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Genetic Variability and Association Studies for Morpho-floral Traits in Backcross Introgression Lines of Rice (*Oryza sativa* L.)

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ABSTRACT

Background: Wild introgressions are pivotal in enriching the genetic diversity of cultivated varieties by introducing novel alleles beneficial for crop improvement. Understanding insights into genetic variability and association studies of morphological and floral traits are crucial for enhancing hybrid seed production in rice.

Methods: 290 backcross introgression lines (BC_4F_2) from inter-specific cross of CRMS 32B (maintainer of male sterile line as low stigma exsertion recipient parent) cv. *Oryza sativa* with *Oryza longistaminata* (wild rice as high stigma exsertion donor parent) were assessed for 12 morpho-floral traits. The experiment was laid out in randomized complete block design (RCBD) at Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, during *Kharif*, 2019.

Result: ANOVA results showed significant differences among lines for all examined traits. The phenotypic coefficient of variation (PCV) was slightly greater than genotypic coefficient of variation (GCV) for each trait. High PCV, GCV, heritability and genetic advance as percentage of mean were observed for dual stigma exsertion percentage (DSE%) and total stigma exsertion percentage (TSE%), whereas moderate to high values of these parameters were noted for single stigma exsertion percentage (SSE%), grain yield per plant (GYPP), effective tillers per plant (ETPP) and grains per panicle (GPP) traits. Most traits showed positive skewness, except traits SFP (spikelet fertility percentage), SSE% and TSE%. Most traits recorded leptokurtic kurtosis, whereas plant height (PH), GYPP and TSE% exhibited platykurtic kurtosis. TSE% displayed a highly significant positive association with SSE% and DSE%, while GYPP showed a significant positive correlation with ETPP and GPP, presenting a promising opportunity for simultaneous selection of these traits.

Key words: Backcross introgression lines, Genetic advance, Genetic variability, Heritability, Total stigma exsertion percentage.

INTRODUCTION

Rice (*Oryza sativa* L.) stands as the primary cereal grain consumed by over half of the global population, playing a crucial role in ensuring food security. The global population is projected to surpass nine billion, raising concerns about the potential emergence of significant food insecurity worldwide by 2050 (Alexandratos and Bruinsma, 2012). To meet the increasing demands of the global population, doubling rice production by 2050 is imperative. However, production rates of released rice varieties have stagnated due to limited genetic diversity in breeding programs. In this context, hybrid rice has shown potential in increasing yields by up to 20% but its wide spread adoption faces challenges such as high seed costs due to low seed yield, annual seed replacement and reliance on external seed sources. Effective approach to increase hybrid seed yield and minimize seed production costs is to develop male sterile lines with a high outcrossing rate. The degree of outcrossing is influenced by various flowering behavior and morphological traits of both female and male parents (Marathi and Jena, 2015). Among these traits, stigma exsertion is highlighted as a crucial factor for enhancing pollination and seed set. Wild rice species, with their higher outcrossing rates ranging from 3.2% to 70.0%, serve as reservoirs of genetic diversity, offering adaptability and novel alleles in crop improvement programs (Prahalada *et al.*, 2021). Backcross inbred lines (BILs), derived from crossing between cultivars and wild species, are valuable tools for breeding research, aiding in pinpointing QTLs or genes associated with yield improvement (Malathi *et al.*, 2017), as well as in breeding male sterile lines with increased outcrossing potential.

Advancement in crop breeding for yield and associated traits are influenced by polygenic control, environmental factors and genetic variability. Evaluating variability estimates for morpho-floral traits is essential for designing

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effective breeding strategies for hybrid seed production. This involves heritable and non-heritable elements through parameters like GCV, PCV, broad-sense heritability and genetic advance. Additionally, skewness and kurtosis provide insights into gene action and trait distribution patterns. Correlation studies are valuable in assessing interrelationships among traits, aiding in selection. Considering the perspectives, the present research was performed to estimate the genetic variability parameters, descriptive statistics and to unravel the degree of association between morpho-floral traits in backcross introgression lines.

MATERIALS AND METHODS

Experimental set up and plant material

The research experiment was executed at the research farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during the *Kharif* season of 2019. The experimental field was located at 25.18° N latitude to 83.03° E longitude in the Northern Eastern Plain Zone (NEPZ). The experimental material comprised of 290 backcross introgression lines (BC_4F_2) from inter-specific cross of CRMS 32B cv. *Oryza sativa* (maintainer of male sterile line; as low stigma exsertion recipient parent) with *Oryza longistaminata* (wild rice; as high stigma exsertion donor parent). The recurrent backcrosses were made with CRMS 32B to get BC_4F_1 and advance to BC_4F_2 generation. The BC_4F_2 introgression lines along with parents were evaluated in RCBD with three replications. Each of the experimental unit comprised an area of 3 m^2. Seedlings of twenty-five days old were transplanted into each puddled experimental unit with a spacing of 20×15 cm. The standard package of practices was followed during the cropping period to ensure the good crop stand.

Phenotypic measurements

The data were recorded for twelve morpho-floral traits from five randomly chosen plants within each line in every replication following standard evaluation system (SES) for rice (IRRI, 2013) under field conditions. Fig 1 depicts different types of stigma exsertion in the spikelets.

Statistical analysis

The analysis of variance was done by Microsoft Excel 2007 (Microsoft Crop., Redmond, WA, USA) to access the significance of differences among treatments on mean data. Estimates of PCV and GCV were calculated by Burton (1952) approach. Coefficients of variations were categorized following the classification outlined by Sivasubramanian and Madhavamenon (1973). Broad sense heritability estimates were computed by methods of Lush (1940) and Allard (1960). While the genetic advance and classification of heritability and GA% was determined as per Johnson *et al.* (1955) procedure. The third-degree statistics, skewness and fourth-degree statistics, kurtosis was calculated in accordance with Snedecor and Cochran (1980) to comprehend the distribution nature of BC_4F_2 introgression lines concerning morpho-floral traits. Descriptive statistics, frequency distribution and Pearson's correlation coefficient analyses were performed using XLSTAT (2014) software.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance revealed that all the examined traits indicating presence of highly significant ($P \leq 0.01$) variability in the BC_4F_2 introgression lines including recurrent parent for all examined traits (Table 1). This variability is critical for the success of selection in breeding programs aimed at enhancing hybrid seed production, particularly in the context of improving stigma exsertion traits. Priyanka *et al.* (2017); Bhattacharjee *et al.* (2020) and Hasan *et al.* (2022) also observed significant genetic variations for different yield and yield-related traits in their rice research.

Genetic variability parameters

The mean, range, variability estimates including GCV, PCV, heritability, genetic advance and descriptive statistics like standard deviation, skewness and kurtosis are provided in Table 2. A broad range of variations was found in the analysis of different traits, specifically for GPP, which ranged from 108.00 to 248.00, with the mean of 166.0 while a narrow

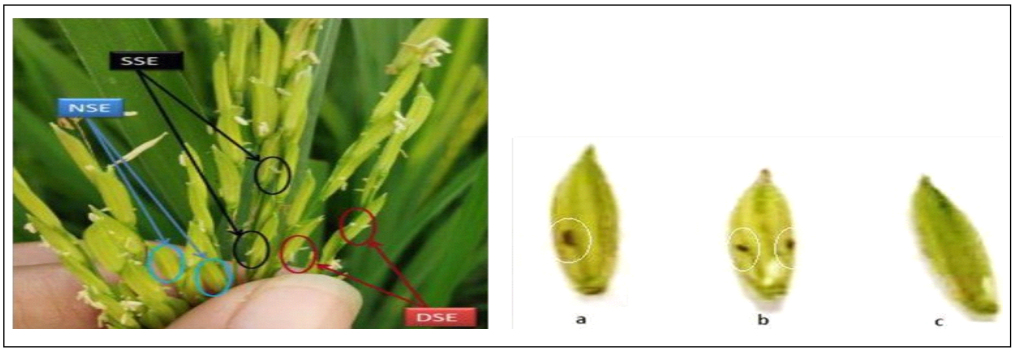


Fig 1: Spikelets depicting A: SSE (Single stigma exsertion), B: DSE (Dual stigma exsertion), C: NSE (No stigma exsertion).

range was noted for PL, varying from 21.4 to 28.2 with the mean of 24.5. The range of variability in morpho-floral traits within the BC_4F_2 introgression lines is clearly illustrated by the box plot in Fig 2.

Genotypic and phenotypic coefficient of variation

The GCV exhibited lower values compared to the PCV for all studied traits suggesting that the observed variations were controlled by both genetic and environmental factors in shaping trait expression, though genetic factors are the primary drivers. Similar results were obtained by Chandramohan *et al.* (2016). Among the traits examined, effective tillers per plant showed a larger discrepancy between PCV and GCV compared to other traits, suggesting that the environment has a greater impact on this trait. These findings are consistent with the results reported by Ratnam *et al*. (2024). The PCV values ranged from 2.40% to 42.17%, whereas GCV ranged from 1.97% to 34.95%. High PCV and GCV estimates were recorded for DSE% and TSE% indicate significant variability within the population, suggesting that simple selection methods can effectively enhance these traits, as supported by El-Namaky, (2018) and Hasan *et al.* (2018). High PCV and moderate GCV were found for ETPP, GYPP and SSE% suggesting potential for improvement through selection in subsequent generations (Govintharaj *et al.*, 2016). Moderate PCV and GCV were noted for GPP, while low PCV and GCV were observed for DF, DM, PH, PL, SFP and TW indicate limited variability, making selection less effective for these traits. These findings align with earlier studies by Hossain *et al.* (2015) and Raghavendra and Hittalmani (2015).

Heritability and genetic advance

Heritability serves as a reliable indicator for the transmission of traits from parents to their offspring. High heritability estimates combined with high GA% of mean provides a more accurate prediction of genetic gain under selection compared to heritability estimates alone. All studied traits

Table 1: Analysis of variance for morpho-floral traits in backcross introgression (BC_4F_2) lines with recurrent parent.

Traits	Mean sum of squares		
	Replication (d.f.=2)	Treatment (d.f.=290)	Error (d.f.=580)
Days to 50% flowering (DF)	4.49	20.64**	2.80
Days to maturity (DM)	3.86	21.96**	3.01
Plant height (PH) (cm)	22.83	52.39**	14.06
Effective tillers per plant (ETPP) (no.)	0.31	5.19**	1.86
Panicle length (PL) (cm)	2.72	4.16**	1.74
Grains per panicle (GPP) (no.)	688.20	1500.38**	431.56
Spikelet fertility % (SFP)	26.00	51.80**	17.09
Grain yield per plant (GYPP)(g)	3.57	24.10**	6.50
Test weight (TW) (g)	1.35	4.59**	0.65
Single stigma exsertion % (SSE%)	35.86	127.27**	15.59
Dual stigma exsertion % (DSE%)	4.56	48.18**	6.36
Total stigma exsertion % (TSE%)	61.78	251.88**	26.46

**Significant at 1% levels.

Table 2: Genetic variability parameters and descriptive statistics for morpho-floral traits in backcross introgression (BC_4F_2) lines with parents.

Traits	Mean			Range		PCV	GCV	$h^2_{(b.s.)}$	GA% of	Skew-	Kurtosis
	P1	P2	BC_4F_2	Min	Max	(%)	(%)	(%)	mean	ness	
DF	98	140	102	96	111	2.9	2.4	68.0	4.1	0.6	1.1
DM	30	168	128	122	143	2.4	2.0	67.7	3.3	1.4	5.3
PH	92.2	210.0	90.4	79.1	104.2	5.7	4.0	47.6	5.6	0.2	-0.1
ETPP	7	15	7	4	11	23.9	14.6	37.4	18.4	0.4	0.1
PL	26.0	38.0	24.5	21.4	28.2	6.5	3.7	31.5	4.2	0.2	0.1
GPP	95	60	166	108	248	16.9	11.3	45.2	15.7	0.3	0.2
SFP	82.1	33.3	82	67.6	91.8	6.5	4.2	40.4	5.4	-0.4	0.4
GYPP	16.7	10.0	13.4	8.4	22.2	26.3	18.1	47.5	25.7	0.5	-0.3
TW	19.5	15.2	18.5	13.0	24.4	7.6	6.2	66.8	10.4	0.5	3.6
SSE%	27.1	10.5	30.9	10.5	47.6	23.5	19.8	70.5	34.2	-0.5	0.1
DSE%	7.1	85.0	10.7	3.7	25.5	42.2	35.0	68.7	59.6	0.6	0.0
TSE%	34.2	95.5	41.6	14.6	64.1	24.3	20.9	74.0	37.0	-0.2	-0.2

P1-CRMS 32B cv. *Oryza sativa*; P2- *Oryza longistaminata.*

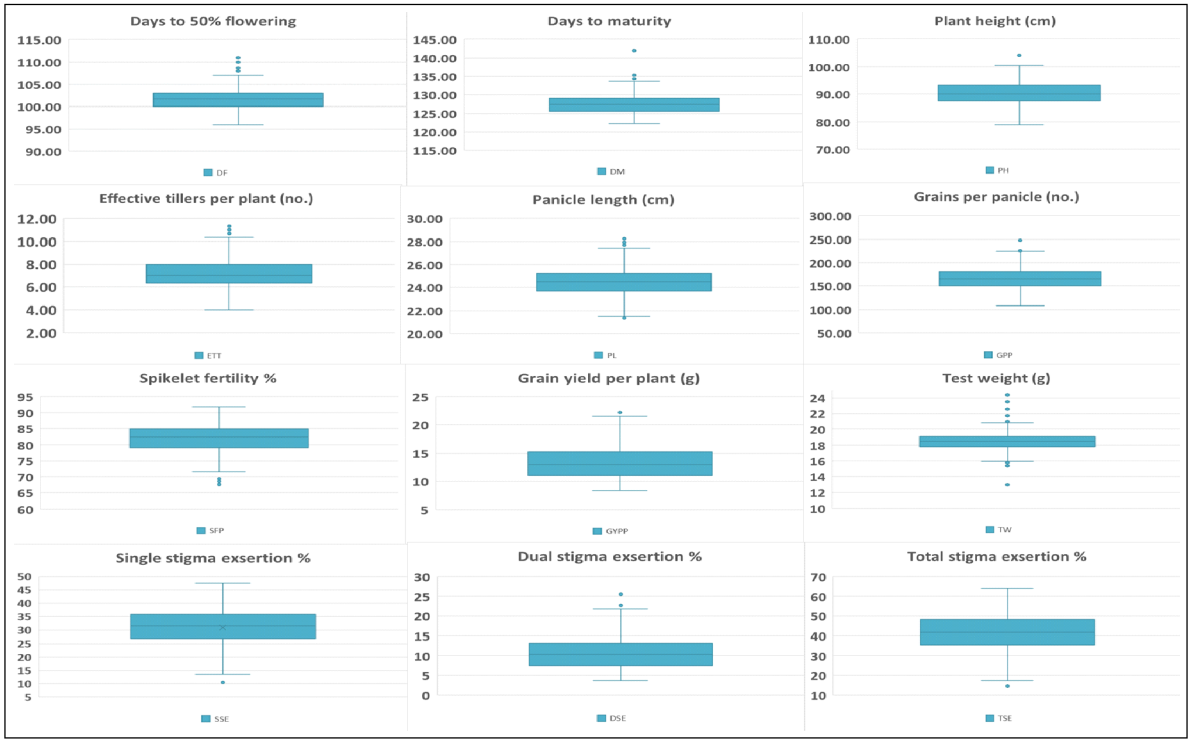


Fig 2: Box plot depicting the range of variability in morpho-floral traits within backcross introgression (BC_4F_2) lines.

displayed moderate to high heritability values, ranging from 31.50 to 73.96%. The GA% of mean ranged from 3.34 to 59.63%. The high heritability estimates, combined with high genetic advance, were recorded for TSE%, DSE% and SSE% suggests that these traits are primarily influenced by additive gene effects and can be efficiently improved through simple selection methods. These results corroborate earlier findings by Yan *et al.* (2009) and Priyanka *et al.* (2017), who emphasized the importance of high heritability coupled with high genetic advance in breeding for enhanced outcrossing traits in rice. Moderate heritability with high genetic advance was observed for GYPP (Vaibhav *et al.*, 2019). The high heritability with a moderate genetic advance noticed for TW, indicates both additive and non-additive gene actions influence this trait thus selection can be advanced to later generations for desirable improvements (Prathiksha *et al.*, 2022). On the other hand, DF and DM showed high heritability accompanied by low genetic advance indicating non-additive gene action and significant environmental impact, which limits the effectiveness of selection (Rambabu *et al.*, 2022). Moderate heritability with moderate genetic advance was noted for ETPP and GPP as also reported by Govintharaj *et al.* (2018). Moderate heritability coupled with low genetic advance was obtained for PH, PL and SFP suggests a substantial environmental influence, thus selection for these traits may prove ineffective (Renuprasath *et al.*, 2023).

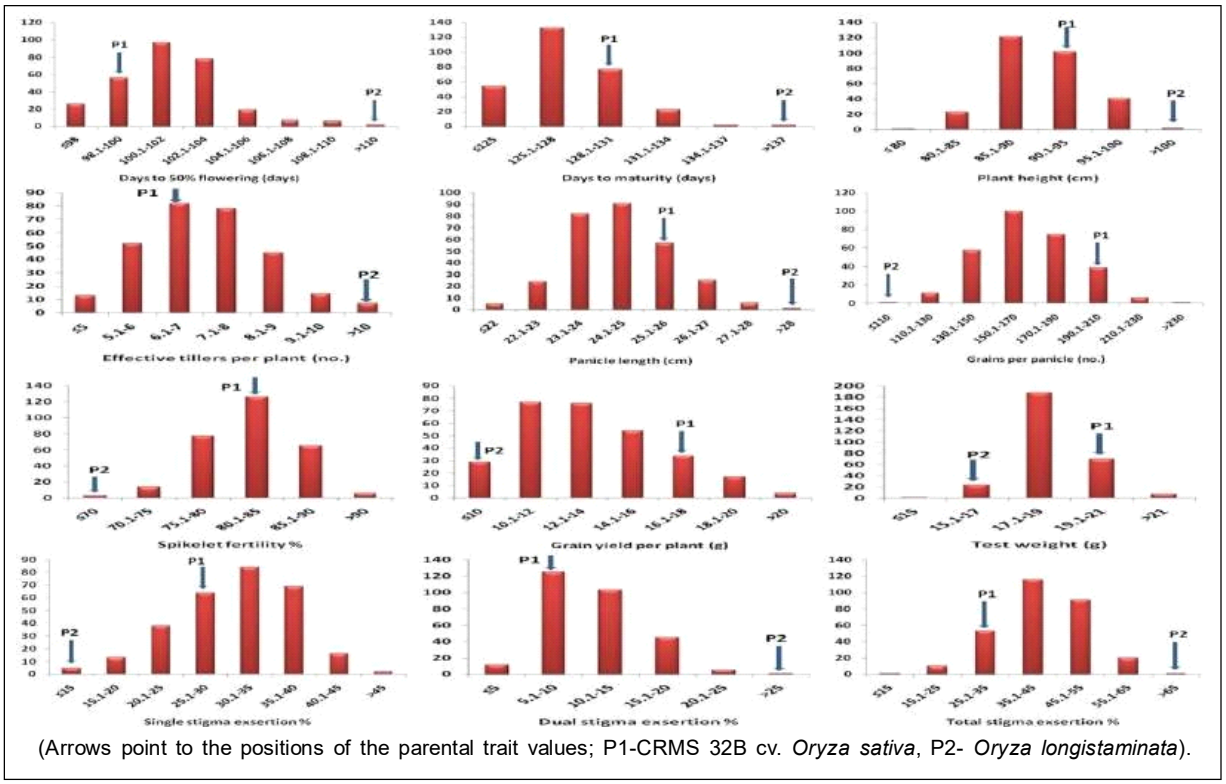
Skewness and kurtosis

The analysis of skewness and kurtosis provides further insights into the genetic architecture of the studied traits. The skewness and kurtosis values for each character are provided in Table 2 with the corresponding frequency distribution graphs in Fig 3. Most traits exhibited positive skewness and leptokurtic distribution, suggesting influence by a small number of genes with complementary gene interactions. Thus, intensive selection from existing variability is necessary for rapid genetic advancement in these traits. Conversely, traits SFP, SSE% and TSE% showed negative skewness, indicating presence of duplicate gene actions (additive × additive) thus mild selection could lead to swift genetic enhancement in these traits. These findings align with those of Beerelli *et al.* (2022), who noted similar trait distributions in F_2 population of wild introgression lines of rice. Additionally, platykurtic distribution observed for traits like PH, GYPP and TSE%, suggests that these traits are influenced by a large number of genes, which could complicate selection efforts.

Correlation coefficient

The phenotypic correlations between 12 analyzed traits were calculated using Pearson's correlation coefficient and are presented in Table 3. In Fig 4, a visual representation is provided for correlation analysis concerning morpho-floral traits within BC_4F_2 lines. Most traits showed correlations with each other, except for TW, which showed no correlation with

any other trait (Luu *et al.*, 2022). TSE% displayed a highly significant positive correlation with SSE% (0.924**) and DSE% (0.784**) suggesting that lines with higher SSE% values are more likely to show increased DSE% values, consequently resulting in an increase in TSE%. This relationship is crucial for developing male sterile lines with improved outcrossing potential, as higher stigma exsertion rates facilitate better pollen capture and thus greater seed set. These associations align with the results documented by Rahman *et al.* (2016) and Zhou *et al.* (2017). Additionally, TSE% had a significant positive association with DF, but a significant negative correlation with PH. Variations in remaining traits were independent of the expression of stigma exsertion. Similarly, SSE% revealed a significant positive correlation with DSE% (0.488**), DF (0.187**) and DM (0.137*) while significant negative correlation with PH (-0.146*). GYPP demonstrated positive and significant correlation with GPP (0.413**), PL (0.442**), ETPP (0.704**)



(Arrows point to the positions of the parental trait values; P1-CRMS 32B cv. *Oryza sativa*, P2- *Oryza longistaminata*).

Fig 3: Frequency distribution of morpho-floral traits in backcross introgression (BC_4F_2) lines.

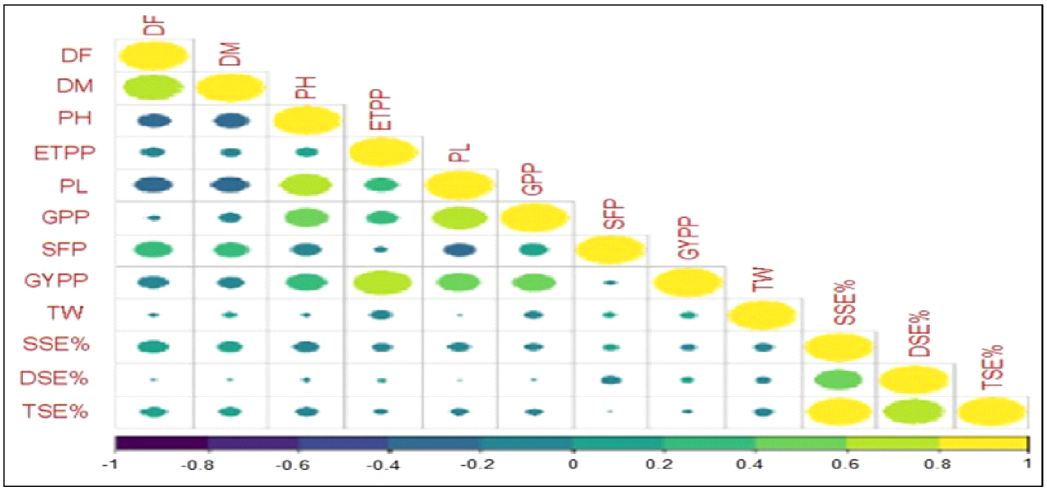


Fig 4: Visual depiction of phenotypic correlation analysis for morpho-floral traits in backcross introgression (BC_4F_2) lines.

Table 3: Phenotypic correlation coefficient estimates among different pairs of morpho-floral traits in backcross introgression (BC_4F_2) lines.

Traits	DM	PH	ETPP	PL	GPP	SFP	GYPP	TW	SSE%	DSE%	TSE%
DF	0.745**	-0.222**	-0.110	-0.300**	-0.035	0.309**	-0.181**	-0.026	0.187**	-0.014	0.126*
DM		-0.258**	-0.096	-0.308**	-0.101	0.259**	-0.176**	0.043	0.137*	0.011	0.102
PH			0.115	0.607**	0.445**	-0.189**	0.370**	-0.025	-0.146*	-0.029	-0.116*
ETPP				0.237**	0.214**	-0.047	0.704**	-0.102	-0.085	0.022	-0.051
PL					0.621**	-0.215**	0.442**	0.007	-0.109	0.005	-0.076
GPP						0.199**	0.413**	-0.072	-0.071	-0.014	-0.056
SFP							-0.040	0.049	0.067	-0.099	0.004
GYPP								0.057	-0.064	0.044	-0.026
TW									-0.085	-0.063	-0.088
SSE%										0.488**	0.924**
DSE%											0.784**

**and* indicate correlation are significant at 1% (99% probability) and 5% (95% probability) levels, respectively.

and PH (0.370**) suggests that selection for these yield-related traits could indirectly improve grain yield in rice. While concurrently GYPP exhibited negative and significant correlation with DF (-0.181**) and DM (-0.176**) suggests that earlier flowering and maturity could be advantageous for yield improvement. This is supported by the work of Devi *et al.* (2017); Karthika *et al.* (2017) and Faysal *et al.* (2022). Inter-correlations among studied traits further revealed associations, such as SFP positively correlated with DF, DM and GPP, but negatively correlated with PH and PL (Sabri *et al.*, 2020). Moreover, positive associations were observed between GPP and PH, ETPP and PL, while PL positively correlated with PH and ETPP but negatively with DF and DM (Mohan *et al.*, 2023). Conversely, PH exhibited negative associations with DF and DM, while DM and DF demonstrated highly significant positive inter-correlations. These results suggested that traits showing positive and significant correlations among them could potentially undergo simultaneous improvement through selection.

CONCLUSION

Backcross breeding is a powerful technique that restores recurrent parent genomes while introducing genetic diversity through gene introgression, ultimately enhancing trait improvement. A comprehensive understanding of genetic variability in morphological and floral traits is crucial for enhancing hybrid seed production in rice. The present study revealed significant genetic variability across various morpho-floral traits, with moderate to high values of PCV, GCV, heritability and GA% of mean observed for traits such as TSE%, DSE%, SSE%, GYPP, ETPP and GPP, indicating the influence of both additive and non-additive genetic effects on these traits. Furthermore, these traits exhibited a skewed leptokurtic and platykurtic distribution, suggesting their complex genetic basis influenced by few to numerous genes with both duplicate and complementary gene actions. A highly significant positive correlation was noted between SSE%, DSE% and TSE% while GYPP exhibited a positive and significant correlation with ETPP and GPP. Thus, simultaneous selection for these traits would prove highly effective in advancing breeding programs and presents a promising avenue for boosting hybrid seed production in rice.

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Conflict of interest

The authors confirm that there are no conflicts of interest to declare.

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