

AGRONOMIC PERFORMANCE AND GRAIN QUALITY ASSESSMENT OF SMALL-GRAIN AROMATIC RICE ADVANCED BREEDING LINES IN BANGLADESH

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ABSTRACT

In Bangladesh, where rice plays a crucial role in both sustenance and economy, aromatic rice varieties have gained prominence due to their distinct taste and fragrance. This study evaluated the agronomic performance, grain quality, and cooking properties of four advanced breeding lines of small-grain aromatic rice that we developed at the Advanced Seed Research and Biotech Centre (ASRBC), ACI Limited, Bangladesh, alongside eighteen locally popular aromatic rice varieties and three check varieties. The study identified ASRBCR 1017-B-12-3 and ASRBCR 1017-B-13-2 as superior genotypes, exhibiting excellent results in terms of effective tillers, grains per panicle, aroma and overall yield. A greater plant height (168cm) was observed in Chinigura, while ASRBCR 1017-B-13-1 had the lowest height (90cm). ASRBCR 1017-B-13-2 had the maximum tiller count (12), and ASRBCR 1017-B-18-2 had the highest number of grains per panicle (350). Bashful (Golden) had the highest thousand-grain weight (19.17g). Detailed assessments of grain quality parameters revealed significant variations in grain length, length-to-breadth ratio, aroma intensity, and cooked rice elongation among the tested genotypes. The Bashful variety (golden) had the longest grain length (7.13mm), and the Madhumala variety had the highest length-to-breadth ratio. All four advanced breeding lines exhibited a grain length of approximately 4mm and a grain breadth ranging from 2.12 to 2.48mm, with a cooked rice elongation ratio ranging from 1.72 to 1.85. In addition, aroma/fragrance genotyping using SSR marker BADEX7-5 showed the presence of fragrance in all the test advanced lines, with common banding patterns, but absence of bands for aroma in Madhumala and BRRI dhan90. Results provide insights into trait interactions, choice of genotypes, and breeding for optimum yields as well as quality in aromatic rice.

Keywords: Principal component analysis, Grain quality, Fragrance genotyping, Small grain rice

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1. INTRODUCTION

Rice (*Oryza sativa*) is among the world's most essential dietary commodities. Over fifty percent of the global population relies on it for dietary sustenance (Zhou et al. 2003; Ghadge and Prasad 2012). The Bangladeshi populace is extremely reliant on rice as its primary meal, which has a significant impact on the country's economy (Islam et al. 2024). Rice alone accounts for 97% of the dietary grain production in Bangladesh (BBS 2013). According to Hossain et al. (2008), over 80% of the calories and approximately 50% of the protein in the diet are provided by the average Bangladeshi citizen. Aroma, or scent, is an intrinsic quality of rice. Aromatic rice, while comprising a relatively minor proportion of the total rice consumption, is considered to be of the highest quality (Singh et al. 2000). Bangladesh, the fourth largest rice producer globally, dedicates 75% of its total cropped area to rice cultivation. Among these areas, aromatic fine rice varieties account for approximately 27% of the total (BBS 2015). The superior flavor, culinary qualities, and aroma of fine rice varieties increase consumer demand (Hossain et al. 2008). Bangladesh is renowned for its production of a diverse range of high-quality aromatic rice varieties. These varieties are suitable for everyday consumption, as well as for the preparation of special dishes such as polao, biryani, jarda, firni, and more.

In recent years, aromatic rice cultivation in Bangladesh has grown in prominence due to the enormous demand for this crop, which is supported by both domestic and international trade (Das and Baqui 2000). According to Rashid et al. (2017), the price of aromatic rice is usually two to three times higher than the price of coarse rice. Aromatic rice has been able to create more profits than other rice cultivars owing to its high price and cheap cultivation cost (Islam et al. 1996). In the majority of Asian countries, rice serves as the principal source of nutrients; hence, fragrant rice varieties are very important to the worldwide commerce within the rice industry. These rice varieties are gaining popularity

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in the United States, Europe, and the Middle East, where they have long been favored. Bangladesh exhibits promising potential in the export of aromatic rice. Since 1992, Bangladesh has been exporting modest amounts of aromatic rice, primarily to the United States. The aromatic rice varieties that are exported most frequently are Kalijira, Kataribhog, and Chinigura (Rahman 2000). Presently, there is a prevalent belief that Bangladesh has the potential to generate significant foreign exchange through the export of aromatic rice (Islam et al. 2012).

Regrettably, the fragrant rice cultivated in Bangladesh often exhibits unfavorable agronomic traits, including sensitivity to photoperiod, poor productivity, vulnerability to pests and diseases, and excessive shedding (Golam et al. 2011). The fluctuation in the amount of daylight over different seasons, often known as the photoperiod, is an important environmental signal that triggers the process of floral induction (Hayama and Coupland 2004; Yano et al. 2001). The duration of blooming is a crucial agricultural characteristic because it affects both effective reproduction and the optimal balance between the length of vegetative growth and reproductive development (Xu et al. 2014). Rice plants, which can adapt to different day lengths, undergo a phase shift from the vegetative to the reproductive phase in response to decreasing day length, as shown by Itoh et al. (2010). Hence, the ability to remain unaffected by changes in day length is a significant characteristic in agriculture that may be used to develop a lucrative kind of rice that can thrive at many latitudes, altitudes, and seasons (Khush 2001; Izawa 2007).

A multitude of more than one hundred aromatic rice landraces have been cultivated across various regions of Bangladesh. The majority of aromatic rice varieties are traditional varieties cultivated in rain-fed lowland ecosystems during the Aman season (Talukder et al. 2004; Das and Baqui 2000). But from November to May, during the Boro season, rice plants get more sunshine for longer periods of time, which means they absorb more solar energy and may produce more rice (Shahidullah et al. 2009). One possible way to increase the output of fragrant rice during the Boro season might be to choose cultivars that provide better yields with even a modest scent (Shahidullah et al. 2009).

The extraordinary taste and look of exquisite rice are driving up its demand on a worldwide and local scale. Improving rice's morphological features and culinary capabilities should be a top priority (Golam et al. 2011; Behera and Panda 2023). Plant height, heading days, days to maturity, panicle length, number of fertile grains per panicle, number of fertile tillers, thousand grain weight, and panicle number are some of the agronomic traits that affect rice grain production (Surek and Beser 2005). Both the number of grains per panicle and the quantity of effective tillers per panicle are quantifiable during the vegetative and reproductive phases, respectively. The stage with the greatest number of tillers is the most critical stage for determining the quantity of panicles (Feng et al. 2007). Typically, the weight of one thousand grains, an essential characteristic, is ascertained throughout the maturation process. As a quantitative characteristic, environmental fluctuations have a large impact on yield. The investigation of factors that impact yield places increased emphasis on addressing those factors (Kole and Hasib 2008; Prasad et al. 2001).

The selection of the appropriate variety is a crucial component in maximizing rice yield (Sarker 2002). Through our aromatic rice breeding program, we successfully created four advanced aromatic lines that are suitable for cultivation throughout the Aman and Boro seasons. These lines are not affected by changes in day length and possess a modern plant type, allowing them to be used as high-yielding varieties. The purpose of this research was to analyze and assess the productivity, milling, physical, and cooking features of our four small-grain aromatic rice lines in comparison to those of other indigenous aromatic rice cultivars.

2. MATERIALS AND METHODS

2.1. Source Materials

At ASRBC, we developed four advanced breeding lines of small grain aromatic/scented rice and evaluated them along with 18 regionally popular aromatic/scented rice varieties using three check varieties (Table 1). The experiment was conducted in three different locations in Bangladesh: Gazipur, Cumilla, and Rajshahi during the transplanted aman season (July–December 2022).

Table 1: List of contributing rice genotypes in this study

Sl.	Designation	Sl.	Designation	Sl.	Designation
1	ASRBCR 1017-B-12-3	10	Begun Mala	19	Kataribhog (Desi)
2	ASRBCR 1017-B-13-1	11	Bhatir Chikon	20	Kataribhog (Philippines)
3	ASRBCR 1017-B-13-2	12	BR5	21	Madhumala
4	ASRBCR 1017-B-18-2	13	BRRI dhan34	22	Radhuni pagal
5	Badshabhog	14	BRRI dhan90	23	Shakhorkora
6	Baoi Jhak	15	Chiniatap	24	Tulshimala
7	Baoibhog	16	Chinigura	25	Zirabhog
8	Bashful (Golden)	17	Chinikanai		
9	Begun Bitchi	18	Kalizira		

2.2. Experimental Design and Cultural Practices

The experiment was carried out using a randomized complete block (RCB) design, and there were three replications in the experiment. The area was 5.4 meters in length and had 5 rows (5.4 square meters). The plants were transplanted at a density of two to three plants per hill, with a distance of 20cm between each plant. Fertilizers doses of 120:19:60:20:3.6kg NPKSZn/ha (260-97-120-110-11kg/ha Urea-TSP-MOP-Gypsum-ZnSO₄, respectively) were applied in split applications of N at 15, 30, and 50 days after transplanting. Application of total P, K, S, and Zn was done in the final stage of land preparation. Weed management and irrigation were used in accordance with the requirements of the situation.

2.3. Phenotyping of Rice Germplasm

Phenotypic data analysis was conducted using five plants of each rice line or variety, which were selected at random. The statistical analyses were performed on the collected data: plant height (cm), number of effective tillers per plant, number of filled grains per panicle, thousand grain weight (g), days to maturity, and grain yield per plot (metric tons per hectare). R software was utilized for these analyses.

2.4. Grain Property Analysis

Following the harvesting process, the grains of each aromatic rice line or variety were dehulled to assess the quality of the grains, including grain morphology (length-to-width ratio), cooking time, fragrance (aroma), and cooked rice elongation. On the basis of their dimensions, the grains were categorized into various categories, as stated by Dela Cruz and Khush (2000). For each genotype, 40 seeds were submerged for ~1 h in 10mL of a 1.7% KOH solution in a conical flask placed at room temperature. The samples were graded as a score (1-4) for the level of aroma: 1 = if there was no aroma; 2 = if there was a low aroma; 3 = if there was a moderate aroma; and 4 = if there was a high aroma. The three individual scores were recorded, which were given by an expert panel of aromatic rice breeders.

2.5. Fragrance Genotyping in Rice

For the purpose of DNA isolation, leaves obtained from 25-day-old seedlings cultivated in ASRBC research fields were utilized. The modified potassium acetate method was utilized to extract genomic DNA (Sun et al. 2010). We performed a 10µL reaction with 10X PCR buffer, 25-30ng of template DNA, 1 U of Taq DNA polymerase (Invitrogen, USA), and 1.5mm MgCl₂. For the tested primer pair BADEX7-5 (Sakthivel et al., 2009), Table 2 shows the sequences, the best annealing temperature, and the expected product sizes. The PCR cycling conditions were as follows: initial denaturation for 3 min; 35 cycles of denaturation at 94°C for 30s, 30s of annealing at 54°C, and 1 minute of extension at 72°C; and a final extension at 72°C for 7min. gels were visualized using a Transilluminator (UVP gelSolo series, Analytik Jena) and amplified PCR product separation was performed using 8% acrylamide gel then stained with ethidium bromide. Gels were classified as fragrant and nonfragrant following the banding pattern.

Table 2: SSR marker for analysis of fragrance (aroma) in rice

Marker	Sequence	Annealing Temp (°C)	Allele no.	Chrm. locus	Expected fragment size in fragrant /non-fragrant rice varieties (bp)
BADEX7-5	F 5'-TGT TTT CTG TTA GGT TGC ATT-3' R 5'-ATC CAC AGA AAT TTG GAA AC-3'	54	2	5	95/103

2.6. Statistical Analysis

Principal component analysis and trait correlation were performed in R software using ggplot2 and Gally packages. Biplot and scree plots analyses were also performed in R using factoextra package. In addition, the data were subjected to analysis via ANOVA, and any mean discrepancies were determined using Duncan's multiple range test (DMRT) in R software using agricolae package.

3. RESULTS

3.1. Principal Component Analysis (PCA)

PCA was used to decrease the intricacy of the dataset while preserving the maximum amount of variation within the dataset. Table 3 displays the standard deviation, percentage of variance contribution, and variable loadings for the major four principal components (PCs). The first three principal components explained 86.06% of the total variability in yield-contributing traits in rice lines. The PCA demonstrated a high level of discriminatory ability for PC1 (with an eigenvalue of 3.255) and a lower level of discriminatory ability for PC4 (with an eigenvalue of 0.416).

Table 3: The eigenvalues, percentage of variance contribution, and variable loadings

	PC1	PC2	PC3	PC4
PH	0.412	0.259	0.467	-0.727
ET	-0.268	0.699	0.219	0.182
DM	-0.438	-0.109	-0.486	-0.656
GP	-0.464	0.285	0.221	-0.040
TGW	-0.275	-0.592	0.659	-0.001
YLD	-0.525	-0.004	0.120	-0.079
Standard deviation	1.804	1.125	0.802	0.645
Eigenvalue	3.255	1.266	0.643	0.416
Proportion of Variance	54.25	21.09	10.72	6.93
Cumulative Proportion	54.25	75.34	86.06	93.00

Legend: PH - Plant height (cm), ET - Number of effective tillers/plant, DM - Days to maturity, GP - Number of filled grains/panicle, TGW - 1000 seed weight (g), and YLD - Grain yield/plot (MT/hectare).

The scree plot shows the percentage of variance that was linked to each principal component because it shows the link between the eigenvalues and the principal component values. The first three principal components explained 86.06% of the total variation. Therefore, working with three principal components may result in the loss of only 13.94% of the information. After three PCs, a semi-curve line is created that fluctuates little between each PC and tends to become straight (Fig. 1). The graph clearly shows that PC1 experienced the most variance when compared to the other 5 PCs. Hence, choosing a few rice lines from these results will be helpful. The principal components based on the multiple eigenvalue data that captured the greatest variability exhibited by the rice lines were selected.

To better understand the association of the yield as well as quality parameters of the advanced rice lines developed by ASRBC with each other, a biplot was generated by plotting the first two principal components (PC1-54.3% and PC2-21.1%). Biplot results are shown in Fig. 2, where genotypes 1 (ASRBCR 1017-B-12-3), 3 (ASRBCR 1017-B-13-2), and 14 (BRRI dhan90) were placed at the upper right corner of the plot. All these genotypes had positive values for both the PCs and for the traits of effective tiller (ET), grains per panicle (GP), and yield per plot (YLD).

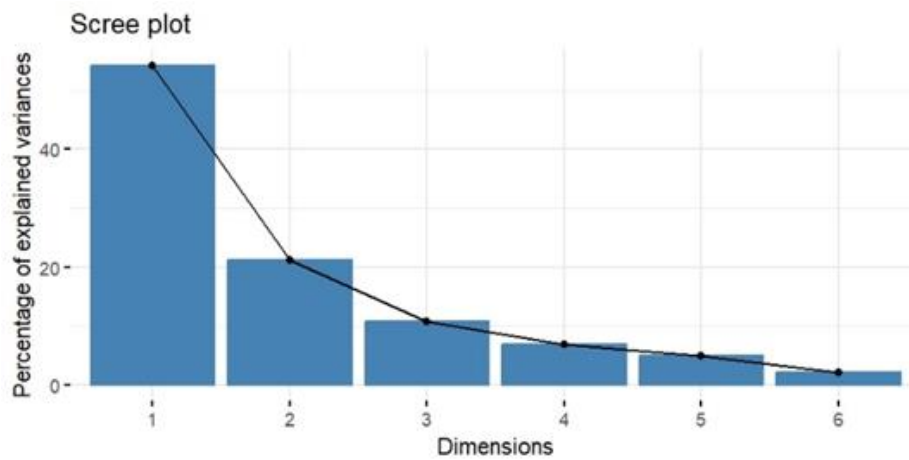


Fig. 1: Scree plot of principal component analysis of 25 small grain aromatic rice germplasm between eigenvalue and principal components

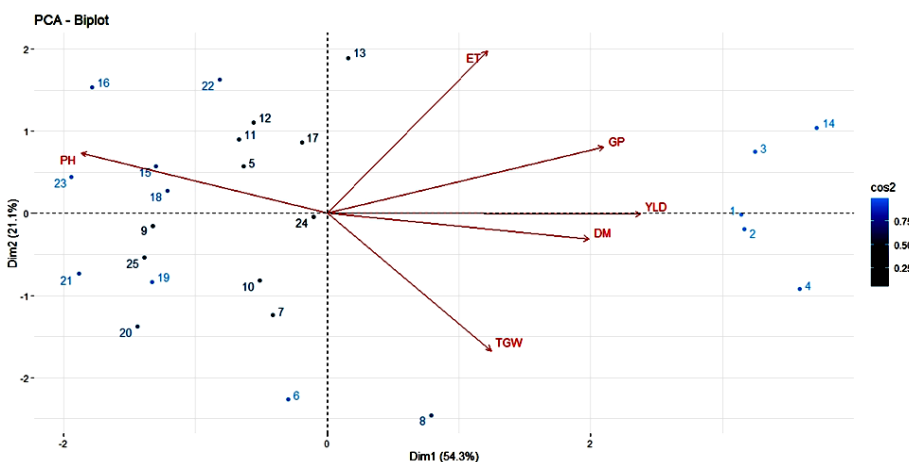


Fig. 2: The biplot of 25 small grain aromatic rice germplasm for PC1 and PC2

3.2. Yield Contributing Attributes and Yield

The plant height at maturity differed substantially among the studied rice genotypes. The greatest plant height was 168cm in Chinigura, while the lowest was 90cm in ASRBCR 1017-B-13-1 (Table 4). The total number of tillers per hill varied from 8 to 12. ASRBCR 1017-B-13-2 has the highest number of tillers per hill (12), closely followed by BRRI dhan90. The variance in tiller numbers might be attributed to genetic traits and varietal inheritance. Table 4 shows that ASRBCR 1017-B-18-2 had the most grains per panicle (350), whereas Madhumala had the fewest (82). It was noted that varieties with short, densely arranged, bold grains had a greater number of grains per panicle. The number of grains per panicle is an important criterion for high yields in rice cultivars. The highest weight of 1,000 grains (19.17g) was found in Bashful (Golden), whereas the lowest weight (9.83g) was observed in Begun bitchi. The weight of the thousand grains is an important parameter for the determination of the grain size, and it exerts a decisive influence on the total crop yield. Among 25 small grain rice genotypes, ASRBCR 1017-B-13-2 exhibited the highest grain yield (6.67 t ha⁻¹), as shown in Table 4. The increase in crop yield was due to the increase in the number of effective tillers per hill. The higher number of efficient tillers per panicle and grains per panicle contributed to the increased yield of the BRRI dhan90 (Fig. 3). In contrast, Shakhorkora produced the lowest grain yield of 1.40 t ha⁻¹ under irrigated conditions (Fig. 3). The results emphasize the significance of choosing appropriate genotypes to get greater yields and improving cultivation techniques based on the distinct traits of each variety.

Table 4: Yield and yield contributing attributes of 25 small grain aromatic rice genotypes of Bangladesh

Sl.	Designation	PH	ET	DM	GP	TGW	YLD
1	ASRBCR 1017-B-12-3	104r	11cd	158d	319b	15.11e	5.84b
2	ASRBCR 1017-B-13-1	90t	11bc	159c	269d	14.47f	5.91b
3	ASRBCR 1017-B-13-2	112o	12a	156e	309c	14.57f	6.67a
4	ASRBCR 1017-B-18-2	106q	10de	165a	350a	16.77b	5.90b
5	Badshabhog	145f	10f	151f	127fg	10.57n	2.69h
6	Baoi Jhak	113n	8i	146i	120h	16.28c	1.86no
7	Baoibhog	109p	8i	144k	117h	11.97j	3.17f
8	Bashful (Golden)	111o	9gh	140n	96jk	19.17a	4.79c
9	Begun Bitchi	107q	9gh	132p	87lm	9.83q	2.50i
10	Begun Mala	131i	10ef	143l	90kl	15.47d	2.20kl
11	Bhatir Chikon	129j	11cd	147h	83mn	10.30op	2.30jk
12	BR5	123l	11bc	130q	246e	11.98j	1.63p
13	BRRI dhan34	152d	11cd	134o	346a	11.25l	3.85d
14	BRRI dhan90	100s	12a	164b	325b	12.60h	6.68a
15	Chiniatap	140h	10de	145j	95k	10.21op	1.78o
16	Chinigura	168a	11b	134o	122gh	10.77m	2.33j
17	Chinikanai	116m	11cd	148g	134f	10.37o	2.03m
18	Kalizira	153cd	10de	142l	115h	12.47hi	2.17l
19	Kataribhog (Desi)	144fg	9gh	142m	79n	13.93g	1.93n
20	Kataribhog (Philippines)	161b	8i	140n	102ij	15.13e	2.80g
21	Madhumala	143g	8i	143k	82mn	11.25l	1.53p
22	Radhuni pagal	150e	12a	139n	131f	11.53k	2.47i
23	Shakhorkora	154c	10f	134o	105i	12.09j	1.40q
24	Tulshimala	125k	10f	144k	129f	12.42i	3.47e
25	Zirabhog	140h	8i	151f	104i	10.16p	1.80o
	Level of significance	0.001	0.001	0.001	0.001	0.001	0.001
	Var	458.7	2.2	9031.8	5.9	97.3	3.3
	SD	21.42	1.48	95.04	2.42	9.86	1.80
	CV	6.79	14.75	58.23	16.59	18.91	56.54

Legend: PH - Plant height (cm), ET - Number of effective tillers/plant, GP - Number of filled grains/panicle, TGW - 1000 seed weight (g), DM - Days to maturity and YLD - Grain yield/plot (MT/hectare).

3.2. Grain Quality

The grain size and shape of all the genotypes were similar, and the plants were characterized as small and round (Fig. 4). However, the husk color varied from variety to variety. Some varieties had black husks, such as Kalizira, while others had gray husks, and most varieties had a golden husk color (Table 5).

Bashful (Golden) had the longest grain length, measuring 7.13mm, while BRRI dhan90 had the shortest length, at 3.96mm. The length of grains is a significant quality criterion that might impact the visual appeal and commercial worth of rice. The studied types exhibited varying length-to-width ratios, which suggest differences in grain morphology. Madhumala had the greatest ratio of 3.6, whilst ASRBCR 1017-B-18-2 had the lowest ratio of 1.6

(Table 5). A greater ratio of length to width indicates a slender and elongated form of the grain. The cooked rice elongation of the tested rice genotypes ranged from 1.5 to 2.07. Varieties with greater cooked rice elongation may be preferred for specific culinary purposes. The cooking times of the tested varieties varied from 12.0 to 18.0 min. The longest cooking time of 18.0 min was required for both the Bashful (Golden) and BR5 varieties (Table 5). The grain gelatinization temperature and coarseness are two variables that affect the cooking time. Different varieties may require different cooking times to achieve the desired texture and taste.

Table 5: Quality performances of 25 small grain aromatic rice genotypes

Sl.	Designation	GL	GB	LBR	CT	CRE	Aroma	Husk color
1	ASRBCR 1017-B-12-3	4.13fg	2.15de	1.9i	15e	1.75gh	Scented	Golden
2	ASRBCR 1017-B-13-1	4.07fg	2.12ef	1.9i	13h	1.83f	Scented	Golden
3	ASRBCR 1017-B-13-2	4.15fg	2.14de	1.9i	15e	1.72hi	Scented	Golden
4	ASRBCR 1017-B-18-2	4.08fg	2.48a	1.6j	15e	1.85f	Scented	Golden
5	Badshahbog	4.00g	2.02ij	2.0hi	12i	2.07a	Scented	Golden
6	Baoi Jhak	4.28efg	2.27b	1.9i	15e	2.05abc	Lightly scented	Brown
7	Baoibhog	4.40efg	1.96kl	2.2ef	16c	1.74hi	Scented	Golden
8	Bashful (Golden)	7.13a	2.18cd	3.3bc	18a	1.64j	Scented	Golden
9	Begun Bitchi	4.13fg	2.05hi	2.0ghi	14f	1.71i	Lightly scented	Black
10	Begun Mala	4.39efg	2.25b	2.0i	14f	1.78g	Scented	Golden
11	Bhatir Chikon	4.27efg	1.79n	2.4e	15e	1.70i	Scented	Golden
12	BR5	4.03g	2.06ghi	2.0i	18a	1.51k	Scented	Golden
13	BRR1 dhan34	4.31efg	1.97jk	2.2efgh	15d	2.03c	Scented	Golden
14	BRR1 dhan90	3.96g	1.91lm	2.1fghi	15e	1.67j	Non-scented	Golden
15	Chiniatap	4.17fg	2.22bc	1.9i	13h	1.90e	Scented	Dark Brown
16	Chinigura	4.29efg	1.80n	2.4e	13h	2.02c	Scented	Golden
17	Chinikanai	4.03g	2.10efg	1.9i	16c	1.78g	Scented	Golden
18	Kalizira	4.63de	1.98jk	2.3e	12i	2.07ab	Scented	Black
19	Kataribhog (Deshi)	4.97cd	1.61o	3.1c	14g	1.91de	Scented	Golden
20	Kataribhog (Philippines)	4.51ef	1.87m	2.4e	16c	1.91de	Scented	Golden
21	Madhumala	5.71b	1.57o	3.6a	12i	1.94d	Non-scented	Golden
22	Radhuni pagal	5.33c	1.61o	3.3b	14g	2.08a	Scented	Golden
23	Shakhorkora	4.02g	2.09fgh	1.9i	12i	2.03c	Scented	Golden
24	Tulshimala	5.16c	1.87m	2.8d	16b	1.73hi	Scented	Grey
25	Zirabhog	4.01g	1.81n	2.2efg	12i	2.03bc	Lightly scented	Brown
	Level of significance	0.001	0.001	0.001	0.001	0.001		
	Var	0.647	0.050	0.308	3.115	0.026		
	SD	0.8056	0.224	0.555	1.764	0.162		
	CV	17.93	11.24	24.21	12.21	8.74		

Legend: GL-Grain length (mm), GB-Grain breadth (mm), LBR-Grain length-breadth ratio, CT-Cooking time (minute), CRE-Cook rice elongation

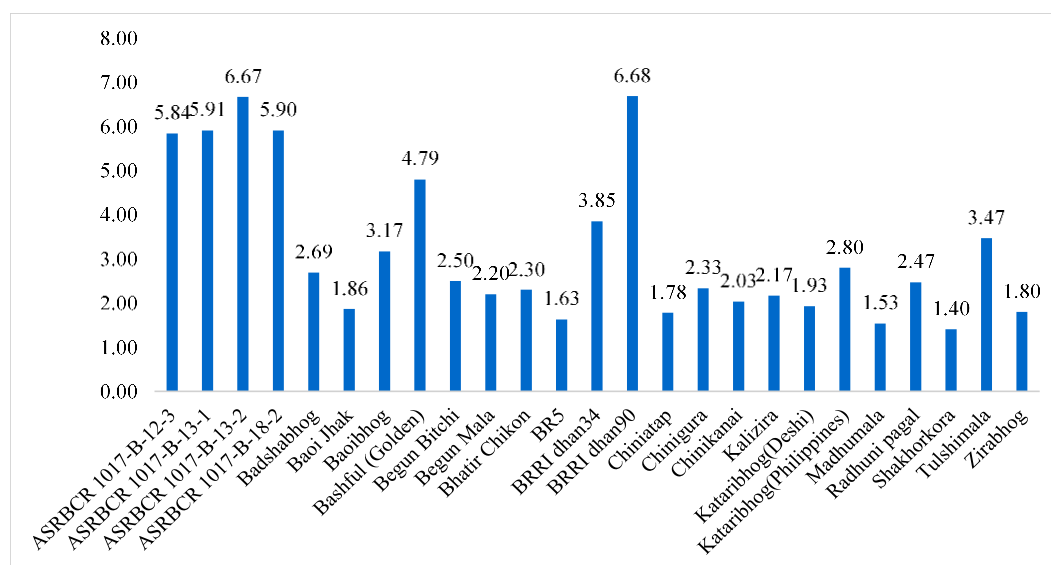


Fig. 3: Yield performance of 25 small grain aromatic rice genotypes

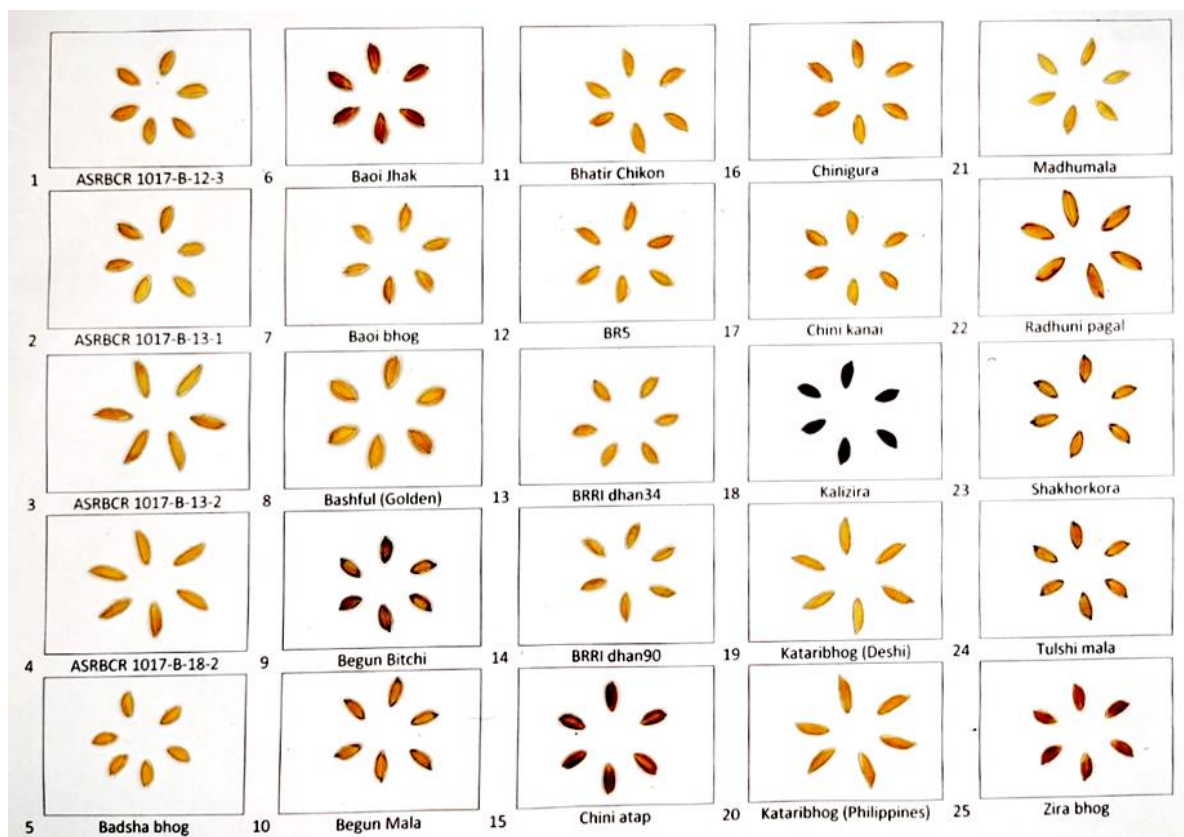


Fig. 4: Grain view of the 25 small grain aromatic rice genotypes

The aroma intensity differed among the tested varieties. Kalizera, Badshabhog, BRRI dhan34, Chinigura, and Radhuni pagal contained higher levels of aroma, while the remaining varieties had a moderate type of aroma (Table 5). Aroma is an important quality characteristic of rice because it contributes to the flavor profile of cooked rice and is preferred in certain culinary traditions. The findings highlight the diversity in grain characteristics, such as length, shape, aroma, cooking time, and husk color, among the tested varieties. These quality parameters are important considerations for consumers, culinary preferences, and the market value of rice.

3.3. Trait Correlation

To estimate the correlation between traits, the phenotypic values of one trait were regressed against those of another trait. The trait correlations between pairs are illustrated in Fig. 5. $ET \times DM$ (0.217), $ET \times GP$ (0.569), $ET \times YLD$ (0.439), $DM \times GP$ (0.521), $DM \times TGW$ (0.298), $DM \times YLD$ (0.702), $GP \times TGW$ (0.248), $GP \times YLD$ (0.785), and $TGW \times YLD$ (0.401) were among the significantly positively correlated characteristics. Significant negative correlations were found between the variables PH and ET (-0.129), PH and GP (-0.446), PH and TGW (-0.372), PH and DM (-0.579), PH and YLD (-0.632), and ET and TGW (-0.139). Research examining the correlation between plant height (PH) and grain yield has established a negative correlation between these two variables. Additionally, grain yield is negatively correlated with aroma.

3.4. Detecting *frg* Genes in Local and Advanced Rice Lines

The results showed that an SSR marker (BADEX7-5) was useful for PCR analysis of genomic DNA from various types of rice. The PCR marker BADEX7-5 produced two different banding patterns, indicating the presence or absence of aroma compounds in the rice lines. A band measuring 95bp indicated the existence of an aroma while a band measuring 103bp showed the lack of an aroma.

Each of the advanced lines that were evaluated consistently exhibited a uniform banding pattern around 95bp, which suggests the presence of aroma compounds (Fig. 6). These results indicate that these advanced lines exhibit the genetic characteristic linked to aroma. Out of the local accessions that were analyzed, the majority showed banding at 95bp, which suggests the existence of an aroma. However, one specific local line, known as Madhumala, and one particular check variety, called BRRI dhan90, did not exhibit a band at 95bp (Fig. 6). This finding suggested that these particular accessions lack the genetic trait associated with aroma.

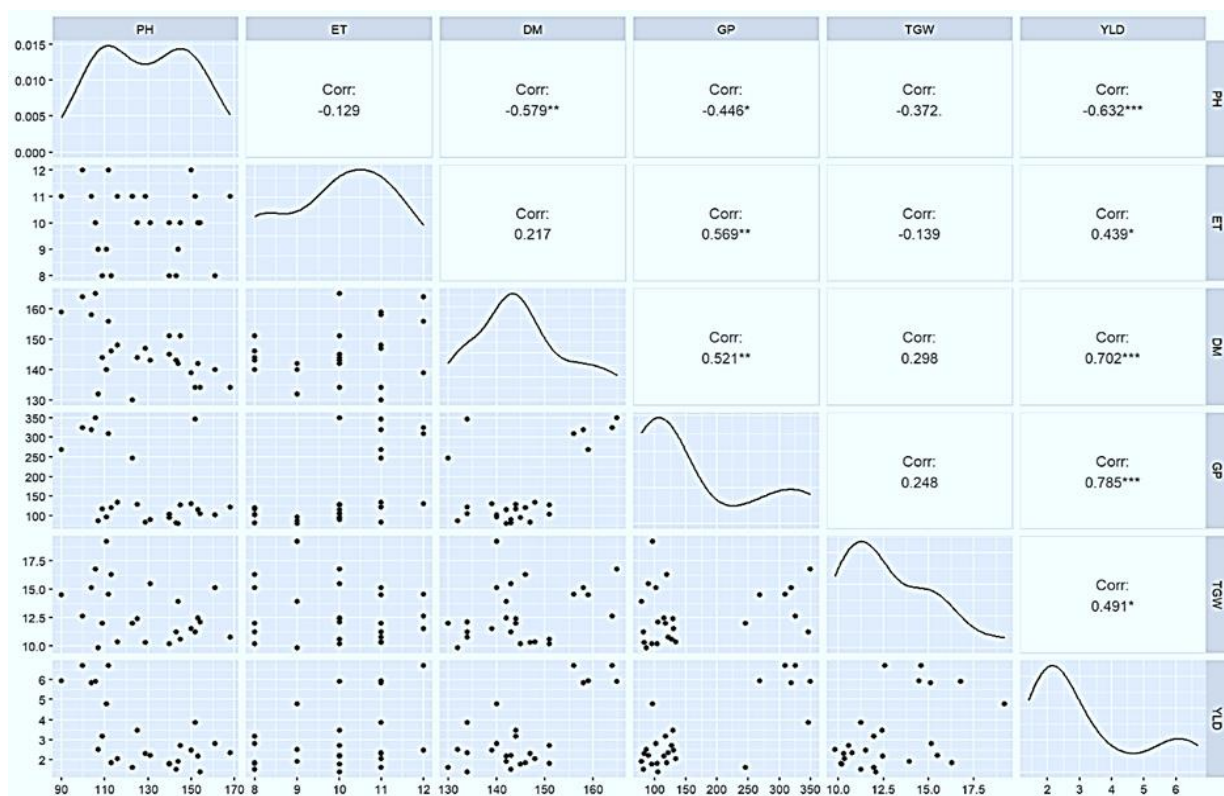


Fig. 5: Correlation matrix of the different variables of 25 small grain aromatic rice genotypes

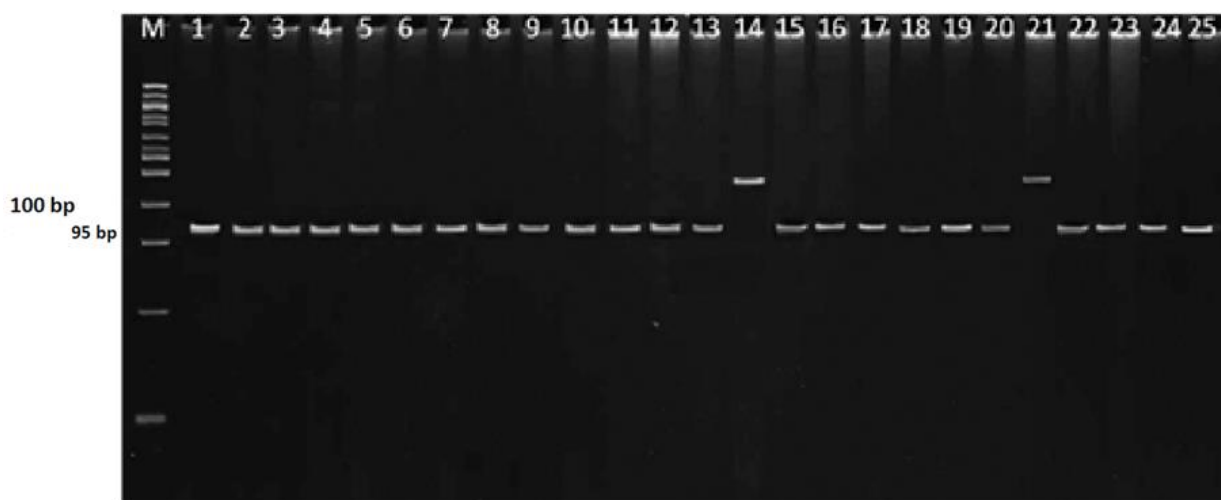


Fig. 6: Amplification of *badh2* gene using marker BADEX7-5 in fragrant and non-fragrant rice genotypes; Lane 1: 1000bp DNA ladder (Promega, USA), and Lane 2-26: 25 small grain rice genotypes showed in Table I. All the fragrant rice genotypes amplified a 95bp fragment, while the non-fragrant genotypes amplified a 103bp fragment

The results showed that the advanced lines that were tested consistently had aroma traits. However, one local accession, which included Madhumala and BRRI dhan90, did not show the banding pattern that is usually associated with aroma. This information can be valuable in selecting and breeding rice varieties with specific aromatic characteristics.

4. DISCUSSION

4.1. Principal Component Analysis (PCA)

Principal component analysis (PCA) is a well-known multivariate statistical method that helps to organize

genotypes according to their PC scores and finds the minimum components that explain most of the total variability (Ringner 2008; Shoba et al. 2019).

Using principal component analysis (PCA), we analyzed quantitative and qualitative traits in rice germplasm. Principal component 1 (PC1) displayed a significant amount of variability at 54.25%, exceeding the 28.99% that Kiani recorded in 2012. In contrast, Principal Components 2 and 3 (PC2 and PC3) contributed 21.09 and 10.72%, respectively (Table 3), to the observed diversity among genotypes for the examined parameters. These findings shed light on the utility of multivariate analysis in scrutinizing rice germplasm, emphasizing the importance of specific principal components in discerning variations in traits and genetic diversity (O'Rourke et al. 2013).

The scree plot facilitated the observation of the variance explained by each principal component, elucidating the clear link between eigenvalues and principal component numbers. Following the initial three PCs, minimal variation between subsequent PCs suggested a more linear relationship. This observation underscores the potential advantages of purposefully selecting a limited set of genetically diverse parental rice genotypes.

The outcomes presented in this study resulted from a thorough exploration of the relationships among advanced rice germplasm lines concerning yield and quality attributes. Using principal component analysis (PCA) as the research method, the study focused on constructing a biplot of the first two principal components, PC1 and PC2, to show the relationships between the variables and observations in a two-dimensional space. Within the generated biplot, a distinct cluster, encompassing three lines (ASRBCR 1017-B-12-3, ASRBCR 1017-B-13-2, and the check variety BRRI dhan90), prominently positioned itself in the top right quadrant. The genotypes exhibited positive values (54.72%) for both PC1 and PC2, indicating their superiority over other lines in terms of yield and quality-related variables (Turin et al. 2021). This collection of genotypes had favorable correlations with many variables, including plant height (PH), days to 50% flowering (FLW), days to maturity (DM), plot yield (YLD), and grain breadth (GB). The strong connections highlight the considerable capacity of these genes to improve both the yield and quality of rice.

4.2. Yield Contributing Attributes and Yield

The yield-contributing characteristics and yield of aromatic rice were substantially impacted by variety. The maturity plant heights of the rice genotypes that were examined exhibited notable variability. The rice line with the shortest plant height (90cm) was ASRBCR 1017-B-13-1, while Chinigura exhibited the greatest plant height (168cm) (Table 4). The results indicated that the effective tiller count varied between eight and twelve. The highest number of tillers, twelve, was acquired from ASRBCR 1017-B-13-2, and BRRI dhan90 produced the same result. This variation may have resulted from heredity, which was directly associated with genetic characteristics. Kataribhog (Desi) contained the fewest grains, panicle⁻¹ (79), whereas ASRBCR 1017-B-18-2 contained the greatest number of grains, panicle⁻¹ (350). A greater number of short, robust granules (tiny) were observed to be densely arranged within a panicle. According to Zhao et al. (2020), the most critical determinant of high productivity in rice cultivars is the quantity of grains per panicle. Similar results were reported by Jayhoon et al. (2023). Begun bitchi had the lowest weight (9.83 g), while Bashful (golden) had the greatest weight 19.17g/thousand grains). Among the twenty-five aromatic rice genotypes, ASRBCR 1017-B-13-2 produced the highest cereal yield (6.67 t ha⁻¹). The increased yield was ascribed to the higher number of efficient tillers per hill, while BRRI dhan90 achieved a bigger yield owing to the larger number of grains per panicle and effective tillers per hill. Shakhorkora, cultivated with the assistance of irrigation, had the lowest grain production of 1.40 t ha⁻¹ (Fig. 3).

4.3. Grain Quality Parameters

The variety had a considerable impact on all the grain quality metrics, as shown in Table 5. The variety Bashful (Golden) had the longest grain length, measuring 7.13mm, while the variety BRRI dhan90 had the shortest grain length, measuring 3.96mm. The length-breadth ratio was rated first in the variety Madhumala (3.6) and low in the line ASRBCR 1017-B-18-2 (1.6). The elongation values of the examined rice varieties varied between 1.72 and 2.08. Similarly, Gimhavanekar et al. (2020) also found that cooked rice elongation of aromatic rice ranged from 1.18 to 1.75. The genotypes that were tested had cooking times varied between twelve and eighteen min. Both the Bashful (Golden) and BR5 varieties needed the longest cooking time, 18.0 min. The cooking duration of rice is contingent upon the grain's coarseness and gelatinization temperature (Singh et al. 2012; Shozib et al. 2022). The aroma strength varied according to the variety. The examined varieties, namely, Kalizera, Badshabhog, BRRI dhan34, Chinigura, and Radhuni pagal, exhibited a greater degree of fragrance than did the other plant types, which had a moderate scent. All the genotypes have the same grain size and form particles, which are tiny and spherical (Fig. 4). The color of the husks varies from one kind to another. Some individuals, such as Kalizera, have a black coloration, while others have a gray coloration. The majority of the variations, however, are mostly golden in hue.

4.4. Trait Correlation

During our analysis, we discovered some significant positive relationships among the essential characteristics. The correlations between various factors, including effective tiller (ET), days to maturity (DM), grains per panicle (GP), thousand grain weight (TGW), and yield (YLD), were evaluated. Specifically, the correlations between ET and DM, ET and GP, ET and YLD, DM and GP, DM and TGW, DM and YLD, GP and TGW, GP and YLD, and TGW and YLD. These positive correlations indicate simultaneous growth in one feature together with another. The positive correlation between effective tiller (ET) and yield (YLD) indicates that an increase in effective tiller number is linked to a greater yield.

During the analysis of correlations in our research, significant negative relationships were found among certain features (Fig. 5). The correlations included the relationships between plant height (PH) and effective tiller (ET), plant height (PH) and grains per panicle (GP), plant height (PH) and thousand-grain weight (TGW), plant height (PH) and days to maturity (DM), plant height (PH) and yield (YLD), and the relationship between effective tiller weight (ET) and thousand-grain weight (TGW). Negative correlations show that the qualities are inversely related, meaning that when one characteristic increases, the other often decreases. As an illustration of this phenomena, consider the inverse connection between pH and yield: as plants grow taller, they produce less grain (Sarker 2002). In contrast to coarse and medium rice varieties, aromatic rice cultivars produce a comparatively low yield (Sinha et al. 2018). These results provide useful insights into the interactions and possible compromises among several plant features in our specific research area.

4.5. *frg* Genes Detection in Local and Advanced Rice Lines

The genomic DNA of 18 local accessions, 4 advanced lines, and 3 check varieties was subjected to PCR analysis using an SSR marker named BADEX7-5. The PCR marker BADEX7-5 produced a banding pattern at 95bp, which indicates the presence of aroma in the rice lines, and 103bp, which indicates no aroma (Sakthivel et al. 2009; Kaewmungkun et al. 2022). All the advanced lines produced the same band at 95bp, which indicates the presence of aroma compounds. Most of the local lines showed band patterns at 95bp, but some did not, which were from Madhumala and one check variety, BRR1 dhan90 (Fig. 6).

5. CONCLUSION

This research showed the effect of various aromatic rice varieties on different characteristics and attributes. A significant difference was observed among the varieties tested for these characteristics, such as plant height, effective tillers per hill, and grains per panicle, 1000 grain weight, and grain yield. ASRBCR 1017-B-13-2 had the highest effective tillers per hill, which resulted in more grain numbers per tiller. Shakhorkora showed the lowest grain yield. Genotypes differed strongly with respect to traits related to grain quality, particularly the length-to-breadth ratio, cooking time, aroma/fragrance intensity, and cooked rice elongation. Grain length, shape, smell, and cooking time, as well as the husk color, matter to customers both in a culinary sense and in terms of market value. The genotypes studied were characterized by their grain quality measures. We also found associations among traits: a negative association between plant height and grain yield, and aroma and grain yield. To identify the aroma of the tested rice lines, we used the SSR marker BADEX7-5. All the tested advanced lines showed the aroma associated banding pattern. One local line, Madhumala, and also the check variety, BRR1 dhan90, did not show any aroma associated banding pattern. Such discoveries could be useful for selecting and breeding aromatic rice varieties with desirable characteristics.

Author Contributions

All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by *Md. Ariful Islam* and *Md. Moniruzzaman Hasan*. The first draft of the manuscript was written by *Md. Ariful Islam* and *Md. Moniruzzaman Hasan*. All the authors commented on the draft version of the manuscript and read and approved the final manuscript.

Conflict of Interests

The authors declare that they have no conflicts of interest regarding this manuscript or research. The authors declare that no funds, grants, or other support was received during the preparation of this manuscript.

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