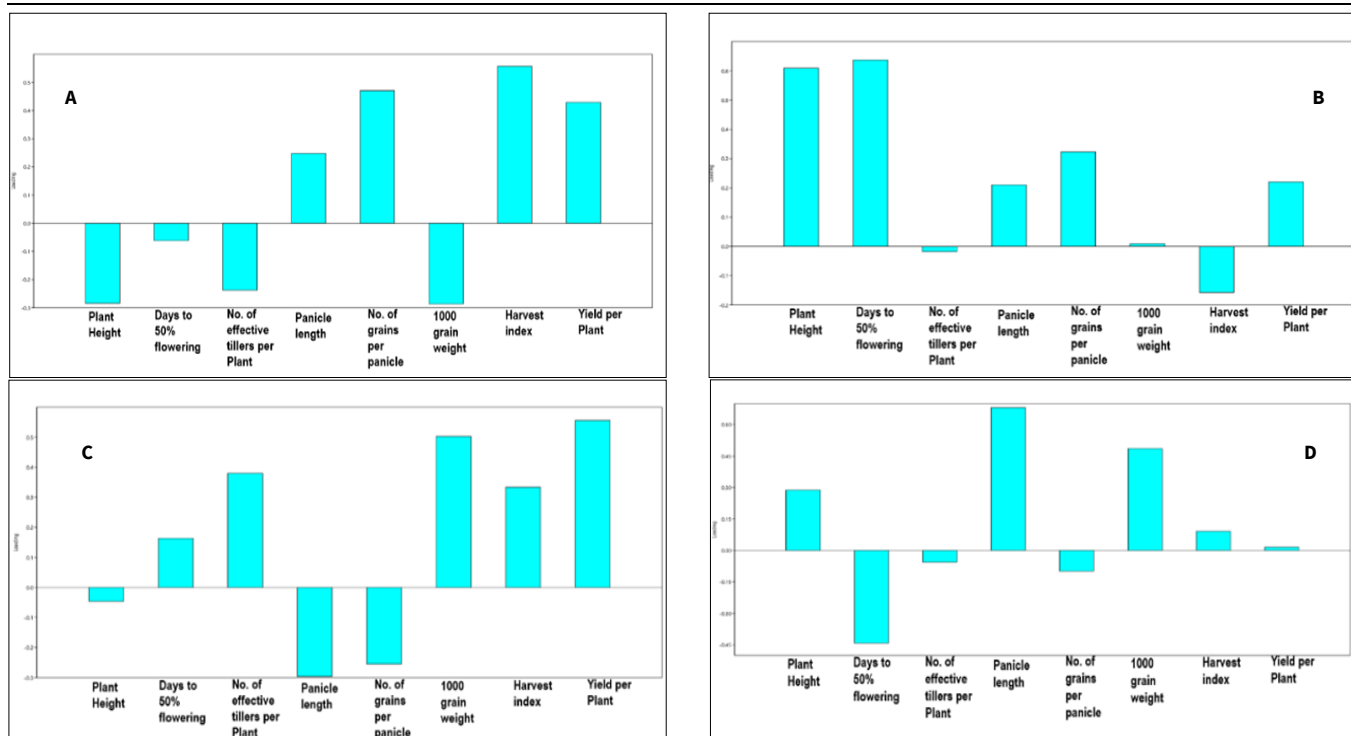
Table 6. Principal component analysis representing eigenvalues, percentage of variance and cumulative variance for eight yield-attributing traits of 52 rice genotypes

PC	1	2	3	4	5	6	7	8
Eigenvalue	2.28	1.52	1.45	1.03	0.89	0.40	0.32	0.09
% variance	28.55	19.03	18.16	12.87	11.18	5.06	4.05	1.11
Cumulative variance (%)	28.55	47.58	65.74	78.61	89.79	94.85	98.90	100.00

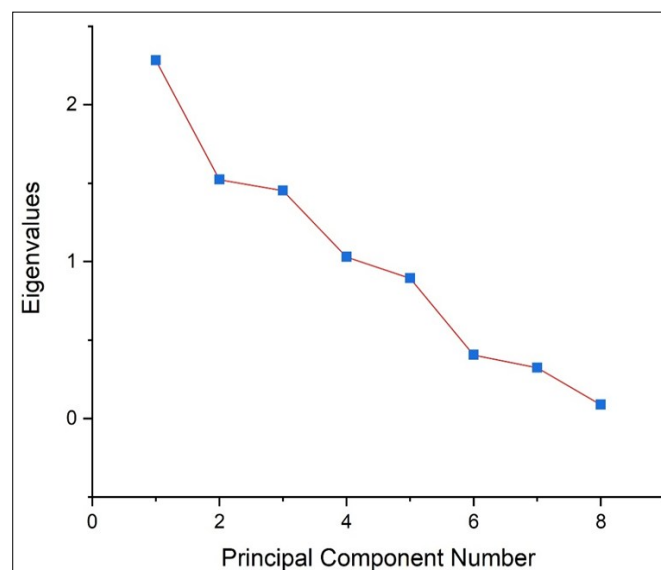
Table 7 explored that PC1 was positively influenced by HI (0.56), NGPP (0.47), GYP (0.43) and PL (0.25). At the same time, traits like TGW (-0.29), PH (-0.28), NETP (-0.24) and DFF (-0.06) were negatively associated (Fig. 4A). Similarly, PC2 highlighted positive contributions from DFF (0.64), PH (0.61), NGPP (0.32), GYP (0.22), PL (0.21) and TGW (0.01) and negative influences from HI (-0.16) and NETP (-0.02) (Fig. 4B). PC3 highlighted GYP (0.56), TGW (0.50) and other factors as positive contributors, whereas PL (-0.30) and NGPP (-0.25) had adverse effects (Fig. 4C). Similarly, PC4 demonstrated positive effects from PL (0.68), TGW (0.49) and others, with negative contributions from traits such as DFF (-0.44) (Fig. 4D). These findings similar with previous study (37) and emphasized that these traits contribute most significantly to the genetic diversity and variability, indicating considerable opportunities for genetic improvement of these traits.

Table 7. Principal components and their factor loadings associated with yield and contributing traits in 52 rice genotypes

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
PH	-0.28	0.61	-0.05	0.29	-0.10	0.37	-0.54	0.15
DFF	-0.06	0.64	0.16	-0.44	-0.10	-0.49	0.25	0.23
NETP	-0.24	-0.02	0.38	-0.06	0.85	0.13	0.04	0.24
PL	0.25	0.21	-0.30	0.68	0.32	-0.48	0.11	0.01
NGPP	0.47	0.32	-0.25	-0.10	0.12	0.59	0.48	0.03
TGW	-0.29	0.01	0.50	0.49	-0.33	0.14	0.55	0.03
HI	0.56	-0.16	0.33	0.09	-0.17	0.00	-0.23	0.68
GY_P	0.43	0.22	0.56	0.02	0.07	-0.02	-0.22	-0.63

**Fig. 4.** Depicts loading plot of PC1 (A), PC2 (B), PC3 (C) and PC4 (D) for different traits.

A scree plot (Fig. 5) illustrated the relationship between eigenvalues and eight principal components (PCs). Among the eight PCs, the first four viz. PC1, PC2, PC3 and PC4 exhibited an eigenvalue of 2.28, 1.52, 1.45 and 1.03, respectively (each PC with an eigenvalue > 1), followed by a gradual decline in eigenvalues for the remaining PCs (eigenvalue < 1). This pattern confirmed that the first four

**Fig. 5.** Scree plot of principal component analysis (PCA) for eight yield-attributing traits of 52 rice genotypes, illustrating the relationship between eigenvalues and principal components.

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