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Selection of Stable and High-Yielding Short-Duration Inbred Rice (*Oryza sativa* L.) Genotypes for the Boro Season in Bangladesh Using WAASB and MTSI Indices

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Abstract

Rice (*Oryza sativa* L.) is the staple food of Bangladesh, with its production tripling since independence. However, declining cultivable land and climate change, particularly erratic rainfall, have led to prolonged water scarcity during the Boro season. To ensure future rice security, genetic improvements for short-duration, high-yielding varieties are essential. In response, the Bangladesh Rice Research Institute (BRRI) developed genotypes with a life cycle of ≤ 145 days, requiring less water while maintaining productivity. This study evaluated four advanced fixed lines (V1 = BR11318-5R-63, V2 = BR11337-5R-72, V3 = SVIN109, V4 = IR17A1723) alongside two reference varieties (V5 = BRRI dhan81, V6 = BRRI dhan96) across 11 locations in Bangladesh during the 2022–23 Boro season. Combined ANOVA revealed significant genotype, environment, and genotype-environment interaction (GEI) effects ($p \leq 0.001$) on yield and related traits. Broad-sense heritability (h^2_b) ranged from 0.05 to 0.50, with most traits showing high selection accuracy (>0.80). Stability analysis using weighted average of absolute scores biplots (WAASB) identified V1 and V3 as the most stable, while GGE biplots highlighted V2's superior grain yield (GY) in multiple locations. Multi-trait stability index (MTSI) ranked V2 as the most stable based on GY and seven yield-contributing traits. Factor analysis further confirmed its agronomic superiority, with GD and TGW showing the highest broad-sense heritability (0.92), followed by PHT (0.885) and GY (0.833), with selection gains ranging from 1.48% to 9.54%. Overall, BR11337-5R-72 (V2) emerged as the most promising short-duration, high-yielding, and stable genotype for Boro rice cultivation, requiring further evaluation and promotion for large-scale adoption.

Key words: BRRI; Rice; Yield; stability; GEI.

1. Introduction

Rice (*Oryza sativa* L.) holds a predominant position as the primary grain crop in Bangladesh's agricultural sector. Given the dietary patterns, ensuring "rice security" plays a pivotal role in achieving food security, a situation mirrored in numerous other nations (Brolley, 2015). Rice production has increased by three times since the liberation of Bangladesh and reaching about 39.1 MT in 2023 (Kabir et al., 2020) and hold third-largest producer of rice globally (Sun et al., 2023). However, future projections indicate a rising demand for rice due to population growth. (Kabir et al., 2015) estimated that approximately 44.6 million metric tons of rice will be needed by 2050. On the other hand, the cultivable land area will be 11.07 M ha in 2050 compared to 12.10 M ha in 2020 (Kabir et al., 2020). Additionally, the country is grappling with the impacts of climate change, including erratic rainfall



patterns and prolonged droughts. Therefore, enhancing the genetic potential of rice crops to increase yield remains crucial for meeting the future rice demand in Bangladesh.

Bangladesh experiences distinct wet and dry seasons, with the dry season, known as the boro season, posing a challenge for rice cultivation due to water scarcity. Therefore, the resilience of short-duration boro rice (BR-SD) varieties becomes particularly valuable. According to research by Rabbi et al. (Rabbi et al., 2020), rice varieties in Bangladesh are classified based on their ecological adaptation and product requirements to better understand the changing potential yield over time. Favorable boro rice (FBR) varieties are categorized as short duration (SD), cultivated during the boro season in favorable environments with minimal abiotic stresses apart from natural disasters. The SD varieties typically have a lifespan of up to 145 days. Conversely, long-duration boro rice types include cultivars grown in similarly favorable environments but with a lifespan exceeding 145 days. FBR-SD varieties have proven to be well-suited for the conditions, requiring less water and completing their life cycle within the limited duration of the dry season. It's important to note that the Bangladesh Rice Research Institute (BRRI) is the leading institution for rice research in the country, having released 108 inbred and 08 hybrid rice varieties. Additionally, the Adaptive Research Division (ARD) of BRRI is tasked with conducting validation and adaptation trials across various agro-ecological zones (AEZs) to identify stable cultivars for release in Bangladesh. While scientists are actively working on genetically enhancing crops by selectively incorporating elite alleles and traits into desired genetic backgrounds, conducting multi-environment trials (METs) is a crucial step in breeding programs before releasing varieties to farmers. Selecting superior genotypes based on yield across diverse AEZs can be highly effective, given that yield is a complex quantitative trait heavily influenced by environmental variations. However, choosing the best genotype from a genetic pool based on their observable traits presents a significant challenge (Alam, 2019; Alam, Akter, Khan, Alam, et al., 2023; Alam, Akter, Khan, Amin, et al., 2024). In the pursuit of stable genotypes, the additive main effects and multiplicative interaction (AMMI) model plays a pivotal role. Moreover, a novel index called weighted average absolute scores (WAAS), derived from the AMMI model, is integrated into METs analysis indices (Alam, Akter, Khan, Alam, et al., 2024). Alongside AMMI, the genotype-genotype environment (GGE) biplot graphical model is commonly utilized to identify environments and winning genotypes within those environments (Akter et al., 2015). The GGE biplot method is also employed to pinpoint stable genotypes and evaluate their interaction with yield and environments. On the flip side, linear mixed models (LMMs) are commonly utilized to improve selection efficiency in various studies (Ahsan et al., 2024; Alam, Akter, Khan, Alam, et al., 2024; Alam, Akter, Khan, Amin, et al., 2024; Alam, Akter, Khan, Rashid, et al., 2023; Khan et al., 2024; Olivoto & Nardino, 2021; Pimentel et al., 2014; Schmidt et al., 2019). Yet, comprehending the genetic mechanisms dictating trait inheritance and the impact of genetic elements on their manifestation is crucial for the triumph of a breeding program (Fotirić-Akšić et al., 2011). An efficient method for analyzing and estimating genetic parameters using agronomic traits is through the restricted maximum likelihood (REML) model (Marchi et al., 2019). This method has been employed across diverse crop breeding endeavors, such as sweet potato (Alam, Akter, Khan, Alam, et al., 2024), white Guinea yam (Norman et al., 2022), soybean (Follmann et al., 2019), and others. An exposition by Olivoto et al. (Olivoto et al., 2019) delves into the theoretical foundations of the Multi-Trait Stability Index (MTSI), facilitating the selection of superior genotypes in METs based on high yield and stability, while considering multiple traits.

Introducing advanced breeding methods and employing METs in conjunction with modern stability analysis models like AMMI, GGE biplot, and MTSI in Bangladesh's rice farming can facilitate the discovery and choice of resilient genotypes with strong yield capabilities. This approach will aid in addressing the growing need for rice production amid environmental constraints and shrinking arable land. The present study was undertaken to examine the four advanced lines with two released varieties from BRRI at diverse environment to check the stability and yield advantages of advanced lines compared to released varieties.

2. Materials and methods

2.1. Description of the study area

The study was conducted over the 2022-23 growing season across 11 districts in Bangladesh: Rangpur, Gopalganj, Faridpur, Barishal, Natore, Sirajganj, Feni, Kushtia, Habiganj, Manikganj, and Gazipur, as illustrated in Figure 1. The experimental fields were chosen in collaboration with the Department of Agriculture Extension (DAE), taking into account variations in soil texture (BRRI, 2024) and elevation, which are detailed in Table 1. Additionally, monthly data on maximum and minimum temperatures, and relative humidity were collected from November to May 2022-23 at the research sites, covering the Boro growing season (BMD, 2023) (Figure 2).

2.2 Plant materials

This research included four advanced lines of FBR-SD: BR11318-5R-63, BR11337-5R-72, SVIN109, and IR17A1723. Two varieties released by BRRI were also included as checks to compare their yield and related characteristics. One check was BRRI dhan81, with the pedigree BR7358-5-3-2-1-HR2. The other check, BRRI dhan96, has the pedigree BR(Bio) 9787-BC2-63-2-2. All six genotypes exhibited uniform flowering, grain maturity, and good wrapping quality with culms. The flag leaves of the studied rice genotypes were erect. Detailed information about these six rice genotypes is presented in Table 2, while a visual representation of the raw rice grain is depicted in Figure 3.



Table 1. Description of the eleven selected locations during the Boro 2022-23 season

Location	Agro-ecological representation	Latitude	Longitude	Elevation	Soil texture
Rangpur	AEZ3	25°44'17"N	89°17'01"E	34	Loam- clay loam
Gopalganj	AEZ14	23°01'78"N	89°81'05"E	3.1	Clay loam-sandy loam
Faridpur	AEZ12	23°25'10"N	89°53'56"E	11	Clay loam-sandy loam
Barishal	AEZ13	22°42'35"N	90°20'00"E	2	Silty clay- clay
Natore	AEZ11	22°36'22"N	89°22'19"E	17	Clay loam
Sirajganj	AEZ4	24°39'34"N	89°65'72"E	13	Clay to loamy
Sonagazi	AEZ23	22°81'28"N	91°38'89"E	5	Clay
Kushtia	AEZ10	23°88'36"N	89°13'28"E	27	Clay-loam
Habiganj	AEZ19	24°31'05"N	91°21'46"E	6	Clay to loamy
Manikganj	AEZ8	23°86'56"N	90°00'98"E	15.92	Loamy/Clay
Gazipur	AEZ28	23°59'26"N	90°24'09"E	34	Silty clay loam

Table 2. The detailed information of FBR-SD genotypes

Genotype code	Genotypes	Year of release	Grain type
V1	BR11318-5R-63	NR	Medium slender
V2	BR11337-5R-72	NR	Medium slender
V3	SVIN109	NR	Medium slender
V4	IR17A1723	NR	Medium slender
V5 ^{CK}	BRR1 dhan81	2017	Medium slender
V6 ^{CK}	BRR1 dhan96	2020	Medium bold

^{NR} Not released yet

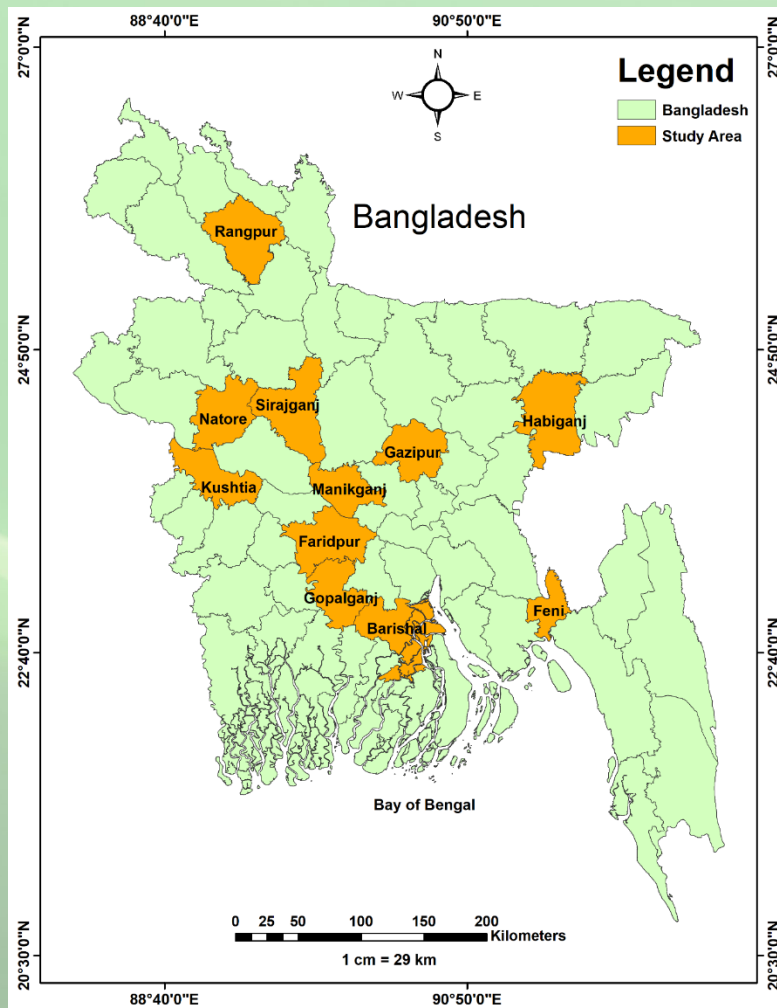


Figure 1. Locations of the study area in different districts in Bangladesh.



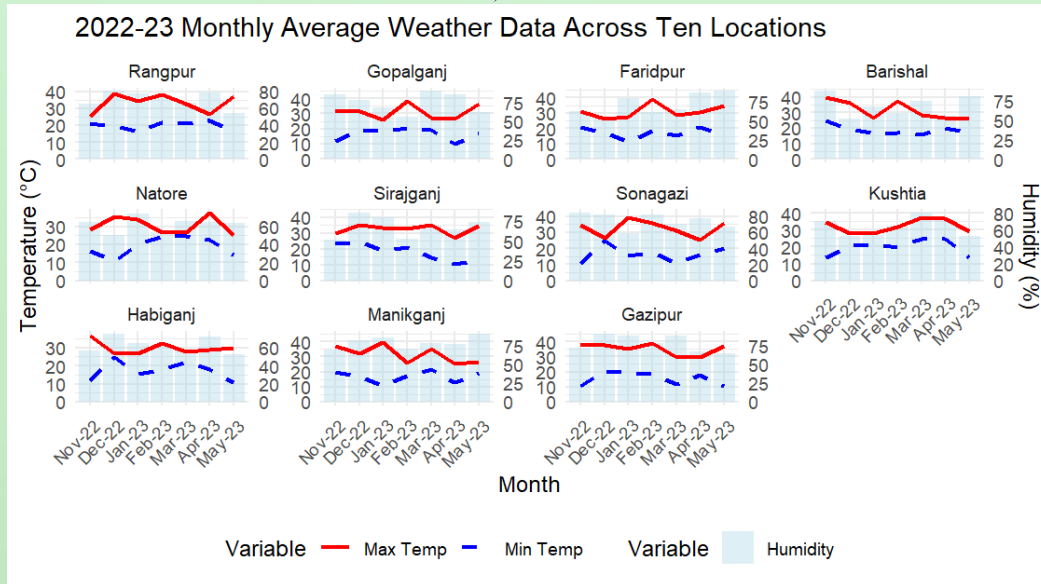


Figure 2. Monthly mean Maximum Temperature (°C), Minimum Temperature (°C) & Relative Humidity (%) of selected eleven (11) experiment locations in Bangladesh during 2022-23 Boro season.



Figure 3. A pictorial view of raw grain for the six FBR-SD genotypes. ^{CK} check

2.3 Experimental design

In every location, the entire experimental field was partitioned into three blocks, each comprising six plots, making a total of eighteen plots. These trials were replicated three times in each location, adhering to a randomized complete block design (RCBD). Each plot had a size of 20 m² (measuring 4.0 m by 5.0 m). Seedlings aged between 35 to 40 days were transplanted with a spacing of 20 cm by 20 cm.

2.4 Crop husbandry

The research site was plowed until achieving a finely textured seedbed for seedbed preparation. Seeding occurred between the last week of November and the last week of December 2022 across eleven locations. Fertilizers were applied at a ratio of 260:100:120:110:10 kg/ha of Urea:DAP:MoP:Gypsum:ZnSO₄, respectively (Ahmmmed et al., 2018). All fertilizers except urea were applied as basal, while urea was split into three equal portions applied at 10 days, 30 days after transplanting, and 5 days before the panicle initiation stage. Weeding and irrigation followed BRRI's recommended management practices as needed, and appropriate measures were implemented for pest control (BRRI, 2022).



2.5 Data collection

Data was recorded of grain yield (GY) (t/ha), growth duration (GD), plant height (PHT) (cm), thousand grain weight (TGW) (g), panicle per m² (PPM), filled grains per panicle (GPP), unfilled grains per panicle (UFG), and sterility percentage (STR%). Harvesting commenced when 80% of the grains were fully ripened of panicle. A designated area of 3 m by 3 m, totaling 9 m², was demarcated excluding the border lines of the experimental plot. Any missing hills within the sample area were carefully counted, and an equivalent number of hills from other areas outside the plot's border lines were added to the harvested sample hills to ensure accurate yield estimation for the 9 m² area. Subsequently, the GY was converted into t/ha. Threshing was carried out using either a pedal thresher or manually by removing leaves, straws, and other debris from the grains. The weight was accurately recorded while simultaneously measuring the moisture content of the grains, adjusting the grain yield to 14% moisture content. To determine PHT, data were collected from ten plants in each plot, measuring from the soil level to the tip of the panicle in centimeters, and then averaged. For measuring TGW, 1000 grains were manually counted from the harvested site of the experimental plot and weighed using a scale of grams. For PPM estimation, a 1 m by 1 m area was marked, and the number of panicles within the marking was counted. Ten panicles were randomly selected outside the marking for both GY and PPM, and the total number of filled grains and unfilled grains was counted. Subsequently, the total number of filled and unfilled grains was averaged to calculate the GPP and UFG. STR% was calculated by following formula,

$$\text{STR}\% = \frac{\text{Total unfilled grain}}{\text{Total filled grain} + \text{Total unfilled grain}} \times 100$$

2.6 Statistical analysis

The study employed a combined two-way analysis of variance (ANNOVA) and mean separation for genotypes, environments, and GEI using Statistics 10. The least significant difference (LSD) test was utilized to distinguish mean values at a significance level of $p < 0.05$. The genetic parameters of studied traits were analyzed using "metan" package of R software. The coefficient of correlation (Pearson's Correlation) of studied traits of rice genotypes was performed using 'metan' package of R statistical software. A heatmap for genotypes and studied traits was prepared by using "pheatmap" package of R. The AMMI analysis of variance, WAAS biplot and GGE biplot were analyzed using 'metan' package of R. The MTSI analysis was performed utilizing the "metan" package within the R statistical software environment (R studio, 2020), employing a positive selection criterion for all traits with a selection pressure set at 15%.

3. Results

3.1 Analysis of variance

Table 3 displays the combined analysis of variance (ANOVA) outcomes for eight yield and yield-related traits in six FBR-SD genotypes across eleven locations of Bangladesh during the 2022-23 boro growing season. The results indicated that all sources of variation, including replication (R), genotypes (G), locations/ environments (E), and genotype-environment interactions (GEI), had a significant impact on the traits studied ($p \leq 0.001$).

3.2 Genetic parameters of studied traits

The effect of G, E and GEI on the studied traits was found significant at $p < 0.05$ based on the likelihood ratio test (LRT) of restricted maximum likelihood (REML) model (Table 4). In the linear mixed model (LMM), Table 4 presents the genetic parameters of various traits in six FBR-SD rice genotypes. The genetic variance (σ^2_g) percentages were highest for GD (50.24%) and TGW (44.27%), while environmental variance (σ^2_e) percentages were highest for GY (39.80%) and GPP (59.60%). PHT, PPM, UFG, and STR% showed the highest variance percentages for genotype-environment interaction (σ^2_{GEI}) at 53.06%, 51.37%, 49.46%, and 65.82%, respectively. The broad-sense heritability (h^2_b) ranged from 0.05 to 0.50 across the studied genotypes for the eight traits. The genetic advance in a percent of mean (GAPM) ranged from 0.47 to 0.92. Selection accuracy (SA) was high (>0.80) for all traits except UFG (0.68). The ratio of genotypic (CV_g) to residual coefficient of variation (CV_r) ranged from 0.34 to 4.13.

3.3 Mean performance of FBR-SD genotypes

Table 5 presents the mean values of eight yield and yield-contributing traits for six rice genotypes. The GY was recorded for V1 at 7.29 t/ha, followed by V2 at 7.20 t/ha and V3 at 7.25 t/ha. Regarding other yield-contributing traits, V2 exhibited significantly higher values for PPM (303.73) and GPP (144.76) compared to the other genotypes, while TGW was highest in V3 (23.48 g). V4 had the shortest mean GD (138.50 days), followed by V1 (139.88 days). Additionally, V1 displayed the tallest PHT (109.81 cm), while V2 had the lowest PHT (93.33 cm). V5 exhibited the highest STR% (23.17%), while the lowest was in V6 (15.47%). The UFG was found highest in V5 at 34.74 and the lowest was in V6 (24.48).

Table 6 depicts the GEI effects on GY. Variability in GY is evident across E and G, with significant differences observed in mean yield values. The highest GY was recorded in the Natore region for the V2 genotype, reaching 9.48 t/ha, followed by V1 at 9.31 t/ha and V3 at 8.64 t/ha. Conversely, the lowest yield was observed in V5, measuring 3.77 t/ha in the Sonagazi region. The highest GY was recorded in Natore at 8.29 t/ha, followed by Sirajganj at 8.21 t/ha and Faridpur at 7.89 t/ha.



Table 3. Combined ANOVA of six FBR-SD rice genotypes during the 2022-23 boro season studied across 11 locations of Bangladesh

SV	df	Mean sum of squares							
		GY	GD	PHT	TGW	PPM	GPP	UFG	STR%
R	2	0.2023***	0.136***	7.77***	0.2377***	850.2***	305.56***	170.57***	4.157***
G	5	7.3806***	210.870***	1285.82***	44.3092***	4650.3***	3865.54***	754.60***	387.254***
E	10	17.4576***	397.371***	1426.97***	18.3079***	31984.6***	6907.52***	1457.29***	428.365***
GEI	50	1.2341***	16.785***	147.57***	3.5301***	1574.0***	802.12***	401.16***	128.658***
Error	130	0.3279***	0.321***	7.37***	0.5430***	337.4***	394.36***	93.10***	12.439***
Total	197								

^R replication, ^G genotype, ^E environment/ locations, ^{GEI} genotype-environment interaction, *** significant at 0.1% level of probability, ^{df} degrees of freedom, ^{GY} grain yield, ^{GD} growth duration, ^{PHT} plant height, ^{TGW} thousand grain weight, ^{PPM} panicle per m², ^{GPP} grains per panicle, ^{UFG} unfilled grain, ^{STR%} sterility percentage SV: Source of variation

Table 4. Genetic parameters of yield and yield attributing traits of six FBR-SD rice genotypes studied under 11 locations in LMM

Parameters	GY	GD	PHT	TGW	PPM	GPP	UFG	STR%
σ^2_g (%)	22.9	50.24	39.05	44.27	11.34	15.53	5.17	13.30
σ^2_{GEI} (%)	37.30	46.81	53.06	35.36	51.37	24.87	49.46	65.82
σ^2_e (%)	39.8	2.94	7.90	20.37	37.30	59.60	45.37	20.88
h ² _b	0.23	0.50	0.39	0.44	0.11	0.16	0.05	0.13
SA	0.91	0.96	0.94	0.96	0.81	0.89	0.68	0.82
CV _g	6.20	1.71	5.78	5.03	3.29	7.35	10.00	14.00
CV _r	8.18	0.41	2.60	3.41	5.96	14.40	29.70	17.50
CV ratio	0.76	4.13	2.22	1.47	0.55	0.51	0.34	0.80
GAPM	0.83	0.92	0.89	0.92	0.66	0.79	0.47	0.67
LRT	*	*	*	*	*	*	*	*

σ^2_g genotypic variance, σ^2_e environmental variance, σ^2_{GEI} variance of genotype-environment interaction, h²_b broad sense heritability, GEI₂ coefficient of determination of the interaction effects, SA selection accuracy, CV_g genotypic coefficient of variation, CV_r residual coefficient of variation, CV ratio ratio of CV_g and CV_r, GAPM genetic advance in a percent of mean, LRT likelihood ratio test, GY grain yield, GD growth duration, PHT plant height, TGW thousand grain weight, PPM panicle per m², GPP grains per panicle, UFG unfilled grain, STR% sterility percentage

Table 5. Mean values of yield and yield traits for six FBR-SD rice genotypes

Genotypes	GY (t/ha)	GD (days)	PHT (cm)	TGW (g)	PPM	GPP	UFG	STR%
V1	7.29a	143.58c	109.81a	22.92b	293.83b	134.97b	26.12b	16.23d
V2	7.20a	144.97a	102.73c	21.37d	303.73a	144.76a	32.36a	20.03bc
V3	7.25a	144.38b	105.16b	23.48a	284.13c	130.97b	33.83a	20.32c
V4	6.20c	138.50f	96.67d	22.89b	288.19bc	118.34c	34.45a	22.17ab
V5	5.98c	139.88e	96.34d	22.10c	268.00d	119.68c	35.74a	23.17a
V6	6.79b	140.38d	93.33e	20.28e	289.63bc	135.25ab	24.48c	15.47d
CV%	8.23	0.40	2.67	3.33	6.25	15.16	29.52	17.58
LSD _{0.05}	0.28	0.28	1.32	0.36	8.95	9.67	4.7	1.72

LSD least significant differences among mean values at 5% level of significant, ^{CV} coefficient of variations, ^{V1} BR11318-5R-63, ^{V2} BR11337-5R-72, ^{V3} SVIN109, ^{V4} IR17A1723, ^{V5} BRRI dhan81, ^{V6} BRRI dhan96, ^{GY} grain yield, ^{GD} growth duration, ^{PHT} plant height, ^{TGW} thousand grain weight, ^{PPM} panicle per m², ^{GPP} grains per panicle, ^{UFG} unfilled grain, ^{STR%} sterility percentage

Table 6. Mean values of grain yield (GY) (t/ha) for interaction effect of genotypes and locations during 2022-23 boro season

Locations	GY (t/ha)						
	V1	V2	V3	V4	V5	V6	LM
Barishal	6.09v-z	6.28s-x	6.36q-x	5.35yzab	4.46bc	5.81w-za	6.42d
Faridpur	8.52b-f	8.75a-d	8.61a-e	7.61f-m	7.60f-m	7.83d-l	7.89b
Gazipur	8.33c-i	7.81e-l	8.22c-j	6.92l-v	7.23l-r	7.20l-s	7.85bc
Gopalganj	8.17c-k	7.31j-p	7.75e-m	6.61n-w	7.14l-s	7.08l-t	7.50c
Habiganj	6.13u-z	6.36q-x	6.48p-x	5.58x-za	5.28zab	6.48p-x	6.09de
Kushtia	6.32r-x	6.43p-x	6.54n-w	6.00v-za	5.83w-za	6.48p-x	5.92e
Manikganj	7.25k-q	7.05l-u	7.58g-m	6.61n-w	6.43p-x	6.87m-v	6.15de
Natore	9.31ab	9.48a	8.64a-e	8.39b-g	7.79e-l	8.86abc	8.29a
Rangpur	6.59n-w	6.43p-x	6.54n-w	6.21t-y	6.43p-x	6.49o-x	5.79e
Sirajganj	8.35c-h	8.39b-g	8.50b-g	7.43h-n	7.42i-o	7.79e-l	8.21ab
Sonagazi	6.09v-z	5.87w-za	6.01v-z	4.62bc	3.77c	5.08ab	6.43d
CV%	15.29	15.38	14.18	21.81	27.11	15.68	13.82
LSD	0.92						0.38

LSD least significant differences among mean values at 5% level of significant, ^{CV} coefficient of variations, ^{LM} locational mean, ^{V1} BR11318-5R-63, ^{V2} BR11337-5R-72, ^{V3} SVIN109, ^{V4} IR17A1723, ^{V5} BRRI dhan81, ^{V6} BRRI dhan96



3.4 Correlation analysis of among traits

Figure 4 presents the Pearson's correlation coefficients between the studied traits of rice genotypes. The traits STR and UFG exhibited a significant positive correlation ($r=0.95$ at $p<0.001$). GY showed a significant positive correlation with growth duration (GD) ($r=0.89$ at $p<0.05$). Additionally, GY and GPP were significantly positively correlated ($r=0.84$ at $p<0.05$). There was no significant negative correlation was found between studied traits.

3.5 Heatmap of genotypes' contribution to the studied traits

Figure 5 illustrates the trait contributions of different rice genotypes using a heatmap. Genotypes V1, V2, V3, and V6 positively contribute to GY. Among them, genotype V2 stands out as the highest contributor to PPM, GPP, and GD, while negatively affecting TGW. Genotypes V5 and V4 are the primary contributors to UFG and STR. Genotype V1 has the highest contribution to PHT and GY, while V3 contributes significantly to TGW. Although genotypes V4, V5, and V6 negatively influence GD, genotype V6 still positively contributes to GY.

3.6 AMMI analysis of variance

Table 7 presents the AMMI ANOVA, highlighting the contributions of different sources of variation to the total sum of squares (TSS) observed in the dataset for GY. The analysis of variance revealed that GY was significantly affected by E, G and GEI, with a p-value less than 0.001. The majority of the total sum of squares (TSS) in the model was attributed to E (174.576), followed by GEI (61.708) and G (36.903). The interaction of the first two principal components (PCs) accounted for approximately 77.5% of the variance. Among the PCs of the AMMI ANOVA, the first three PCs were significant at ≤ 0.01 probability, contributing to a total of 90.7% of the variance.

3.7 Stability analysis through WAAS biplot

Figure 6 presents a WAAS biplot showing the performance of six rice genotypes across eleven locations. The vertical axis represents the average GY across all environments, with genotypes to the right indicating higher GY and those to the left indicating lower GY. The horizontal axis represents the mean WAAS, dividing the biplot into four quadrants. Genotypes in different quadrants are classified based on their stability and yield performance. Quadrant I typically contain genotypes with low stability and yield, including V6, V8, and V5. Genotype V2 in quadrant II shows high WAAS values, indicating high GEI influence and high yield. Quadrants III and IV have low WAAS values, indicating high stability. Genotypes V1 and V3 in quadrant IV exhibit high GY and stability across environments. There were no genotypes fall into the quadrant III.

3.8 GGE polygonal biplot analysis for MET

Within the GGE biplot, a polygon is formed by connecting the vertex genotypes (V1, V2, V6, V4, and V5) (Figure 7). This polygon is then divided into five distinct sectors by rays (dotted lines) originating from the plot's origin and extending perpendicular to the polygon's sides. In regions where environments are located and genotypes are positioned above them, it indicates that these specific genotypes perform optimally in those particular environments.

Table 7. AMMI ANOVA of rice grain yield (GY) (t/ha) for six FBR-SD genotypes studied under 11 locations during 2022-23 boro season

Source of variations	df	TSS	MSS	PC Contribution	Total contribution
E	10	174.58	17.46***	-	-
R (E)	22	7.41	0.34	-	-
G	5	36.90	7.38***	-	-
GEI	50	61.71	1.23***	-	-
PC1	14	26.97	1.93***	43.7	43.7
PC2	12	20.87	1.74***	33.8	77.5
PC3	10	8.12	0.81**	13.2	90.7
Noise (PC)	8	5.75	0.75	9.3	-
Residuals	110	35.62	0.32	-	-
Total	247	377.92	1.53	-	-

^E environment/ location, ^R replication, ^G genotype, ^{GEI} genotype-environment interaction, *** significant at 0.001% level of probability, ** significant at 0.01% level of probability, ^{df} degrees of freedom, ^{TSS} total sum of square, ^{MSS} mean sum of square, ^{PC} principal component

Table 8. Broad-sense heritability (h^2) and selection gain (SG) (%) based on factor analysis obtained through MTSTI for grain yield (GY) of six rice genotypes

VAR	Factor	X _o	X _s	h^2	SG (%)	Sense	Goal
GY	FA1	6.96	7.29	0.833	3.93	increase	100
GD	FA1	142	145	0.92	1.88	increase	100
GPP	FA1	131	147	0.792	9.54	increase	100
TGW	FA2	22.1	21.4	0.92	-3.04	increase	0
UFG	FA2	32.7	35.2	0.468	3.62	increase	100
STR	FA2	20.1	21.3	0.668	4.22	increase	100
PPM	FA3	294	311	0.662	3.91	increase	100
PHT	FA4	102	103	0.885	1.48	increase	100

X_o observed mean, X_s mean of selected genotypes, FA factor, GY Grain yield, GD Growth Duration, PHT Plant Height, TGW thousand grain weight, PPM Panicle per m², GPP Grains per panicle, UFG Unfilled grain, STR% Sterility percentage



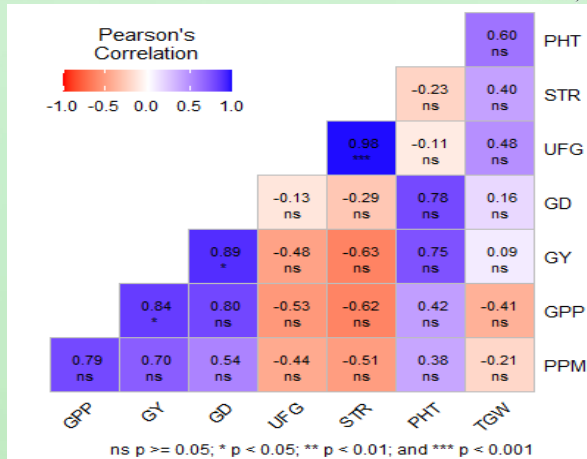


Figure 4. Co-efficient of correlation among traits of six FBR-SD rice genotypes. ^{GY} grain yield, ^{GD} growth duration, ^{PHT} plant height, ^{TGW} thousand grain weight, ^{PPM} panicle per m², ^{GPP} grains per panicle, ^{UFG} unfilled grain, ^{STR} sterility percentage

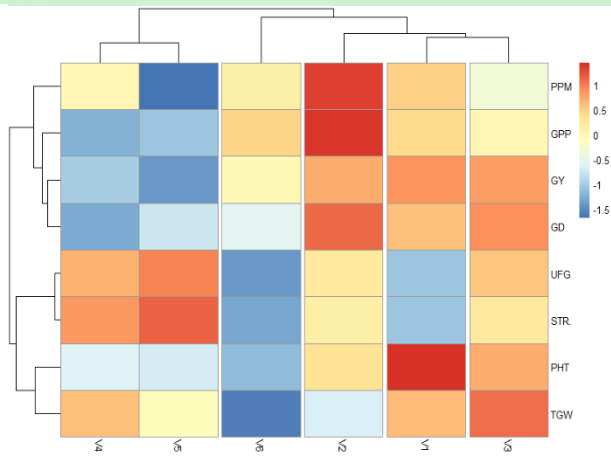


Figure 5. Heatmap of six rice genotypes and eight yield and yield related traits. ^{GY} grain yield, ^{GD} growth duration, ^{PHT} plant height, ^{TGW} thousand grain weight, ^{PPM} panicle per m², ^{GPP} grains per panicle, ^{UFG} unfilled grain, ^{STR}% sterility percentage

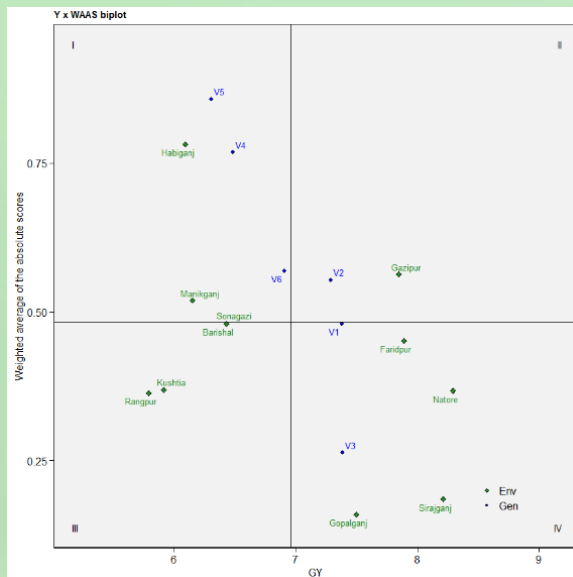


Figure 6. WAAS biplot of AMMI stability model with stability and mean performance of grain yield (GY) (t/ha).

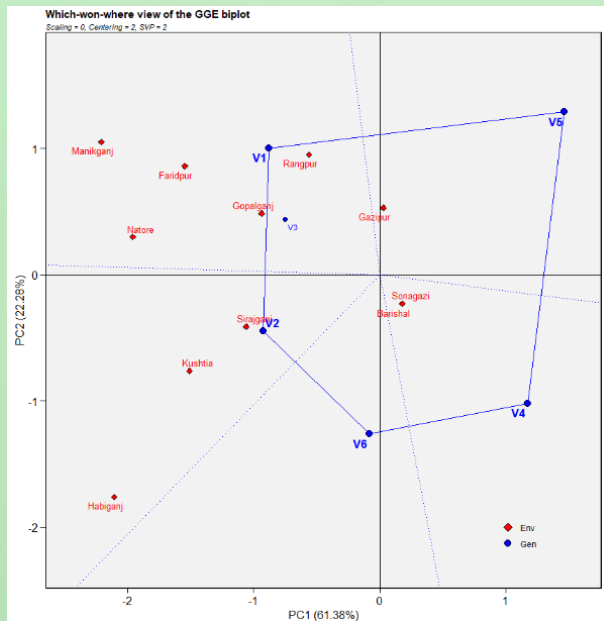


Figure 7. Polygonal view of GGE biplot for winner genotype in respective environment/ location for grain yield (GY) (t/ha).

Genotypes located in areas without any environments are considered unsuitable for cultivation in the examined environments and are classified as weak genotypes in most environments. Genotype V2 demonstrated superior GY performance in Sirajganj, Kushtia, and Habiganj, while Genotypes V1 and V3 showed desirable GY in Manikganj, Faridpur, Natore, Gopalganj, and Rangpur. Additionally, V4 performed well in the Sonagazi and Barishal environments, while V5 excelled in the Gazipur region. However, Genotype V6 was positioned without any associated environment.

3.9 Multitrait stability index (MTSI)

Table 8 displays the results of the factor analysis, which reveal the broad sense heritability (h^2), selection gain percentage (SG) (%), desired selection sense, and the achievement status of the goal based on factor analysis of selected rice genotypes using eight traits. The factor analysis revealed that contributing factors formed into three distinct groups consisting of eight traits under study. The desired selection sense was to increase for all traits. The goal was successfully achieved for all traits, with the exception of TGW. Among the studied traits, the h^2 was highest for GD and TGW (0.92), followed by PHT (0.885) and GY (0.833). Furthermore, the SG (%) for studied traits was ranged from 1.48 to 9.54 %. The selected genotype, however, possesses a negative SG (%) in TGW (-3.04%).



Figure 8 presents the representation of the order of the most stable genotype determined by the multi-trait stability indexing (MTSI). Within this context, the red circle symbolizes the threshold for selecting the stable genotype. The rice genotype named V2 stands out as the most stable compared to other genotypes.

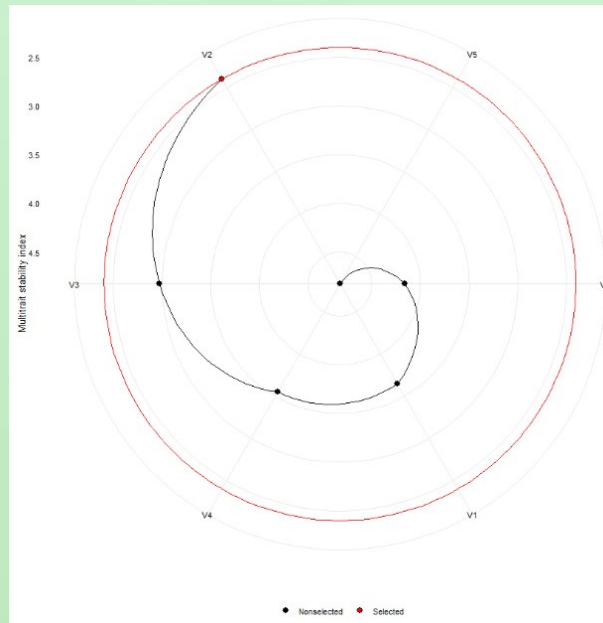


Figure 8. Ranking of genotype stability based on MTSI index

4. Discussion

The study observed significant variations in yield and its attributing traits among different genotypes (G), environmental/locational conditions (E), and genotype-environment interactions (GEI) (Table 3). The percentages of genotypic (σ^2_g), environmental (σ^2_e), and GEI variance (σ^2_{GEI}) in Table 4 confirm the differences observed in the studied traits. The success of crop breeding programs largely depends on genetic diversity and the transmission of desirable characteristics. Analyzing the genetic diversity of a species with the involvement of plant breeders can lead to the enhancement of commercially desirable traits (Sarker, 2020). To assess genetic diversity, researchers often utilize σ^2_g , σ^2_e , and σ^2_{GEI} , as well as coefficients of variation (CV) (Baraki et al., 2020; Khan et al., 2024). A CV ratio of genotypic CV (CV_g) and residual CV (CV_r) higher than 1 indicates high genetic diversity, while a lower ratio suggests low genetic diversity (Sharma et al., 2021). In this study, the CV ratios for growth duration (GD), plant height (PHT), and thousand grain weight (TGW) were all above 1, indicating high genetic diversity. Moreover, these traits exhibited high broad-sense heritability (h^2_b) and selection accuracy (SA) (Table 4). Traits with high genetic diversity, h^2_b , and SA suggest potential for improvement through trait-based selection (Alam, Akter, Khan, Alam, et al., 2024; Alam, Akter, Khan, Amin, et al., 2024; Alam, Akter, Khan, Rashid, et al., 2023; Hasan et al., 2020). High h^2_b combined with a high percentage of genetic advance relative to the mean (GAPM) is important for trait selection in breeding programs. In this study, GD, PHT, and TGW showed both high h^2_b and high GAPM, indicating additive gene action, thus making them suitable targets for rice improvement. Previous studies have also reported similar findings regarding high h^2_b and GAPM for various yield-related traits (Sarker, 2020). Except for grain yield (GY), the traits showed high GAPM, suggesting the influence of both additive and non-additive gene actions in trait expression. This aligns with findings from other research on rice (Faysal et al., 2022).

Yield and its contributing traits of rice are influenced by various factors (Kiniry et al., 2001; Kumar et al., 2017). According to Sarker et al. (Sarker, 2020), genotype plays a significant role in determining rice yield and its contributing characteristics, although numerous factors such as meteorological conditions, soil texture, nutrient composition, and genetic traits also influence these traits (Dou et al., 2016; Xuan et al., 2019). Boro rice cultivation, typically practiced in irrigated ecosystems due to low rainfall during the boro growing season (Mainuddin et al., 2021), tends to perform better in coarse-textured soils compared to fine-textured soils, despite careful farmer management practices (Bhuyan et al., 2023; Frimpong et al., 2023). In our study, locations like Natore, Sirajganj, and Faridpur exhibited the highest average grain yield for FBR-SD rice genotypes, indicating the presence of favorable environmental conditions in those areas. Rice varieties exhibit substantial variation in the duration from sowing to harvest (Vergara et al., 1966). In our study, the average GD significantly differed among rice genotypes, with the V4 genotype displaying the shortest GD. Opting for a short-duration crop offers numerous advantages over a long-duration one, even if their total grain yields are equivalent. A shorter-duration crop requires less water per cycle, faces reduced exposure to risks such as pests, diseases, droughts, and storms, and extends the window



for subsequent plantings of rice or other crops (Vergara et al., 1966). PHT also varied among rice genotypes, consistent with findings by Zhang et al. (Zhang et al., 2017), who highlighted the role of the OsMPH1 gene in regulating plant height. They observed that high OsMPH1 expression in rice nodes and pulvini, and its nuclear localization, led to increased plant height through cell elongation in internodes. TGW exhibited significant variability across rice genotypes, as reported by Zuo et al. (Zuo et al., 2022), who identified OsMADS56 as the gene responsible for a QTL affecting grain weight and size. Wang et al. (Wang et al., 2024) proposed increasing panicle numbers as a viable strategy to enhance rice yield potential, noting that the Lemont genotype showed a substantial increase in panicle count, albeit with a decrease in grain numbers. Discrepancies in filled grain per panicle (GPP) and panicles per m² (PPM) underscore the genetic basis of these traits, aligning with our study's findings. Unfilled grain (UFG) and grain sterility (STR%) varied significantly with genotype and environmental conditions. Elevated temperature coupled with high humidity at heading induces nearly complete grain sterility in rice, with a 30% reduction in relative humidity at high temperatures resulting in decreased grain sterility (De Abeysirwardena et al., 2002; Osada et al., 1973). Puteh et al. (Puteh et al., 2014) observed significant genotype-dependent variation in UFG and STR%.

The correlation among traits facilitates the identification of specific traits that, through indirect selection, can enhance overall yield performance. Heatmap analysis provides a practical evaluation of the direct and indirect influences of each trait associated with the genotypes. The primary economic trait, GY, demonstrated a significant positive correlation with GPP in our study, consistent with previous findings (Kayastha et al., 2022; Wang et al., 2024). In the present study, there was no significant correlation between GPP and Additionally, GD showed a significant positive correlation with GY. Genotypes V1, V2, V3, and V6 were identified as the top contributors to GY (Figure 5), suggesting that enhancing GPP in these genotypes may lead to improved GY performance. According to Huang et al. (Huang et al., 2018), rice yield tends to increase with prolonged GD, although some rice cultivars with shorter GD also exhibit high GY (Chen et al., 2019), indicating the potential for high GY in short-duration cultivars as well. In our study, V6 negatively contributed to GD but positively contributed to GY. Okamura et al. (Okamura et al., 2018) found a positive and significant correlation between STR% and UFG, consistent with our study findings.

AMMI analysis revealed that the error mean sum of squares for GY was lower compared to G, E, and GEI, indicating the model's accuracy. Studies by Abdelrahman et al. (Abdelrahman et al., 2022) and Sharifi et al. (Sharifi et al., 2017) supported this observation. Additionally, Fathi Sadabadi et al. (Sadabadi et al., 2018) and Hassani et al. (Hassani et al., 2023) found that the first and second principal components (PCs) of GEI collectively explained 71.60% of the variability, aligning closely with our study's findings (77.5%). Understanding GEI assists in decision-making regarding adaptation to different environmental conditions, as noted by Ghazy et al. (Ghazy et al., 2024) and Lee et al. (Lee et al., 2023). The WAAS biplot differs from the AMMI model in that it incorporates all scores of the PCs rather than focusing solely on the first two. Genotypes with low WAAS values falling into quadrant IV in the biplot are identified as high yielding and stable, according to Ghazy et al. (Ghazy et al., 2024). In our WAAS biplot analysis, considering both GY and WAAS values, genotypes V1 and V3 emerge as superior performers with stable performance. In a similar rice genotype study, Ghazy et al. (Ghazy et al., 2024) categorized the WAAS biplot into four categories: high-yielding stable, high-yielding not stable, low-yielding stable, and low-yielding unstable genotypes. This classification aids rice breeders in predicting the genotypic performance of each genotype under study before integrating them into different programs, which aligns with our study findings.

Utilizing a GGE polygonal biplot, as recommended by Gauch and Zobel (Gauch & Zobel, 1997), assists in suggesting suitable genotypes for specific environments. In our study, the biplot identified five mega-environments among 11 tested locations for GY. Among these, five locations (Manikganj, Natore, Faridpur, Gopalganj, and Rangpur) were deemed favored environments for genotype V1, characterized by conducive environmental conditions and soil properties for optimal rice growth. Akter et al. (Akter et al., 2015) also observed similar outcomes and identified appropriate genotypes for specific environments using GGE biplot analysis. Moreover, among the 11 environments assessed, genotype V2 exhibited superior performance in three environments (Sirajganj, Kushtia, and Habiganj), while V5 showed favorable performance only at Gazipur, and V4 displayed good performance in Sonagazi and Barishal. These results underscore the significant influence of GEI on the performance of tested genotypes. GEI negatively impacted the performance of evaluated genotypes by compromising their yield under unfavorable conditions. This observation is corroborated by the findings of other studies, such as those by Liang et al. (Liang et al., 2015) and Singh et al. (Singh et al., 2023). Conversely, V6 demonstrated poor performance in all tested environments, indicating its unsuitability across the eleven locations studied. This finding aligns with the conclusions drawn by Ghazy et al. (Ghazy et al., 2024).

The selection process considers variations among characteristics in relation to their level of hereditary influence. The relationship between broad sense heritability (h^2) and genetic gain (SG) is a crucial factor in breeding programs, as there is a direct association between the h^2 of traits and the increase in SG (Alam, Akter, Khan, Amin, et al., 2024). Chattopadhyay et al. (Chattopadhyay et al., 2011) indicated that traits with h^2 exceeding 60% are considered highly heritable, those with 30 to 60% are moderately heritable, and those with 0 to 30% are lowly heritable. Our findings reflect this pattern, with h^2 demonstrating high values for all traits except UFG.



Additionally, the SG for the studied traits was also notably high. This suggests that direct selection of genotype V2 could effectively enhance the studied traits in rice cultivation. However, for TGW, although the aim of selection was positive, the selection goal was not met, resulting in a negative SG, which is considered desirable for rice varietal selection in the context of Bangladesh (Choudhury, 1979; Mottaleb & Mishra, 2016). The slender grain type exhibits low TGW due to the reduced individual weight of rice grains. The MTSI index was computed based on eight attributes related to yield and its contributing characteristics to rank stable genotypes with higher mean trait values. Several researchers have employed the MTSI index for selecting stable genotypes of rice and other crops (Alam, Akter, Khan, Rashid, et al., 2023; Lee et al., 2023; Olivoto et al., 2019; Sharifi et al., 2017). These findings align with the results obtained from our investigation, where genotype V2 emerged as the top-ranked stable genotype.

5. Conclusions

The extensive evaluation of advanced rice lines and reference varieties across various locations during the 2022-23 boro season unveiled significant impacts of genotype, environment, and their interactions on yield and associated traits. V1 exhibited the highest grain yield, closely trailed by V2 and V3, with V3 showing remarkable performance. Nature stood out as the location with the highest yield, and grain yield showed a positive correlation with growth duration and filled grain per panicle. Heatmap analysis revealed favorable contributions to yield from V1, V2, V3, and V6. The WAAS and GGE biplot emphasized the stability and adaptability of different genotypes across locations, with V2 emerging as the top stable genotype using MTSI. Factor analysis showcased promising results for most traits, with significant broad-sense heritability observed for growth duration, plant height, and grain yield. Overall, this study offers valuable insights into genotype performance and stability across diverse environments, aiding in the selection and breeding of high-yielding boro rice varieties. It's important to acknowledge the study's limitation of being conducted for a single growing season, potentially overlooking annual variability in environmental conditions such as climate, soil, and pest pressures. Hence, a comprehensive, multi-seasonal investigation incorporating identified genotypes is recommended to enhance the reliability and precision of results. Considering the alignment of traits with stability analysis, ARD, BRRI proposes advancing the V2 genotype for proposed variety trials with MEs before finalizing its release.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the authority of Bangladesh Rice Research Institute, 1701, Gazipur, Bangladesh.

Author Contribution Statement

*Mir Mehedi Hasan: Data collection, supervision, conceptualization, methodology, Analyzed and interpreted the data, writing the original draft, review and editing

Shamsunnaher: Data collection, supervision, conceptualization, methodology and investigation

Md. Habibur Rahman Mukul, Afruz Zahan, Md. Niaz Morshed, Khandoker Khalid Ahmed, Md. Romel Bishwas, Md. Abdul Gaffar, Aparajita Badhan, Sanjida Akter, Biswajit Karmakar, and Md Humayun Kabir: Contributed data collection and materials.

Fund Statement

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Conflict of Interest

“The authors declare no conflict of interest.”

Data availability statement

Data will be made available on request.

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Abbreviations and Units

BRRI: Bangladesh Rice Research Institute, BRR: Blast resistant rice, RCBD: Randomized complete block design, GEI: Genotype-environment interactions, GGE: Genotype and Genotype-Environment, PCA: Principal component analysis, ANOVA: Combined analysis of variance, PBD: Plant Breeding Division, GY: Grain yield, PHT: Plant height, PPM: panicle per meter², DTM: Growth duration, GPP: Grains per panicle, TGW: 1000-grain weight, FGP: Filled grains per panicle, UFG: Unfilled grains per panicle, STR: sterility percentage, CV: Coefficient of variation, AMMI: Additive Main Effects and Multiplicative Interaction, WAASB: Weighted Average of Absolute Scores from the BLUPs, MTSTI: Multi-Trait Stability Index.

