

Trait Variability, Genotypic Relationships, and Cluster Analysis of Aromatic Rice RILs for Agronomic Improvement

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ABSTRACT

Aromatic rice is renowned for its premium grain quality and export potential, yet breeding efforts are often hampered by low yield and limited genetic diversity. This study evaluated 48 recombinant inbred lines (RILs), derived from a cross between Rajendra Bhagwati and Pusa Sugandh-5, to identify elite genotypes with superior agronomic and physiological traits. Field experiments were conducted during the *Kharif* seasons of 2023 and 2024 using a randomized block design with three replications. Twelve traits were assessed: days to 50% flowering (DFF), flag leaf area (FLA), days to physiological maturity (DPM), plant height (PH), number of tillers per plant (NOT), panicle length (PL), number of spikelets per panicle (NSP), number of grains per panicle (NGP), spikelet fertility percentage (SF%), test weight (TW), biological yield per plant (BY), and grain yield per plant (GYP). Substantial phenotypic variability was observed, with high coefficients of variation for traits like FLA, PH, NSP, and GYP. Genotypic correlation analysis identified strong positive associations between GYP and traits such as BY, NGP, and PL, indicating their significance in indirect selection. Rajendra Bhagwati exhibited early flowering and superior grain yield, while Pusa Sugandh-5 showed higher tiller number and test weight. Hierarchical cluster analysis grouped genotypes into three distinct clusters, with Cluster III containing transgressive segregants outperforming both parents in yield-contributing traits. The study demonstrates the effectiveness of integrating phenotypic screening and multivariate analysis to identify promising RILs for aromatic rice improvement and varietal development under diverse agro-climatic conditions.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, with aromatic rice occupying a unique segment due to its sensory quality and export potential (Khush, 1997; IRRI, 2013). In India, the demand for high-yielding, quality aromatic rice cultivars is increasing steadily to meet both domestic consumption and international market expectations. However, the productivity of aromatic rice is often constrained by its narrow genetic base and susceptibility to abiotic and biotic stresses (Bisne & Sarawgi, 2008; Singh *et al.*, 2018). Therefore, enhancing yield potential while maintaining grain quality traits remains a central challenge in aromatic rice breeding.

Recombinant inbred lines (RILs), developed through the single seed descent (SSD) method, provide a stable and genetically diverse platform for dissecting trait inheritance and identifying superior genotypes (Brim, 1966; Collard & Mackill, 2008). Such populations are invaluable for evaluating phenotypic variability, understanding inter-trait relationships, and exploiting transgressive segregation for agronomic enhancement (Manickavelu *et al.*, 2007). Earlier studies have emphasized the effectiveness of RILs in mapping quantitative trait loci and trait-based selection in rice (Mohammadi & Prasanna, 2003; Yadav *et al.*, 2013).

Phenotypic evaluation of morphological and yield-contributing traits is critical for genetic improvement and varietal selection.

Traits such as days to 50% flowering, flag leaf area, plant height, panicle length, spikelet fertility, and grain yield per plant have been identified as key contributors to yield variability in rice (Kumar *et al.*, 2015; Prakash *et al.*, 2021; Ahmed *et al.*, 2022). Moreover, genotypic correlations among these traits offer insights into indirect selection strategies, facilitating the simultaneous improvement of multiple complex traits (Singh *et al.*, 2019; Verma *et al.*, 2021).

Cluster analysis based on multivariate data is a powerful approach to classify genotypes and understand genetic divergence within breeding populations. By grouping genotypes with similar trait profiles, breeders can select promising lines for targeted improvement programs (Kumar *et al.*, 2020). Such trait-based clustering is particularly valuable in aromatic rice, where balancing grain quality with yield stability is essential.

Given the above considerations, the present study aimed to evaluate a population of 48 RILs derived from a cross between two contrasting aromatic rice varieties—Rajendra Bhagwati and Pusa Sugandh-5. The objectives were to (i) assess variability in key agronomic and physiological traits, (ii) determine genotypic correlations to identify yield-contributing traits, and (iii) perform cluster analysis to classify genotypes for selection in breeding programs. This integrated approach is expected to identify elite lines with desirable trait combinations for the development of improved aromatic rice cultivars.

2. Material and Methods

2.1. Plant Materials and Development of RILs Population

The experimental material comprised 48 recombinant inbred lines (RILs) derived from a cross between two contrasting aromatic rice varieties: Rajendra Bhagwati (RB), a semi-dwarf line with dark green foliage and long slender aromatic grains, and Pusa Sugandh-5 (PS-5), a high-yielding variety with extra-long grains and resistance to shattering (Bisne & Sarawgi, 2008; Singh *et al.*, 2018). The initial cross and F₁ generation were developed in 2018 at the Student's Instructional Farm (SIF), Acharya Narendra Deva University of Agriculture and Technology (ANDUAT), Kumarganj, Ayodhya. The RIL population was advanced using the single seed descent (SSD) method (Brim, 1966; Singh, 2016), culminating in stable F₆ lines. These lines, exhibiting broad genetic variation, were evaluated for morphological and diversity analyses during the Kharif seasons of 2023 and 2024 (Khush, 1997; Collard & Mackill, 2008).

2.2. Experimental Design and Field Management

A Randomized Block Design (RBD) with three replications was implemented to evaluate the RILs along with their parents. Each plot consisted of six rows (1.5 m × 5 m), with data collection limited to the central four rows to mitigate border effects. Spacing was standardized at 0.25 m between rows, 0.5 m between plots, and 1 m between blocks. Uniform agronomic practices were followed. Nitrogen was supplied via urea at 100 kg N/ha in three splits—at sowing, tillering, and panicle initiation—while phosphorus was supplied using DAP at 124.5 kg/ha as a basal dose. Manual weeding was conducted three times, and no pesticides were applied to allow for natural pest and disease interactions (FAO, 2006; Gomez & Gomez, 1984; IRRRI, 2013).

2.3. Phenotypic Trait Evaluation

Phenotypic assessments were carried out in accordance with the DUS (Distinctness, Uniformity, and Stability) guidelines under the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPV&FR Authority, 2001). Twelve agro-morphological traits were recorded: days to 50% flowering (DFF), flag leaf area (FLA), days to physiological maturity (DPM), plant height (PH), number of tillers per plant (NOT), panicle length (PL), number of spikelets per panicle (NSP), number of grains per panicle (NGP), spikelet fertility percentage (SF%), 1000-grain weight (TW), biological yield (BY), and along with grain yield per plant (GYP). For each entry, three randomly selected plants were observed, and their mean values were used in the analysis.

2.4. Statistical Analysis and Data Visualization

All statistical computations and visualizations were conducted in R (version 4.4.0) using RStudio (version 2024.02). Descriptive statistics such as mean, standard deviation, and range were calculated using dplyr and psych (Wickham *et al.*, 2019; Revelle, 2024). Correlation coefficients among traits were generated and visualized using the corrplot package (Wei & Simko, 2021). Hierarchical cluster analysis was performed with Euclidean distance and Ward's method using FactoMineR, factoextra, and cluster (Le *et al.*, 2008; Charrad *et al.*, 2025). Visualization of

distributions and group-wise comparisons was accomplished using ggplot2 and ggpubr (Kassambara, 2018; Wickham & Sievert, 2009). Data from Excel files were read and processed using readxl and openxlsx to ensure workflow reproducibility (Ooms, 2024).

3. Results and Discussion

3.1. Phenotypic Evaluation and Trait-Based Variability Among Rice Genotypes

The assessment of aromatic rice genotypes revealed substantial variability across important agronomic and physiological traits, reflecting considerable genetic diversity (Figure 1). Days to 50% flowering (DFF) ranged from 85.0 days in 'RB' to 97.66 days in 'QRR 16', highlighting variability in flowering duration that can be exploited for early-maturing varieties in drought-prone or intensive cropping systems (Kumar *et al.*, 2020). Flag leaf area (FLA), a critical determinant of photosynthetic potential, was highest in 'QRR 15' (42.68 cm²), followed closely by 'QRR 16' (40.59 cm²), while the lowest was observed in 'RB' (20.83 cm²). Larger flag leaves are associated with higher assimilate production and grain filling efficiency (Kumar *et al.*, 2018). The duration from flowering to physiological maturity, expressed as days to physiological maturity (DPM), varied slightly among genotypes, with the highest in 'QRR 15' (127.66) and the lowest in 'RB' (114.66), showing diversity in growth periods. Plant height (PH) ranged from 103.20 cm in 'PS-5' to 127.06 cm in 'QRR 17', indicating the availability of both tall and semi-dwarf plant types, which is critical for selecting lodging-resistant and high-yielding cultivars (Sarkar *et al.*, 2019). The number of tillers per plant (NOT) ranged from 9.33 ('QRR 16') to 13.66 ('PS-5'), while panicle length (PL) varied between 22.36 cm ('QRR 16') and 27.36 cm ('RB'), indicating strong yield component variability (Meena *et al.*, 2021). Number of spikelets per panicle (NSP) and number of grains per panicle (NGP) were also variable, with 'QRR 16' showing the highest NSP (240.33) and NGP (211.33), although this did not translate into the highest yield. Spikelet fertility (SF%) was highest in 'QRR 17' (88.10%) and lowest in 'QRR 15' (82.17%), directly impacting grain filling. Test weight (TW), indicative of grain density and size, was highest in 'RB' (23.06 g), followed by 'PS-5' (22.73 g), and lowest in 'QRR 16' (13.94 g). Biomass yield per plant (BY) ranged from 31.66 g to 53.33 g, while grain yield per plant (GYP) ranged from 14.32 g ('QRR 15') to 22.74 g ('RB'). Among all, 'RB' emerged as the most promising genotype, combining early flowering, high test weight, large panicle length, and the highest grain yield, making it a valuable parent for future breeding programs targeting yield enhancement in aromatic rice. The performance of 'PS-5' was also notable for tiller number and grain yield. These findings are in line with previous research, which emphasized the critical role of traits like flag leaf area, tiller number, spikelet fertility, and test weight in determining rice yield potential (Singh *et al.*, 2023; Prakash *et al.*, 2021; Ahmed *et al.*, 2022). Therefore, genotypes such as 'RB' and 'PS-5' are recommended for further evaluation and incorporation in breeding pipelines aimed at enhancing productivity in aromatic rice.

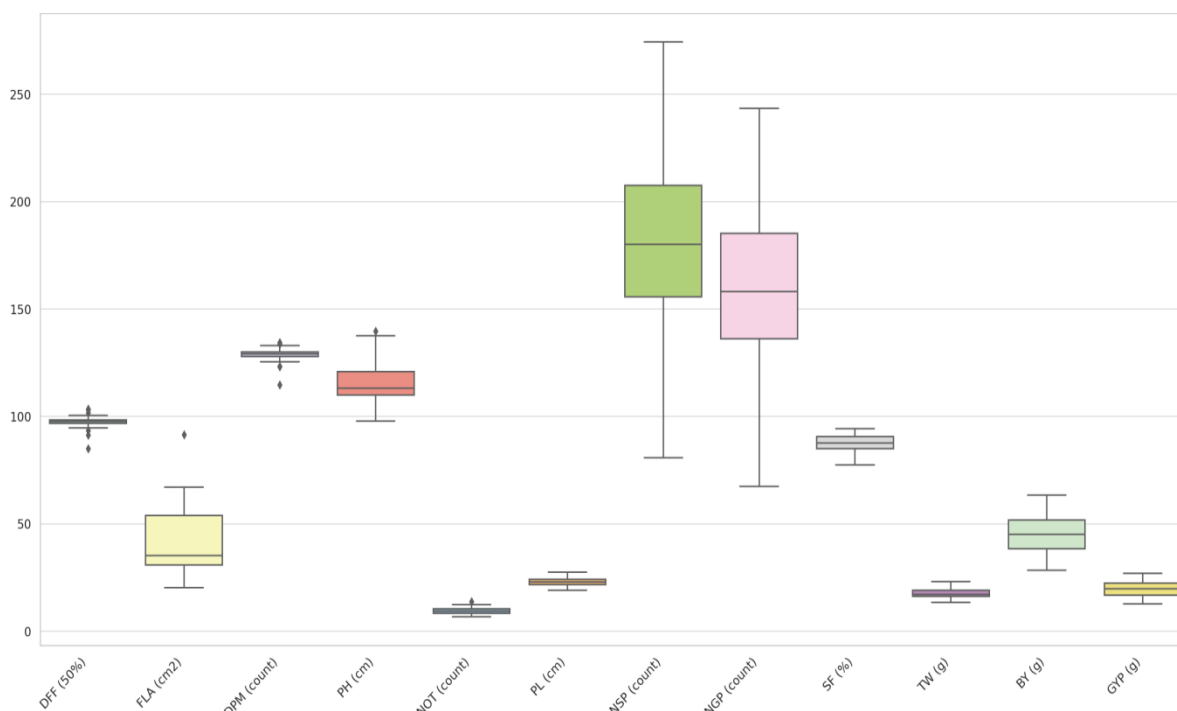


Figure 3.1. Comparative analysis of agronomic and physiological traits among selected aromatic rice genotypes.

3.2. Variability Analysis of Morphological and Yield Traits

The analysis of variability for thirteen morphological and yield-related traits among the evaluated rice genotypes revealed substantial differences, suggesting the presence of considerable genetic variability (Table 1; Figure 1). The mean days to 50% flowering (DFF) was 97.52 days, ranging from 85.00 to 104.00 days, indicating moderate variability, which is crucial for the selection of early-maturing genotypes. Flag leaf area (FLA) showed wide variation, with a mean of 41.18 cm² and a coefficient of variation (CV) of 235.98%, suggesting a highly diverse population in terms of photosynthetic potential. The number of days to physiological maturity (DPM) had a mean of 128.77 days, reflecting moderate variability with a CV of 8.95%. Plant height (PH) ranged from 97.83 cm to 134.20 cm, showing high variability (CV = 101.14%), which could be exploited for developing ideal plant types.

The number of tillers per plant (NOT) had a relatively lower CV (2.44%), indicating lesser environmental influence and higher heritability potential. Panicle length (PL) and number of

spikelets per panicle (NSP) showed moderate variability (means of 22.71 cm and 185.29, respectively), while the number of grains per panicle (NGP) exhibited a higher mean (162.20) and wide range (67.33 to 275.33), reflecting strong selection scope for yield traits. Spikelet fertility percentage (SF) averaged 87.45% with a CV of 15.62%, indicating stable reproductive efficiency.

Test weight (TW) and biological yield per plant (BY) had means of 17.65 g and 45.03 g, respectively, with CVs of 4.51% and 72.16%, signifying good variability. Importantly, grain yield per plant (GYP) demonstrated high diversity with a mean of 19.75 g, a wide range (12.63-32.87 g), and a CV of 12.45%, highlighting the potential of elite genotypes for yield improvement. These findings are consistent with earlier studies that emphasized the importance of variability in yield-contributing traits in rice for successful breeding (Kumar *et al.*, 2015; Singh *et al.*, 2018; Verma *et al.*, 2021).

Table 3.1. Descriptive statistics (Mean, SE(d), C.D. at 5%, Min, Max, and C.V.%) for agronomic and yield traits in rice genotypes.

	DFF (50%)	FLA (cm ²)	DPM (count)	PH (cm)	NOT (count)	PL (cm)	NSP (count)	NGP (count)	SF (%)	TW (g)	BY (g)	GYP (g)
Mean	97.52	41.18	128.77	115.21	9.39	22.71	185.29	162.20	87.45	17.65	45.03	19.76
SE(d) ±	0.38	2.17	0.42	1.42	0.22	0.26	5.65	5.19	0.56	0.30	1.20	0.50
C.D. at 5%	2.72	15.36	2.99	10.06	1.56	1.80	39.93	36.70	3.95	2.12	8.49	3.53
C.V. (%)	7.38	235.98	8.95	101.14	2.44	3.26	1594.60	1346.75	15.62	4.51	72.16	12.45
Min	85.00	20.25	114.67	97.83	6.67	18.90	80.67	67.33	77.32	13.27	28.33	12.63
Max	103.33	91.51	134.33	139.67	13.67	27.37	274.33	243.33	94.31	23.06	63.33	26.94

Where, S.E.= standard error, C.D.= critical difference, C.V.= coefficients of variation, DFF= days to 50% flowering, FLA= flag leaf area, DPM= days to physiological maturity, PH= plant height, NOT= number of tillers per plant, PL= panicle length, NSP=

number of spikelets per panicle, NGP= number of grains per panicle, SF= spikelet fertility, TW= test weight, BY= biological yield, GYP= grain yield per plant.

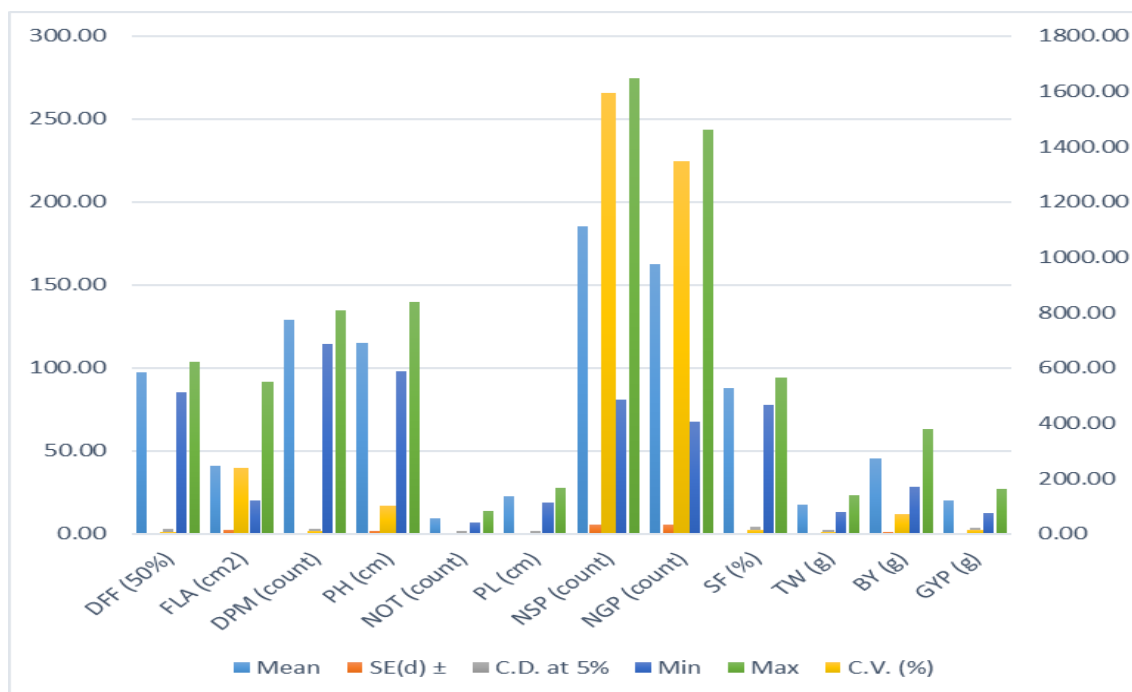


Figure 3.2. Variability graph showing Mean, SE(d), C.D. at 5%, Minimum, Maximum, and Coefficient of Variation (%) for 12 morphological and yield traits in rice genotypes.

3.3. Genotypic Correlation Analysis Among Morphological and Yield-Related Traits

Understanding the interrelationship among agronomic traits is vital in rice breeding programs to identify key yield-contributing components and formulate effective indirect selection strategies. The genotypic correlation matrix among twelve morphological and yield-related traits in the present study revealed several significant and biologically meaningful associations, indicating the presence of strong genetic linkages that can be effectively harnessed for the simultaneous improvement of multiple traits (Table 2).

A highly significant and positive correlation was observed between days to 50% flowering (DFF) and days to physiological maturity (DPM) ($r = 0.960$), suggesting that early-flowering genotypes tend to reach maturity earlier. This relationship highlights the potential for selecting early-maturing lines based on phenological indicators, such as flowering time, particularly in time-bound or water-limited environments (Kumar *et al.*, 2016). Additionally, DFF exhibited a moderate positive correlation with flag leaf area (FLA) ($r = 0.273$), indicating that genotypes with larger photosynthetic area tend to flower later, possibly due to prolonged vegetative growth. Since flag leaf area plays a pivotal role in grain filling, this relationship may influence yield indirectly.

Conversely, number of tillers per plant (NOT) showed a negative correlation with DFF ($r = -0.344$), DPM ($r = -0.344$), and plant height (PH) ($r = -0.324$), implying that early-maturing and semi-dwarf genotypes may produce more tillers, which is advantageous for sink development and biomass accumulation. These negative correlations suggest that breeders aiming for higher tillering genotypes should prioritize early-maturing and moderately short plant types to avoid excessive lodging and

resource competition (Verma *et al.*, 2021). Moreover, PH had a weak and negative correlation with DFF and DPM ($r = -0.027$ and -0.066 , respectively), suggesting limited direct influence of plant stature on crop duration.

Among yield-attributing traits, grain yield per plant (GYP) demonstrated a strong positive association with biological yield (BY), indicating that greater biomass production generally translates into increased grain output. Likewise, GYP was positively correlated with the number of grains per panicle (NGP), reflecting the critical role of panicle fertility and grain setting efficiency in enhancing final yield. Supporting this, NGP also correlated positively with the number of spikelets per panicle (NSP) and panicle length (PL), suggesting that longer panicles bearing more spikelets contribute to higher grain numbers, thereby serving as vital yield determinants (Singh *et al.*, 2019).

Interestingly, spikelet fertility (SF) and test weight (TW) displayed weaker or variable correlations with most traits, indicating that these may be governed by distinct genetic pathways or influenced by specific environmental cues. Nonetheless, their stability and direct influence on grain quality and yield merit consideration during varietal selection.

The overall findings from the genotypic correlation analysis suggest that traits such as NGP, NSP, PL, and BY exhibit strong and positive associations with grain yield, making them ideal selection indices in breeding programs aimed at yield enhancement. These results are in agreement with earlier reports highlighting the effectiveness of indirect selection through positively correlated component traits (Kumar *et al.*, 2016; Singh *et al.*, 2019; Verma *et al.*, 2021). Integrating such correlations into selection strategies can significantly improve genetic gain in rice improvement programs, especially under aromatic or high-value genotype evaluation frameworks.

Table 3.2. Genotypic Correlation Matrix Among Morphological and Yield-Related Traits in Rice Genotypes.

	DFF (50%)	FLA (cm ²)	DPM (count)	PH (cm)	NOT (count)	PL (cm)	NSP (count)	NGP (count)	SF (%)	TW (g)	BY (g)	GYP (g)
DFF (50%)	1.000											
FLA (cm ²)	0.273	1.000										
DPM (count)	0.960	0.293	1.000									
PH (cm)	-0.027	0.036	-0.067	1.000								

NOT (count)	-0.344	-0.023	-0.344	0.32 4	1.000							
PL (cm)	-0.274	0.057	-0.289	0.16 7	0.185	1.00 0						
NSP (count)	0.018	-0.131	-0.004	0.15 0	-0.228	0.13 3	1.000					
NGP (count)	0.013	-0.050	-0.004	0.14 6	-0.267	0.07 9	0.984	1.000				
SF (%)	0.002	0.389	0.031	0.01 3	-0.306	0.25 5	0.175	0.341	1.00 0			
TW (g)	-0.276	0.165	-0.306	0.20 2	0.078	0.15 8	-0.408	-0.374	0.04 9	1.00 0		
BY (g)	-0.205	-0.040	-0.190	0.13 1	0.382	0.20 2	0.012	0.031	0.07 6	0.10 0	1.00 0	
GYP (g)	-0.156	0.038	-0.151	0.14 5	0.393	0.21 0	-0.050	-0.033	0.05 0	0.17 1	0.98 0	1.00 0

Where, DFF= days to 50% flowering, FLA= flag leaf area, DPM= days to physiological maturity, PH= plant height, NOT= number of tillers per plant, PL= panicle length, NSP= number of spikelets per panicle, NGP= number of grains per panicle, SF= spikelet fertility, TW= test weight, BY= biological yield, GYP= grain yield per plant.

3.4. Genetic Divergence and Trait-Based Clustering of RILs

The hierarchical cluster analysis using Ward's method grouped 48 recombinant inbred lines (RILs) and their two parental lines into three distinct clusters based on twelve agro-morphological and yield-related traits: days to 50% flowering (DFF), flag leaf area (FLA), days to physiological maturity (DPM), plant height (PH), number of tillers (NOT), panicle length (PL), number of spikelets per panicle (NSP), number of grains per panicle (NGP), spikelet fertility (SF%), test weight (TW), biological yield (BY), and grain yield per plant (GYP).

The dendrogram (Figure 1) revealed significant genetic diversity and grouped the genotypes into three major clusters at a Euclidean distance of approximately 12.

Cluster I, which included the parent Rajendra Bhagwati and 17 RILs, showed early flowering (low DFF), moderate plant height, and relatively higher spikelet fertility (SF%), which are desirable traits under short-season or water-limited environments. These genotypes also demonstrated intermediate performance in grain yield (GYP) and biological yield (BY), suggesting stability across multiple traits.

Cluster II comprised 13 RILs along with the parent Pusa Sugandh-5. This group showed moderate DFF and FLA, higher values for

panicle length (PL) and spikelet number (NSP), indicating their genetic closeness to the Pusa Sugandh-5 background. These lines were characterized by balanced trait combinations, especially test weight (TW) and days to maturity (DPM), indicating their potential for quality grain and adaptability.

Cluster III included 18 RILs that recorded superior performance in yield-related traits, notably GYP, BY, NOT, and NGP. These lines exhibited longer panicles, higher test weight, and greater spikelet number, traits directly contributing to improved yield. This cluster likely includes transgressive segregants, which outperform both parents in certain traits due to recombination.

The clustering revealed that grain yield (GYP) and its associated traits such as TW, NSP, NGP, and BY had a significant role in defining groupings, reaffirming their importance in genotype differentiation and selection. These results are in agreement with previous studies where hierarchical clustering based on multivariate agro-morphological traits has been effectively used to exploit genetic diversity and identify elite recombinants in rice (Kumar *et al.*, 2020; Mohammadi & Prasanna, 2003; Yadav *et al.*, 2013).

The presence of both parents in separate clusters also highlights the transgressive segregation among RILs and demonstrates the utility of recombinant inbred populations for creating novel genetic variability (Manickavelu *et al.*, 2007). Cluster III, having the most desirable yield traits, presents a valuable source of genetic material for further breeding and hybridization programs aimed at developing high-yielding rice cultivars with adaptability and resilience.

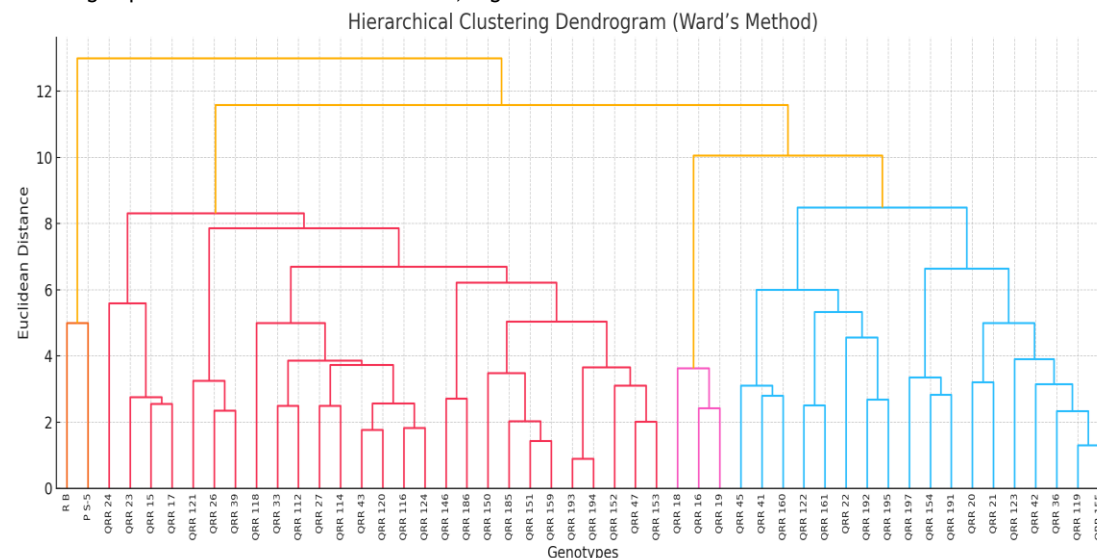


Figure 3.3. Hierarchical clustering dendrogram of 48 rice recombinant inbred lines (RILs) and their parents based on twelve agro-morphological and yield-related traits using Ward's method and Euclidean distance.

Conclusion

The comprehensive evaluation of 48 aromatic rice recombinant inbred lines (RILs) and their parents, Rajendra Bhagwati and Pusa Sugandh-5, revealed substantial phenotypic and genetic variability across key agronomic and physiological traits. Traits such as days to 50% flowering, flag leaf area, plant height, tiller number, panicle length, and grain yield per plant demonstrated wide variation and high coefficients of variation, indicating strong potential for selection and improvement. Genotypic correlation analysis further highlighted the significance of grain number per panicle, panicle length, and biological yield as major contributors to grain yield, offering useful indirect selection indices. Rajendra Bhagwati exhibited early flowering and superior grain yield, while Pusa Sugandh-5 stood out for its high tillering and favorable test weight, making both parents valuable contributors to the RIL population's diversity.

The hierarchical cluster analysis using Ward's method grouped the genotypes into three major clusters, clearly illustrating the underlying genetic divergence among the RILs. Cluster III comprised genotypes with superior performance in yield-contributing traits and likely included transgressive segregants surpassing both parents. The identification of elite genotypes with desirable combinations of early maturity, high biomass, and improved grain yield underscores the potential of this population for advancing aromatic rice breeding programs. These findings validate the effectiveness of phenotypic selection and trait-based clustering in identifying promising genotypes for genetic improvement and varietal development under diverse agro-climatic conditions.

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