RESEARCH ARTICLE

Genetic insights and principal component analysis of inter-subspecific derivatives in rice (*Oryza sativa* L.)

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Abstract

Rice is a staple crop in Southeast Asia and increasing its productivity through hybrid breeding is essential to meet the growing population demand. The development of new restorers is a key goal in hybrid rice breeding to increase heterosis. Studying newly developed breeding lines and their trait associations is crucial for their effective use in future breeding activities. The newly developed lines, derived from *indica* and tropical *japonica* crosses, were evaluated for eight quantitative traits across four locations to study genetic variation and trait associations. Single plant yield (20.92) had the highest genotypic coefficient of variation (CVg), while total spikelets per panicle (17.71), productive tillers (15.35) and plant height (13.31), recorded moderate CVg. Further, all traits except spikelet fertility, exhibited high broad-sense heritability spikelet fertility. The first three principal components (PCs), with eigenvalues greater than one, explained 67 % of the total variation. The biplot of the first two PCs, accounting for 53 % of the variation, showed significant divergence among genotypes, with plant height contributing most, followed by single plant yield and spikelet fertility. Spikelet fertility (0.44) and the total number of spikelets per panicle (0.25) showed a strong correlation with single plant yield in the combined analysis and across locations, making them promising traits for selection. Thus, greater emphasis must be placed on spikelet fertility and the number of spikelets per panicle when selecting inter-subspecific cross derivatives. The newly developed inter-subspecific breeding lines in the present study showed significant variation in yield and other traits, making them valuable resources for heterosis breeding.

Keywords: rice; *indica*; tropical *japonica*; PCA; heritability

Introduction

Rice is the world's most essential food crop, which feeds more than 3.5 billion people and contributes to 20 % of their daily caloric intake (1). Over the past decade, rice production and consumption have increased worldwide and the crop serves as a main food source for developing countries in Southeast Asia (2). The effect of growing population has made it necessary to double the production by 2050 to feed a global projection of more than nine billion people (3). Climate change and water availability have limited the expansion of arable land, thus improved rice hybrids and cultivars are needed to meet the increased demand (4).

Harnessing heterosis is a crucial strategy for improving rice crop yields (5, 6). Significant hybrid vigour was observed in rice hybrids resulting in a 20 % increase in grain yield over that of their parental lines (7). To exploit heterosis fully, diversifying *indica* restorers through tropical *japonica* germplasm was proposed, as *indica*/*indica* hybrids exhibit lower heterosis

compared to *indica*/tropical *japonica* crosses (8). However, *indica*/tropical *japonica* cross derivatives need to be assessed for genetic variation of traits and spikelet fertility for their effective utilization in breeding programs (9, 10).

The availability of genetic variation within a population is essential for its improvement and for making decisions about its utilization in crop breeding (11). The presence of morpho-genetic variation in the agronomic characteristics and understanding the genetic variation of newly developed inter-subspecific derivatives is crucial for their deployment in hybrid development (12, 13). Previous reports have suggested that the presence of genetic variability in advanced breeding lines plays a significant role and provides an opportunity in selecting superior lines (14, 15). Additionally, measuring heritability and genetic advance is critical for the effectiveness of selection and transmission of quantitative traits in breeding programs (16). In inter-subspecific cross derivatives, studying the major traits contributing to yield is crucial, as traits like spikelet fertility,

panicle length, productive tillers and number of spikelets per panicle play key roles (17). Identifying the traits that directly affect yield in each breeding population will help breeders to develop effective selection strategies (18, 19).

Principal Component Analysis (PCA) is the most useful statistical tool for estimating and visualizing the genetic variance of a population/breeding material and allows us to understand the differences and similarities among individuals (20). This approach also aids in distinguishing the relationships between quantitative traits and between traits and genotypes (21).

In the current experiment, 76 advanced breeding lines derived from crosses between *indica* and tropical *japonica* lines were evaluated, along with checks and landraces across four different environments, to assess their genetic variability for agronomic traits *via* principal component analysis (PCA) and correlations among traits. The goal was to understand the genetic structure of various traits in the population, for utilization in hybrid breeding.

Material and Methods

Experimental description

The experimental material for the present study consisted of eighty-eight rice lines, which included 76 newly developed breeding lines derived from *indica*/tropical *japonica* crosses obtained from the parental diversification program of the corresponding author at the Paddy Breeding Station, TNAU, Coimbatore, five commercial checks, two commercial restorers and five landraces collected from the Department of Plant Genetic Resources, TNAU, Coimbatore. The study was conducted at four different locations in Tamil Nadu: Coimbatore (CBE), Gudalur (GUD), Aduthurai (ADT) and Madurai (MDU). An alpha lattice design (11 × 8) with three replications was employed for evaluation. The recommended agronomic practices were followed in all environments to obtain a normal crop stand (22).

Data collection

Data on eight quantitative traits were measured in all the rice lines across the environments in each replication. The studied characters included, days to fifty percent flowering (DFF), plant height (PH in cm), number of productive tillers (NPT), panicle length (PL in cm), total number of spikelets per panicle (TNG), spikelet fertility (SF in %), hundred seed weight (HSW in grams) and single plant yield (SPY in grams). The traits were recorded as per rice standard evaluation system (23).

Statistical analysis

The genetic parameters and genotypic values were calculated *via* the restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP) methods (24), with the following model;

$$y = Xb + Zg + Wc + \varepsilon$$

where, y is the responsible variable vector; b is the vector of fixed effect coefficients; g is the genotype effect (considered random); c is the genotype-by-environment interaction effect (considered random); and ε is the random error. X, Z and W, are design matrices of 0s and 1s relating y to b, g and c respectively. The genetic parameters were estimated by considering both genotype and genotype-environment interaction effects as random, following the above model. The REML model and genetic parameters were evaluated using the 'metan' package of R studio (25).

The associations among the traits were assessed using the Karl Pearson correlation coefficient and the correlogram was plotted using the 'GGally' package in R Studio. Principal component analysis (PCA) was performed with the 'FactoMineR' package in R Studio. The scree plot, contribution plot and biplot of PC1 *vs.* PC2 were created using the 'factoextra' package to visualize the results.

Results and Discussion

Mean performance

This study assessed the genetic variation and associations of eight quantitative traits of newly developed inter-subspecific breeding lines at four locations. The combined analysis of genotypes across environments was conducted using a mixed model. The mixed model analysis is preferred over conventional ANOVA because it resolves issues related to the assumption of homogeneous error variance and normal distribution, which are often disrupted by high genotype-environment interaction (GEI) (26). The mixed model analysis, which considers genotype and the GEI as random effects and the environment as a fixed effect, revealed that all genotypes, the GEI and the environment were significant for all the studied quantitative traits (Table 1, 2). This suggests that the population used in this study exhibited a wide genetic variation for all traits, highlighting the importance of the environment in determining trait effects (27). The broader variation and the environmental influence on genotypes could be leveraged to improve adaptability of lines through selection (28).

The mean, maximum and minimum values of each

Table 1. Likelihood ratio analysis of quantitative traits in rice inter- subspecific derivatives using REML mixed model for random effects

Source	DFF	PH	NPT	PL	TNG	SF	HSW	SPY
Genotype	105.97**	232.70**	68.48**	107.93**	70.57**	9.87**	52.40**	55.37**
Block	4.70	4.23	9.63	0.01	0.95	0.00	2.19	0.00
Genotype × Environment	399.07**	326.13**	705.40**	263.75**	1073.09**	346.83**	775.61**	1123.80**

** Significant of likelihood ratio test by the Chi-square test at 1%.

DFF - Days to fifty per cent flowering, PH - Plant height, NPT - Number of productive tillers, PL - Panicle length, TNG - Total number of spikelets per panicle, HSW - Hundred seed weight and SPY - Single plant yield

Table 2. Mean sum of squares analysis of quantitative traits using REML mixed model for fixed effects

Source	*df*	DFF	PH	NPT	PL	TNG	SF	HSW	SPY
Environment	3	769.25**	2888.04**	24.30**	54.46**	293.93**	378.15**	0.07**	47.95**
Replication	8	17.82	39.09	1.26	3.05	110.28	23.43	0.01	6.88

** - Significant at 1 % level of probability, *df* – degree of freedom.

DFF - Days to fifty per cent flowering, PH - Plant height, NPT - Number of productive tillers, PL - Panicle length, TNG - Total number of spikelets per panicle, HSW - Hundred seed weight and SPY - Single plant yield

trait are presented in Table 3. The mean DFF among the genotypes was 97 days, ranging from 84 days (G-86) to 117 days (G-36). Among the environments, the minimum DFF was recorded at ADT, whereas the maximum was observed at GUD. The mean PH of the studied lines was 107.12 cm, ranging from 76.62 cm (G-61) to 142.49 cm (G-72). The NPT varied from 3.77 (G-80) to 16.58 (G-58), with a mean value of 12.67, whereas the panicle length (PL) exhibited a maximum of 28.54 cm (G-38) and a minimum of 20.52 cm (G-86), with a mean of 24.21 cm. Among the environments, ADT had the highest values for PH, NPT and PL, while the minimum PH and NPT were observed at GUD and the minimum PL was recorded at CBE. The TNG ranged from 77.25 (G-55) to 208.85 (G-39) and the SF varied from 76.12 % (G-24) to 93.83 % (G-15), with mean values of 118.68 and 83.38 %, respectively. Both traits had their fullest expression at CBE where the other extreme was at MDU among the four environments. The average SPY was 22.07 g, with a maximum of 43.51 g (G-68) and a minimum of 11.72 g (G-24), while the HSW ranged from 1.45 g (G-13) to 2.45 g (G-76), with a mean of 1.97 g. Both traits expressed minimum mean values at MDU, whereas the SPY and HSW were highest at CBE and GUD, respectively. These results implied that CBE provided a favourable environment with the highest mean value for SPY and major yield contributors such as TNG and SF. In contrast, MDU was a poor location, with lower mean values for yield and its attributes.

Genetic parameters

The heritability and variance components for all traits across locations, presented in Fig. 1, were estimated *via* REML. In the combined analysis of all environments, the residual coefficient of variance (CVr) was <10 for all traits studied, indicating high precision in the experimental values, which is consistent with previous findings (29). A high genotypic coefficient of variation (CVg) reflects greater genetic variance within the population. In this study, SPY presented a high CVg, whereas NPT, PH and TNG displayed moderate CVg values, indicating that the observed variance is primarily

genetic and can be leveraged to improve these traits through selection (30). It was suggested that moderate to high genetic variability, particularly in key traits like grain yield (10), in newly developed lines, indicates significant genetic diversity, making them an important resource for exploitation in heterosis breeding (31). The heritability analysis across all environmental data revealed high values for PH and moderate values for SPY, TNG, PL, NPT and DFF, suggesting that these traits can be tapped through selection. The wide genetic variation and moderate heritability observed in the combined analysis indicated that grain yield and major yield attributes, such as NPT and TNG, have the potential to be improved in the current population (31). A high magnitude of genotypic coefficient of variation and heritability for yield and moderate variation with high heritability for plant height, productive tillers and grains per panicle was reported in a previous study (32), like the findings of the present study. In contrast, it was observed high CVg and heritability for most traits in newly developed rice restorers (33). The moderate CVg and heritability observed in this study could be due to the influence of environmental and GEI interactions, as the combined data analysis indicated that the environment plays a significant role in determining traits, as evidenced by the significant environmental and GEI effects (34). Therefore, the observed variation could potentially be utilised through the implementation of novel statistical tools for effective selection strategies, minimising environmental influences.

Association analysis

Grain yield in rice is a complex trait influenced by multiple yield-related factors and is greatly affected by environmental conditions (19). Therefore, identifying the key traits that are strongly correlated with grain yield is essential for improving selection strategies aimed at enhancing yield (21). The correlation results for each environment and the combined data are presented in Fig. 2. The traits SF and TNG were significantly correlated with SPY in both the combined and individual environments, indicating their key role in

Table 3. Descriptive statistics of different quantitative traits of rice inter- subspecific breeding lines studied at four environments

Parameters	DFF	PH	NPT	PL	TNG	SF	HSW	SPY
Mean	97.66	107.12	12.67	24.21	118.68	83.38	1.97	22.07
SE	0.31	0.6	0.1	0.09	1.03	0.25	0.01	0.24
SD	10.16	19.54	3.33	2.8	33.38	7.97	0.26	7.93
Min. (Environment)	ADT (93.53)	GUD (93.05)	GUD (11.65)	CBE (23.01)	MDU (112.96)	MDU (79.45)	MDU (1.9)	MDU (19.7)
Max. (Environment)	GUD (104.04)	ADT (117.65)	ADT (14.32)	ADT (25.27)	CBE (124.27)	CBE (86.47)	GUD (2.05)	CBE (24.49)
Min. (Genotype)	G-86 (84.75)	G-61 (79.62)	G-80 (3.77)	G-86 (20.52)	G-55 (77.25)	G-24 (76.12)	G-13 (1.45)	G-24 (11.72)
Max. (Genotype)	G-36 (117.00)	G-72 (142.49)	G-58 (16.58)	G-38 (28.54)	G-39 (208.85)	G-15 (93.83)	G-76 (2.45)	G-68 (43.51)

DFF - Days to fifty per cent flowering, PH - Plant height (cm), NPT - Number of productive tillers (no.), PL - Panicle length (cm), TNG - Total number of spikelets per panicle, HSW - Hundred seed weight (g), SPY - Single plant yield (g), ADT - Aduthurai, CBE - Coimbatore, GUD - Gudalur, MDU - Madurai
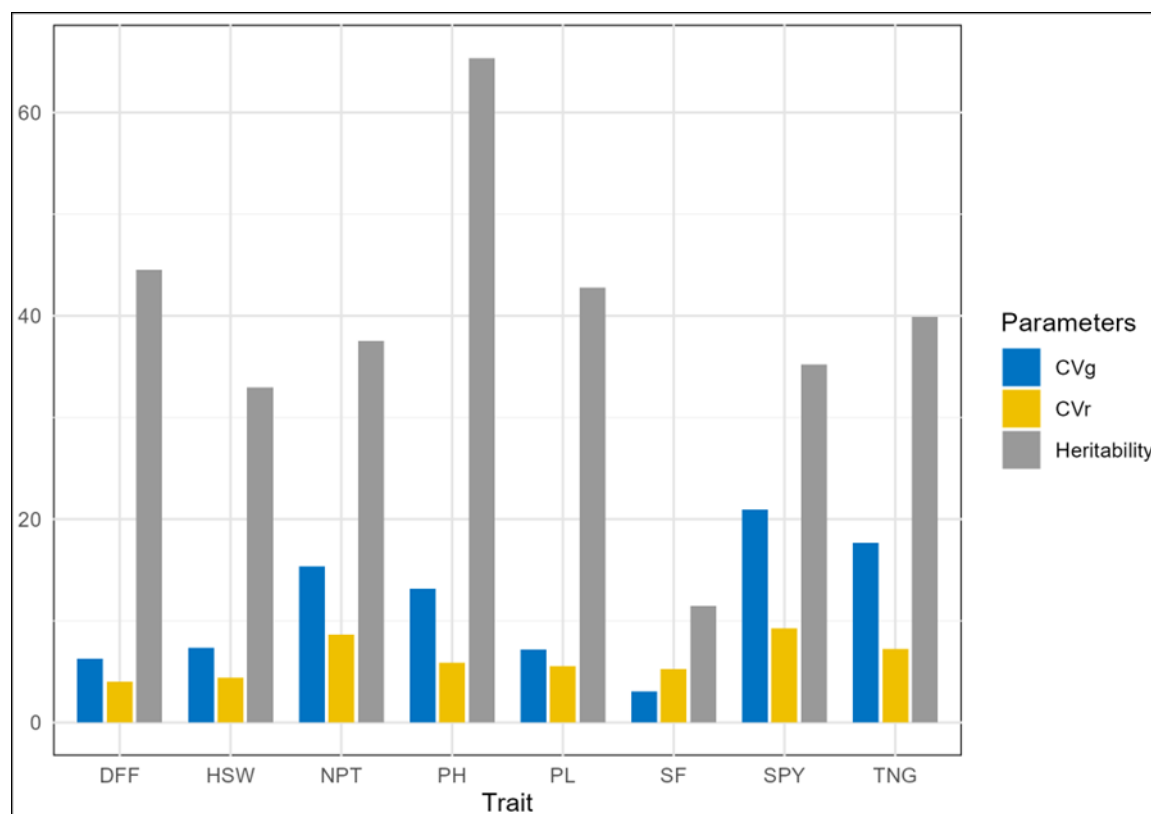
Fig. 1. Estimates of heritability, genotypic coefficient of variation (CVg) and residual coefficient of variation (CVr) for different quantitative traits in rice inter-subspecific breeding lines studied at four locations. DFF - Days to fifty per cent flowering, PH - Plant height (cm), NPT - Number of productive tillers (no.), PL - Panicle length (cm), TNG - Total number of spikelets per panicle, HSW - Hundred seed weight (g) and SPY - Single plant yield.

determining grain yield in the population. Earlier studies highlighted the strong correlation between SF and TNG with SPY (35, 36). SF was also reported to be a major trait in restorers and inter-subspecific crosses (10, 14, 37-38). Despite this, SF presented a lower CVg and heritability, suggesting limited genetic variation, with values ranging from 76.12 % to 93.83 % in the combined data. This could be a result of selection for high spikelet fertility in earlier generations. The distribution curve in Fig. 2 shows that the values are skewed to the left, indicating that most of the studied lines fall on the higher end across all environments, with varying degrees of skewness in each environment. This further reiterates that SF is a major trait influenced primarily by environmental factors. Nonetheless, SF and TNG should be given greater emphasis when selecting lines from the inter-subspecific populations (39-42). By minimizing the influence of environmental factors and understanding the dynamics of these traits, we can enhance the selection process, leading to improvements in both these traits and overall yield in inter-subspecific populations of rice.

Principal component analysis

Principal component analysis (PCA) is a dimension reduction technique that transforms the original data, generating principal components by minimizing the less important information (43). The results of the PCA are shown in Fig. 3. The first three principal components (PCs) had eigenvalues greater than one, together explaining 67 % of the total variation. The first principal component (PC1) accounted for 31 % of the variation, followed by PC2 with 22 %. The first two PCs together explained 53 % of the total variation and these

were used to develop the biplot for visualizing the variation. Traits such as PH, SPY and SF contributed the most to the total divergence in the population. Specifically, PH, NPT and PL had the greatest contribution to PC1, whereas SPY, SF and TNG were the major contributors to PC2 (Table 4). In a previous study, the significant contribution of single plant yield to the total variation was noted (44). Similarly, a greater contribution to genetic divergence from SF and TNG was also reported (45, 42). Further, PL and PH were important contributors in the first PC (46), as observed in the present study.

The relationships among the studied traits are visualized in the PCA biplot. An acute angle (<90°) between the vectors indicates a positive correlation, whereas an obtuse angle (>90°) suggests a negative correlation (47). Thus, SPY positively correlated with both SF and TNG with an angle that was less than 90°, aligning with the correlation results. The biplot revealed a highly scattered distribution of the newly developed lines, indicating significant genetic variation among them for the traits studied (48). PCA was used to study the variation in newly developed restorers and Awad-Allah (10) suggested that the observed greater genetic variation could be effectively harnessed to exploit heterosis in hybrid breeding. Furthermore, in the PCA biplot, the genotypes grouped along the trait vector in the same quadrant are considered to exhibit greater performance for those traits (49). The breeding lines G-68, G-15, G-17 and G-82 have shown good performance for traits such as SPY and SF. Similarly, G-39, G-50 and G-88 have exhibited strong performance for TNG. Owing to their superior traits, the above genotypes
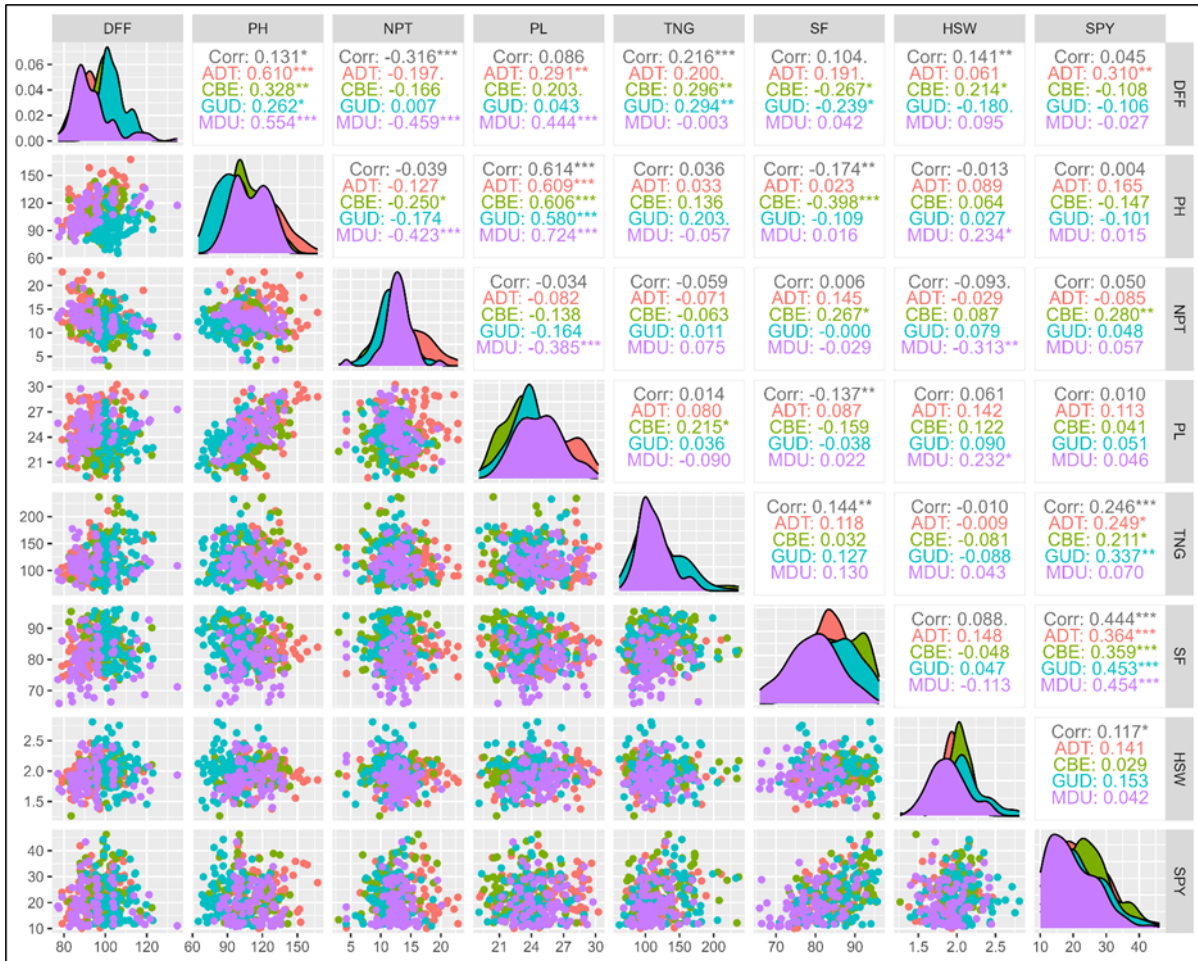
Fig. 2. Pearson's correlation coefficient of various quantitative traits studied in rice breeding lines at four locations.

DFF - Days to fifty per cent flowering, PH - Plant height, NPT - Number of productive tillers, PL - Panicle length, TNG - Total number of spikelets per panicle, HSW - Hundred seed weight and SPY - Single plant yield.
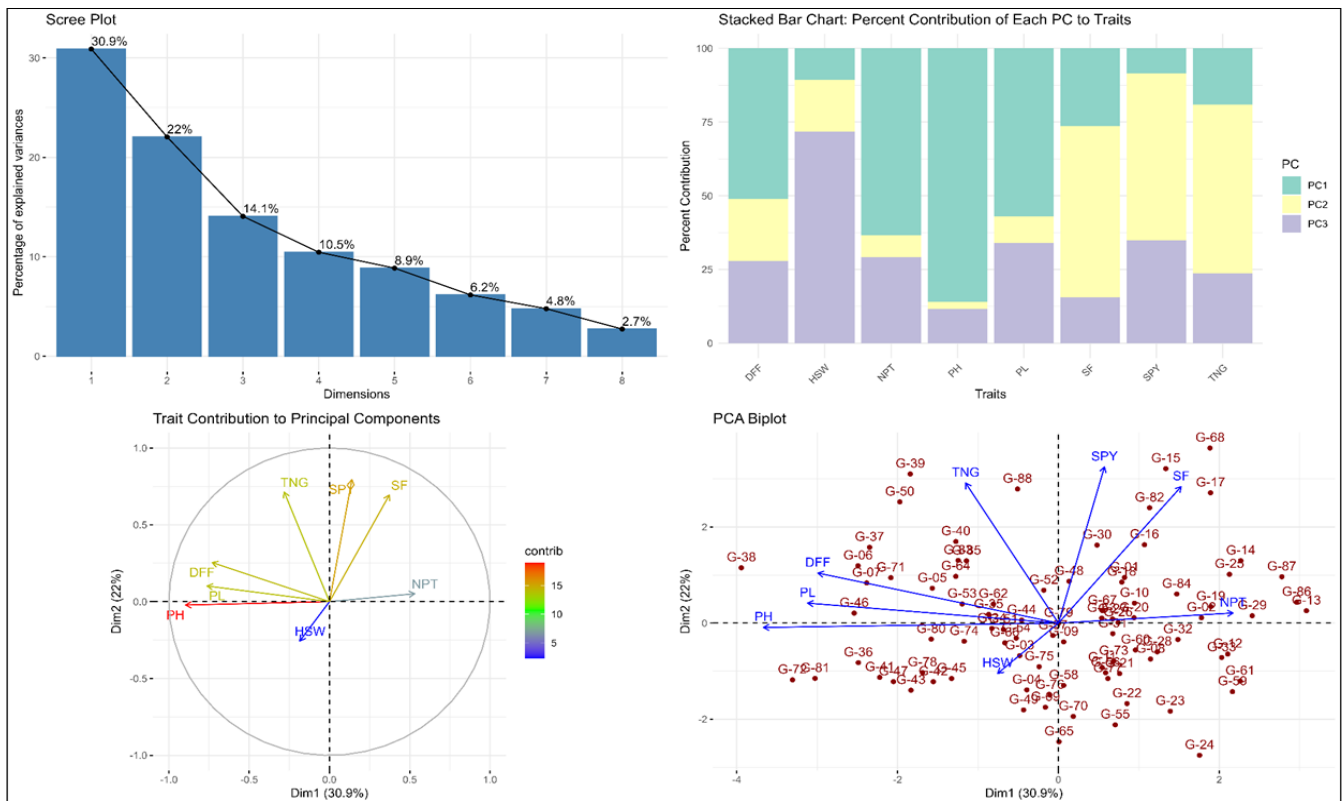

Fig. 3. Principal component analysis of rice inter-subspecific breeding lines evaluated at four locations.

DFF - Days to fifty per cent flowering, PH - Plant height, NPT - Number of productive tillers, PL - Panicle length, TNG - Total number of spikelets per panicle, HSW - Hundred seed weight and SPY - Single plant yield.

Table 4. Summary of principal component analysis and eigenvalue for various parameters in inter- subspecific breeding lines of rice

Particulars	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
DFF	-0.46	0.19	-0.25	-0.09	-0.24	0.72	-0.16	0.27
PH	-0.57	-0.02	0.08	-0.22	0.15	0.00	0.11	-0.76
NPT	0.34	0.04	0.16	-0.84	-0.34	0.04	-0.16	-0.08
PL	-0.48	0.08	0.29	-0.34	0.34	-0.38	-0.02	0.55
TNG	-0.18	0.54	-0.22	0.15	-0.49	-0.51	-0.31	-0.09
SF	0.24	0.52	0.14	0.04	0.57	0.20	-0.51	-0.15
HSW	-0.12	-0.19	0.79	0.30	-0.34	0.12	-0.34	-0.03
SPY	0.09	0.60	0.37	0.05	-0.11	0.14	0.68	0.02
Standard deviation	1.57	1.33	1.06	0.91	0.84	0.70	0.62	0.47
Proportion of Variance	0.31	0.22	0.14	0.10	0.09	0.06	0.05	0.03
Cumulative Proportion	0.31	0.53	0.67	0.77	0.86	0.92	0.97	1.00

DFF - Days to fifty per cent flowering, PH - Plant height, NPT - Number of productive tillers, PL - Panicle length, TNG - Total number of spikelets per panicle, HSW - Hundred seed weight and SPY - Single plant yield

could be potentially used in breeding programs to improve yield.

Conclusion

The new breeding lines, checks and landraces presented significant genetic variation in yield and other yield-contributing traits. The greatest genetic variation was observed in SPY, followed by TNG, NPT and PH. Heritability was high for PH and moderate for the other traits, except for SF. The positive correlation of SF and TNG with SPY across locations and in the combined analysis suggested their priority during selection. PCA revealed high divergence among the studied lines for the quantitative traits recorded in this study. Furthermore, the genotypes G-68, G-15, G-17 and G-82 were strongly associated with high SPY and SF, making them potential candidates for utilization in breeding programs. Thus, the observed genetic variation and results provide valuable insights into these newly developed inter-subspecific derivatives in rice. These lines need to be further screened molecularly for the presence of restorer genes to ensure their effective use in either three-line or two-line system. Additionally, evaluating these lines with CMS or TGMS lines based on marker profiles could help to identify the most heterotic combinations for exploiting expected heterosis.

Acknowledgements

Authors' contributions

BAJ conducted the experiments, collected data, performed statistical analyses and wrote the manuscript. SR conceptualized the experiment, provided materials, reviewed the manuscript and supervised all activities, including field research, data analysis and manuscript preparation. MS, RJ, RPR and UD participated in field research and provided technical support for manuscript preparation. All authors have read and agreed to the publication of the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

The author used the "Curie" AI language editing tool to check grammar. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the publication's content.

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