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RESEARCH ARTICLE

Analysis of genetic variability and trait association in rice (*Oryza sativa* L.)

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Abstract

Rice is a staple food for billions of people worldwide. In recent years unpredictable weather patterns, making rice cultivation more challenging and threatens its productivity. To ensure food security, developing high-yielding and climate-resilient rice varieties is essential. This requires a deep understanding of genetic variability and the relationships between key agronomic traits. Thus, the present study explores the genetic variability, as well as the major yield attributes of a segregating population derived from black rice genotypes, to formulate an effective selection strategy. In this research, 188 F_2 plants derived from crosses between the improved *Chakhao Amubi* and improved *Co 51* varieties were analysed and superior progenies were forwarded to F_3 generation. The variability study in the F_2 population revealed substantial variation for single plant yield and related yield attributes such as productive tillers and panicle length. High heritability estimates across all traits, along with a significant genetic advance over the mean for yield and major yield components, indicate that phenotypic selection would be effective. Principal Component Analysis (PCA) further confirmed the extensive variability present in the F_2 population. Correlation and path analysis highlighted panicle length and flag leaf length as key contributors to single plant yield, emphasizing their importance in selection strategies. The combined analysis of the F_2 and selected F_3 families revealed that single plant yield is predominantly governed by additive gene action, as evidenced by significant inter-generational correlation and high narrow-sense heritability. Furthermore, the observed positive selection response for yield suggests that simple phenotypic selection can lead to substantial genetic gain in rice

Keywords: black rice; intergeneration correlation; principal component analysis; selection gain

Introduction

Black rice (*Oryza sativa* L. indica) is a special cultivar of rice which contains remarkably high anthocyanin pigments in the aleurone layer than white and red rice which account for its violet or dark purple colour (1). Black rice is locally known as 'Chakhao', means delicious rice in Manipuri language is cultivated mainly by Meitei farmers of Manipur. Black rice is packed with nutrients and health's benefits, making it a standout among rice varieties. It has nearly six times more antioxidant activity than other types of rice and boasts a high protein content (8.16 %) while being extremely low in fat (0.07 %) (2). Naturally gluten-free and gentle on the gut, it also acts as a natural detoxifier with various medicinal properties (3). This nutrient-rich rice contains essential amino acids like lysine and tryptophan, along with functional lipids, dietary fibre and vitamin B1, vitamins B2, vitamins E and folic acid. It also has powerful phenolic compounds, including γ-oryzanols, tocopherols and tocotrienols, which contribute to its health benefits. When cooked, black rice has a mildly nutty flavour and a slightly sticky texture. It's loaded with essential macro and micronutrients like iron, zinc, calcium, phosphorus and selenium, yet remains low in calories (4).

Among the rice varieties grown in Northeast India, black rice stands out for its superior nutritional value and high protein content. Though, its cultivation is becoming increasingly difficult due to climate change. It led to rising temperatures, unpredictable rainfall and salt intrusion in coastal areas (5-7). These environmental challenges situate rice production at risk, emphasizing the need for resilient rice varieties and adaptive farming practices to sustain yields.

Grain yield is influenced by genotype, environment and cultural practices, along with their interactions (8). Still, the most significant variations in yield are primarily driven by the interaction between genotype and environment, even under consistent cultural practices. Additionally, planting time plays a crucial role in determining yield outcomes (9).

Differences in yield are evident across different rice genotypes, environmental conditions and their interactions (10). To analyse these variations, multivariate statistical techniques like PCA and cluster analysis are commonly used (11).

PCA helps identify patterns within large datasets by reducing redundancy and highlighting key relationships among variables, making it useful for classifying genotypes. On the other hand, cluster analysis focuses on grouping previously uncategorized materials based on their similarities. PCA allows researchers to visualize relationships between variables and observations, with 'eigenvalues' indicating how much each principal component contributes to overall variance. Meanwhile, eigenvector coefficients show amount variable influences a given component. The main advantage of PCA is its ability to pinpoint the most critical dimensions responsible for variation within a dataset. It is particularly useful when studying plant traits related to yield (12-14). The global population rising and challenges like climate change, shrinking arable land and water scarcity, improving rice productivity and resilience has become a major focus in agricultural research (15).

To enhance genetic improvement through breeding, researchers must understand variability in rice genotypes, their trait associations and the factors affecting yield and quality (16). Advanced statistical tools have proven effective in identifying key traits that contribute to higher productivity and can help guide breeding strategies for better-performing rice varieties.

Materials and Methods

To study genetic variability crosses were made between the improved *Chakhao Amubi* [Drought-tolerant backcross inbred lines of Chakhao Amubi were successfully developed through marker-assisted breeding by introgressing the drought-responsive QTLs *qDTY1.1*, *qDTY2.1* and *qDTY6.1* from the donor parent Apo] and improved *Co51* rice varieties [Disease resistant line with multiple resistant genes for bacterial blight (*xa5, xa13 and Xa21*) and blast (*Pi54*)]. True hybrids in the F_1 generation were identified using polymorphic markers and then advanced to the F_2 generation. These hybrids were self-pollinated to produce the F_2 population of 188 F_2 plants to assess genetic diversity and variability. The F_2 plants with desirable genes (both drought QTLs and biotic stress resistant genes) were forwarded to F_3 and raised in a family row method.

A total of nine quantitative traits were recorded in individual F_2 plants and all the plants in F_3 family rows. Those are, days to fifty percent flowering (DFF, days), plant height (PH, cm), number of productive tillers (NPT, count), panicle length (PL, cm), FLL (cm), flag leaf width (FW, cm), number of grains per panicle (NGP, count), hundred seed weight (HSW, g) and single plant yield (SPY, g).

The experiment was conducted at the Paddy Breeding Station, TNAU and Coimbatore. It is located at an elevation of 426.72 meters at 11°N latitude and 77°E longitude. The station covers 12.96 hectares of cultivable land with clayey soil and a pH of 7.8. The region receives an average annual rainfall of 670 mm, providing controlled conditions for studying trait variation.

Statistical analysis

The collected data were evaluated for genetic variability parameters using MS Excel software. The variability analysis and parent progeny regression were performed according to Singh and Narayanan (17). The PCA was performed using 'FactoMineR' R package and visualized using 'factoextra' R package. The correlation and path analysis were performed using 'agricolea' R package.

Results and Discussion

Genetic variability

The Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) values showed that SPY had the highest genetic variability, with GCV and PCV recorded at 41.31 % and 43.61 %, respectively. This close proximity between GCV and PCV also indicated low environmental influence and a high degree of heritable variation. High GCV and PCV were also noted for flower length (FL) and pod length (PL), whereas days to 50 % flowering (DFF) and number of green pods (NGP) exhibited the lowest GCV (1.24 % and 2.37 %, respectively), implying limited genetic variability and strong environmental influence. All the studied traits showed low coefficient of variation (CV) which suggesting more precise measurements and lower environmental fluctuation.

Heritability estimates ranged from moderate to high across traits. Flower length (FL) exhibited the highest heritability (0.95), followed closely by SPY (0.90) and hundred seed weight (HSW) (0.91). These high values indicate that the observed variation is largely due to genetic factors and can be effectively transferred to the next generation. Conversely, Number of Pods per Plant (NPT) recorded the lowest heritability (0.45), reflecting higher environmental influence and reduced selection efficiency.

Genetic Advance (GA) and Genetic Advance as Percent of Mean (GAM) results showed that the SPY had the highest GA (22.09) and GAM (80.60 %), reinforcing its suitability for selection in breeding programs. Similarly, high GAM values were recorded for flower length (63.50 %) and pod length (39.01 %), indicating traits governed primarily by additive gene action. Traits such as number of green pods (NGP) and DFF showed low GAM values (3.7 4 % and 1.94 %, respectively), suggesting limited potential gain through selection (Table 1).

Correlation

The correlation matrix provides valuable insights into the relationships between various agronomic traits in rice, highlighting key interactions that can guide to formulation of breeding strategies. Strong positive correlations, such as between FLL and PL ($r = 0.72$, $p < 0.001$), suggest that an increase in FLL is associated with longer panicles, which may contribute to higher yield potential. These relationships play a crucial role in selecting desirable traits for breeding programs to improve rice productivity (18). For instance, that flag leaf size is directly linked to grain yield, influencing photosynthetic efficiency and assimilate translocation. Similarly highlighted the role of yield contributing traits in determining overall grain production. Understanding such correlations aids in targeted selection and genetic improvement strategies to enhance the

Table 1. Genetic variability parameters for agronomic traits in selected populations

	DFF	PH	NPT	PL	FL	FW	NGP	HSW	SPY
Ve	1.26	13.90	3.55	4.07	3.61	0.00	7.21	0.01	14.67
Vp	2.94	89.57	6.50	23.78	70.64	0.01	17.50	0.06	142.79
Vg	1.68	75.67	2.95	19.71	67.02	0.01	10.29	0.05	128.12
Mean	104.26	110.56	12.22	21.34	25.87	1.21	135.36	2.44	27.40
GCV	1.24	7.87	14.06	20.80	31.64	6.53	2.37	9.44	41.31
PCV	1.64	8.56	20.87	22.85	32.49	7.60	3.09	9.88	43.61
CV	1.08	3.37	15.42	9.45	7.35	3.90	1.98	2.90	13.97
heritability	0.57	0.84	0.45	0.83	0.95	0.74	0.59	0.91	0.90
GA	2.02	16.47	2.38	8.33	16.43	0.14	5.07	0.45	22.09
GAM	1.94	14.90	19.51	39.01	63.50	11.54	3.74	18.59	80.60
Max	109	141	21	32	44	1.6	147	3.81	67
Min	100	55.7	7	12	2	1.1	121	2.1	7

yield and adaptability of rice varieties (19). The study reveals negative correlations between PL and tillering capacity (r = -0.17, p < 0.05), suggesting a trade-off between these factors (20). These findings have implications for rice breeding, as traits like FL, PL and FW can be prioritized for yield improvement. The negative correlation between PL and tiller number highlights the complexity of genetic interactions and the need for multi-trait selection strategies (21, 22).

The correlation coefficient measures the strength and direction of the relationship between two traits, making it a valuable tool for selecting and improving correlated traits simultaneously (23). The study indicates a strong positive correlation between traits like plant height, flag leaf size, leaf width and grain yield per plant (24). Additionally, panicle length and other factors like total tillers, effective tillers, PL , grains per panicle, fertile grains per panicle, 1000 grain weight and kernel breadth also contribute positively significantly to grain yield. These findings suggest these traits are valuable targets for improving grain yield in crop breeding (25-31). Grain yield per plant is negatively correlated with days to fifty percent flowering reported by (32). Spikelet fertility has a negative correlation with grain yield (33) and 1000 grain weight has a negative correlation (Fig. 1).

Path analysis

Path coefficient analysis was used to determine the importance of different traits in improving yield (34). Grain yield per plant is negatively correlated with days to flowering, while traits like spikelet fertility, kernel length and L/B ratio show a positive but non-significant correlation. Spikelet fertility has a negative correlation with grain yield, while 1000 grain weight has a negative correlation. Path coefficient analysis was used to determine the importance of different traits in improving yield (35) (Table 2).

Principal Component Analysis (PCA)

PCA was employed to assess the extent to which individual agronomic traits contributed to the total variability within the dataset. The scree plot shows the percentage of explained variance for each principal component (PC), helping determine the optimal number of PCs to retain for analysis (Fig. 2). The first three principal components (PC1, PC2 and PC3) together explained 60 % of the total variation, with PC1 alone accounting for 31 %. Traits like single plant yield, FLL and PL had the strongest influence on PC1, suggesting they play a key role in overall trait variation (36). Plant height and the number of grains per panicle were most important in PC2, while the number of productive tillers and 100-seed weight stood out in

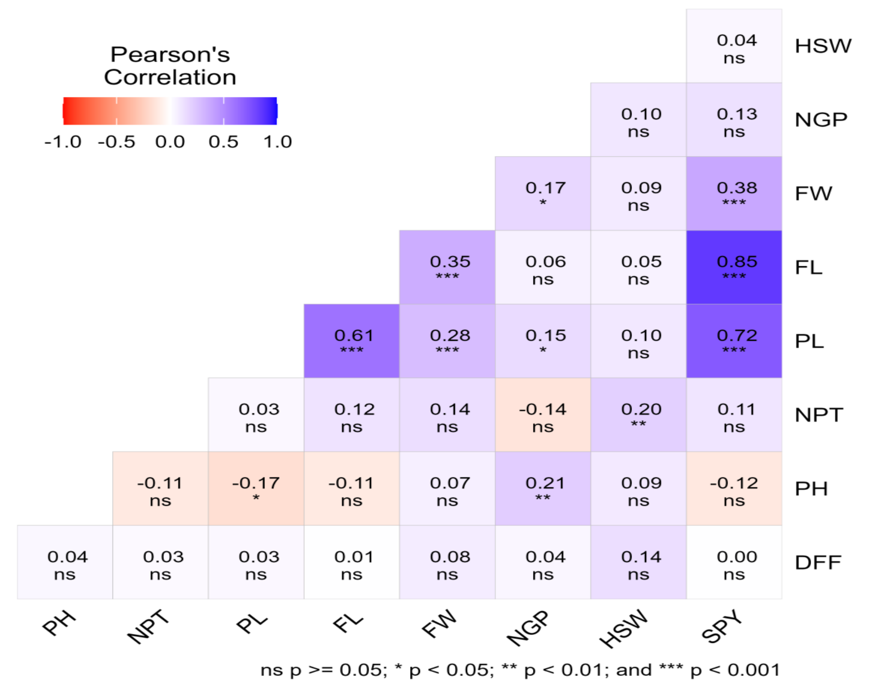

Fig. 1. Heatmap of Pearson's correlation coefficients among agronomic traits.

Table 2. Path coefficient analysis indicating direct and indirect effects of agronomic traits

	DFF	PH	NPT	PL	FL	FW	NGP	HSW
DFF	-0.0183	-0.0002	0.0009	0.0094	0.0063	0.0052	0.0017	-0.005
PH	-0.0007	-0.0039	-0.0032	-0.0531	-0.0696	0.0045	0.0091	-0.0032
NPT	-0.0005	0.0004	0.0291	0.0094	0.0759	0.0091	-0.0061	-0.0072
PL	-0.0005	0.0007	0.0009	0.3123	0.3857	0.0181	0.0065	-0.0036
FL	-0.0002	0.0004	0.0035	0.1905	0.6323	0.0227	0.0026	-0.0018
FW	-0.0015	-0.0003	0.0041	0.0874	0.2213	0.0648	0.0074	-0.0032
NGP	-0.0007	-0.0008	-0.0041	0.0468	0.0379	0.011	0.0434	-0.0036
HSW	-0.0026	-0.0003	0.0058	0.0312	0.0316	0.0058	0.0043	-0.0359
Residual								
0.205228								

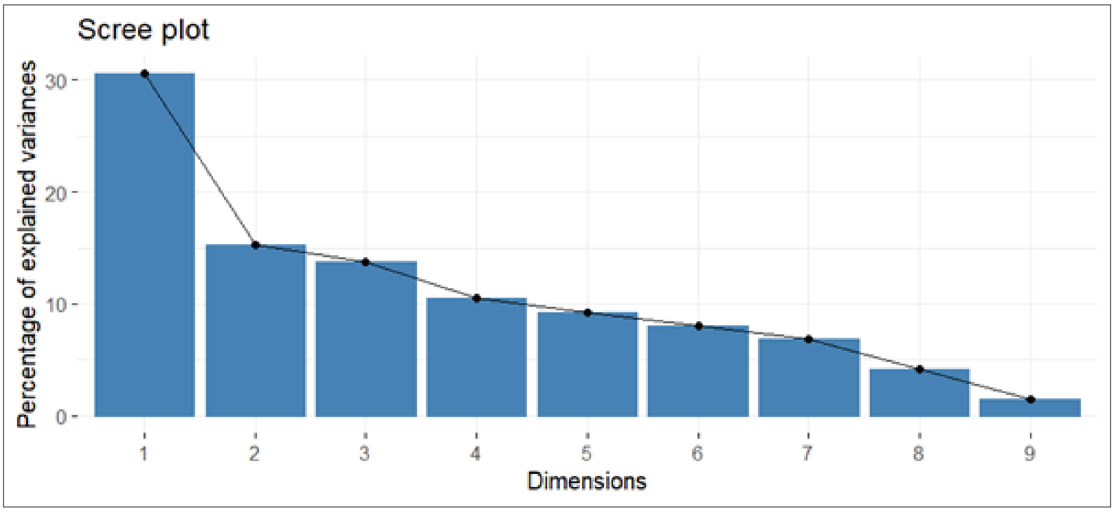

Fig. 2. Scree plot showing the percentage of explained variance across principal components.

PC3. Interestingly, days to 50 % flowering had a strong negative impact on PC4. By the time six components are considered, nearly 87 % of the variability is captured, meaning most of the important differences among traits can be understood with just a few key patterns. This makes it easier to focus on the traits that matter most in breeding and selection efforts.

However, Retaining PC1 and PC2 is a reasonable approach for dimensionality reduction and trait selection in rice breeding programs, as they capture the most critical variations among agronomic traits (37). Thus, PCA biplot generated using first two PCs. Principal components with eigenvalues greater than 1.0 indicate high variability among rice genotypes, making them useful for selecting diverse parental lines Similar findings were reported by (38). Recent genetic analysis highlights key parameters shaping trait inheritance and selection effectiveness. Traits like seed yield per plant and number of productive tillers show high selection gains (46.45 and 75.02, respectively), indicating potential for genetic improvement. Conversely, days to 50 % flowering and flag leaf length show negative selection gains (-4.11 and -20.43), suggesting a generational decline in these traits (39).

The study reveals stable inheritance patterns for FLL (0.74) and hundred-seed weight (HSW) (0.76), as well as strong positive correlations at p <0.01, for SPY (0.61) and NPT (0.67), DFF (72.83) are showed high inheritable, while PL (9.06) and FW (10.66) show lower heritability, suggesting stronger environmental influence (40). These findings suggest that three traits should be prioritized in breeding programs for sustained genetic improvement. The F_3 families show significant variation in key agronomic traits, such as days to DFF, PH, NPT, PL, FLL and SPY providing valuable insights for breeding programs (41).

PC1 explains the highest variance, accounting for over 30 %, followed by PC2 which contributes around 20 %. A sharp decline in variance is observed after PC2, indicating that the first two PCs capture a substantial proportion of the total variance in the dataset. This pattern aligns with the Kaiser criterion. The gradual decrease in explained variance across subsequent PCs indicates diminishing contributions to overall data variation (42-43). A scree plot shows the percentage of explained variance for each principal component, with PC1 exhibiting the highest variability.

The PCA results reveal that FL, SPY and PL are the most influential in explaining variability (8), while path analysis confirms FL and SPY as key contributors to productivity, emphasizing their role in breeding programs. Additionally, the selection gain for SPY (+12.71) indicates promising yield improvement, further supporting its importance in genetic enhancement strategies (44). High heritability traits (FL, SPY, NGP) indicate strong genetic control, making them ideal for selection (45). Similarly, high GAM values for SPY, FL and PL suggest significant improvement potential under selection pressure. CA simplified the analysis of agronomic traits by identifying key contributors to variation in rice. The first three components explained 60 % of the total variance, with PC1 (31 %) driven by traits like single plant yield, flag leaf length and panicle length-highlighting their importance for yield improvement. PC2 and PC3 emphasized structural and reproductive traits, while days to 50 % flowering showed an independent trend in PC4. The scree plot supported focusing on the top components, suggesting that a few key traits can effectively guide selection and breeding decisions. The PCA results reveal that FL, SPY and PL are the most influential in explaining variability (46), while path analysis confirms FL and SPY as key contributors to productivity, emphasizing their role in breeding programs. Additionally, the selection gain for SPY (+12.71) indicates promising yield improvement, further supporting its importance in genetic enhancement strategies (47) (Table 3).

Genetic studies in F_3

Family 143, 151 and 141 of the F_3 family showed the highest SPY (45.55), (44.85) and (44.72) respectively indicating superior yield potential. Panicle length varied from 22.20 cm (F_3-169) to 24.99 cm (F_3-143) and flag leaf length showed significant genetic variability, with F_3-58 having the longest FLL (24.58 cm) and F_3-56 having the shortest (16.69 cm). FDFF ranging from 96.57 days (F_3-151) to 103.35 days (F_3-74), indicating genetic differences in growth duration. Plant height also showed considerable variation, with F_3-56 reaching the tallest height (118.84 cm) and F_3-151 the shortest (103.24 cm), pointing to differences in plant architecture (Table 4). These variations in yield-related traits highlight the genetic diversity within the F_3 families, making entries like 143, 151 and 141 strong candidates for further breeding selection (48).

Among the selected, families 143, 151 and 141 exhibited the highest seed yield per plant, making them strong candidates for future breeding efforts. Additionally, differences in panicle length, flag leaf length, flowering time and plant height demonstrate the genetic variability within these families, offering breeders a wide selection pool for trait improvement (49). Interestingly, while traits like seed yield per plant (SPY) and the NPT show high selection gains, others such as days to 50 % flowering (DFF) and FLL exhibit negative selection gains, indicating a potential decline over generations. This suggests that while some traits are strongly inheritable and stable, others may require careful selection to prevent undesirable changes over time (50, 51). Overall, these findings highlight the importance of multi-trait selection strategies in rice breeding (Table 5). By prioritizing high-yielding families, maintaining genetic diversity and balancing trade-offs between correlated traits, breeders can enhance rice productivity while ensuring long-term genetic stability. High heritability traits (FL, SPY, NGP) indicate strong genetic control, making them ideal for selection (52). Similarly, high GAM values for SPY, FL and PL suggest significant improvement potential under selection pressure (53, 54).

Conclusion

Table 3. Principal component contributions to agronomic trait variability

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
DFF	0.04	0.28	-0.34	-0.84	0.24	-0.01	0.17	-0.03	-0.01
PH	-0.09	0.60	0.12	0.29	0.28	-0.61	0.24	0.16	0.01
NPT	0.11	-0.15	-0.67	0.38	0.18	0.21	0.53	0.11	0.03
PL	0.50	-0.05	0.11	-0.13	-0.24	-0.05	0.03	0.78	0.22
FL	0.53	-0.10	0.07	0.01	0.03	-0.24	0.10	-0.53	0.59
FW	0.33	0.25	-0.09	0.18	0.59	0.32	-0.58	0.07	0.02
NGP	0.12	0.57	0.28	0.07	-0.27	0.61	0.33	-0.13	0.04
HSW	0.09	0.35	-0.55	0.10	-0.59	-0.18	-0.39	-0.11	-0.04
SPY	0.56	-0.08	0.10	0.00	-0.01	-0.16	0.13	-0.18	-0.77
Standard deviation	1.66	1.17	1.11	0.97	0.91	0.85	0.79	0.61	0.36
proportion of variance	0.31	0.15	0.14	0.10	0.09	0.08	0.07	0.04	0.01
cumulative proportion	0.31	0.46	0.60	0.70	0.79	0.87	0.94	0.99	1.00

Table 4. Selected mean performance F3 lines

F3 family name	DFF	PH	NPT	PL	FLL	FW	NGP	HSW	SPY
2	101.51	116.23	21.74	22.62	22.79	1.17	138.67	2.31	41.03
18	101.91	103.39	20.11	22.41	22.54	1.20	137.10	2.31	37.56
56	98.06	118.84	22.00	22.73	16.69	1.18	137.19	2.31	28.65
58	102.08	108.75	20.84	23.68	24.58	1.22	139.73	2.20	44.53
73	102.60	117.48	21.44	24.26	17.91	1.19	138.72	2.18	37.78
74	103.35	105.84	20.40	22.52	17.01	1.16	137.67	2.19	43.49
141	98.43	106.84	21.51	23.90	18.97	1.18	137.82	2.20	44.72
143	100.15	113.86	21.91	24.99	21.06	1.17	137.61	2.18	45.55
151	96.57	103.24	21.56	22.25	20.69	1.16	136.85	2.17	44.85
169	96.66	109.33	21.66	22.20	19.86	1.17	129.68	2.19	37.37
171	98.57	116.68	22.06	23.98	24.39	1.20	139.51	2.23	35.54

Table 5. Selection response and heritability estimates in F_3 families

	mean F2	Mean F3	Selection gain	Selection differential	inter generation correlation	parent progeny regression	narrow sense heritability
DFF	104.26	99.97	-4.11	-4.29	-0.02	-0.04	72.83
PH	110.56	110.94	0.35	0.39	0.03	0.02	33.79
NPT	12.22	21.39	75.02	9.17	0.67*	0.13	9.81
PL	21.34	23.23	8.83	1.89	0.19	0.03	9.06
FLL	25.87	20.58	-20.43	-5.29	0.74**	0.24	16.20
FW	1.21	1.18	-2.31	-0.03	-0.07	-0.01	10.66
NGP	135.36	137.28	1.42	1.92	-0.15	-0.10	31.70
HSW	2.44	2.22	-8.95	-0.22	0.76**	0.25	16.12
SPY	27.37	40.08	46.45	12.71	0.61*	0.43	35.08

This study provides meaningful insights into the relationships among key agronomic traits in rice through correlation analysis, path coefficient analysis and PCA. The strong positive association between FLL and PL highlights their potential as reliable traits for selection in breeding programs focused on yield enhancement. In contrast, the negative correlation between PL and tillering capacity points to the need for careful consideration of trade-offs when selecting multiple traits simultaneously. Path analysis helped identify both direct and indirect effects of various traits, revealing the complex roles of spikelet fertility, kernel traits and 1000 grain weight in determining grain yield. The genetic variability and PCA results, indicated that tremendous genetic variation presented in the segregating population, making the selection process more efficient. Genetic variability and heritability observed among F_3 families and genetic correlation of F_2 with F_3 suggested the predominance of additive gene action in single plant yield implying phenotypic selection would be more effective. The mean performance of F_3 families indicated that lines 143, 151 and 141, as potential candidates need to be forwarded further to isolate superior line with improved yield for climate resilience.

Authors' contributions

ST conducted the experiments, collected data, performed statistical analyses and wrote the manuscript. JBA performed statistical analyses, wrote and edited the manuscript. JR conceptualized the experiment, provided materials, reviewed the manuscript and supervised all activities, including field research, data analysis and manuscript preparation. RPG, SM, CG and MR 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

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