

## Original Research Article

# Unraveling promising genetic variability associated with strong culm and yield parameters in inter sub-specific cross derived recombinant inbred lines in rice (*Oryza sativa* L.)

### ABSTRACT

Strengthening the rice culm through genetic improvement has been an important target in breeding to abate the lodging problem in rice. The present investigation was attempted to study the variability, genetic parameters and trait association in recombinant inbred lines (RILs) derived from inter sub-specific cross of elite *indica* mega variety ‘Swarna’ and Tropical *japonica* strong culm accession ‘IRGC 39111’. Analysis of Variance (ANOVA) displayed significant variation in RILs for all the traits studied. The investigation revealed high estimates of GCV and PCV along with high heritability and genetic advance as percent of mean (GAM) for all culm strength (CS) traits revealing the role of additive gene effect and selection of these traits shall be effective in rice improvement. Correlation studies revealed that the internode breaking weight (IBW) and bending moment at breaking (M) had a high significant positive correlation with all the CS traits, panicle weight and grain yield. Thus, the direct selection of positively associated traits can improve the lodging resistance in rice. The principal component analysis (PCA) showed that, seven principal components (PCs) out of 23 traits attributed approximately 77.4% of the total cumulative variability. The traits culm thickness (CT), Internode breaking weight (IBW), section modulus (SM), bending stress (BS), Bending moment at breaking (M) contributed maximum to the total diversity and the lines with higher trait values for the aforementioned traits could be utilized as donors to improve the culm strength and its attributing traits in future breeding programs. From this study, it is evident that tropical *japonica* lines can be a great source of genetic diversification for rice improvement.

**Keywords:** Rice, tropical *japonica*, inter sub-specific cross, PCV, GCV, heritability, GAM, correlation, PCA.

### Introduction

Rice (*Oryza sativa* L.) is the second most cultivated cereal crop in the world. It is estimated that 40% of the world’s population consume rice and is the main stay food for people of Asia. To further enhance the yield of rice varieties amidst climate change and burgeoning population, it is necessary not only to improve yield component traits but also to strengthen the culms bearing heavy panicles. The present deceleration drift in production and yield is a cause of concern and has to be upturned to meet the growing demand (Akshay et al. 2022). It is therefore necessary to harness the available resources and knowledge on rice and engage large genetic variability from outside because the available genetic diversity of the cultivated *indica* pool has already been utilized to greater extent. In the evolutionary process, two cultivated sub-species of rice, *indica*

and *japonica* have become genetically isolated. This has stifled the genetic diversity within each sub-species. *Indica* sub-species harboring the greatest genetic diversity, is grown across the world and thus has been the focus of most genetic research and utilization (Eizenga et al. 2021). However, the exploration of diversity panels has revealed that *indica* is relatively less diverse than the *japonica* (Singh et al. 2022). *Japonica* rice consists of two subtypes: temperate and tropical. Tropical *japonica* (TrJ) initially considered as a third sub-species called *javanica*, represents an intermediary between *indica* and *japonica* and can hybridize with both *indica* and *japonica*, overcoming the cross-incompatibility barrier between the two sub-species. Consequently, TrJ serves as a bridge for *indica-japonica* hybridization and a valuable source for increasing genetic base in rice (Singh et al. 2022). Tropical *japonica* landraces were used in breeding program for new plant type (NPT) rice at IRRI (International rice research institute) to increase the yields of modern *indica* cultivars (Fujita et al. 2013). New plant type concept is gaining significant momentum in the context of doubling the farmer's income by enhancing the yield potential of the existing cultivars and focus is now on breeding for high yielding lines (Jyothi et al. 2018). In this context, it is imperative to increase culm strength that may provide scope for attaining targeted high yields in rice (Bagudam et al. 2021).

Moreover, the introduction of genes *Gn1a* and *APO1* for number of grains (Ashikari et al. 2005; Ikeda- Kawakatsu et al. 2009, Terao et al. 2010); *DEP1* and *WFP* for panicle architecture (Huang et al. 2009; Miura et al. 2010); *GS3*, *GW2*, and *qSW5* for grain size (Fan et al. 2006; Song et al. 2007; Shomura et al. 2008; Fujita et al. 2013) has resulted in heavier panicles, but the culms supporting those panicles have not improved, and there is the concern of lodging again (Hirano et al. 2017). Furthermore, erratic precipitation patterns, severe typhoons and alarming surge in the cyclonic turbulence in India and East Asia are expected to pose imminent threats to rice production. To cope with this situation, enhancing the mechanical strength of the culm is becoming inevitably important to develop the rice varieties having strong culm and lodging resistance to bear heavy panicles for yield improvement. To understand the physiological and morphological basis of culm strength and improve it further, prior knowledge of the genetic control of mechanisms that regulate culm strength is a prerequisite (Badri et al. 2024).

The presence of adequate genetic variability is regarded as the fundamental pre-requisite to conduct any crop improvement programme. Hence, it is very crucial to understand the species' variability, the nature of character relationships and the role of various traits in increasing rice production through breeding programme (Khan et al. 2020). Indonesian tropical *japonica* landraces are characterized by large panicles, large leaves, strong culms, fewer tillers and vigorous root architecture, contributing to their adaptability and resilience in diverse environments (Singh et al. 2022). Being an unexplored germplasm, Tropical *japonica* offers an abundant scope to dwell into and harness the desirable alleles for agronomically important traits like strong culm. To capture these desirable alleles, merging the genetic diversity across sub-species could bring in increased variability and therefore increased heterosis, for which tropical *japonica* would be useful.

Sometimes phenotypic selection based on their performance may not be effective because these genotypes may perform poor in further segregating generations, so it is essential to select the genotypes based on heritability and genetic advance (Akshay et al. 2022). The genotypic coefficient of variation (GCV) measures the magnitude of genetic variability and reflects the heritable portion of variation. Genetic variability along with heritability estimates would provide the amount of genetic gain expected out of selection (Burton, 1952). Therefore, assessment of variability is essential to formulate selection criteria for the improvement of culm strength and constructive augmentation of yield. The rice strong culm trait is influenced by the intricate interplay of many factors. To improve the complicated trait like strong culm in rice, it is crucial to understand how attributing traits interact with one another and helps to increase the grain yield. One of the requirements for directing selection is to know the degree of relation between strong culm and its contributing traits through correlation studies (Singh et al. 2018). Since strong culm is a challenging complex trait that depends on several variables and the environment, to determine and reduce the number of characteristics for efficient selection, a method known as Principal Component Analysis (PCA) can be effective. Principal component analysis (PCA), a multivariate technique, reduces data with a large number of correlated variables into a substantially smaller set of new variables through a linear combination of the variables that accounts for most of the variation present in the original variables (Yasaswini et al. 2023). By keeping the aforesaid points in mind, the present investigation was undertaken to elucidate information on variability, heritability, and genetic advance in RILs derived from inter sub-specific cross to identify effective selection criteria for strong culm and yield attributes.

## **MATERIAL AND METHODS**

A set of 353 RILs and six checks were evaluated during dry season (DS), 2022-23 at ICAR-IIRR, Hyderabad. The RILs were sown in the raised bed and transplanted into the main field 27 days after sowing with the spacing of 20 x 15 cm in Augmented randomized complete block design (ARCBD). RILs were planted only once while the checks were replicated in all the seven blocks and a single seedling per hill was maintained. All the package of practices were followed for the good establishment of the crop. RILs were evaluated and observations were recorded on random four plants in each line for morphological traits viz., plant height (PH) (cm), internode length (IL) of fourth internode (cm), culm length (CL) (cm), outer diameter of major axis (ODMa)- $b_1$  (mm), outer diameter of minor axis (ODMi)- $a_1$  (mm), inner diameter of major axis (IDMa)- $b_2$  (mm), inner diameter of minor axis (IDMi)- $a_2$  (mm), culm diameter (CD) (mm), culm thickness (CT) (mm), section modulus (SM) ( $\text{mm}^3$ ), bending stress (BS), bending moment at breaking (M), Internode breaking weight (IBW) (g), pushing resistance (PR), culm with leaf sheath weight (CLSW)(g), tiller number (TN), panicle number (PN), panicle length (PL) (cm), panicle weight (PW) (g), Days to 50 percent flowering(DFF), total number of grains/panicle(GN), 1000 grain weight(TW) (g), grain yield (GY) (g). The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were estimated according to the method Burton (1952) and the estimates for variability were treated as per the categorization proposed by Sivasubramanian and Madhavamenon, 1973. The broad sense heritability ( $h^2$ ) and GAM were calculated as per Lush (1940) and Johnson et al. (1955). The degree and direction of association between variables was

estimated by using Karl Pearson's Coefficient of correlation (r) estimated from variance and covariance of the variables and PCA was calculate using *ver* 4.2.1 software.

The following traits were determined as per Ookawa et al. (2010) and Badri et al. (2024)

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

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

$$\text{Section modulus (SM)} = \pi / 32 \times (a_1^3 b_1 - a_2^3 b_2) / a_1$$

$$\text{Bending stress (BS)} = (TR \div 40) \times (1000 \div TN)$$

$$\text{Bending moment at breaking (M)} = \text{Section modulus (SM)} \times \text{Bending stress (BS)}$$

Where, TR is the prostrate tester reading value (measure of pushing resistance) and TN is the tiller number. The prostrate tester (DIK-7400, Daiki Rika Kogyo Co. Ltd., Tokyo, Japan) was used to measure the pushing resistance of the culm (Badri et al. 2024).

## RESULTS AND DISCUSSION

The identification of the key traits that can be used in crop improvement through various breeding techniques is extremely important. The detailed knowledge of variability, heritability, magnitude and direction of relationship between strong culm and its attributes along with PCA forms the foundation for effective selection of the traits in crop plants. In the present study, the tropical *japonica* line IRGC 39111, possessing a strong culm was used as a parent to develop RILs. The RILs elicited great phenotypic variability, particularly related to NPT phenology.

The Analysis of variance (ANOVA) displayed that the lines used in the study were significantly different for all the characters (Table 1). The variability analysis revealed that the PCV was higher than GCV for all the traits in the study (Table 2) indicating the apparent variation is due to the environmental influence apart from genotypes. This result was in accordance with the findings of Akshay et al. (2022). The PCV and GCV were higher in the following traits viz., PR (27.5, 23.29), CT (42.45, 38.76); SM (56.22, 53.04); BS (35.58, 33.17), M (68.67, 67.89), IBW (32.71, 28.45) CLSW (28.82, 25.86), TN (31.08, 30.73), PN (31.03, 26.59), GN (21.73, 20.7), TW (26.36, 24.96) and similar findings were also reported by Reddy et al. (2019), Bagudam et al. (2021). The observed variability among RILs probably be attributed to the inherent genetic differences and the environment in which they were grown. The findings are presented in Fig. 1.

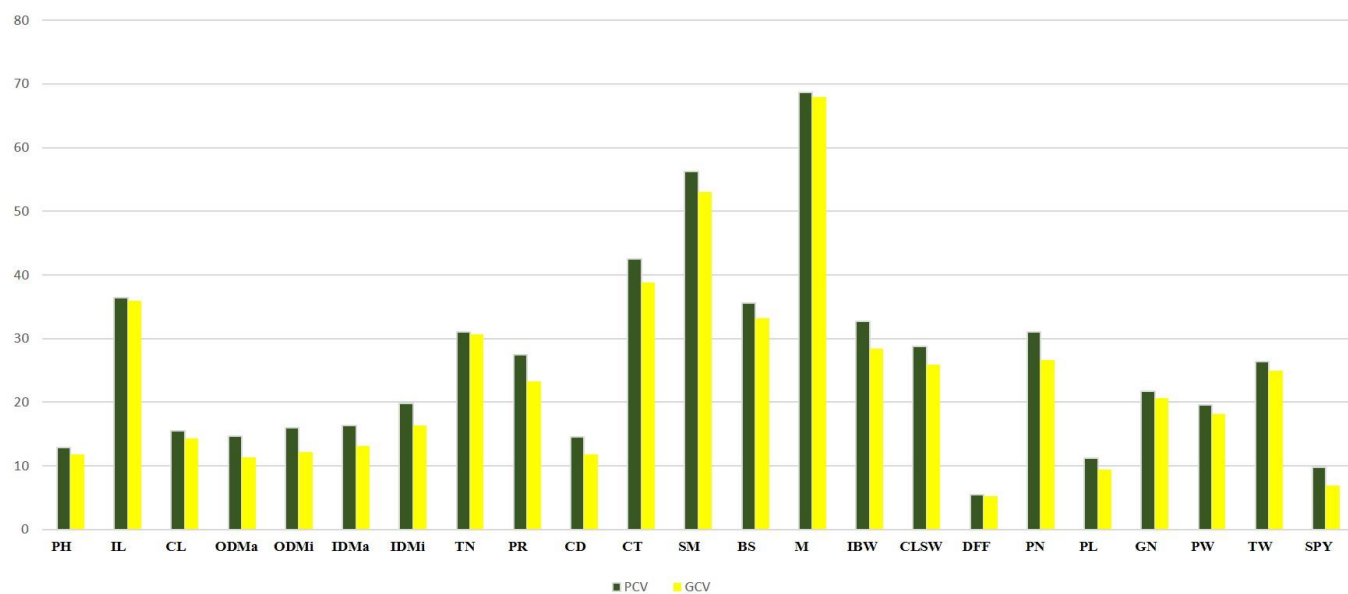
The heritability for all the characters studied was high (>70%) except for ODMa, ODMi, GY which were medium and it ranged from 49.93 % to 97.74 % (Table 3). Heritability coupled with GAM was found to be more useful for selection (Johnson et al. 1955). In the present study, GAM ranged from 10.06 (GY) – 138.45 (M), which is a measure of culm strength. Most of the CS traits including PH, IL, CL, IDMa, IDMi, PR, BS, M, IBW and CLSW recorded high GAM and high heritability indicating that most likely the heritability is due to additive gene action and these traits were less influenced by the environment. Thus, can be easily be selected based on phenotype and can be effective. These results confirm that there is a possibility of direct selection for these traits, similar results were observed by Reddy et al. (2019). DFF and PL recorded high heritability

(66.90 %) and moderate GAM (17.7) indicating the role of both additive and non-additive gene action (Figure 2).

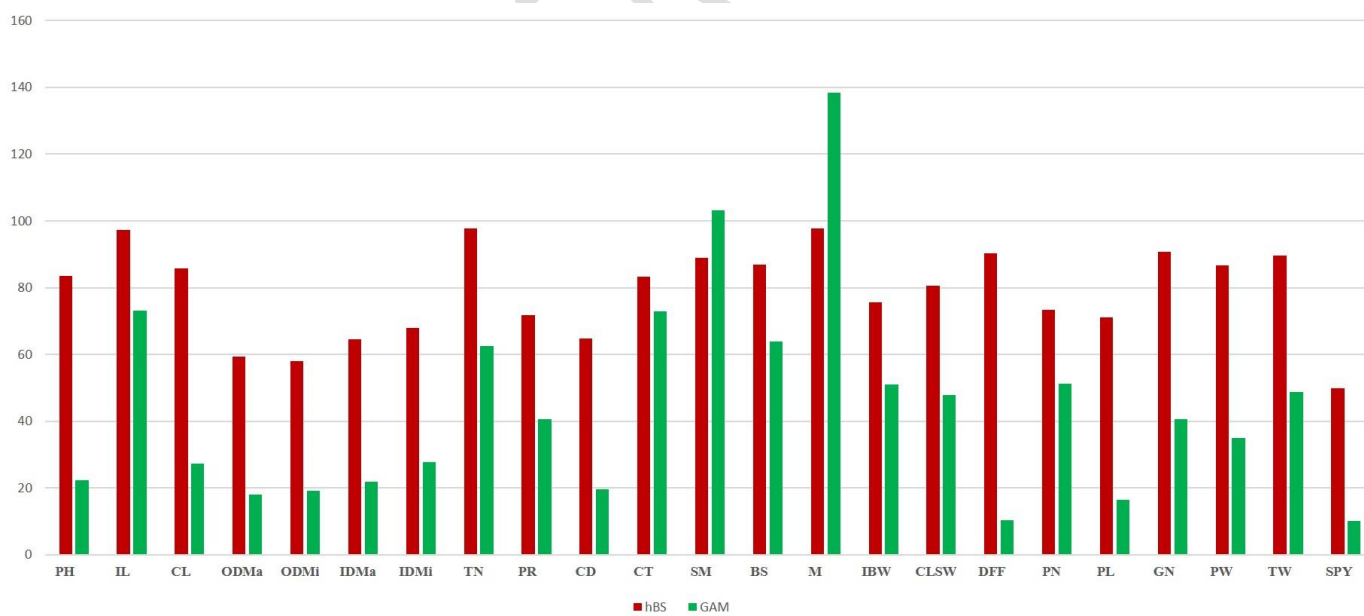
Pearson's correlation is the measure of strength of linear relationship between the characters (Reddy et al. 2019). The correlation analysis disclosed that the IBW and M had a high significant positive correlation with all the culm related traits, PW and GY (Figure 3). Correlation studies allow a complete measure of the relationship between two traits. The positive correlation of strong culm with various traits was in concomitant with the reports of Ookawa et al. (2010); Zhang et al. (2010); Yang et al. (2012); Zhang et al. (2016) and Badri et al. (2024). Such strong positive correlations among traits, heritability and genetic advance suggest that they are heritable and genetically controlled which could be simultaneously transferred to the desirable lines. The panicle length was negatively correlated with grain number. This may be due to longer panicle length but with a smaller number of filled grains. The distribution of traits was approximately continuous and indicated that the traits were controlled by a few minor genes and are typical quantitative traits.

Analysis of association among the strong culm attributing traits revealed that the IBW had the positive and highly significant correlation with panicle weight and grain yield. Hence selecting the plants with a higher IBW strongly improves the grain yield *via* panicle weight. Similarly, all the culm related traits were significantly and positively correlated with each other indicating that the selection may be positive direction for these traits. The above findings showed that strong selection for any one of the aforementioned attributes concurrently enhances the culm strength and grain yield and these findings are concomitant with the previous findings of Chigira et al. (2023). Panicle number per plant had a negative and significant correlation with the IBW, CD and CT. This clearly shows that increasing panicle number eventually reduces the strength of culm *via* partitioning of biomass to produce greater number of panicles by the plant. A critical analysis of the character association results revealed a favorable correlation between the grain yield per plant and IBW, CD, CT, CL (Yadav et al. 2019). As a result, choosing for these characteristics may improve the yield components and culm strength.

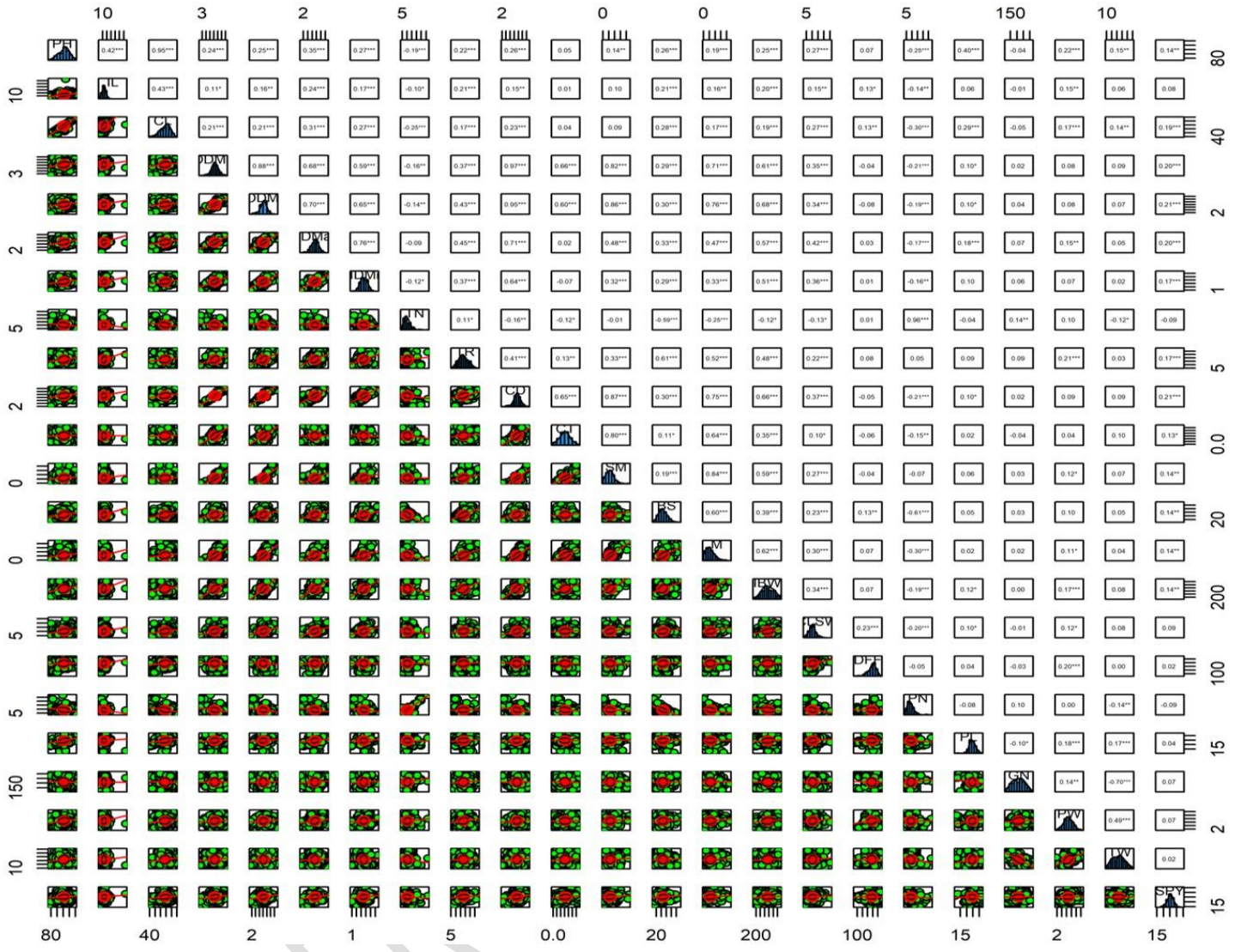
Principal component (PC) analysis was carried out to identify the minimum number of components which can explain maximum variability out of total variability and also rank genotypes on the basis of PC scores Reddy et al. (2019). Among the 23 PCs, seven PCs had eigen values greater than 1.00 and accounted for approximately 77.4% of the total cumulative variability. The scree plot (Figure 4) showed the amount of variance described by each PC and showed that PC1 had the largest eigen value contributing highest amount of variability, at about 32.1%. Additionally, PC2, PC3, PC4, PC5, PC6, and PC7 showed a continuous decrease in variability, with comparable values of 12.1%, 9%, 8%, 6%, 5.7% and 4.5% respectively.



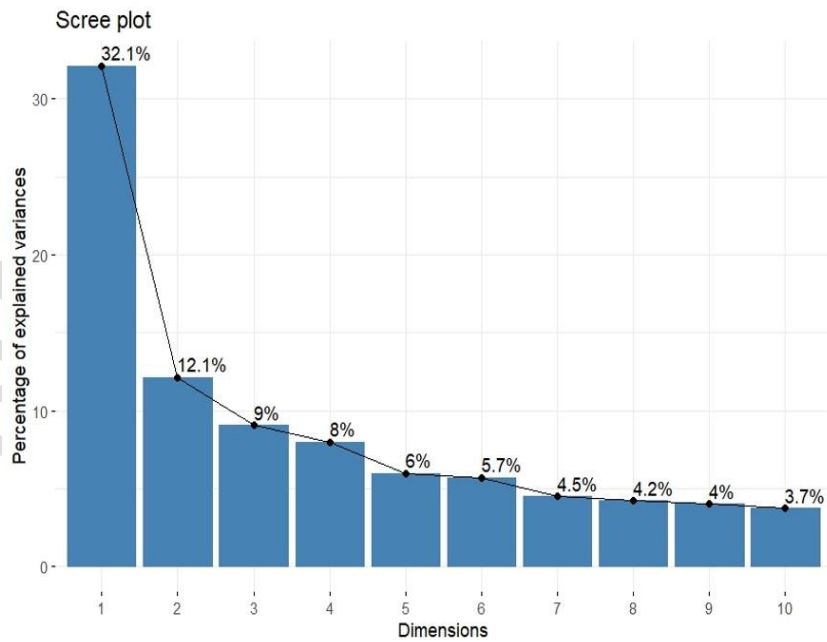
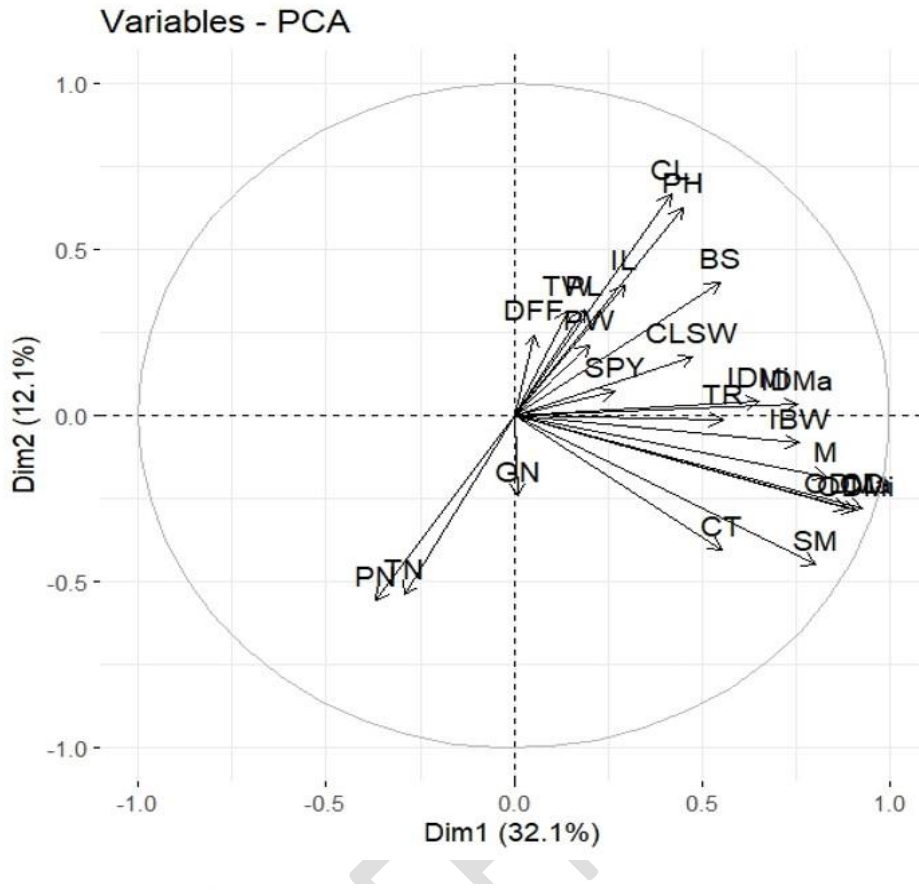
**Figure 1: Representation of Variability parameters for strong culm and yield components**



**Figure 2: Representation of Heritability and genetic advance as per cent of mean for strong culm and yield**



**Figure 3. Correlation matrix with significant values and trait distribution among 23 characters**



**Figure 4: Representation of Biplot diagram of PC1 and PC2 and Scree plot explaining variance**

The first two primary PCs' biplot (Figure 4) demonstrated how the features affected the divergence. The length of a trait's vector in PCA represents its contribution to the overall divergence; the longer the vector, the larger the contribution. All culm and yield related traits except for DFF, GN and GY exhibited maximum length and contributed maximum to the total diversity. These results were in conformity with the findings of Reddy et al. (2019), Gupte et al. (2023).

## Conclusion

In summary, high PCV and GCV, together with heritability combined with GAM, were reported by the traits PR, IL, CT, SM, BS, M, IBW, CLSW, TN, PN, GN, and TW. Therefore, while selecting genotypes to increase culm strength, these attributes should be considered. Correlation analysis revealed a positive and substantial relationship between the GY and PW and the culm strength (IBW, M). In the PCA analysis, SM, ODMi, M, IBW, and CT had the longest vector lengths and contributed the most to overall diversity and the lines with high mean values for these traits might be utilized as donors to increase the culm strength and its attributing traits in a future breeding programs. In this study, we present the synergistic associations between culm strength and grain yield traits and tropical *japonica* germplasm can be a great source of genetic diversification for rice improvement. The variability and character associations identified in the present study demonstrated that introgression of desirable traits particularly thick culm with greater physical strength associated with grain yield traits from tropical *japonica* would broaden the genetic base of elite *indica* cultivars with enhanced yield potential.

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**Table 1: Analysis of variance for strong culm and yield components in recombinant inbred lines of rice**

Source	Df	PH	IL	CL	ODMa	ODMi	IDMa	IDMi	TN	PR	CD	CT	SM
<b>Block (ignoring Treatments)</b>	6	1080.62**	34.58**	1055.09**	1.9**	2.23**	2.85**	3.15**	22.72*	104.96**	2.23**	0.65**	71.57**
<b>Treatment (eliminating Blocks)</b>	358	325.48**	28.42**	370.64**	1.19**	0.94**	0.78**	0.6**	14.02*	27.11**	0.98**	0.48**	57.19**
<b>Treatment: Check</b>	5	2383.28**	84.21**	982.8**	23.92**	21.7**	16.02**	8.54**	182.71**	340.5**	20.86**	3.49**	745**
<b>Treatment: Test and Test vs. Check</b>	353	296.33**	27.63**	361.97**	0.87**	0.65**	0.56**	0.49**	11.63*	22.68**	0.7**	0.44**	47.44**
<b>Residuals</b>	30	42.28	0.67	33.75	0.35	0.28	0.21	0.16	0.22	6.88	0.24	0.07	5.34
			<b>BS</b>	<b>M</b>	<b>IBW</b>	<b>CLSW</b>	<b>DFE</b>	<b>PN</b>	<b>PL</b>	<b>GN</b>	<b>PW</b>	<b>TW</b>	<b>GY</b>
			456**	194770**	241952**	63.43**	289**	16.05*	35.1**	10370**	4.12**	110**	25.88**
			341**	187328**	75993**	10.07**	76.19**	11.04*	7.77**	2126**	0.97**	33.34**	6.61**
			3157**	1482436**	1379709**	104**	1762**	135**	46.11**	15518**	21.6**	127.44**	40.79**
			301**	168984**	57526**	8.73**	52.3**	9.27*	7.23**	1936**	0.68**	32**	6.13*
			37.69	3901.87	14743	1.76	4.91	1.93	2.19	193.67	0.1	3.37	3.09

\* P ≤ 0.05; \*\* P ≤ 0.01

**Table 2: Estimates of variability, heritability and genetic advance for strong culm and yield contributing traits**

<b>Trait</b>	<b>PV</b>	<b>GV</b>	<b>PCV</b>	<b>GCV</b>	<b>h(BS)</b>	<b>GAM</b>
<b>PH</b>	256.71	214.43	12.91	11.8	83.53	22.25
<b>IL</b>	25.54	24.87	36.39	35.91	97.38	73.11
<b>CL</b>	235.49	201.74	15.5	14.35	85.67	27.4
<b>ODMa</b>	0.85	0.51	14.67	11.29	59.25	17.93
<b>ODMi</b>	0.66	0.39	16	12.19	57.99	19.14
<b>IDMa</b>	0.58	0.37	16.35	13.14	64.58	21.79
<b>IDMi</b>	0.5	0.34	19.77	16.31	68.04	27.75
<b>TN</b>	9.74	9.52	31.08	30.73	97.74	62.54
<b>PR</b>	24.36	17.48	27.5	23.29	71.74	40.7
<b>CD</b>	0.69	0.45	14.62	11.77	64.83	19.55
<b>CT</b>	0.45	0.37	42.45	38.76	83.36	73
<b>SM</b>	48.65	43.3	56.22	53.04	89.02	103.25
<b>BS</b>	288.82	251.14	35.58	33.17	86.95	63.82
<b>M</b>	171202.8	167300.9	68.67	67.89	97.72	138.45
<b>IBW</b>	60529.84	45785.87	32.71	28.45	75.64	51.04
<b>CLSW</b>	9.06	7.3	28.82	25.86	80.56	47.89
<b>DFE</b>	50.47	45.56	5.52	5.25	90.27	10.28
<b>PN</b>	7.26	5.33	31.03	26.59	73.41	51.26
<b>PL</b>	7.6	5.41	11.21	9.46	71.21	16.47
<b>GN</b>	2096.75	1903.08	21.73	20.7	90.76	40.68
<b>PW</b>	0.73	0.63	19.52	18.18	86.72	34.92
<b>TW</b>	32.62	29.25	26.36	24.96	89.68	48.76
<b>GY</b>	6.17	3.08	9.77	6.9	49.93	10.06

PV: Phenotypic variance; GV: Genotypic variance; PCV: Phenotypic coefficient of variance; GCV: Genotypic coefficient of variance; h(BS): Heritability in broad sense; GAM: Genetic advance as percent of mean, PH-Plant height, IL-Internode length, CL-Culm length, ODMa-Outer diameter of major axis, ODMi-Outer diameter of minor axis, IDMa-Inner diameter of Major axis, IDMi- Inner diameter of minor axis, TN-Tiller number, PR-Pushing resistance, CD- Culm diameter, CT-Culm thickness, SM- Section modulus, BS-Bending stress, M-Bending moment at breaking, IBW- Internode breaking weight, CLSW- Culm with leaf sheath weight, DFF-Days to 50% flowering, PN-Panicle number, PL-Panicle length, GN-No of filled grains per panicle, PW- Panicle weight, TW-Test weight, GY-grain yield.

**Table 3. Eigen value, contribution of variability for PCA in RILs.**

Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigen value	2.71	1.66	1.44	1.35	1.17	1.14	1.02
Percentage of variance	32.1	12.1	9.0	8.0	6.0	5.7	4.5
Cumulative percentage of variance	32.1	44.2	53.2	61.2	67.2	72.9	77.4
PH	0.17	0.38	0.23	-0.04	0.32	-0.21	0.03
IL	0.11	0.24	0.17	0.04	0.04	-0.21	0.00
CL	0.15	0.40	0.19	-0.01	0.31	-0.21	-0.03
ODMa	0.33	-0.17	-0.03	-0.05	0.12	0.06	-0.06
ODMi	0.33	-0.17	0.00	-0.02	0.10	0.08	0.01
IDMa	0.28	0.02	0.21	0.13	0.02	0.34	0.00
IDMi	0.24	0.03	0.19	0.18	0.04	0.47	0.00
TN	-0.11	-0.32	0.51	-0.12	-0.05	-0.07	0.02
TR	0.20	-0.01	0.20	0.13	-0.41	-0.07	0.37
CD	0.34	-0.17	-0.02	-0.05	0.12	0.07	-0.04
CT	0.20	-0.24	-0.25	-0.25	0.12	-0.40	-0.08
SM	0.30	-0.27	-0.06	-0.15	0.07	-0.17	-0.07
BS	0.20	0.24	-0.22	0.24	-0.36	-0.07	0.24
M	0.31	-0.11	-0.16	0.01	-0.16	-0.20	0.02
IBW	0.28	-0.05	0.05	0.01	-0.14	0.06	0.01
CLSW	0.18	0.11	0.10	0.04	-0.09	0.14	-0.52
DFE	0.02	0.15	0.12	0.02	-0.39	-0.18	-0.63
PN	-0.14	-0.33	0.46	-0.12	-0.02	-0.04	0.06
PL	0.07	0.19	0.17	-0.19	0.20	-0.04	0.08
GN	0.00	-0.14	0.16	0.51	0.03	-0.34	0.01
PW	0.07	0.13	0.24	-0.27	-0.37	-0.21	0.09
TW	0.05	0.19	-0.03	-0.61	-0.20	0.20	0.14
GY	0.10	0.04	0.04	0.07	0.06	-0.09	0.28

PH-Plant height, IL-Internode length, CL-Culm length, ODMa-Outer diameter of major axis, ODMi-Outer diameter of minor axis, IDMa-Inner diameter of Major axis, IDMi- Inner diameter of minor axis, TN-Tiller number, PR-Pushing resistance, CD- Culm diameter, CT-Culm thickness, SM- Section modulus, BS-Bending stress, M-Bending moment at breaking, IBW- Internode breaking weight, CLSW- Culm with leaf sheath weight, DFF-Days to 50% flowering, PN-Panicle number, PL-Panicle length, GN-No of filled grains per panicle, PW- Panicle weight, TW-Test weight, GY-grain yield.