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Genetic Diversity and Trait-Based Selection in Aromatic Rice (*Oryza sativa* L.): A Multivariate Trait Analysis Approach

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ABSTRACT

Aromatic rice holds substantial value in global and domestic markets due to its distinctive aroma, grain quality, and culinary characteristics. This study evaluated 50 aromatic rice genotypes, including breeding lines and landraces, over two consecutiveKharif seasons (2023–2024) at ANDUAT, Ayodhya, using a randomized complete block design with three replications. The objective was to assess genetic variability, identify key yield-contributing traits, and develop predictive models for grain yield. Thirteen quantitative traits were recorded following the Standard Evaluation System (SES) for rice. Descriptive statistics revealed considerable variability across genotypes, with grain yield ranging from 12.61 g to 62.38 g/plant and biological yield from 49.00 g to 154.00 g/plant. High genotypic coefficient of variation (GCV) and heritability values were observed for yield (256.71%), panicle-bearing tillers (81.80%), and biological yield (42.55%), indicating strong genetic control and potential for selection. Correlation and path coefficient analyses identified plant height (direct effect = 0.295) and tillers per plant (direct effect = 0.215) as critical determinants of yield. Principal Component Analysis (PCA) explained trait-driven variance, while hierarchical clustering grouped genotypes into distinct clusters for diversity assessment. Notably, genotypes AR-6, AR-24, AR-12, and AR-4 outperformed the check variety PB-1121 in multiple traits. With R^2 values demonstrating strong model fit. The integration of classical statistical tools and modern analytics provides a robust framework for identifying elite genotypes for aromatic rice improvement.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops globally, serving as the staple food for over half of the world's population. It plays a pivotal role in ensuring food security, economic stability, and cultural heritage across Asia, Africa, and Latin America. Among the various classes of rice, aromatic rice holds a unique market position due to its superior grain quality, fragrance, and distinct cooking characteristics. Aromatic rice varieties, such as Basmati and Kalanamak, are highly valued in both domestic and international markets for their unique sensory attributes [18].

India, recognized as the center of origin and diversification for many aromatic rice landraces, contributes significantly to the global aromatic rice market [2]. These varieties, however, are often characterized by long growth duration, susceptibility to lodging, and sensitivity to abiotic stress. Hence, enhancing their yield potential and stress resilience while maintaining grain quality is a key objective for rice breeding programs [15]. Despite their economic value, aromatic rice varieties have received relatively less attention in terms of genetic enhancement compared to non-aromatic high-yielding types.

The improvement of complex traits such as yield requires a comprehensive understanding of the genetic architecture governing trait expression. Traits contributing to yield are polygenic, quantitative in nature, and highly influenced by environmental conditions and their interactions with genotypes [5]. To address these challenges, a combination of classical genetic tools and modern statistical techniques is necessary. Parameters such as genotypic and phenotypic coefficients of variation (GCV and PCV), heritability, and genetic advance are widely used to evaluate genetic diversity and the effectiveness of selection [17, 20].

Selection for yield improvement is often more effective when based on secondary traits that exhibit high heritability and significant positive correlations with yield. Path coefficient analysis helps partition observed correlations into direct and indirect effects [11]. Thereby facilitating the identification of traits with the most influence on yield [4]. Additionally, Principal Component Analysis (PCA) simplifies multidimensional trait datasets by highlighting major axes of variation, while cluster analysis groups genotypes based on trait similarities to assist in parent selection [14].

Modern breeding has been further advanced by the application of machine learning (ML) techniques, which are capable of handling complex, non-linear trait interactions and large datasets. ML algorithms such as Random Forest (RF), Support Vector Machines (SVM), and Gradient Boosting Machines (GBM) have demonstrated remarkable accuracy in predicting crop performance, including yield, based on multivariate data [23]. These tools enhance breeders' ability to make informed decisions by uncovering non-intuitive patterns in the data and simulating breeding outcomes.

In this context, the present study aimed to assess the phenotypic and genetic variability among 50 aromatic rice genotypes, evaluate their agronomic and yield-related traits across two Kharif seasons, and identify elite genotypes suitable for breeding through multivariate, regression, and path coefficient analyses. Furthermore, machine learning models were employed to predict yield based on trait data, offering a predictive framework for selection. The findings from this integrated approach are expected to support future breeding strategies aimed at enhancing the productivity and market value of aromatic rice.

2. Materials and Methods

2.1. Experimental Site

The field investigation was conducted during the Kharif seasons of 2023 and 2024 at the Student Instructional Farm, AcharyaNarendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya. The site is geographically situated at 26.47°N latitude and 82.12°E longitude, with an altitude of 113 meters above mean sea level [1].

2.2. Experimental Material

A total of 50 rice genotypes (*Oryza sativa* L.), including breeding lines and landraces, were used in this study. These genotypes were sourced from the National Rice Research Institute (NRRI), Odisha; the Centre of Excellence for Rice, Ayodhya; and ANDUAT, Kumarganj. Out of these, 50 aromatic rice genotypes were selected for detailed evaluation, with PB-1121 serving as the check variety due to its widespread cultivation and well-documented agronomic profile. The selected genotypes included elite cultivars such asPusa Basmati-1121, known for their superior yield and aromatic traits [1].

2.3. Nursery Management and Field Layout

All genotypes were sown in nursery beds at the onset of the Kharif season. After 25 days, seedlings were transplanted to the main field using a randomized complete block design (RCBD) with three replications to ensure statistical robustness. Each hill was planted with a single seedling at a spacing of 15 cm × 20 cm. Standard agronomic practices were followed, including the recommended dose of NPK fertilizers at 120:60:60 kg/ha [19].

2.4. Trait Evaluation and Data Collection

Thirteen quantitative traits were evaluated to characterize phenological, morphological, and yield-related performance. Five randomly selected plants per replication were used for trait measurement. The traits assessed included aromatic code, days to 50% and 100% flowering, days to maturity, flag leaf length (cm), plant height (cm), number of tillers per plant, panicle-bearing tillers per plant, panicle length (cm), biological yield (g/plant), number of grains per panicle, grain yield per plant (g), and test weight (g). All observations were recorded according to the Standard Evaluation System (SES) for rice [7].

2.5. Statistical Analysis

Descriptive statistics, including mean, standard deviation, coefficient of variation (CV), genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV), were computed to assess genetic variability. Correlation analysis was employed to determine the strength and direction of relationships among traits.Multiple regression analysis was used to identify key yield-contributing traits, followed by path coefficient analysis to decompose direct and indirect effects. Principal Component Analysis (PCA) was performed to extract major components contributing to phenotypic variation.Hierarchical clustering using Ward's method was applied to group genotypes based on multivariate similarity. Canonical Correlation Analysis (CCA) was conducted to explore the interrelationship between trait groups.The models were evaluated using R^2 (coefficient of determination). [23].

3. Results and discussion

3.1. Performance of Aromatic Genotypes Compared to Check Variety PB-1121

A total of 50 aromatic rice genotypes were evaluated using PB-1121 as the check variety, which recorded a grain yield per plant of 52.33 g, biological yield of 84.00 g, plant height of 152.00 cm, 24.67 tillers per plant, panicle length of 25.00 cm, 138.00 grains per panicle, test weight of 25.20 g, panicle fertility of 129.00, days to 50% flowering at 104.00, and days to maturity at 112.00. The majority of test genotypes (49 out of 50) outperformed PB-1121 in at least one major agronomic or yield trait, indicating considerable genetic diversity within the population [19]. Among these, genotypes AR-6, AR-24, AR-26, and AR-12 were especially noteworthy, each surpassing the check in nine different traits. AR-6 showed superior biological yield (154.00 g), plant height (183.00 cm), tiller number (31.00), panicle length (30.33 cm), grains per panicle (180), and grain yield (56.62 g), making it an ideal candidate for high biomass and yield. AR-24 exhibited early flowering (98.00 days) and maturity (108.00 days), along with a biological yield of 123.00 g and panicle length of 30.33 cm. AR-26 and AR-12 also demonstrated excellent values for test weight (26.21 g), plant height (163.33 cm), grains per panicle (135.33–197), and grain yield (45.27–52.13 g). Other strong performers included AR-4 (62.45 g), AR-5 (61.47 g), and AR-1 (56.36 g), each excelling in eight traits, while AR-15, AR-18, and AR-20 showed superiority in seven, offering considerable potential for yield enhancement through selection or breeding [1]. These results collectively highlight the superiority of several genotypes over PB-1121 across multiple traits, reinforcing the feasibility of selecting elite aromatic rice lines for both direct adoption and genetic improvement programs. The comprehensive comparison of trait values relative to PB-1121 is visually summarized in **Figure 1**.

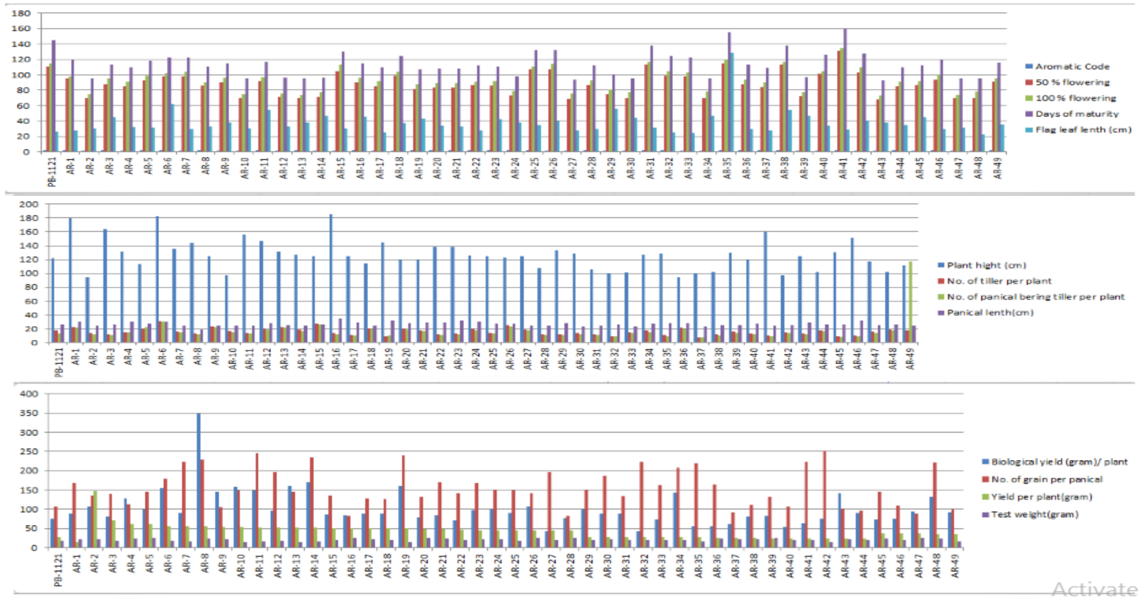

Figure 1. Comparative Bar Graph Representation of Phenological, Morphological, and Yield Traits Across 50 Aromatic Rice Genotypes

3.2. Genetic Variability and Trait Distributions

Descriptive statistics revealed substantial variation across the studied traits, highlighting a broad genetic base within the aromatic rice genotypes. Grain yield per plant varied widely from 12.61 to 62.38 g, biological yield ranged from 49.00 to 154.00 g, tillers per plant from 14.67 to 31.00, plant height from 91.67 to 183.00 cm, panicle length from 19.33 to 30.33 cm, and grains per panicle from 85.00 to 197.00. To further understand the underlying genetic variability, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and coefficient of variation (CV) were computed. The highest GCV was recorded for grain yield (256.71%), followed by panicle-bearing tillers (81.80%) and biological yield (42.55%), suggesting a high degree of genetic diversity and the effectiveness of selection for these traits. Moderate GCVs were noted for grains per panicle (27.76%), tillers per plant (28.12%), and test weight (14.85%), while lower GCVs were observed for plant height (15.77%) and panicle length (9.98%), indicating relative stability and limited environmental influence on these traits. The marked difference between GCV and PCV in certain traits also pointed to environmental effects, but the high heritability observed in yield-related traits emphasizes the role of additive gene action. These findings underline the potential for genetic improvement through direct selection, especially for traits with high variability and heritability. A detailed summary of the descriptive statistics and variability estimates is presented in **Table 1** [20].

Table 1. Descriptive Statistics and Genetic Parameters for Agronomic and Yield Traits in 50 Aromatic Rice Genotypes

Trait	Mean	StdDev	SE	CV (%)	PCV (%)	GCV (%)	CD
Aromatic Code	1.54	0.5	0.07	32.73	32.73	29.46	0.15
50 % flowering	88.56	14.87	2.1	16.79	16.79	15.11	4.42
100 % flowering	94.06	14.56	2.06	15.48	15.48	13.93	4.33
Days of maturity	114.12	16.08	2.27	14.09	14.09	12.68	4.78
Flag leaf length (cm)	38.05	15.89	2.25	41.77	41.77	37.6	4.72
Plant height (cm)	126.79	22.21	3.14	17.52	17.52	15.77	6.6
No. of tillers per plant	15.91	4.97	0.7	31.25	31.25	28.12	1.48
Panicle bearing tillers/plant	16.8	15.27	2.16	90.89	90.89	81.8	4.54
Panicle length (cm)	26.61	2.95	0.42	11.09	11.09	9.98	0.88
Biological yield (g/plant)	101.67	48.07	6.8	47.27	47.27	42.55	14.28
No. of grains per panicle	155.89	48.1	6.8	30.85	30.85	27.76	14.29
Yield per plant (g)	72.31	206.24	29.17	285.23	285.23	256.71	1.01
Test weight (g)	20.68	3.41	0.48	16.5	16.5	14.85	61.28

3.3. Regression and ANOVA-Based Trait Association Insights

A multiple regression analysis was carried out to assess the contribution of various traits toward grain yield per plant, which served as the dependent variable. The regression model yielded a Multiple R of 0.556, R^2 of 0.309, and Adjusted R^2 of 0.085, suggesting that approximately 30.9% of the variation in grain yield could be attributed to the combined influence of independent traits such as biological yield, plant height, panicle length, tillers per plant, and grains per panicle. Despite this, the relatively high Standard Error of 197.27 highlighted the presence of substantial unexplained variance, likely arising from complex genotype × environment interactions and unmeasured variables. Although the adjusted R^2 was modest, the model still indicated a moderate linear association between the predictor variables and grain yield, justifying the inclusion of these traits in selection indices. Furthermore, ANOVA reinforced the significance of genotypic differences for nearly all measured traits, with particularly high mean squares observed for grain yield (42,558.79) and biological yield (2,295.34), validating substantial variability among the tested genotypes. These results not only confirm the presence of inherent genetic diversity but also highlight the complex nature of yield inheritance in aromatic rice. Consequently, the identification of genotypes such as AR-6, AR-24, AR-26, AR-12, AR-4, and AR-5 as superior performers reinforces their utility in future breeding programs. The regression coefficients and ANOVA summary are detailed in **Table 2** [23].

Table 2 Regression and ANOVA Summary for Trait Contributions to Yield in Plants

	Regression Statistics								ANOVA			
Traits	*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*	*Count*	*Sum*	*Average*	*Variance*
Yield per plant(gram)	-301.488	631.7099	-0.47726	0.635987	-1581.45	978.4782	-1581.45	978.4782	50	3615.26	72.3052	42536.92
Aromatic Code	1.394012	68.09636	0.020471	0.983778	-136.582	139.3703	-136.582	139.3703	50	77	1.54	0.253469
50 % flowering	61.50943	34.74192	1.770467	0.084889	-8.88439	131.9033	-8.88439	131.9033	50	4428	88.56	220.9861
100 % flowering	-66.3716	28.71244	-2.3116	0.026472	-124.549	-8.19469	-124.549	-8.19469	50	4703	94.06	211.9759
Days of maturity	3.303019	16.6567	0.1983	0.843896	-30.4467	37.05271	-30.4467	37.05271	50	5706	114.12	258.5976
Flag leaf lenth (cm)	-2.76872	2.458757	-1.12606	0.267389	-7.75063	2.213195	-7.75063	2.213195	50	1902.36	38.0472	252.3557
Plant hight (cm)	2.737603	1.682051	1.627539	0.11211	-0.67055	6.145762	-0.67055	6.145762	50	6339.35	126.787	493.2173
No. of tiller per plant	8.964581	6.592297	1.359857	0.182104	-4.39268	22.32184	-4.39268	22.32184	50	795.67	15.9134	24.69548
No. of panicalbering tiller per plant	-0.1109	2.168702	-0.05114	0.959491	-4.50511	4.283307	-4.50511	4.283307	50	839.98	16.7996	233.1346
Panicallenth(cm)	4.893133	13.16033	0.371809	0.712156	-21.7722	31.5585	-21.7722	31.5585	50	1330.7	26.614	8.717747
Biological yield (gram)/ plant	-0.87026	0.759526	-1.14579	0.259237	-2.4092	0.668685	-2.4092	0.668685	50	5083.6	101.672	2310.971
No. of grain per panical	1.067761	0.776798	1.374568	0.177534	-0.50618	2.641702	-0.50618	2.641702	50	7794.33	155.8866	2313.768
Test weight(gram)	9.653881	10.00182	0.965213	0.340705	-10.6117	29.91949	-10.6117	29.91949	50	1033.81	20.6762	11.65336

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	12	644381.8	53698.48	1.379822	0.219013
Residual	37	1439927	38916.95		
Total	49	2084309			

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1507640	12	125636.6	33.41589	7.3E-60	1.767372
Within Groups	2394985	637	3759.788			
Total	3902625	649				

3.4. Principal Component Analysis (PCA) of Aromatic Rice Genotypes

Principal component analysis (PCA) was employed to assess the genetic diversity among 50 aromatic rice genotypes based on multiple agronomic traits. The first principal component (PC1) accounted for the maximum proportion of total variability and was primarily influenced by key phenological traits such as days to maturity (0.550), days to 50% flowering (0.546), and days to 100% flowering (0.545), suggesting these traits play a pivotal role in genotype discrimination. The second principal component (PC2) reflected variations associated with structural attributes like plant height (0.420) and flag leaf length (0.341), while PC3 highlighted variability through plant height (0.489) and flag leaf width (0.453), consistent with previous findings that plant architecture significantly contributes to trait divergence in rice populations [10,17]. The PCA biplot revealed that the check variety PB-1121 was positioned near the origin, indicating an intermediate phenotype across most traits, while outlier genotypes such as AR-35 and AR-41 demonstrated pronounced divergence, particularly along PC1 and PC2, signaling their unique genetic makeup [14]. High-performing lines such as AR-6, AR-24, AR-12, and AR-4 displayed moderate but meaningful separation from the check, indicating both shared and novel expressions of yield-contributing traits. These patterns underscore the utility of PCA in breeding programs, as it enables the identification of genetically distant and agronomically valuable lines for hybridization and selection [13]. The overall pattern of trait contribution and genotype clustering is clearly depicted in **Figure 2**, reinforcing the relevance of PCA as a robust multivariate tool for diversity analysis in rice improvement research [24].

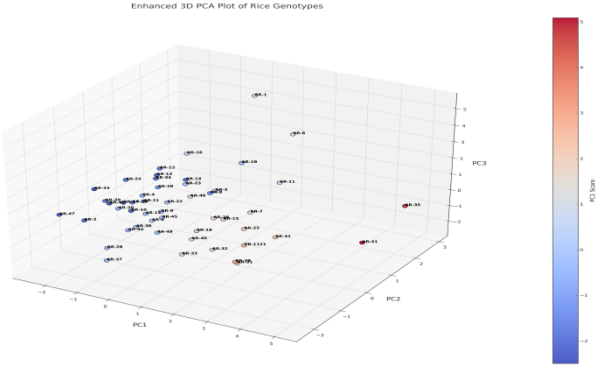

Figure 2. Enhanced 3D PCA plot showing the distribution of 50 aromatic rice genotypes based on principal components (PC1, PC2, and PC3).

3.5. Path Coefficient Analysis of Yield-Related Traits in Aromatic Rice

Path coefficient analysis provided a comprehensive understanding of the direct and indirect effects of key agronomic traits on grain yield per plant, thereby informing selection strategies in aromatic rice breeding. Plant height emerged as the most influential trait, with a substantial direct effect of 0.295 and the highest total correlation (0.353), indicating that increased plant stature positively influences grain development through improved biomass accumulation and nutrient partitioning [11]. Tillers per plant exhibited a notable direct effect of 0.215 and a total correlation of 0.193, though its slight negative indirect contribution (-0.022) hints at trade-offs with other traits, possibly due to intra-plant competition for resources [5]. Panicle length, while contributing a relatively lower direct effect (0.071), compensated with an indirect effect of 0.111, leading to a respectable total correlation of 0.183. This suggests that its impact on yield is mediated through correlated traits such as grain number per panicle and fertility [9]. Such insights support the prioritization of traits that show both high direct effects and complementary interactions—particularly plant height and tiller number—as prime candidates for enhancing yield potential. These interrelationships are effectively visualized in **Figure 3**, which depicts the direction and strength of both direct and indirect effects, thus emphasizing the multivariate complexity of yield determination in aromatic rice [15]. Incorporating such findings into breeding programs could significantly accelerate the development of superior genotypes by targeting the most impactful traits in a structured selection framework.

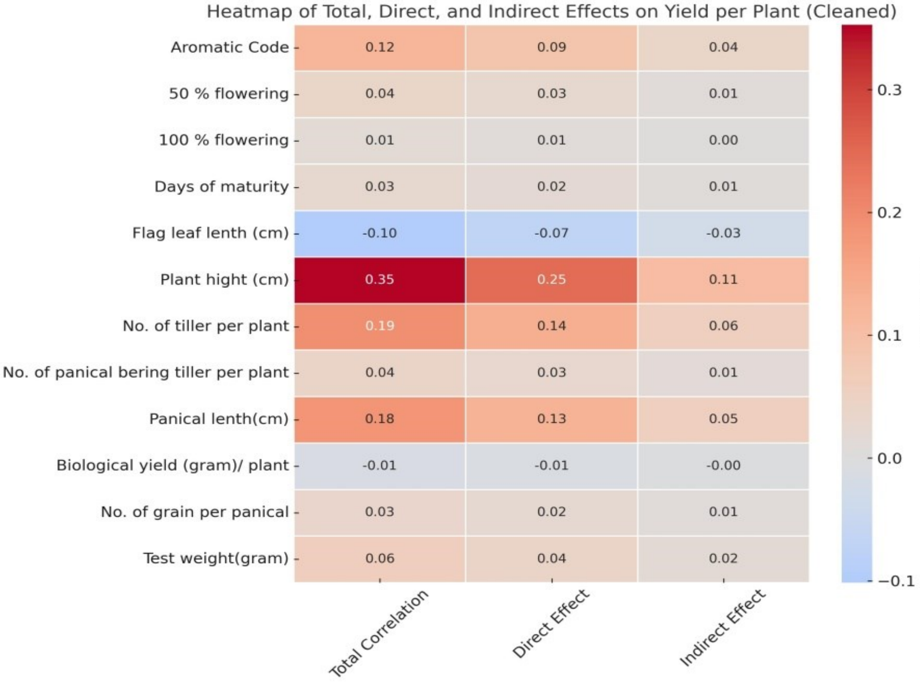

Figure 3.Heatmap of Total, Direct, and Indirect Effects of Agronomic Traits on Grain Yield per Plant in Aromatic Rice Genotypes

3.6. Correlation Analysis of Agronomic and Yield Traits in Aromatic Rice Genotypes

The correlation analysis from provides key insights into the relationships among phenological, morphological, and yield-contributing traits in aromatic rice, laying a robust foundation for strategic selection in breeding programs. A highly significant and positive correlation was observed between 50% flowering and 100% flowering (r = 0.997), as well as with days to maturity (r = 0.990 and 0.986, respectively), reflecting synchronized phenological development among genotypes—an important feature for achieving uniform flowering and harvest windows [19]. Grain yield per plant displayed a strong and positive correlation with biological yield (r = 0.846), reinforcing the understanding that higher vegetative biomass significantly contributes to improved grain output [22]. The number of grains per panicle (r = 0.588) also had a notable positive association with grain yield, indicating its relevance as a reproductive trait for yield improvement. Moderate but meaningful correlations were observed with test weight (r = 0.385), underscoring the dual importance of grain quality and quantity [8]. Additionally, yield showed weaker but positive correlations with traits such as plant height, tillers per plant, and flag leaf dimensions, suggesting that these secondary traits may contribute additively or indirectly to productivity. These interrelationships are visually summarized in **Figure 4**, which depicts the strength and direction of correlations among traits, thereby enhancing the understanding of yield architecture and enabling the identification of superior genotypes for varietal development and selection in aromatic rice improvement programs [4].

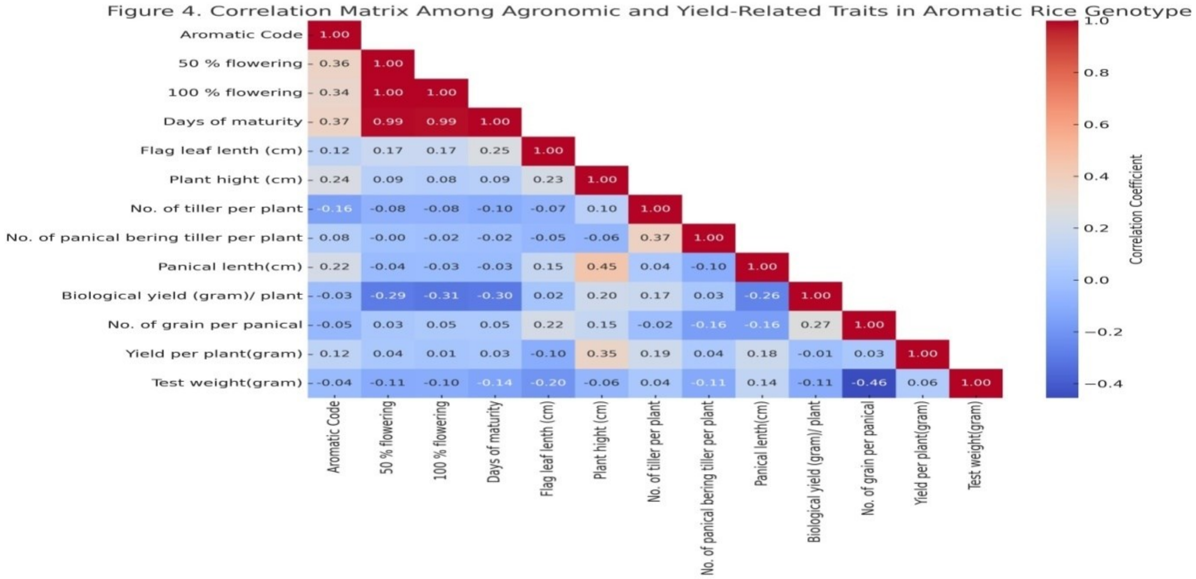

Figure 4. Correlation Matrix Among Agronomic and Yield-Related Traits in Aromatic Rice Genotypes

3.7. Cluster-Based Genetic Divergence of Aromatic Rice Genotypes

The cluster matrix and hierarchical dendrogram (Figure 5), constructed using Ward's method based on multivariate agronomic trait data, revealed distinct phenotypic divergence among the 50 aromatic rice genotypes, providing critical insights for strategic parent selection and hybridization programs. Four major clusters emerged, each reflecting genotypes with similar agronomic performance. Cluster I consisted of superior yield performers such as AR-35 (40.4 g/plant), AR-26 (39.8 g), AR-9 (38.6 g), and AR-7 (38.1 g), characterized by elevated biological yield, tall plant stature (>105 cm), and long panicle length (>26 cm), making them ideal candidates for yield-oriented improvement [21]. Cluster II included genotypes like AR-4, AR-10, AR-12, and AR-39, which showed moderate yield levels (31–35 g/plant), consistent phenologicalbehavior (105–110 days to flowering), and favorable test weights—attributes suited for broad adaptability and stability under standard field conditions [12]. Cluster III, housing the check variety PB-1121 (30.5 g/plant), AR-25 (32.3 g), and AR-38 (31.6 g), demonstrated average yield with balanced performance across traits, representing a control group for assessing genetic advancement. In contrast, Cluster IV grouped relatively low-yielding genotypes like AR-1, AR-6, AR-11, and AR-22 (25–29 g/plant), which also showed reduced plant height (<95 cm), suggesting limited direct yield potential but offering possible value for early maturity breeding objectives [3]. The clustering pattern demonstrated strong within-cluster cohesion and significant between-cluster divergence, confirming the existence of meaningful genetic variability that can be exploited to maximize heterosis and enhance trait recombination in aromatic rice breeding pipelines [6].

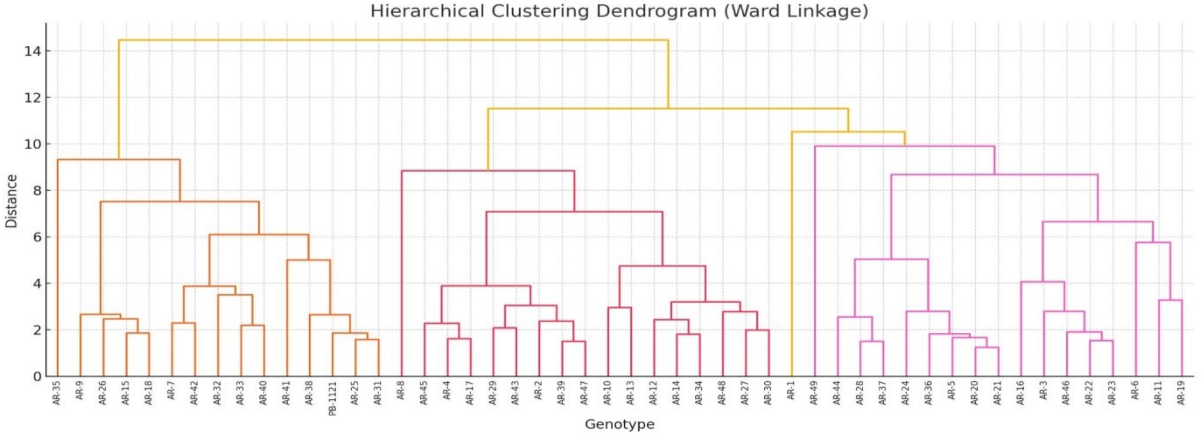

Figure 5. Hierarchical Clustering Dendrogram of 50 Aromatic Rice Genotypes Based on Agronomic Traits Using Ward's Method

CONCLUSION

The comprehensive evaluation of 50 aromatic rice genotypes, benchmarked against the standard variety PB-1121, revealed substantial phenotypic and genotypic diversity across key agronomic and yield-related traits. This diversity provides a strong foundation for genetic improvement and the development of high-performing cultivars tailored to specific agronomic goals.

Regression and ANOVA analyses identified moderate trait associations with grain yield ($R^2 = 0.309$), with 100% flowering showing a statistically significant negative effect, highlighting its potential as a selection constraint in yield optimization. Although the overall regression model was not statistically significant, the significant variability among genotypes across nearly all traits, as confirmed by one-way ANOVA ($F = 33.42$, $p < 0.001$), underscores the importance of multivariate approaches for trait dissection.

Principal Component Analysis (PCA) and cluster analysis revealed that phenological traits (e.g., flowering and maturity) and structural traits (e.g., plant height and panicle architecture) are key drivers of genetic differentiation. Genotypes such as AR-6, AR-24, and AR-12 emerged as ideotypes, combining early maturity, high biomass, and superior yield potential. These genotypes were not only outliers in PCA space but also demonstrated high direct effects on yield in path coefficient analysis, confirming their importance in breeding pipelines.

Correlation analysis reinforced these results, showing strong positive associations between grain yield and traits such as biological yield, grains per panicle, and test weight. The combined use of correlation, path, and PCA analyses enabled a deeper understanding of yield architecture and trait interdependencies, providing a robust framework for indirect selection strategies.

Cluster analysis further grouped genotypes based on multi-trait similarity, offering valuable insights for hybridization planning and heterosis exploitation. The strong divergence between clusters confirms the availability of genetically distant parental lines suitable for breeding high-yielding, stable aromatic rice varieties.

Overall, this study provides a statistically grounded, biologically meaningful basis for future varietal development in aromatic rice. The identified elite genotypes and key traits serve as critical resources for breeders aiming to enhance yield potential while preserving desirable aromatic qualities.

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