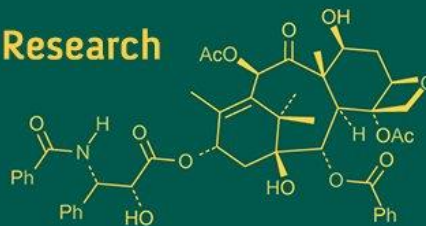
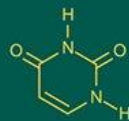


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Multivariate analysis of phenotypic diversity among advanced breeding lines for culm and yield related traits in rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) is the staple food for more than half of the global population, making simultaneous improvement of grain yield and lodging resistance a major breeding objective. However, both traits are quantitatively inherited, highly influenced by the environment, and governed by several interrelated component traits, complicating effective selection. Therefore, the present study employed multivariate analysis to evaluate 30 advanced breeding lines derived from diverse parental backgrounds for identifying superior lines combining high yield and lodging resistance. A total of 13 lodging resistance and yield related traits were assessed during *Kharif* 2025 and analyzed using principal component analysis (PCA). Twelve principal components were extracted, of which the first four accounted for 68.76% of the total phenotypic variation. The analysis revealed that culm length, plant height, culm strength, culm thickness, culm diameter, grain number per panicle and panicle weight were the major contributors to genetic divergence, highlighting their importance as key selection criteria for simultaneous improvement of lodging resistance and grain yield. PCA also effectively elucidated the relationships among the measured traits and facilitated the identification of superior breeding lines. Among the evaluated breeding materials, lines *viz.*, ABL-1, ABL-7 and ABL-8 exhibited desirable combinations of lodging resistance and yield-related attributes, indicating their potential for multilocation evaluation and use as donor parents in future breeding programs.

Keywords: Eigen value, principal components, lodging resistance, variability

Introduction

Rice (*Oryza sativa* L.) is the primary staple food for more than half of the world's population and plays a pivotal role in global food and nutritional security. Sustaining rice productivity under changing climatic conditions and increasing labour scarcity remains one of the foremost challenges confronting modern agriculture. While substantial progress has been achieved through the development of high-yielding semi-dwarf cultivars, further genetic improvement is constrained by complex interactions among yield components, plant architecture, and environmental factors. (Peng *et al.*, 2000; Hirano *et al.*, 2017) [11, 18]. Consequently, breeding strategies have increasingly shifted towards the development of superior genotypes possessing an optimal combination of high grain yield, lodging resistance and agronomically desirable traits rather than improving individual characters independently.

Lodging is among the most detrimental constraints limiting rice productivity, particularly under intensive cultivation systems where high fertilizer inputs, improved irrigation, and favourable growing conditions promote vigorous vegetative growth. Lodging causes irreversible stem bending or breakage, leading to substantial reductions in grain yield, grain quality, harvest efficiency and economic returns. Yield losses due to lodging have been reported to range from 10% to over 80%, depending on the severity, growth stage, genotype, and prevailing environmental conditions (Setter *et al.*, 1997; Berry *et al.*, 2004). The increasing frequency of extreme weather events, including heavy rainfall and strong winds associated with climate change, has further intensified the occurrence of lodging in rice-growing regions, underscoring the necessity of developing cultivars with improved structural stability while maintaining high yield potential (Lang *et al.*, 2012; Luo *et al.*, 2022) [5, 14, 15, 20].

Grain yield and lodging resistance are quantitatively inherited traits governed by numerous component characters and is profoundly influenced by genotype $\times$ environment interactions. These traits are highly interrelated and often exhibit antagonistic or compensatory relationships, making simultaneous selection particularly challenging in breeding programmes (Akshay *et al.*, 2024a) ^[1]. Understanding the collective contribution of lodging associated and yield related traits is essential for designing effective selection strategies aimed at developing climate-resilient, high-yielding rice cultivars with superior structural stability. Conventional univariate analyses evaluate each trait independently and therefore may overlook the underlying relationships among correlated variables, resulting in inefficient selection decisions when multiple complex traits are involved (Varanasi *et al.*, 2023) ^[23].

Advanced breeding lines developed from multiple elite parental background provide an enriched genetic base by combining favorable alleles from diverse genetic backgrounds. Such breeding populations generally exhibit wide phenotypic variability, increased recombination and novel trait combinations, thereby offering greater opportunities for identifying superior lines with desirable combinations of lodging resistance and grain yield. However, the large number of correlated agronomic traits measured in advanced breeding populations necessitates robust statistical approaches capable of simultaneously evaluating multiple variables and identifying the principal sources of variation. Multivariate statistical techniques have become indispensable tools in modern plant breeding because they enable comprehensive evaluation of complex datasets involving numerous correlated traits. Among these approaches, principal component analysis (PCA) is one of the most widely adopted dimension-reduction techniques for summarizing the total variability into a few orthogonal principal components without substantial loss of information (Jolliffe, 1986) ^[12]. PCA facilitates the identification of key traits contributing to overall phenotypic variation, reveals relationships among traits, classifies breeding materials based on multivariate similarity, and assists breeders in selecting ideotypes possessing favorable combinations of economically important characteristics (Akshay *et al.*, 2024b, Anderson, 1972) ^[2, 3]. By reducing redundancy among correlated variables, PCA provides valuable biological insights into trait associations and enhances the efficiency of selection in advanced breeding populations.

Therefore, the present study was undertaken to evaluate advanced rice breeding lines developed from multiple parental combinations using a randomized block design (RBD) under field conditions. Multivariate analysis through principal component analysis was employed to quantify phenotypic diversity, identify the major traits contributing to overall variability, elucidate relationships among lodging resistance and grain yield attributes, and identify promising breeding lines possessing desirable trait combinations for future varietal development and parental selection in rice improvement programmes.

2. Materials and methods

A total of 30 advanced breeding lines (ABLs) developed from diverse parental combinations, were evaluated during the Kharif 2025 season at the Rice Research Unit (RRU), Agricultural Research Institute (ARI), PJTAU, Rajendranagr. The experiment was conducted under irrigated field

conditions using a Randomized Block Design (RBD) with two replications. Seeds of each breeding line were initially raised in a nursery and transplanted to the main field 27 days after sowing at a spacing of 20 $\times$ 15 cm. All recommended agronomic and crop management practices were followed uniformly throughout the crop growth period to ensure optimum plant establishment and minimize environmental variation. Observations were recorded on three randomly selected plants from each experimental plot for lodging related morphological and yield contributing traits. The traits evaluated includes days to 50% flowering (DFF), plant height (PH, cm), culm length (CL, cm), internode length (INL, cm), culm diameter (CD, mm), culm thickness (CT, mm), panicle length (PL, cm), panicle weight (PW, g), number of grains per panicle (GNP), test weight (TW, g), and grain yield per plant (GYP, g).

Plant height (cm) was measured from the plant base to the panicle tip. panicle number, grain number, and panicle weight, Internode length, culm length (cm) were recorded as the panicle-bearing tillers, filled grains per panicle, and weight of a fully matured panicle, the length of the fourth basal internode, the distance from the plant base to the base of the panicle respectively. Pushing resistance was measured by applying a prostrate tester perpendicular to the plant at 20-25 cm above the ground and pushing it to a 45° angle.

The fourth basal internode was used for the measurement of culm morphological characteristics. Culm diameter (CD) and culm thickness (CT) were determined following the procedures described by Ookawa *et al.* (2010), Badri *et al.* (2024) and Mamidi *et al.* (2025) ^[4, 16, 17] using Vernier calipers. Culm diameter was calculated as:

$$\text{Culm diameter (CD)} = (a_1 + b_1)/2$$

Culm thickness was calculated as

$$\text{Culm thickness (CT)} = [(a_1 + b_1)/2] - [(a_2 + b_2)/2]$$

Where, a_2 and b_2 denote the inner diameters of the minor and major axes, respectively and a_1 and b_1 represent the outer diameters of the minor and major axes, respectively.

Analysis of phenotypic diversity among the advanced breeding lines was performed using Principal Component Analysis (PCA) to identify the major traits contributing to variation in lodging resistance and grain yield. Mean values of all measured traits were subjected to PCA after standardization to eliminate scale differences among variables. The analysis was carried out using R software (Ver. 4.3.2). Scree plots were generated to determine the proportion of variance explained by each principal component, while PCA biplots were used to visualize genotype dispersion and trait associations.

3. Results and discussion

3.1 Principal component analysis revealed the major sources of phenotypic variation

Principal component analysis (PCA) was employed to elucidate the multivariate relationships among lodging related and yield associated traits evaluated in thirty advanced breeding lines. The analysis generated twelve principal components, of which the first four possessed eigenvalues greater than unity, satisfying the Kaiser criterion, and collectively explained 68.76% of the total phenotypic variation (Table 1). The first principal component (PC1)

exhibited the highest eigenvalue (2.75) and accounted for 22.94% of the total variation, followed by PC2 (2.47; 20.61%), PC3 (1.71; 14.22%) and PC4 (1.32; 11.00%). Together, the first two principal components explained 43.54% of the total variation, indicating that a substantial proportion of the phenotypic diversity among the evaluated breeding lines could be effectively represented in a two-dimensional ordination space (Figure 1). The scree plot displayed a distinct elbow after PC4, followed by a gradual decline in eigenvalues, confirming that the first four principal

components adequately captured the biologically meaningful variation within the breeding population. Similar patterns have been reported in rice and other cereal crops, (Deepika *et al.*, 2021; Harnal *et al.*, 2024; Reddy *et al.*, 2019) [8, 19] where the first few principal components efficiently summarize complex variation among correlated agronomic traits. The relatively high cumulative variance explained by the retained components reflects the broad phenotypic diversity and provides a strong basis for identifying key selection traits and superior breeding lines.

Table 1: Eigenvalues, proportion of variance explained, and cumulative variance of the principal components derived from principal component analysis (PCA) of lodging-related and yield-associated traits in thirty advanced rice breeding lines

Principal Component	Eigen value	Percent Variance%	Cumulative Variance%
PC1	2.75	22.94	22.94
PC2	2.47	20.61	43.54
PC3	1.71	14.22	57.76
PC4	1.32	11.00	68.76
PC5	0.95	7.93	76.69
PC6	0.89	7.39	84.08
PC7	0.70	5.84	89.92
PC8	0.54	4.50	94.42
PC9	0.32	2.67	97.09
PC10	0.19	1.59	98.67
PC11	0.11	0.93	99.61
PC12	0.05	0.39	100.00

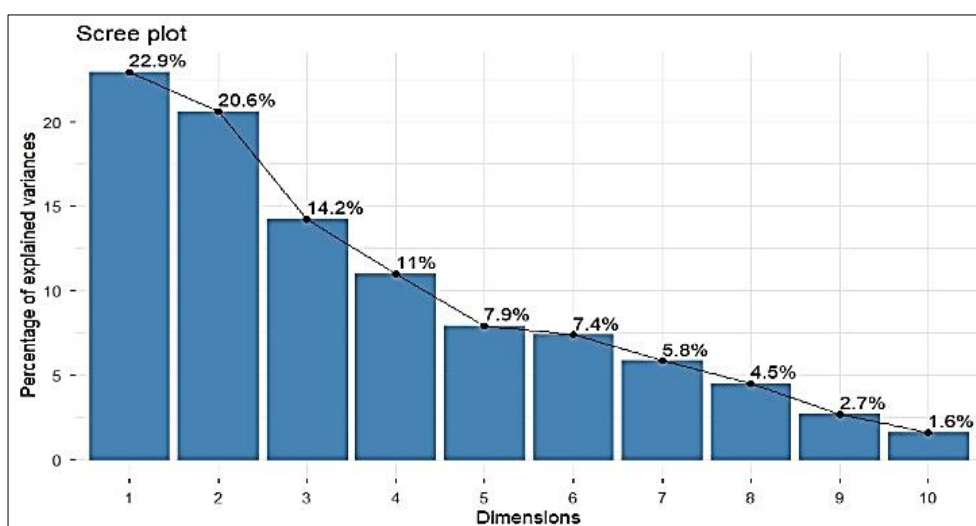


Fig 1: Scree plot showing the eigenvalues and percentage of total phenotypic variation explained by the twelve principal components derived from principal component analysis (PCA)

3.2 Trait loadings, variable contributions and quality of representation identified the principal determinants of genetic divergence

The PCA loading matrix, variable contribution analysis and squared cosine ($\cos^2$) values collectively identified the major traits responsible for phenotypic divergence among the advanced breeding lines (Table 2; Figures 2). The first principal component (PC1) was predominantly associated with plant architectural traits, with culm length (loading = -0.516 ; contribution = 26.66%; $\cos^2 = 0.734$) and plant height (-0.482 ; 23.24%; $\cos^2 = 0.640$) exhibiting the highest loading magnitudes, contributions and quality of representation, followed by days to 50% flowering (-0.339 ; 11.51%; $\cos^2 = 0.317$). In contrast, panicle weight (0.380), grain number per panicle (0.328), culm thickness (0.135), test weight (0.116) and number of productive tillers (0.103) displayed positive loadings, indicating that PC1 represented a gradient

contrasting taller, late-flowering lines with compact plant types possessing superior reproductive performance. The strong influence of plant height and culm length indicates that variation in plant architecture constituted the primary source of phenotypic diversity within the breeding population. This observation is consistent with the rice ideotype concept, where moderate plant stature improves harvest index, reduces lodging susceptibility and enhances grain productivity (Peng *et al.*, 2000) [18].

The second principal component (PC2), accounting for 20.61% of the total variation, was predominantly governed by culm strength (loading = 0.566; contribution = 32.04%; $\cos^2 = 0.792$), followed by panicle length (0.541; 29.26%; $\cos^2 = 0.724$), internode length (0.357; 12.76%; $\cos^2 = 0.315$), grain number per panicle (0.341; 11.6%; $\cos^2 = 0.287$) panicle weight (0.234; 5.49%; $\cos^2 = 0.136$). similar results are reported by (Reddy *et al.*, 2019) [19]. The high loading,

contribution and $\cos^2$ values of culm strength clearly indicated that stem mechanical strength was the most influential trait defining this component and played a pivotal role in discriminating the breeding lines. Plant height (-0.060) and culm length (-0.112) and test weight (-0.174) exhibited negative loadings on PC2, suggesting that improvements in stem strength may be achieved without necessarily increasing plant stature. Similar findings have been reported by (Ookawa *et al.*, 2010)^[17] who demonstrated that strengthening culm mechanical properties significantly enhances lodging resistance without compromising grain yield.

The third principal component (PC3), explaining 14.22% of the total variation, was mainly characterized by culm thickness (loading = -0.670; contribution = 44.92%) and culm diameter (-0.544; 29.62%). Increased culm diameter and thickness improve bending resistance by enhancing the second moment of area and strengthening supporting tissues, thereby contributing directly to lodging resistance

(Kashiwagi and Ishimaru, 2004; Ookawa *et al.*, 2010). Similar results were recorded by Gupte *et al.* (2023)^[9, 13, 17]. Although these traits exhibited comparatively lower $\cos^2$ values on the first two principal components, their substantial contribution to PC3 confirms their biological importance and highlights the need to consider stem anatomical characteristics alongside plant architecture and mechanical strength during selection.

Overall, the loading matrix, contribution percentages and $\cos^2$ analyses consistently identified culm length, plant height, culm strength, culm thickness and culm diameter as the principal contributors to multivariate variation. These traits therefore constitute the most informative selection criteria for simultaneous improvement of lodging resistance and grain yield in advanced rice breeding populations. similar results were reported by susmi *et al.* (2025), Yadav *et al.* (2017), Chakravorty *et al.* (2012) and Reddy *et al.* (2019), Chigira *et al.* (2023), Tiwari *et al.* (2019)^[5, 6, 19, 21, 22, 23].

Table 2: Principal component loadings (PC1-PC4) of lodging-related morphological and yield-associated traits used to characterize advanced rice breeding lines

Trait	PC1	PC2	PC3	PC4
PH	-0.482	-0.060	0.156	0.266
INL	-0.217	0.357	0.063	0.050
CL	-0.516	-0.112	0.141	0.262
CS	-0.202	0.566	-0.074	-0.086
CD	0.049	-0.049	-0.544	0.415
CT	0.135	0.074	-0.670	0.136
DFP	-0.339	0.176	-0.284	-0.122
NPT	0.103	-0.058	0.119	0.501
PL	-0.032	0.541	-0.014	-0.175
PW	0.380	0.234	0.269	0.210
GNPP	0.328	0.341	0.176	0.292
TW	0.116	-0.174	-0.051	-0.483

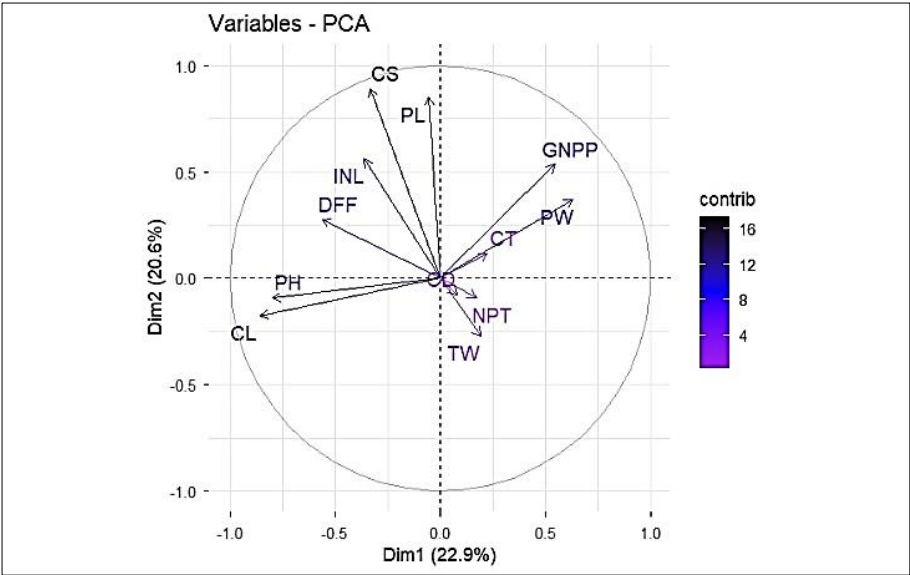


Fig 2: PCA variable factor map illustrating the relationships among lodging-related morphological and yield-associated traits based on the first two principal components.

Table 3: Contribution of Yield and Culm Traits to Principal Components

Trait	PC1	PC2	PC3	PC4
PH	23.24	0.36	2.42	7.07
INL	4.72	12.76	0.39	0.25
CL	26.66	1.25	1.98	6.88
CS	4.06	32.04	0.55	0.75

CD	0.24	0.24	29.62	17.25
CT	1.82	0.54	44.92	1.84
DFF	11.51	3.11	8.08	1.48
NPT	1.07	0.34	1.42	25.13
PL	0.10	29.26	0.02	3.08
PW	14.47	5.49	7.25	4.39
GNPP	10.78	11.60	3.08	8.51
TW	1.34	3.02	0.26	23.37

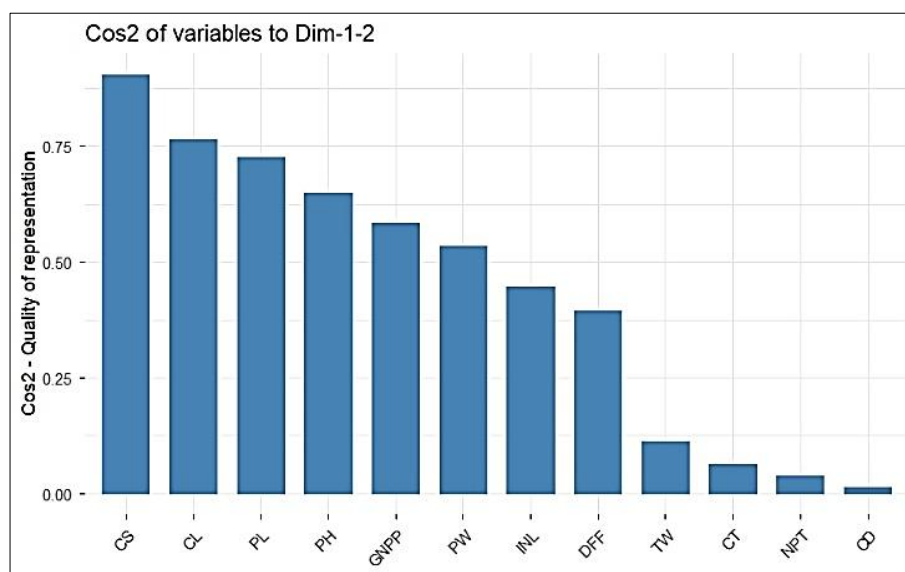


Fig 3: Quality of representation ($\cos^2$) of lodging-related and yield-associated traits on the first two principal components.

PH -Plant height, INL -Basal internode length, CL -Culm length, CS - Culm strength, CD -Culm diameter, CT -Culm thickness, DFF -Days to fifty percent flowering, PN -Panicle number, PL -Panicle length, GN -Grain number, PW -Panicle weight, TW -Thousand grain weight, SPY -Single plant yield.

3.3 Distribution of advanced breeding lines and breeding implications

The PCA score plot revealed a broad distribution of the advanced breeding lines across the principal component space, indicating substantial phenotypic diversity generated through recombination of multiple parental lines (Figure 4). Genotypes positioned farther from the origin exhibited greater divergence, whereas those located near the centre displayed relatively balanced expression of the evaluated traits. The PCA biplot further illustrated the relationships between breeding lines and trait vectors, enabling identification of superior genotypes and potential donor parents (Figure 5).

Among the evaluated materials, three breeding lines *viz.*, ABL-1, ABL-7 and ABL-8 were positioned in close proximity to the vectors representing culm strength, grain number per panicle, panicle weight and panicle length, indicating favourable combinations of lodging resistance and yield-contributing traits. Their association with these key variables suggested superior structural stability coupled with enhanced sink capacity, making them promising candidates for advanced yield evaluation and potential donor parents in future breeding programmes. Conversely four breeding lines *viz.*, ABL-2, ABL-6 and ABL-11 were more closely

associated with plant height and culm length, reflecting that their taller plant architecture may increase lodging susceptibility under intensive cultivation. Several breeding lines, including ABL-14, ABL-15, ABL-16, ABL-18 and ABL-19, were positioned away from the major trait vectors, indicating comparatively lower expression of both lodging-resistant and yield-associated characteristics, whereas lines located near the origin exhibited intermediate performance across the measured traits. Similar observation were recorded by susmi *et al.* (2025)^[21].

The orientation of the trait vectors further revealed important biological relationships among the evaluated variables. The close alignment of grain number per panicle, panicle weight and panicle length indicated strong positive associations among these yield components, while their positive relationship with culm strength suggested that enhanced stem mechanical stability can be achieved without compromising grain productivity. In contrast, the opposite orientation of plant height and culm length relative to the major yield vectors indicates that excessive vegetative growth may adversely affect reproductive efficiency and increase lodging susceptibility. These findings reinforce the importance of selecting genotypes possessing moderate plant stature, stronger culms and superior panicle architecture for the development of high-yielding, lodging-resistant rice cultivars. Overall, the PCA effectively identified the principal traits governing genetic divergence and highlighted three breeding lines *viz.*, ABL-1, ABL-7 and ABL-8 as promising breeding materials for future varietal development and strategic parental selection.

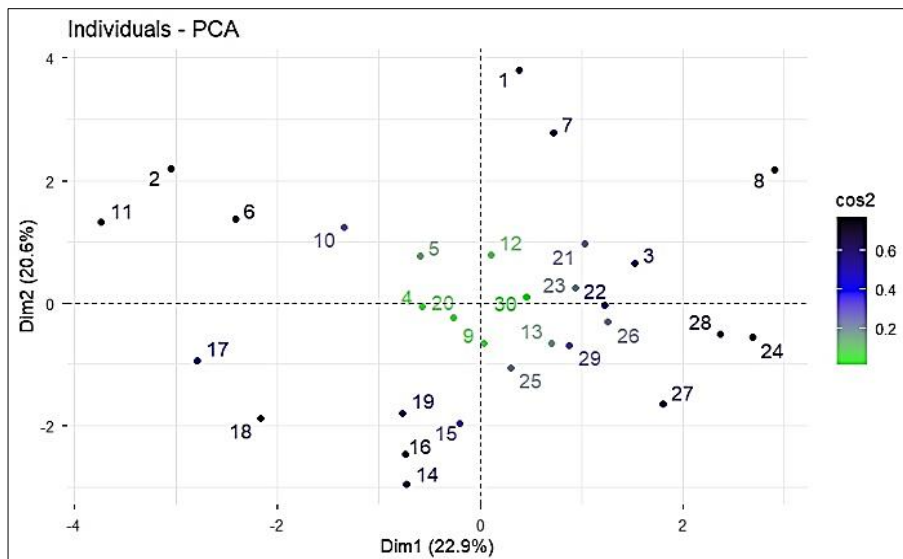


Fig 4: PCA score plot illustrating the distribution of thirty advanced rice breeding lines based on the first two principal components.

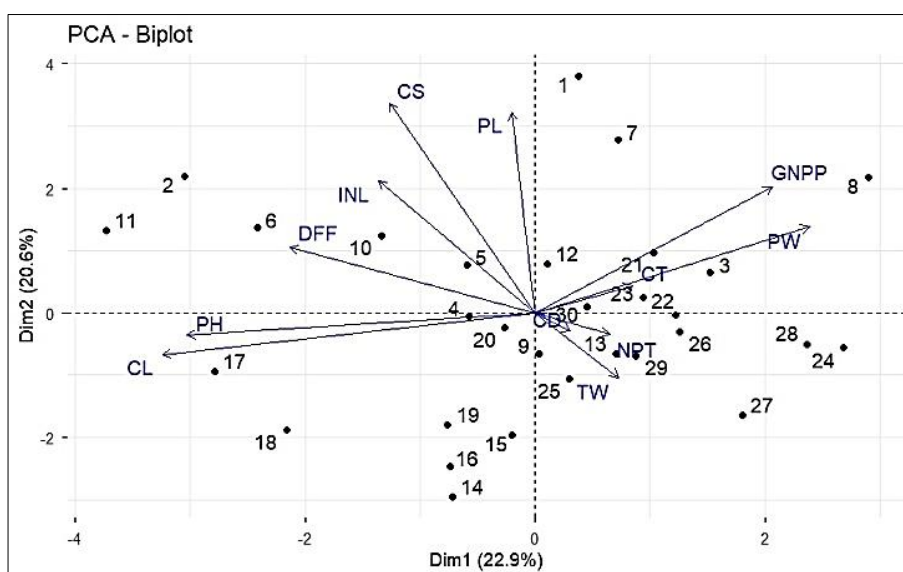


Fig 5: Principal component analysis (PCA) biplot depicting the relationships among advanced rice breeding lines and lodging-related morphological and yield-associated traits based on the first two principal components.

PH -Plant height, IL -Basal internode length, CL -Culm length, CS - Culm strength, CD -Culm diameter, CT -Culm thickness, DFF -Days to fifty percent flowering, PN -Panicle number, PL -Panicle length, GN -Grain number, PW -Panicle weight, TW -Thousand grain weight, SPY -Single plant yield.

4. Conclusion

Principal component analysis effectively unraveled the complex interrelationships among lodging-related and yield-associated traits in the evaluated advanced rice breeding lines, with the first four principal components explaining 68.76% of the total phenotypic variation. The analysis identified culm length, plant height, culm strength, culm thickness, culm diameter, grain number per panicle and panicle weight as the principal contributors to genetic divergence, underscoring their importance as key selection criteria for simultaneous improvement of lodging resistance and grain yield. The positive association of culm strength with major yield-contributing traits demonstrated that enhanced stem mechanical stability can be combined with improved productivity, providing a strong foundation for developing superior rice ideotypes. Among the evaluated

materials, ABL-1, ABL-7 and ABL-8 emerged as promising breeding lines owing to their favourable association with both lodging resistance and yield attributes, making them valuable candidates for advanced multilocation evaluation and potential donor parents in future breeding programmes. Overall, the study demonstrated that PCA is a powerful multivariate tool for trait prioritization, parental selection and ideotype breeding, thereby accelerating the development of high-yielding, lodging-resistant and climate-resilient rice cultivars through efficient multi-trait selection.

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