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Development of Zn and Fe biofortified high yielding premium quality aromatic rice suitable for wet season in Bangladesh

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ABSTRACT

The existing cultivars of aromatic rice are not sufficient to satisfy the demand for aromatic rice because of their limited yield potentials and environmental constraints. High yielding aromatic rice is a significant agricultural focus in Bangladesh due to its potential to enhance food security, economic growth, and export revenues. The research was instigated to develop high yielding, premium quality new aromatic rice variety to satisfy the present market demand. Therefore, single crosses were made and after confirmation of F₁s, generations were advanced through the pedigree selection method until the selection of several superior inbred lines. Among the selected lines, the proposed genotype (BU-103-23-5-3) was derived from ACC 34 X ACC 51 with better qualitative and quantitative attributes than the check variety (BRRI dhan70). The proposed genotype has the modern plant architecture, having tall but robust plant with an erect flag leaf, a long panicle with higher filled grain in addition to insects and diseases resistance. On an average, it requires 120-125 days to mature with a yield potential of 5.0 to 5.5 t ha⁻¹, which is earlier and showed nearly 20 % higher yield than the check variety. It is recognized as long-slender, premium-quality rice with 21.9 g of 1000-grain weight. Amylose and protein content of the polished rice are 26 and 7.56 %, respectively. The proposed genotype is Zn and Fe biofortified aromatic rice containing 23.6 ppm of Zn and 11 ppm of iron in the 11% polished rice. The newly developed genotype has satisfactorily passed the Proposed Variety Trial (PVT) conducted in ten locations during wet (Aman) season of 2023. Consecutively, the National Seed Board (NSB) approved the proposed genotype (BU-103-23-5-3) as GAU dhan 3 for commercial cultivation in the wet season in Bangladesh because of its significant benefits for food security, economic growth, and export revenues.

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Introduction

Aromatic rice constitutes a small but genetically distinct subpopulation within cultivated rice (*Oryza sativa*), which is globally popular because of its pleasant aroma (Roy *et al.*, 2020). Aromatic rice varieties are prized for their distinct, pleasant aroma, taste, and flavor, which enhance the sensory experience of meals. The main aromatic compounds, 2-acetyl-1-pyrroline (2-AP), are responsible for their unique scent (Lu *et al.*, 2023; Wakte *et al.*, 2017; Waheed *et al.*, 2023). Aromatic rice embraces noteworthy cultural, economic, and culinary importance in Bangladesh. The fine grains and delicate texture of scented rice improve the general quality of dishes. The popular aromatic rice varieties, such as Kalijira, Kataribhog, Tulsimala, BRRI dhan34, and Chinigura, are integral to traditional Bangladeshi cuisine (Anik and Talukder, 2002). The unique aroma and quality make them competitive in global markets (Kabir *et al.*, 2020; Kader *et al.*, 2020). Cultivating aromatic rice can be more profitable for farmers due to its higher market price compared to non-aromatic varieties (Tama *et al.*, 2015). This can lead to better income stability for rural households. Consumer demand for rice fragrance is increasing in many countries (Bairagi *et al.*, 2020). However, the pace of improvement of aromatic rice is slower than that of other non-aromatic rice (Roy *et al.*, 2020; Singh *et al.*, 2000; Singh *et al.*, 2019). Therefore, we often experience a shortage of fragrant rice, resulting in higher prices and import

dependencies to meet consumer demand. In Bangladesh, mostly, fragrant rice cultivation was dominated by traditional landraces during the wet (Aman) season. However, several research institutes have developed a good number of high-yielding aromatic rice varieties, such as BRRI dhan50, and BINA dhan25 for the dry (Boro) season. Therefore, a research gap exists in replacing low-yielding aromatic cultivars with high-yielding varieties for the wet season.

Biofortification of aromatic rice with micronutrients presents a strategic approach to combat essential nutrient deficiencies while preserving the unique qualities that make aromatic rice varieties widespread. Zinc and Iron are essential for immune function, growth, and cognitive function (Jin *et al.*, 2024; Wessels *et al.*, 2017; Barua *et al.*, 2023; McCann *et al.*, 2020). The global biofortification pipeline has made remarkable progress, with over 37 biofortified rice varieties now released worldwide (Birol *et al.*, 2015; Senguttuvel *et al.*, 2023). The development of biofortified aromatic rice varieties presents additional challenges due to the complex genetic architecture underlying aroma production and the need to maintain premium grain quality traits that command higher market prices (Islam *et al.*, 2020). Genetic studies have revealed complex relationships between aroma production, grain quality parameters, and micronutrient accumulation in rice. High yielding aromatic rice varieties combined with desirable traits can make them competitive in international

trade. In Bangladesh, researchers have made numerous endeavors to develop and promote aromatic cultivars, like, BRRI dhan50, BRRI dhan80, and BRAC dhan2 (Kader *et al.*, 2020; Kader *et al.*, 2023). Nevertheless, these are not up to the mark because of their limitation to climatic suitability and market competitiveness. Moreover, consumers prefer more premium qualities in aroma, grain shape, and cooking qualities.

There are several approaches to rice biofortification, including conventional cross-breeding, genetic engineering (GM) to alter genetic makeup for Zn and Fe content, and agronomic management, leading to applying Zn and Fe fertilizers to the soil. However, conventional cross-breeding is often considered advantageous for biofortification over other methods for several reasons (Avnee *et al.*, 2023). Quality maintenance represents another critical challenge in aromatic rice biofortification. Premium aromatic varieties must maintain specific amylose content (typically 20-25%), optimal protein levels (7-9%), and characteristic cooking properties, including grain elongation, volume expansion, and texture (Bhattacharya, 2011). In Bangladesh, the rice production system is characterized by distinct seasonal patterns. Wet season (Aman) cultivation presents unique challenges, including variable rainfall patterns, flooding risks, pest and disease pressures, and specific soil conditions that influence both crop performance and nutrient uptake (Mainuddin *et al.*, 2013). Developing biofortified aromatic

varieties adapted explicitly to wet season conditions is crucial for maximizing both production and nutritional impact. Seasonal variation significantly affects micronutrient accumulation in rice grain. Wet season conditions, characterized by higher humidity and variable water management, may alter the physiological processes governing micronutrient transport from roots to grain, necessitating varieties with robust nutrient accumulation mechanisms under these conditions (Gupta *et al.*, 2023).

Considering all the above aspects, the advancement of zinc and iron-biofortified high yielding premium quality aromatic rice varieties optimized for wet-season farming in Bangladesh represents a critical research priority that addresses multiple interconnected challenges. Such varieties could provide a sustainable solution to micronutrient malnutrition while preserving the economic and cultural value of aromatic rice production. Therefore, we aimed to develop high yielding, premium quality Fe and Zn-biofortified aromatic rice varieties to meet multiple goals. By targeting wet-season cultivation, these varieties could reach millions of smallholder farmers who depend on rain-fed rice production and have limited access to alternative nutritional interventions.

Materials and Methods

A good number of previously developed inbred lines were selected from the seed gene bank of the Department of Genetics

and Plant Breeding of Gazipur Agricultural University based on their grain quality (presence of aroma and micronutrients) and high yielding potential. Sixteen crosses were done among the selected parental lines with the aim of developing Zn and Fe-biofortified, high yielding, premium quality aromatic rice genotypes. The crosses were done in 2016, and generations were advanced through the pedigree method. A single cross between ACC 34 and ACC 51 culminated in the development of the proposed genotype, which was initially designated as BU-103-23-5-3. After attaining homozygosity, several selected lines were subject to evaluation with a standard check variety (BRRI dhan70) at the university research farm. In 2020, the selected lines of the advanced genotypes were tested for an Advanced yield trial. A total of six advanced lines with one check were evaluated in the RCB design with 4 replications in the advanced yield trial during wet season of 2021 while five genotypes, including one check with 3 replications were evaluated in the regional yield trial during wet season of 2022. The trials were conducted in five locations under rainfed conditions in Gazipur, Cumilla, Mymensingh, Dinajpur, and Rangpur. Twenty-five to thirty days old seedling with single seedlings per hill was transplanted at a spacing of 20 cm x 20 cm. Fertilizers were applied at the rate of 200 kg urea, 70 kg TSP, 100 kg MoP, 70 kg gypsum, and 6 kg zinc sulphate. Total amount of TSP, gypsum, and two-thirds of MoP was applied at the time of final land preparation. The total amount of zinc sulphate was applied at

the first top dressing. Urea was applied in three equal splits at 10, 25, and 35 days after transplanting. The rest of the one-third MoP was applied during the second top-dressing of urea. Crop management was done as recommended for modern rice cultivation for Aman season. Weeding and other cultural operations were done in when necessary. The selected genotype was evaluated for various physico-chemical properties, cooking qualities, elongation ratio, optimal planting time, and disease-insect resistance under natural conditions. Milling and grain appearance qualities were measured at 14% moisture content using Zaccaria Testing Rice Mill (model PAZ/1-DTA). The weights of brown rice, polished rice, head rice, and broken rice were taken, and calculations were made based on the procedure of IRRI (IRRI 2010) at the grain quality testing laboratory, IRRI Bangladesh office, Gazipur. Whole kernel rice grain parameters (length, width, length-width ratio, and chalkiness) were obtained using the Vibe QM3 Rice Analyzer (Vibe, Capitola, CA, USA) in milled rice. The Zn and Fe content examination of 11% polished rice was executed using a Vanta series pXRF (Olympus, Waltham, MA, USA) spectrometer at the grain quality testing laboratory. Aroma quantification was done in the lab using a standard sensory test by 1.7% KOH (Hine *et al.*, 2006). The protein content was determined by multiplying the nitrogen content by a conversion factor of 5.95, which was identified using a Skalar SAN Plus segmented flow analyzer (Skalar Inc., Breda, The Netherlands). The growth

period was counted from seeding to 80% grain maturity. Grain yield of the genotypes was taken from a 10-square-meter sample plot in each replication. In Aman 2023 season, the proposed genotype, BU-103-23-5-3 was evaluated against check variety (BRRI dhan70) by the NSB at ten locations in Bangladesh as part of the Proposed Variety Trial. The statistical analyses (ANOVA followed by LSD test) of the experiments were performed using the Agricolae package of R software (R Core Team, 2021).

Results

Advanced yield trial

The morphological features of the proposed line (BU-103-23-5-3) are shown in Table 1. The plants of this genotype are taller than the plants of the check variety, resulting in higher biomass production. The proposed genotype (BU-103-23-5-3) has erect green flag leaves at maturity, which simplifies the maximum

solar radiation uptake. The advanced yield trial (AYT) of this line was conducted in the farmer's fields in five rice-growing districts of Bangladesh. The genotype BU-103-23-5-3 has a growth duration of 126 days with 142 cm plant height. The proposed line also showed a higher yield (5.76 t ha⁻¹) than BRRI dhan70 (4.32 t ha⁻¹). In aromatic rice, maximizing grain yield is the topmost target in developing modern rice varieties in addition to other quality traits. It is a strong aromatic rice genotype with higher yield and Zn and Fe-enriched. The proposed genotype showed nearly 25% higher yield than the check variety (BRRI dhan70) in the Aman 2020 season. Besides the proposed line, two other genotypes also showed better yield potentials than the check variety (Table 1). The yield advantages of BU-103-23-5-3 were due to its genetic potential of producing a greater number of grains per panicle, longer grain size, and an elevated number of productive tillers per hill than the check variety.

Table 1. Performance of the BU-103-23-5-3 and other rice genotypes in the AYT during Aman season of 2020

Designation	Plant height (cm)	Growth duration (days)	Location					Pooled
			Gazipur	Dinajpur	Rangpur	Cumilla	Mymensingh	
BU-103-23-5-3	142	126	6.12	5.84	5.40	6.15	5.32	5.76
SE-12-7-25-4	140	152	4.29	4.50	3.75	4.24	3.77	4.11
AS-5-27-12-8	130	145	4.32	4.53	3.78	4.26	3.92	4.16
AB-12-25-14-4	134	132	4.58	4.95	4.12	4.36	4.00	4.40
SA-24-7-22-9	138	142	4.43	4.90	4.14	4.33	4.15	4.39
BU-7-23-10-16	139	143	4.40	4.63	3.95	4.30	3.95	4.27
BRRI dhan 70	126	131	4.36	4.26	4.18	4.70	4.13	4.32
LSD =5.46 LSD=8.70			LSD = 0.355					

Regional yield trial

The selected homozygous advanced lines and the check variety (BRRI dhan70) were assessed in five rice-dominating sites as regional yield trial and the results are shown in Table 2. The genotype BU-103-23-5-3 gave higher yield in five locations compared to the check variety (BRRI dhan70) and other lines. The results show the yield potential of BU-103-23-5-3 (Proposed genotype) is approximately 10 percent higher than the check variety. Nonetheless, the selected line also has fewer insect and disease infestations in all the locations over the growing period. The genotype BU-103-23-5-3 had significantly higher plant height than the check variety, as well as higher stem diameter, producing more biomass than BRRI dhan70, which offers more profit to farmers due to fulfill their need for fodder. Farmers preferred BU-103-23-5-3 for its early maturity, high yielding capability, fine grain, and strong aroma content.

Proposed variety trial

The performance of the proposed genotype (BU-103-23-5-3) at on-farm and on-station

trials in Aman 2023 season are presented in Table 3. Performance of the proposed genotype was evaluated during the proposed variety trial by Technical Committees of the National Seed Board (NSB) of Bangladesh. The maximum grain yield of the proposed genotype was observed as 6.31 t ha⁻¹ at Feni Sadar. The yield potentials of the proposed genotype ranged from 3.72 to 6.31 t ha⁻¹. The average grain yield of 4.83 t ha⁻¹ indicated that the genotype could produce more yield with proper crop management. The genotype BU-103-23-5-3 gave higher yield than the check variety in all ten locations. It produced yield 5.39 t ha⁻¹ in BRRI, Gazipur; 4.87 t ha⁻¹ in BINA, Mymensingh; 6.31 t ha⁻¹ in Feni; 4.52 t ha⁻¹ in Cumilla; 4.48 t ha⁻¹ in Bogura; 4.44 t ha⁻¹ in Jashore; 5.06 t ha⁻¹ in BRRI, Rangpur, 4.45 t ha⁻¹ in BRRI, Faridpur; 3.72 t ha⁻¹ in Rajshahi, and 5.02 t ha⁻¹ in Dinajpur. The average yield potentials of the check variety were 4.02 t ha⁻¹, whereas the proposed genotype BU-103-23-5-3 yielded 4.83 t ha⁻¹, which is 0.81 t ha⁻¹ higher than the check variety BRRI dhan70. The proposed genotype

Table 2. Performance of the proposed line (BU-103-23-5-3) and other genotypes in the regional yield trial during Aman season of 2021.

Designation	Growth duration (days)	Plant height (cm)	Grain yield (t ha ⁻¹)
BU-103-23-5-3	124	139	5.33
AB-12-25-14-4	134	132	4.40
SA-24-7-22-9	138	142	4.39
BU-7-23-10-16	139	143	4.27
BRRI dhan70	132	125	4.80
LSD (0.05)	3.76	3.98	0.221

* Mean of five locations

BU-103-23-5-3 offered nearly 20.10% higher yield than BRRI dhan70 (Table 3). The growth duration of the proposed genotype, BU-103-23-5-3 ranged from 118 days to 128 days, depending on the agro-climatic situation in Aman season of 2023. Mean growth duration of the proposed genotype was 123 days, which is 9 days earlier than the check variety (132 days).

Physicochemical properties

The genotype BU-103-23-5-3 is a long-slender grain having a grain length of 7.70 mm and a width of 1.77 mm. The milling turnout of this genotype is 67%, with a head rice recovery stand at 52% (Table 4). The protein and amylose percentages of the proposed genotype are 7.56% and 26%, respectively (Table 4). The Iron and Zinc content of 11

percent polished rice of this genotype are 11 ppm and 23.6 ppm, respectively. It requires only 15 minutes to be cooked and has an elongation ratio is 1.4. The analysis of sensory properties indicated that the genotype possesses a strong aroma, good texture, and overall moderate acceptability (Table 6). Because of having a sweet, strong aroma and long-slender grains, this line will get a higher market price.

Disease and insect reaction

The novel aromatic rice genotype (BU-103-23-5-3) demonstrated significant ability to resist major diseases and pests. The reaction of each entry to major diseases and insect pests was scored visually following the 0–9 Standard Evaluation System (SES) for rice of IRRI (Hosagoudar *et al.*, 2019). BU-103-23-

Table 3. Performance of the BU-103-23-5-3 in proposed variety trial at ten locations during Aman season of 2023

Region	Trial Location	Duration (Days)		Yield (t ha ⁻¹)		Yield advantage over check
		BU-103-23-5-3	BRRI dhan70	BU-103-23-5-3	BRRI dhan70	
Dhaka	BRRI, Gazipur	126	130	5.39	4.14	30.19%
Mymensingh	BINA	122	132	4.87	4.24	14.86%
Faridpur	BRRI, Faridpur	126	135	4.45	3.58	24.30%
Jashore	Sadar, Jashore	127	136	4.44	3.57	24.37%
Chittagong	Sadar, Feni	128	137	6.31	5.24	20.42%
Cumilla	Laksham	119	126	4.52	3.70	22.16%
Bogura	Bogura, Sadar	119	129	4.48	3.81	17.59%
Rajshahi	Rajshahi	118	130	3.72	3.22	15.52%
Rangpur	BRRI, Rangpur	119	129	5.06	4.16	21.63%
Dinajpur	Sadar, Dinajpur	128	134	5.02	4.53	10.00%
Average		123	132	4.83	4.02	20.10%
		LSD = 6.53		LSD = 0.49		

Table 4. Physical and nutritional properties of the BU-103-23-5-3 from grains harvested in Aman season of 2022

Designation	Physical properties					Nutritional properties			
	MT (%)	LMR (mm)	BMR (mm)	LBR	GS	AP (%)	P (%)	Zn	Fe
BU-103-23-5-3	67.1	7.70	1.77	4.35	Long slender	26	7.56	23.6	11.0
BRR1 dhan70	70.2	6.2	1.4	4.4	Long slender	21.7	9.5	-	-

N. B: MT=Milling outturn, LMR=Length of milled rice, BMR= Breath of milled rice, LBR=L-B ratio, GS=Grain Shape, AP=Amylose, P=Protein, Zn= Zinc (ppm), Fe=Iron (ppm).

*Scale: Grain length is considered very long, when >7.50 mm, long 6.61 to 7.50 mm, medium (5.51-6.60 mm), and short when <5.50 mm. Grain shape is slender, when L/W is >3; medium, when L/W is 2.1 to 3; Bold, when L/W is <2. Chalkiness (0 when % of chalky area is none, 1<10%, 5 for 10 to 20%, 9 for > 20%. Rice is treated as waxy when the Amylose content is 0-2%, very low (3-9) %, low (10-19) %, Intermediate (20-25) %, and High (>25%).

Table 5. Cooking/pasting of grains of the proposed line harvested in Aman season of 2023

Designation	Cooking/Pasting							
	GT	GC	BV	SBV	CT	GE	VE	Aroma
BU-103-23-5-3	Intermediate	Soft	0.0	0.0	15	1.4	3.2	Aromatic
BRR1 dhan70	Intermediate	medium-soft	0.0	0.0	15.3	1.5	3.0	Aromatic

N.B: GT= Gelatinization temp, GC= Gel consistency, BV=Break down viscosity(cP), SBV=Set back viscosity(cP), CT=Cooking time (minutes), GE= Grain Elongation Ratio, VE= Volume Expansion Ratio.

*Scale: Gelatinization Temp is treated as Low at 55-69 0°C, intermediate at 70-740 °C, and high >74°C. Gel consistency is soft, 61 to 100 mm; medium, 41 to 60 mm; and hard, 27 to 40 mm.

Table 6. Sensory properties of the seeds of the proposed genotype BU103-23-5-3

Designation	Sensory properties						Total score	Result
	Appearance	Color	Texture	Taste	Smell	Overall acceptability		
BU-103-23-5-3	6	7	6	8	8	7	7	Like moderately
BRR1 dhan70	6	7	6	7	7	7	7	Like moderately

*Evaluation scale: 9 = enjoy extremely, 8 = like very much, 7 = like moderately, 6 = enjoy a little, 5 = Neutral, 4 = Slightly Dislike, 3 = Moderately Dislike, 2 = Very much Dislike, 1= Extremely Dislike.

5-3 (Proposed genotype) showed resistance to major diseases and insects under the natural field conditions in the field of GAU and various rice-dominating regions. The variety demonstrated a ShB score of 1, BLB score of 1, and a false smut score of 0, meaning that it is resistant to ShB, bacterial leaf blight, and false smut (Table 7). It also showed good resistance against insects. The genotype demonstrated score 1 for stem borer, leaf roller, brown plant hopper (BPH), and rice yellow stem borer (RYSB), indicating it is resistant to stem borer, leaf roller, brown plant hopper, and rice yellow stem borer. Compared to the check variety, the proposed genotype had better performance against false smart, Bacterial leaf blight, and yellow stem borer. BU-103-23-5-3 demonstrated resistance to significant diseases and insects in natural field conditions at GAU and various rice-dominant areas. The genotype demonstrated a ShB score of 1, a BLB score of 1, and a false smut score of 0, indicating its tolerance to ShB, bacterial leaf blight, and false smut (Table 7).

Distinctness from check variety

Distinctive features of the candidate variety BU-103-23-5-3 compared to the check

variety BRRI dhan70 include anthocyanin pigmentation of leaf auricles and collar, Color of stigma, Stem: culm diameter, Panicle length, Spikelet: color of the tip of lemma, awns in the Spikelet, length of the longest awn, Panicle: color of a awns, maturity timing, leaf senescence, decorticated grain: shape (length-breadth ration), decorticated grain (bran): color, endosperm: content of amylase, and Decorticated grain: aroma (Table 8). In addition to that, the proposed variety contains more Fe and Zn compared to the check variety. The proposed genotype has 11ppm Fe and 23.6ppm Zn, while the check variety, BRRI dhan70 has 10 ppm Fe and 22.37ppm Zn. At the 50% flowering date, there were no off-type plants and no segregation in flowering behavior noted. It indicated that the candidate variety is uniform according to the International Union for the Protection of New Varieties of Plants (UPOV) standard. In the trial plots of multiple successive seasons, no significant variation or segregation was observed, indicating the stability of the candidate variety. Following a comprehensive assessment by the National Seed Board of Bangladesh (NSB) across ten farmer fields in Bangladesh, BU-103-23-5-3 has been approved as GAU dhan3 in 2025.

Table 7. Responses of the novel genotype to major diseases and insects under natural conditions at the GAU farm in Aman season of 2021

Designation	BB	ShB	FS	BLS	BLB	YSB	LR	BPH	RYSB
BU-103-23-5-3	2	1	0	2	1	1	1	1	1
BRRI dhan70	2	2	1	2	2	2	1	1	1

N. B: ShB=Sheath Blight, BB=Bacterial Blight, LR=Leaf Roller, FS=False Smut, LB=Bacterial leaf Blight, SB=Stem Borer, BPH=Brown Plant Hopper, YSB=Yellow Stem Borer, RYSB=Rice Yellow Stem Borer, BLS=Bacterial leaf streak. Scoring scale 0 to 9 (IRRI, 2013; Kader *et al.*, 2019).

Table 8. Distinctness of the BU-103-23-5-3 with a similar check variety BRRI dhan70

Characteristics	BRRI dhan70 (Check variety)		GAU dhan3 (BU103-23-5-3)		Remarks
	Code	Status	Code	Status	
Anthocyanin coloration of leaf auricles and collar	9	Present	1	Absent	Distinct
Color of stigma	5	Purple	1	White	Distinct
Stem: culm diameter	5	Large	3	Medium	Distinct
Panicle length	5	Long	7	Long	Distinct
Spikelet: color of the tip of lemma	5	Red	2	Yellowish	Distinct
Spikelet awns in the Spikelet	9	Present	0	Absent	Distinct
Spikelet: length of the longest awn	1	Very short	0	Absent	Distinct
Panicle: color of the awns	4	Reddish	0	Absent	Distinct
Time of maturity	9	Very late	5	Medium	Distinct
Leaf senescence	5	Intermediate	1	Late and slow	Distinct
Decorticated grain: shape (length-breadth ratio)	7	Slender	9	Long slender	Distinct
Decorticated grain (bran): color	1	White	2	Light brown	Distinct
Endosperm: content of amylase	3	Intermediate	5	High	Distinct
Decorticated grain: aroma	5	Lightly present	1	Present	Distinct
Other distinct special character (if any)		Aroma present		Zn and Fe enriched with aroma	Distinct

**Fig. 1. Pictorial view of proposed genotype BU103-23-5-3 in the field condition**



Fig. 2. Pictorial view of rice grain, unpolished rice, and polished rice of the proposed genotype and the check variety. The upper panel of the picture represents samples of the check variety, and the lower panel represents the samples of the GAU dhan3.

Discussion

Rice aroma is a gift of nature, and fragrant rice, a distinct subgroup of rice, is often preferred by consumers and can fetch a higher market price. Incorporating micronutrients with this trait makes the aromatic cultivar more appealing and increases the likelihood of widespread adoption and consumption. The development of new high-yielding, Fe, and Zn-biofortified aromatic rice in Bangladesh is important because it addresses the significant public health issue of micronutrient deficiencies. To develop a new, nutrient-enriched, high-yielding aromatic rice genotype, we selected parents based on their grain quality and the presence of aroma. ACC34, which was Zn and Fe-enriched, strong aromatic, and ACC51, a high-yielding genotype from which

we got our desirable genotype. The F_1 seeds were developed by hybridization between them in the Amon season of 2016. After confirming the cross between the selected parents, the harvested F_1 seeds were grown to produce F_2 seeds. The F_2 and subsequent generation seeds were planted to observe their segregation and selection of desirable genotypes. Pedigree selection methods were used to develop homozygous. The discovery and characterization of a homozygous elite genotype, BU103-23-5-3, from several better performers has created significant momentum in the breeding process to higher-yielding Zn and Fe-biofortified rice. This novel genotype incorporated several sought-after traits, including early maturation, yield advantages, and resistance to insects and pathogens, which are crucial aspects for farmers approval. The

comparative yield trial experiments carried out with the check variety (BRRI dhan70), offer details regarding the efficiency of the advanced line, BU103-23-5-3, under natural conditions.

The proposed genotype (BU103-23-5-3) performed better in both AYT and RYT over the selected genotypes as well as the check variety, BRRI Dhan 70, in all desirable aspects of their evaluation, including earliness, resistance to pests, and higher yield. This genotype possesses all the desirable attributes to be a modern, high-yielding, aromatic variety. Due to its higher plant height and thick stem diameter, the total above-ground biomass production was higher than that of other wet-season rice genotypes, which will provide additional advantages to farmers as a source of cattle feed. In PVT trials, the proposed genotype outperforms the check variety, BRRI dhan70, in terms of yield in all settings (Table 4). The yield potentials of the proposed genotype ranged from 3.72 to 6.31 tha^{-1} . On average, BU103-23-5-3 has 20.10% yield advantages over the check variety in PVT trials. The successful development of BU-103-23-5-3 (proposed variety) demonstrates the feasibility of combining high yield with micronutrient biofortification and premium aromatic quality in rice, which is a significant advancement over the check variety BRRI dhan70. The genetic potential of the line to produce >20% higher grain yields is attributed to its superior plant architecture, taller plants, erect flag leaves, longer panicles, more filled grains per panicle, and more productive tillers per hill.

The early maturation was an additional advantage of the proposed genotype. The maturity duration ranged from 128 to 119 days, with an average of 123 days, which was 9 days earlier than the check variety BRRI dhan70 (Table 4). The proposed genotypes showed higher yield and early maturity over the check variety in all the evaluation processes, indicating their genotypic potential and stability of better performance. Its earlier maturity than the check variety is a distinct advantage for enhancing cropping intensity and fitting into existing cropping calendars, particularly under the wet-season climatic conditions (Kabir *et al.*, 2019; Anshori *et al.*, 2024). Thus, the BU103-23-5-3 genotype may play an imperative role in food and nutritional wellbeing, given its high zinc and iron content, high yield potential, and suitability for wet-season growing, which will help improve cropping intensity. Additionally, the new aromatic rice genotype (BU-103-23-5-3) showed strong potential in defending against major diseases and insects. The wet-season rice of Bangladesh is more susceptible to insects and diseases due to high humidity, warm temperatures, and varying rainfall during the monsoon period, which significantly impacts pest outbreaks in rice fields (Morshed *et al.*, 2023). However, the diseases and insect infestation of the proposed genotype were better than the check variety (Table 7). The proposed genotype has superior grain quality attributes, including long slender grains, intermediate amylose content (26%), acceptable protein content (7.56%), and strong aroma, ensuring

market competitiveness and higher income for farmers, while its short cooking time and good cooking properties add consumer convenience. Together, the genotype demonstrates a successful combination of targeted attributes of a high-yield aromatic rice biofortified with Zn and Fe. The success of the genotype in AYT, RYT, and PVT trials across multiple environments ultimately led to its official release as GAU dhan 3 by the National Seed Board of the Ministry of Agriculture.

Conclusion

The research was initiated with the aim of developing new high yielding biofortified aromatic rice variety. In yield trials across multiple locations, the performance of the proposed line BU103-23-5-3 displayed better results for both short growth duration and higher grain yield. Finally, it was launched as a new aromatic rice variety designated as GAU dhan 3, for farming across Bangladesh based on its superior performance in all the trials compared to the check variety. It is expected that if this variety can be cultivated in the Aman season of Bangladesh, aromatic rice production will increase. In the future, this biofortified aromatic rice variety may ensure stable aromatic rice production in the country by expanding more areas in aromatic rice cultivation. The study emphasizes the importance of breeding approaches, like hybridization and selection, to achieve complex trait integration (biofortification, high yield, aroma, disease resistance) in

a premium aromatic rice genotype for the tropical wet season of Bangladesh.

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Conflict of Interest

The authors declare that they have no conflicts of interest regarding this manuscript or research.

Authors Contribution

Conceptualization, Nasrin Akter Ivy and M. Moynul Haque; methodology, Nasrin Akter Ivy and Liakat Ali; software and Validation Liakat Ali; resources, Nasrin Akter Ivy and M. Moynul Haque; data curation, Nasrin Akter Ivy, Liakat Ali, and Skeikh Maniruzzaman; writing preparation of the initial draft, Liakat Ali and Nasrin Akter Ivy; writing, review and editing, Nasrin Akter Ivy, M. Moynul Haque, Sheikh Maniruzzaman visualization, Liakat Ali; supervision, Nasrin Akter Ivy.; project administration, Nasrin Akter Ivy and Liakat Ali.; revenue acquisition, Nasrin

Akter Ivy. All the authors commented on the draft version of the manuscript and read and approved the final manuscript.

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