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# Development and Identification of Recombinant Inbred Lines (RILs) Exhibiting Reproductive-stage Cold Tolerance in Rice

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## ABSTRACT

Cold injury-induced spikelet sterility and flash flood inundation at pre-ripening to ripening stages of Boro rice are common phenomena in the *haor* areas of Bangladesh. The development and deployment of short-duration and cold-tolerant high-yielding rice varieties can be a sustainable solution to address these issues. This study reports the development of recombinant inbred lines (RILs) using a local cultivar, '*Rata Boro*' collected from *haor* areas, as a donor for cold tolerance. The RILs were screened under cold-stress environment to identify short-duration, cold-tolerant breeding lines at the reproductive stage. A total of 398 RILs were developed from crosses between *Rata Boro* and three elite breeding lines, and advanced to $F_5$ generations through single-seed descent-based rapid generation advance techniques. The first-year evaluation of 425 genotypes including 398 RILs and 27 parental lines under cold-stress conditions in the field at Gazipur during Boro 2023-24 identified 11 breeding lines with minimal reduction in yield and spikelet fertility. The subsequent multi-location testing of these selected entries in Boro 2024-25 revealed a differential adaptation pattern. Genotypes G5 (BR14628-4R-156-Gaz-1), G3 (BR14628-4R-125-Gaz), G7 (BR14628-4R-198-Gaz-1), and G9 (BR14628-4R-50-Gaz-1) exhibited superior performance and specific adaptation to cold-prone *haor* environments. Additionally, G6 (BR14628-4R-180-Gaz-1) and G11 (BR11894-R-R-R-R-169) demonstrated broad adaptability and stability across all test sites. Notably, G3, G6, and G9 consistently produced high yields under both cold-stress and non-stress conditions. These cold-tolerant RILs represent valuable genetic resources for improving cold tolerance in rice and for future studies aimed at elucidating underlying mechanisms of cold tolerance.

**Keywords:** Rice, Cold stress, *Haor* areas, Multilocation, Spikelet fertility, grain yield.

## INTRODUCTION

Rice (*Oryza sativa* L.) serves as the staple food for more than half of the global population and is cultivated across diverse ecosystems worldwide. Although rice originated in the swampy areas of the tropics, it is highly vulnerable to abiotic stresses, including cold stress (Rice Improvement, 2021). In the northeastern haor regions (Bokhtiar *et al.*, 2024) of Bangladesh, Boro rice faces cold stress during its reproductive stages when planted earlier than the usual. Farmers often adopt this early planting strategy to reduce the risk of crop loss from flash

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floods, which typically occur during the ripening stage (Biswas *et al.*, 2020).

In *haor* areas, flash floods usually occur in early to mid-April, when the Boro rice crop remains at the pre-ripening to ripening phase. The risk of crop damage due to flash flood can be minimized by sowing seeds of short-duration varieties immediately after receding the flood water from the seedbed in late October to early November. However, adjusting the sowing date increases the risk of cold injury during the panicle initiation (PI) to booting stages in late January to early February, which can cause extensive spikelet sterility and subsequent yield loss. The early pollen microspore stage (10-12 days before heading) is the most sensitive stage to cold injury (Mitchell *et al.*, 2016). Rice is particularly sensitive to cold stress when daily average temperatures remain below 20°C for 5-6 consecutive days during its reproductive phase. Exposure to such conditions leads to spikelet degeneration, incomplete panicle exertion, pollen abortion, and abnormal microspore development, all of which contribute to higher spikelet sterility and reduced grain yield (Biswas *et al.*, 2018). In 2017, the Boro rice crop of 1.5 million hectares, worth approximately USD 450 million, was damaged due to a huge flash flood that occurred in early April (Hossain *et al.*, 2023). To address the dual challenges of flash floods and cold injury, the development of short-duration and cold-tolerant rice varieties has become essential.

Before the introduction of high yielding varieties in the *haor* areas, landrace cultivars with resilience to cold stress were predominantly grown during the Boro season. The cold-tolerant rice breeding program of Bangladesh Rice Research Institute has used Hbj.B.VI, a landrace-derived improved cultivar (Poshushail) collected from the *haor* areas as a donor parent for cold tolerance, along with some other exotic germplasm. However, the resilience of germplasm to cold stress at the reproductive stage depends on its origin, distribution, and adaptation mechanism. Dependency on a few donors' germplasm might limit or narrow the scope of achieving adapted elite breeding lines out of the breeding programs. Therefore, identification and use of novel genetic resources in the breeding program is critical for the development of resilient cultivars capable of sustaining yield under adverse climatic conditions (El-Refaee *et al.*, 2024). A companion study identified '*Rata Boro*', a landrace cultivar grown in the haor areas, as a potential donor for cold tolerance at the reproductive stage (Akter *et al.*, 2025). The development of recombinant inbred lines (RILs) from a cross combination of elite breeding lines and a donor parent in a shorter period is crucial for accelerating variety development and studying genetics. Single-seed descent (SSD)-based rapid generation advance (RGA) technique is an ideal approach for shortening line fixation time. $F_{4:5}$ RILs can be developed within two years of hybridization between two parents (Biswas *et al.*, 2023).

Accurate characterization of breeding germplasm for cold tolerance is another crucial component of varietal development and study of underlying genetic mechanisms. Previous studies showed that cold-tolerant rice cultivars identified under controlled screening environments demonstrate consistent tolerance when evaluated under natural field conditions (Farrell *et al.*, 2006). However, predicting the intensity and duration of cold-stress in Bangladesh remains highly unstable due to the variability of temperature fluctuations. In certain seasons, cool weather may not reach the threshold, thereby complicating the accurate assessment of genotypic performance. Staggering sowing dates under natural field environments enables the multi-simultaneous exposure of genotypes to variable temperature regimes, thereby facilitating a more reliable characterization of cold tolerance. Although several screening efforts have been reported, the characterization of genotypes for yield under cold stress conditions remains the effective approach to combine cold tolerance with optimum yield potential (Bala *et al.*, 2025). With increasing climate variability, genotypic responses to stress exhibit strong location-specific effects, underscoring the need to identify tolerant cultivars adapted to fluctuating temperature regimes and diverse agro-climatic conditions. In light of these challenges, the present study focuses on the development and evaluation of RILs under both cold stress and non-stress natural field conditions under target population of environments (TPE) in the haor areas for identification of reproductive stage cold-tolerant rice. By integrating cold stress-related morphological trait assessment along with yield under cold-prone environments, this study aims to identify tolerant genotypes that could withstand cold stress at reproductive stage and sustain the productivity, thereby contributing to the long-term stability of rice cultivation in the *haor* ecosystems.

## METHODOLOGY

### Development of recombinant inbred lines (RILs)

Rata Boro, a local *indica* rice landrace grown in *haor* areas, was used as a cold-tolerant donor parent in this study. This cultivar has very low yield potential and is characterized by tall plant height, short-bold grain with awns, and the presence of a light aroma. To develop RILs under elite backgrounds with reproductive stage cold-tolerant characteristics, Rata Boro was crossed with three elite breeding lines, BR10317-5R-25, BR11303-5R-156, and BR12266-44-11-32-5-1-1-HR10-B. The salient features of the parental lines used in the crosses are presented in Table 1. The resulting $F_1$ plants were confirmed through QC genotyping using a 10 QC SNP panel designed by IRRI and deployed at an outsourced genotyping company (Intertek Inc., Australia). The resulting three $F_2$ populations - BR14624 (BR10317-5R-25/Rata Boro), BR14625 (BR11303-5R-156/Rata Boro), and BR14628 (BR12266-44-11-32-5-1-1-HR10-B/Rata Boro) were subsequently advanced to $F_{4:5}$ generation following the SSD-based RGA method (Beredo *et al.*, 2016; Collard *et al.*, 2017; Rahman *et al.*, 2019).

**Table 1. Salient features of the parental lines.**

| Designation | Status | Plant height (cm) | Growth duration (days) | Panicle length (cm) | Yield (t/ha) |
|---|---|---|---|---|---|
| Rata Boro | Donor | 153.13 | 150 | 26.00 | 4.07 |
| BR10317-5R-25 | Recipient | 106.00 | 149 | 21.67 | 6.50 |
| BR11303-5R-156 | Recipient | 95.70 | 147 | 21.42 | 6.81 |
| BR12266-44-11-32-5-1-1-HR10-B | Recipient | 86.20 | 148 | 19.67 | 6.90 |

Fig. 1 shows the schematic diagram of line fixation or development from hybridization to F5 seed production following the SSD-based RGA technique. At the beginning of RGA at each generation, seed dormancy was broken before sowing by oven-drying for 72 hours at 50°C. Germination test was performed to confirm viability of the seeds. Seedlings were raised in Minuro trays (8 × 13 cells; 104 cells per tray). The cells were filled to three-quarters capacity with sterilized, gravel-free soil supplemented with basal fertilizer (1g/kg soil). For each population, four Minuro trays were used. Direct dry seeding was performed using 3-5 seeds per cell. At the 3-leaf stage, approximately 10-14 days after emergence, thinning was performed to allow only one plant to grow. The trays were then placed in blue crates on three PVC pipes installed in them, as shown in Fig. 2. Watering in the trays was performed daily in such a way that the bottom of the Minuro tray touched the water and maintained uniform moisture conditions in the soil. Crop management practices included sequential pruning at 30, 60, and 75 days after sowing to retain only the mother tiller. Minimal basal fertilization (1g/kg soil) was administered by topdressing with iron sulfate, ammonium sulfate, and mixed fertilizers at 21 and 42 days after sowing. Pest management was performed installing sticky traps, routine rat baiting, and scheduled chemical sprays to minimize biotic stress. Plants were harvested 21 days after last flowering of each population. After harvesting seeds were dried and subjected to dormancy-breaking treatment for advancing to the subsequent generation.

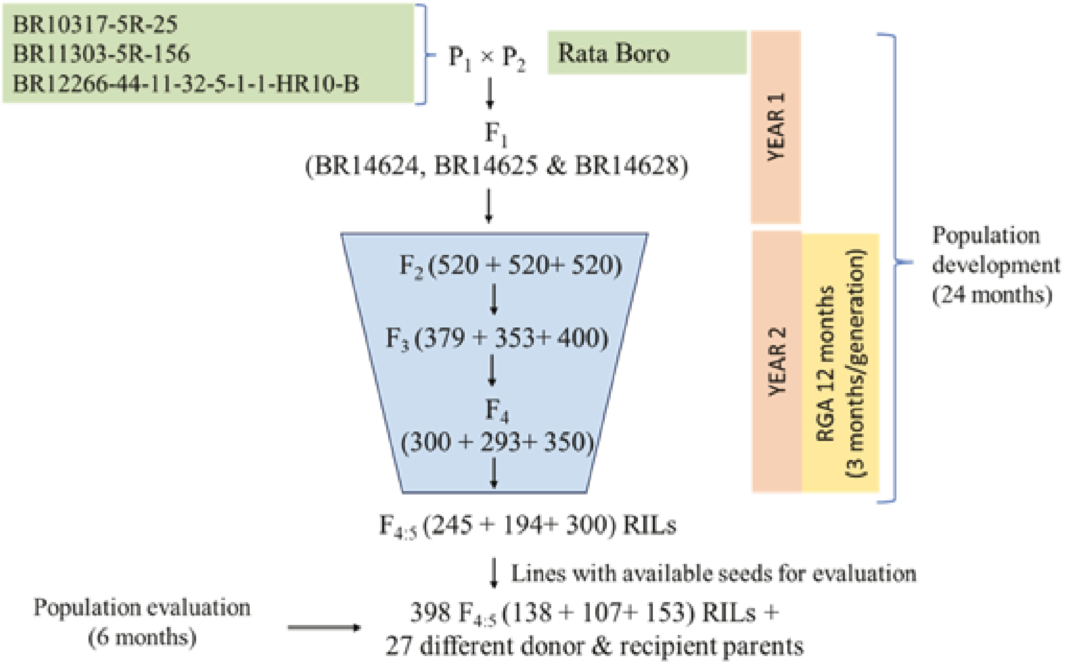


Fig. 1. Schematic representation of population development from hybridization to $F_{4:5}$ seed production using a single-seed descent (SSD) based rapid generation advance (RGA) approach. The diagram illustrates the sequential steps of line fixation and subsequent population evaluation for assessment of cold stress-related morphological traits.

Fig. 2. Advancement of segregating populations ($F_2$-$F_4$) using the Rapid Generation Advance (RGA) method.

### Evaluation of breeding lines for cold tolerance at the reproductive stage

The recombinant inbred breeding lines (RILs) were evaluated under cold-stress and non-stress conditions at the reproductive stage in the field by staggering sowing dates at Gazipur in the 1st year (Boro 2023-24) and at one site in Gazipur and three sites in the *haor* areas in the 2nd year (Boro 2024-25). In the 1st year, the cold stress condition at the reproductive stage was mimicked by sowing seeds on 21 October 2023, while non-stress was modulated through sowing seeds on 25 November 2023. Seed sowing in October allows the panicle initiation (PI) to the booting stages of the rice crop to be exposed to daily average low temperatures (<200C) during the end of January to the first week of February. A total of 425 breeding lines, including 398 RILs from three populations and 27 parental genotypes (cold-tolerant donor, variety, and advanced lines), were evaluated following a row-column design with two replications. Thirty-day-old single seedlings were transplanted in a 5.4 m × 2 rows plot with a spacing of 20 cm × 20 cm. The October sowing trial was considered as the cold-stress (CS) trial, while the November sowing trial represented the non-stress (NS) trial. BRRI dhan28 and BRRI dhan67 were used as susceptible and moderately tolerant check varieties, respectively.

In the 2nd year, 11 genotypes selected from the 1st year trial, along with the standard check varieties BRRI dhan102 as yield check, BRRI dhan28 as susceptible check, BRRI dhan67 and BR11894-R-R-R-R-169 as tolerant checks, were evaluated. The experiment was conducted at BRRI Gazipur and in three *haor* sites (Habiganj, Nikli of Kishoreganj, and Tahirpur of Sunamganj) under two sowing dates (staggered sowing)- cold stress (21–29 October) and non-stress conditions (19 November–12 December). Thirty-five-day-old seedlings of each genotype were transplanted into a 5.4 m × 6 rows plot using two seedlings per hill with a spacing of 20 cm × 20 cm following a row-column design with two replications.

### Data Collection

In the 1st year, observations on vegetative stage score (VegS) were recorded at the maximum tillering stage. Heading date and panicle degeneration scores were recorded when 50% of the hills of a plot had at least one panicle emerged from the flag leaf sheath. Panicle exsertion and spikelet fertility scores were recorded at the hard dough stage. The traits were scored following the Standard Evaluation System (SES; IRRI, 2013) using a 1-9 scale, with VegS (1 = very good, 3 = good, 5 = fair, 7 = poor, 9 = very poor), panicle degeneration score (PDS) (1 = 0%, 3 = 1–10%, 5 = 11–25%, 7 = 26–40%, 9 = >40% panicle degeneration), panicle exsertion score (PES) (1 = >1.0 cm, 3 = 0.5–1.0 cm, 5 = 0–0.5 cm, 7 = <0 cm to one-quarter of the panicle length, 9 = >one-quarter of the panicle length), and spikelet fertility score (SFS) (1 = highly fertile (>90%), 3 = fertile (75–89%), 5 = partly sterile (50–74%), 7 = sterile (≤50% to trace), 9 = highly sterile (0%)). Maturity date was recorded when 80% grains of the panicles became mature and turned yellow to straw color. Plant height, panicle length, filled and unfilled grain counts were recorded from five random hills from each plot following Gomez (1972). Grain yield (g) was recorded from 24 hills per genotype and adjusted to 14% moisture content. Spikelet fertility was estimated in percentage using the formula:

$$\textit{Spikelet fertility (\%)} = \frac{\textit{Number of filled grains}}{\textit{Total number of spikelets}} \times 100$$

In the 2nd year, data on plant height, heading date, maturity date, panicle degeneration score (PDS), panicle exertion score (PES), spikelet fertility score (SFS), and yield (t/ha) were collected across four locations. The percentage reduction in phenotypic trait performance under cold stress was calculated relative to the non-stress conditions, following the method described by Biswas et al. (2020):

$$\textit{Reduction rate (\%)} = \frac{\textit{(Mean value under non-stress-Mean value under cold stress)}}{\textit{Mean value under non-stress}} \times 100$$

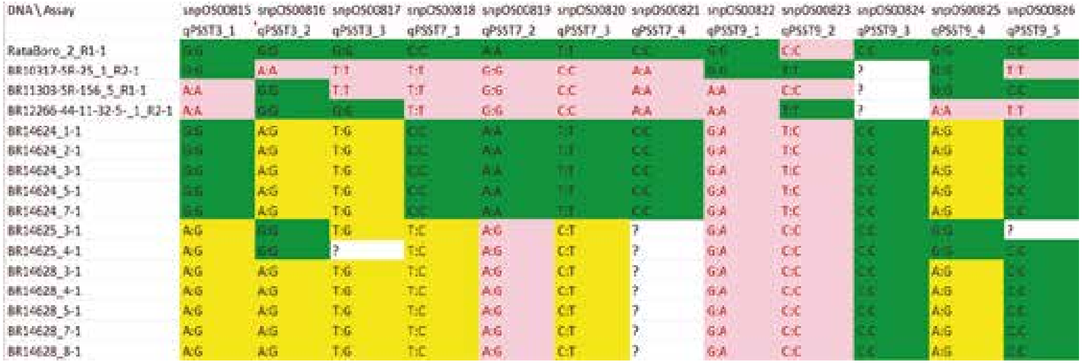
## Statistical analysis

Descriptive statistical measures such as mean, range, and standard deviation were computed using base R functions within the tidyverse framework (packages 'dplyr' and 'tidyr') integrated in R version 3.2.1. A two-stage linear mixed analytical framework was also implemented using the R package 'lme4' and 'emmeans' to estimate heritability, best linear unbiased estimate (BLUE), and phenotypic best linear unbiased prediction (pBLUP). R package agricolae (available at https://CRAN.R-project.org/package=agricolae, accessed on October 9, 2024) was used to estimate least significant difference (LSD). The principal component analysis (PCA) was performed using the prcomp() function, while Spearman's rank correlation coefficients were estimated using cor.test () function. The genotype environment interaction analysis was performed using 'metan' package in R (available at https://CRAN.R-project.org/package=metan, accessed on September 10, 2024).

## RESULTS

## Development of RILs

Three populations (BR14624, BR14625, and BR14628) were developed from crosses between Rata Boro and three elite breeding lines (Table 1).



| DNA\Assay | snpOS00815<br>qPSST3_1 | snpOS00816<br>qPSST3_2 | snpOS00817<br>qPSST3_3 | snpOS00818<br>qPSST7_1 | snpOS00819<br>qPSST7_2 | snpOS00820<br>qPSST7_3 | snpOS00821<br>qPSST7_4 | snpOS00822<br>qPSST9_1 | snpOS00823<br>qPSST9_2 | snpOS00824<br>qPSST9_3 | snpOS00825<br>qPSST9_4 | snpOS00826<br>qPSST9_5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RataBoro_2_R1-1 | G:G | G:G | G:G | C:C | A:A | T:T | C:C | G:G | C:C | C:C | G:G | C:C |
| BR10317-5R-25_1_R2-1 | G:G | A:A | T:T | T:T | G:G | C:C | A:A | G:G | T:T | ? | G:G | T:T |
| BR11303-5R-156_5_R1-1 | A:A | G:G | T:T | T:T | G:G | C:C | A:A | A:A | C:C | ? | G:G | C:C |
| BR12266-44-11-32-5-_1_R2-1 | A:A | G:G | G:G | T:T | G:G | C:C | A:A | A:A | T:T | ? | A:A | T:T |
| BR14624_1-1 | G:G | A:G | T:G | C:C | A:A | T:T | C:C | G:A | T:C | C:C | A:G | C:C |
| BR14624_2-1 | G:G | A:G | T:G | C:C | A:A | T:T | C:C | G:A | T:C | C:C | A:G | C:C |
| BR14624_3-1 | G:G | A:G | T:G | C:C | A:A | T:T | C:C | G:A | T:C | C:C | A:G | C:C |
| BR14624_5-1 | G:G | A:G | T:G | C:C | A:A | T:T | C:C | G:A | T:C | C:C | A:G | C:C |
| BR14624_7-1 | G:G | A:G | T:G | C:C | A:A | T:T | C:C | G:A | T:C | C:C | A:G | C:C |
| BR14625_3-1 | A:G | G:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | G:G | ? |
| BR14625_4-1 | A:G | G:G | ? | T:C | A:G | C:T | ? | G:A | C:C | C:C | G:G | C:C |
| BR14628_3-1 | A:G | A:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | A:G | C:C |
| BR14628_4-1 | A:G | A:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | A:G | C:C |
| BR14628_5-1 | A:G | A:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | A:G | C:C |
| BR14628_7-1 | A:G | A:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | A:G | C:C |
| BR14628_8-1 | A:G | A:G | T:G | T:C | A:G | C:T | ? | G:A | C:C | C:C | A:G | C:C |

Fig. 3. Confirmation of $F_1$ plants in the BR14624, BR14625, and BR14628 populations using trait-specific markers linked to the reproductive-stage cold tolerance QTLs *qPSST3, qPSST7*, and *qPSST9*.

The resulting $F_1$ plants were confirmed through genotyping with trait-specific SNP markers for reproductive-stage cold tolerance QTLs (*qPSST3, qPSST7*, and *qPSST9*) (Fig. 3). These segregating populations (BR14624, BR14625 and BR14628) were advanced through SSD-based RGA method. At maturity in $F_2$ generation, seeds from 379 plants of BR14624, from 353 plants of BR14625, and from 400 plants of BR14628 were collected out of 520 plants grown for each population (Table 2). This indicated that a substantial mortality rate or infertility rate ranging from 23.07% to 32.12% has occurred in advancing from $F_2$ to $F_3$ generations. In the $F_3$ generation, a relatively low mortality rate (12.5% to 20.84%) was observed with survivability of 300, 293 and 350 $F_4$ progenies of three populations. In the $F_4$ to $F_5$ advancement cycle, a total of 138 plants of BR14624, 107 plants of BR14625, and 153 plants BR14628 were identified with optimum amount of seeds available for field evaluation to assess reproductive-stage cold tolerance. The progressive reduction of 43.67 % to 49.0% in population size due to mortality or failure to seed set across generations was observed, resulting in a total of 398 $F_{4:5}$ derived fixed lines used for phenotypic assessment under cold stress. Using this RGA scheme, up to three generations were completed in a year, thereby substantially reducing the time required for line fixation and accelerating the overall breeding pipeline.

**Table 2. Advancement of segregating generations for the development of RILs.**

| Sl | Population | Parentage | $F_2$ gen. | $F_3$ gen. | $F_4$ gen. | $F_{4:5}$ gen. | $F_{4:5}$ used for evaluation |
|---|---|---|---|---|---|---|---|
| 1 | BR14624 | BR10317-5R-25/Rata Boro | 520 | 379 | 300 | 245 | 138 |
| 2 | BR14625 | BR11303-5R-156/Rata Boro | 520 | 353 | 293 | 194 | 107 |
| 3 | BR14628 | BR12266-44-11-32-5-1-1-HR10-B/Rata Boro | 520 | 400 | 350 | 300 | 153 |

**gen.: Generation**

$F_2$: Date of seeding (DS): 15/6/2022, Date of harvesting (DH): 9/9/2022; $F_3$: DS: 9/14/2022, DH: 17/1/2022; $F_4$: 28/1/2023, DH: 12/7/2023.

### Temperature profile and flowering period of 425 genotypes evaluated in the 1st year

The 425 rice genotypes evaluated under cold stress conditions in the first year flowered between 31 January 2024 and 22 March 2024 (Fig. 4B). Therefore, the panicle initiation (PI) to booting stages of the lines were between the 1st week of January and 3rd week of February. The maximum and minimum temperatures during this period were 17°C-34.3°C and 8.7°C–23°C (Fig. 4A), respectively. The average daily temperature below 20°C (mean: 16.4°C), with maximum temperature 17°C – 26.2°C was observed from 5-30 January 2024. These conditions imply that the breeding lines that flowered between January 31 and February 8 were exposed to natural low temperatures below 20°C during their PI and booting stages. Additionally, the breeding lines that flowered between 9 February to 15 February encountered moderate cold stress with temperatures between 21.5°C and 23.6°C during the booting stage.

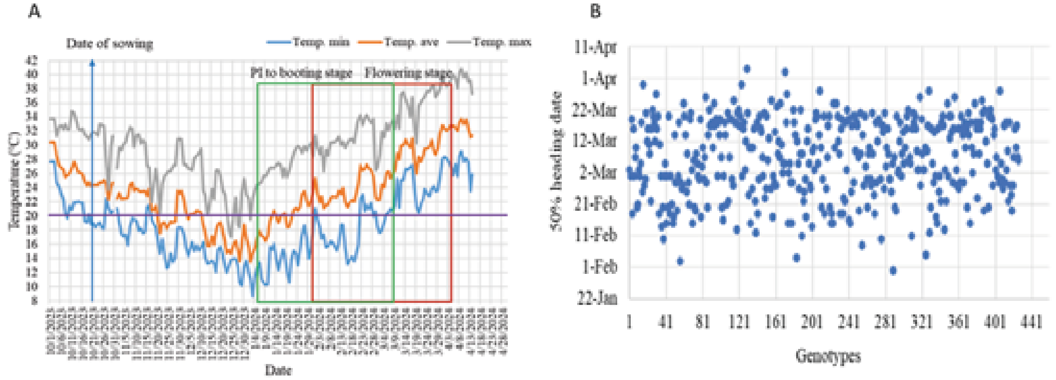


Fig. 4. (A) Seasonal temperature profile during the Boro 2023–24 growing period showing daily minimum, average, and maximum temperatures. The blue vertical line indicates the date of sowing, the green box marks the panicle initiation (PI) to the booting stage, and the red box represents the flowering stage. (B) Distribution of flowering dates for 425 breeding lines evaluated in the 1st year during Boro 2023–24. Flowering across the population occurred between 31 January and 4 April 2024.

### Phenotypic variation under cold stress and non-stress conditions during 1st Year

Significant phenotypic variation was observed in the 1st year among the 425 genotypes for all measured traits under both cold stress and non-stress environments (Table 3). Mean trait performance varied considerably between the two conditions. The score data for the cold related traits- VegS, PDS, PES, and SFS recorded higher values under cold-stress conditions relative to non-stress; whereas agronomic traits such as plant height (PH), panicle length (PL), yield per hill (YLD/hill), and spikelet fertility percentage (SFP) showed pronounced reductions and days to heading date (DTH) was increased under cold stress conditions.

Under cold-stress conditions, PH decreased from 114.27 cm (non-stress) to 105.64 cm (cold stress), and heading date increased from 125.22 (non-stress) to 138.74 days (cold stress). The SFP declined sharply from 64.46% under non-stress to 40.54% under cold stress, corresponding to a substantial yield reduction from 11.93 g to 7.84 g per hill. Conversely, PES and SFS exhibited higher mean values under cold stress, indicating the adverse influence of low temperatures on reproductive development processes. The significance tests confirmed that all trait means differed significantly ($p < 0.001$) between the cold stress and non-stress environments. Broad-sense heritability ($H^2b$) estimates ranged from 0.54 to 0.94 under cold stress and 0.41 to 0.95 under non-stress, implying a strong genetic basis for trait expression in both environmental conditions. Overall, the results indicate that cold stress adversely affected the key agronomic and cold-responsive traits, particularly related to panicle development and yield-related parameters. The observed high heritability suggests ample scope for selecting and developing genotypes with tolerance to cold stress.

**Table 3. Descriptive statistics of phenotypic traits across 425 genotypes under cold stress and non-stress environments during Boro 2023-24.**

| Traits Parameters | VegS | PDS | PES | SFS | PH (cm) | DTH (days) | PL (cm) | YLD/ Hill (g) | SFP |
|---|---|---|---|---|---|---|---|---|---|
| Mean_CS | 4.96± 0.67 | 3.22± 2.64 | 4.31± 1.76 | 5.55± 1.24 | 105.64± 5.82 | 138.74± 3.36 | 21.78± 1.33 | 10.84± 1.71 | 58.54± 2.65 |
| Mean_NS | 4.76± 0.66 | 1.78± 0.64 | 2.62± 1.31 | 4.8± 0.72 | 114.27± 4.44 | 125.22± 1.66 | 23.95± 1.17 | 11.93± 1.45 | 64.46± 3.93 |
| p value | 9.69E-05*** | 2.81E-09*** | 5.20E-81*** | 3.87E-13*** | 6.77E-11*** | 6.87E-11*** | 1.51E-05*** | 2.83E-05*** | 6.09E-10*** |
| $H_2b$_CS | 0.54 | 0.67 | 0.65 | 0.57 | 0.94 | 0.94 | 0.77 | 0.72 | 0.66 |
| $H_2b$_NS | 0.41 | 0.71 | 0.74 | 0.84 | 0.95 | 0.93 | 0.81 | 0.84 | 0.81 |

D/S: 21/10/2023 (Cold stress), DS: 25/11/2023 (Non-stress), CS: Cold stress, NS: Non-stress, VegS: Vegetative score, PDS: Panicle degeneration score, PES: Panicle exsertion score, SFS: Spikelet fertility score, PH: Plant height, DTH: Days to heading, PL: Panicle length, YLD/hill: Yield/hill (g), SFP: Spikelet fertility percentage, SE: Standard error, $H_2b$: Heritability in broad sense.

The association between scored and measured traits under cold-stress conditions aids in the selection of cold-tolerant genotypes. Hence, Spearman's rank correlation analysis was carried out among the evaluated traits in the 1st year, and the results were presented in Fig. 5. A strong and positive correlation was found between YLD/hill and SFP ($r = 0.44, p < 0.001$), indicating that higher spikelet fertility contributed substantially to yield performance under low-temperature stress. YLD/hill was also significantly and positively correlated with PL ($0.15, p < 0.01$) and DTH ($0.20, p < 0.01$). Whereas PES exhibited a positive and significant relationship with SFS ($r = 0.57, p < 0.001$) and PDS ($r = 0.27, p < 0.001$), suggesting that improved panicle exertion enhanced spikelet fertility and reduced panicle degeneration under cold stress conditions. Among agronomic traits, PH was moderately and positively associated with DTH ($r = 0.40$, $p < 0.001$) and showed a similar relationship with PL ($r = 0.50$, $p < 0.001$), reflecting coordinated growth responses under cold stress. While VegS showed no significant correlation with any other traits, indicating that the response of this trait to cold stress was largely independent.

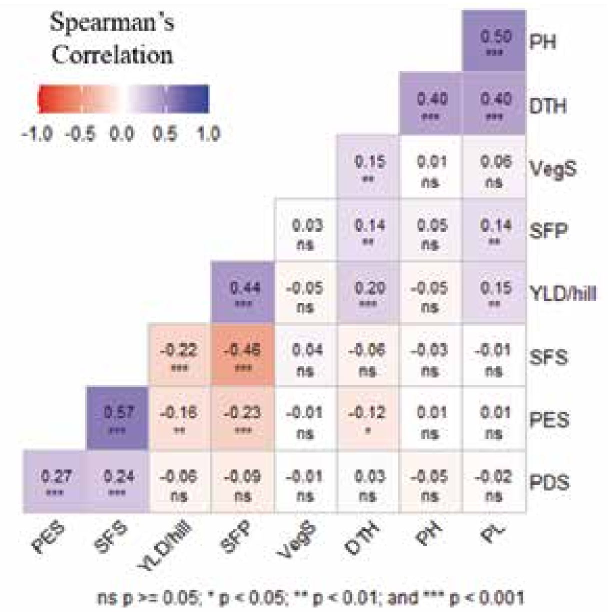


Fig. 5. Spearman's correlation matrix among phenotypic traits of 425 rice genotypes evaluated under cold stress conditions during Boro 2023-24.

The principal component analysis showed that the first two principal components accounted for 54.3% of the total phenotypic variation, with PC1 and PC2 explaining 36.1% and 18.2%, respectively (Fig. 6). Yield reduction rate (YRR), spikelet fertility percentage reduction (SFPR), PDS, PES, and SFS were predominantly explained by PC1. By contrast, DHD contributed mainly to PC2, reflecting variation associated with morphological and phenological performance that are indirectly affected by cold stress. Thus, PC1 primarily captured the extent of cold-induced damage to reproductive traits, while PC2 represented structural growth responses that may modulate tolerance under cold stress conditions. Based on performance metrics including panicle exertion, spikelet fertility, and yield per hill, 11 promising genotypes were selected for further evaluation in the 2nd year (Fig. 6).

### Temperature profile and flowering period of the genotypes evaluated in the 2nd year

In the 2nd year, 11 selected breeding lines flowered between 16 February and 20 March 2025 across four experimental sites: Gazipur, Habiganj, Nikli, and Tahirpur. In Gazipur, flowering was observed from late February to mid-March, with the majority concentrated between 2 March and 10 March (Fig. 7B). In Habiganj, most entries predominantly flowered between 4 and 15 March (Fig. 8B), while in Nikli, the majority of lines flowered between 20 February and 7 March (Fig. 9B). Tahirpur exhibited a narrower flowering window, spanning from 27 February to 8 March (Fig. 10B). Collectively, these results indicate a consistent flowering period across all sites, concentrated in late February to mid-March 2025.

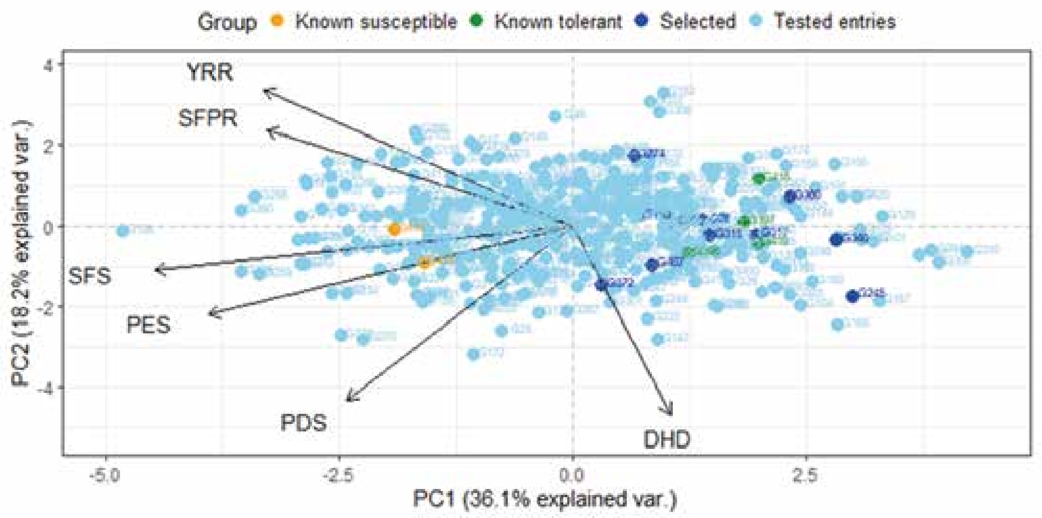


Fig. 6. Principal component biplot illustrating trait associations and genotype responses to cold stress and non-stress conditions during Boro 2023-24. Sky-blue dots represent the tested entries, orange dots indicate known susceptible checks, green dots denote the known tolerant donor, and blue dots represent the selected entries. Highlighted genotypes include: G76 (BR14624-4R-88), G190 (BR14625-4R-73), G245 (BR14628-4R-3), G274 (BR14628-4R-50), G275 (BR14628-4R-51), G317 (BR14628-4R-125), G318 (BR14628-4R-126), G340 (BR14628-4R-156), G360 (BR14628-4R-180), G372 (BR14628-4R-198), G407 (TP16199), G416 (BRRI dhan28; susceptible check), G417 (BRRI dhan81; susceptible check), G396 (Rata Boro), G397 (IR83222-F11-173; tolerant check), G410 (BR11894-R-R-R-169), and G418 (BRRI dhan67).

During the study period, significant spatial variations in air temperature were observed among the four monitoring sites. In Gazipur, the minimum and maximum temperatures ranged from 12.2°C to 21.2°C and 25.4°C to 31.8°C, respectively, with a mean daily temperature of 22.2°C (Fig. 7A). Habiganj exhibited a wider thermal amplitude, with minimum temperatures between 12.9°C and 20.0°C and maximum temperatures ranged from 18.6°C to 28.9°C, yielding an average daily temperature of 21.72°C (Fig. 8A). At Nikli, the minimum and maximum temperatures varied between 14.7°C–19.2°C and 19.8°C–27.3°C, respectively, with a mean daily temperature of 19.4°C (Fig. 9A). Tahirpur recorded the lowest overall temperature among the study sites, with minimum temperatures from 12.3°C to 16.4°C and maximum temperatures between 19.2°C and 24.7°C, resulting in an average daily temperature of 18.38°C (Fig. 10A). Therefore, the majority of the materials experienced severe to moderate levels of cold shock during the PI to booting stage at Nikli and Tahirpur sites. The temperature curves revealed a distinct cold-dip period during the PI to booting stage (denoted by the red dashed boxes) with Tahirpur showing the most pronounced low minimum temperatures, followed by Nikli, noticeably cooler than the other locations. Throughout this stage, the temperature profiles indicated that Tahirpur and Nikli's breeding materials were subjected to significant cold stress, likely affecting early reproductive development. As the crop transitioned into the flowering period (highlighted by green dashed boxes), temperatures climbed steadily across all sites, creating a warmer and more favorable environment for anthesis and grain setting.

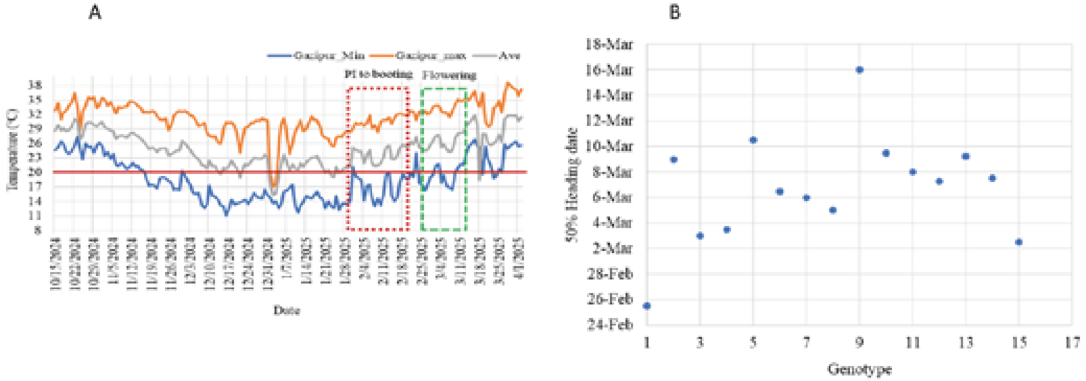


Fig. 7. Temperature profile (A) and flowering window (B) of the selected breeding lines in the 2nd year at Gazipur, Boro 2024-25.

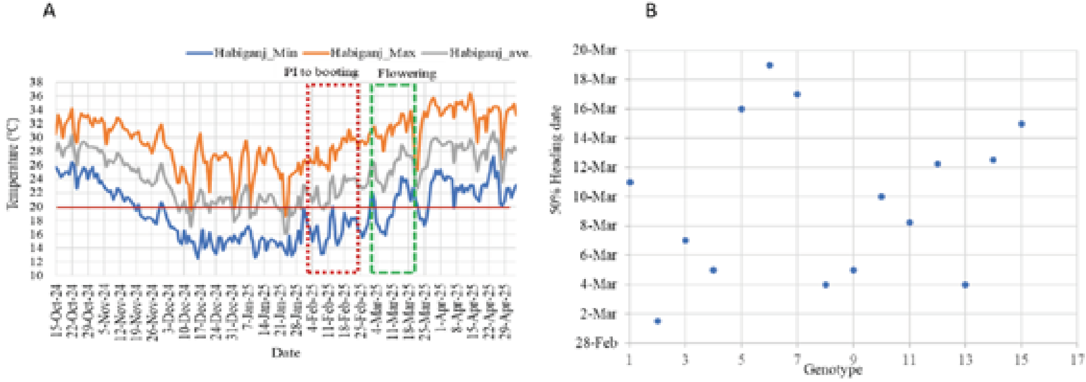


Fig. 8. Temperature profile (A) and flowering window (B) of the selected breeding lines in the 2nd year at Habiganj, Boro 2024-25.

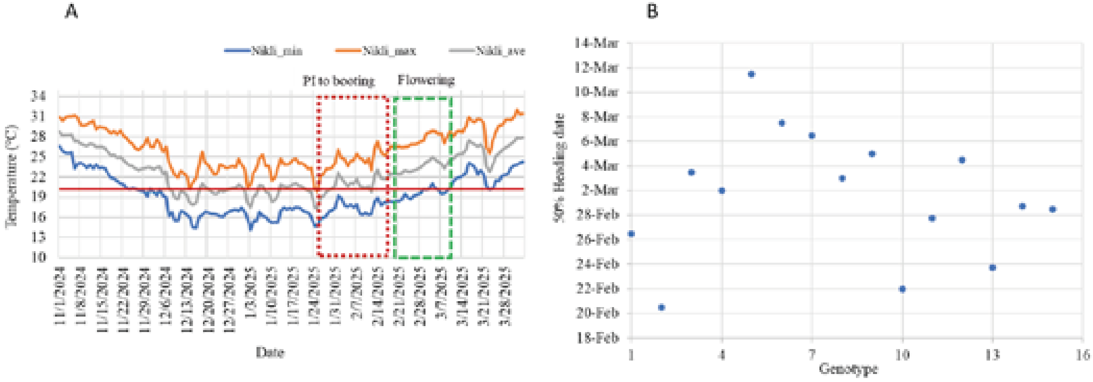


Fig. 9. Temperature profile (A) and flowering window (B) of the selected breeding lines in the 2nd year at Nikli, Boro 2024-25.

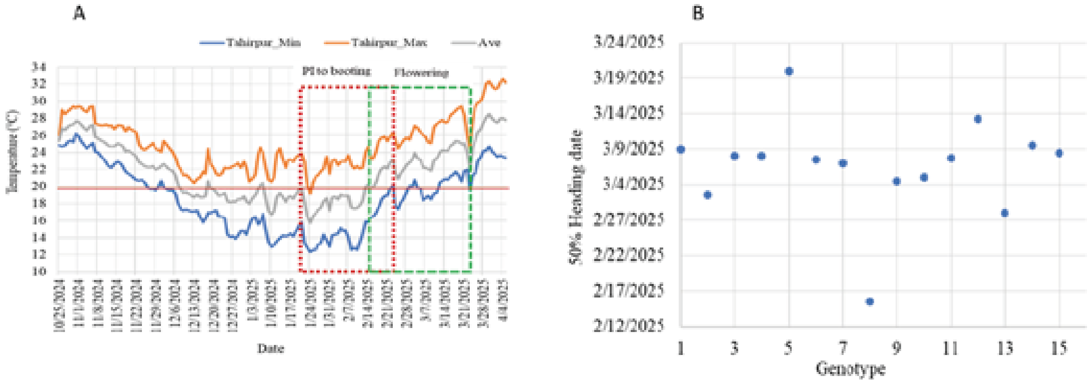


Fig. 10. Temperature profile (A) and flowering window (B) of the selected breeding lines in the 2nd year at Tahirpur, Boro 2024-25.

## Multi-location Performance of the Selected Genotypes in the 2nd year (Boro 2024-25)

Eleven rice genotypes, along with two check varieties (BRRI dhan28 and BRRI dhan67) were evaluated across four locations to determine their performance under cold-stress and non-stress conditions. Genetic correlation analysis across four locations revealed strong positive associations between Nikli and Tahirpur under both cold-stress and non-stress conditions (r = 0.89 under cold-stress and r = 0.66 under non-stress) (Fig. 11A, B). It indicates stable genotypic performance across these two haor environments. The Gazipur site showed weak or negative correlations with other sites under cold stress conditions (r = -0.52) with no correlation under non-stress conditions (r = 0.00), indicating divergent genotype responses and strong environmental influences across stress regimes.

Boxplot analysis further revealed that under cold-stress conditions, Habiganj exhibited the highest mean yield (4.8 t/ha) followed by Nikli (3.49 t/ha) and Tahirpur (3.26 t/ha), while Gazipur produced the lowest grain yield (2.3 t/ha), indicating differential genotype adaptation across environments (Fig. 12A). Under non-stress conditions, yield performance was improved across all locations, with Tahirpur showing the highest mean grain yield (6.73 t/ha), followed by Nikli, while Gazipur and Habiganj performed similar grain yield (5.59 t/ha) (Fig. 12B). Reduced variation and higher median values reflecting better overall genotype performance in favorable environments.

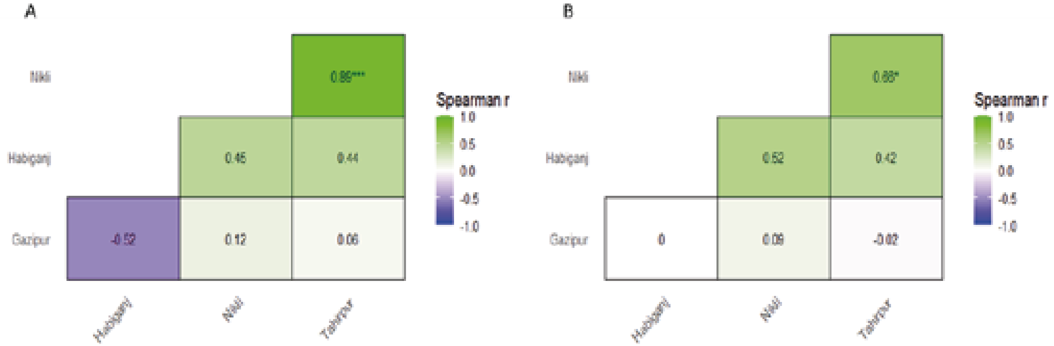


Fig. 11. Genetic correlation among the trial sites for the yield trait of selected genotypes under A) cold stress and B) Non-stress conditions, Boro 2024-25.

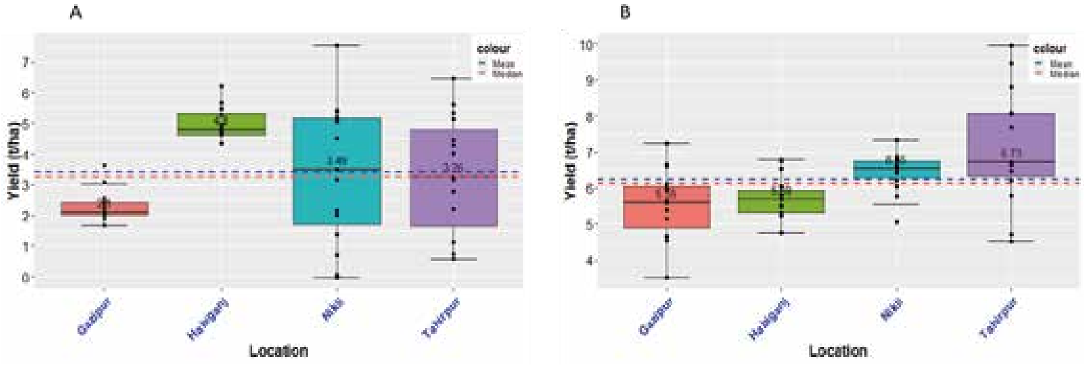


Fig. 12. Location-wise grain yield (t/ha) of selected genotypes under A) cold stress and B) non-stress conditions. The blue line indicates the mean value, and the red line represents the median value across the locations.

**Genotypic variation in different growth traits in the selected genotypes under cold stress and non-stress conditions**

Under cold-stress conditions, significant genotypic variation was observed for plant height, growth duration, and grain yield across Nikli and Tahirpur. Plant height ranged from 88 to 113 cm, while growth duration varied from 143 to 163 days (Table 4).

**Table 4. Genotypic response of selected breeding lines/varieties under cold stress at reproductive stage during Boro 2024-25.**

| Entry ID | Designation | Plant height (cm) | | Growth duration (days) | | Yield (t/ha) | |
|---|---|---|---|---|---|---|---|
| | | Average | Range | Average | Range | Average | Range |
| G1 | BR14624-4R-88-Gaz | 84 | 79-88 | 155 | 151-160 | 4.2 | 3.3-5.2 |
| G2 | BR14625-4R-73-Gaz | 106 | 102-109 | 149 | 143-154 | 0.6 | 0.1-1.1 |
| G3 | BR14628-4R-125-Gaz | 98 | 97-99 | 157 | 154-159 | 5.1 | 5.10 |
| G4 | BR14628-4R-126-Gaz | 100 | 97-103 | 156 | 153-159 | 3.7 | 3.2-4.3 |
| G5 | BR14628-4R-156-Gaz-1 | 111 | 109-113 | 167 | 163-171 | 7.0 | 6.5-7.5 |
| G6 | BR14628-4R-180-Gaz-1 | 102 | 100-103 | 159 | 159.00 | 5.4 | 5.3-5.4 |
| G7 | BR14628-4R-198-Gaz-1 | 100 | 98-102 | 158 | 158.00 | 5.5 | 5.4-5.6 |
| G8 | BR14628-4R-3-Gaz | 103 | 100-106 | 153 | 150-156 | 1.8 | 1.4-2.2 |
| G9 | BR14628-4R-50-Gaz-1 | 97 | 96-98 | 156 | 155-157 | 4.8 | 4.5-5.1 |
| G10 | BR14628-4R-51-Gaz | 89 | 87-90 | 151 | 147-156 | 2.5 | 2.2-2.8 |
| G11 | BR11894-R-R-R-R-169 | 98 | 94-102 | 156 | 153-159 | 3.8 | 3.5-4.0 |
| G12 | BRRI dhan102 | 104 | 102-107 | 160 | 156-164 | 3.8 | 3.2-4.5 |
| G13 | BRRI dhan28 | 86 | 81-91 | 149 | 147-151 | 0.4 | 0.0-0.7 |
| G14 | BRRI dhan67 | 103 | 99-108 | 157 | 153-161 | 4.4 | 4.4-4.5 |
| G15 | TP16199 | 97 | 96-97 | 157 | 153-160 | 1.3 | 0.6-2.0 |
| | LSD (<0.05) | 4.86 | | 2.68 | | 1.51 | |
| | H2B (%) | 92.39 | | 94.95 | | 92.37 | |
| | p value | 8.47E-14 | | 6.90E-09 | | 6.47E-18 | |

DS: 24/10/2024 (Nikli); 25/10/2024 (Tahirpur)

Among the tested entries, genotype G5 (BR14628-4R-156-Gaz-1) produced the highest mean grain yield (7.0 t/ha) with a growth duration of 167 days, exhibiting superior stress-specific performance. Other promising genotypes, including G7 (BR14628-4R-198-Gaz-1), G6 (BR14628-4R-180-Gaz-1), and G3 (BR14628-4R-125-Gaz) showed significantly higher mean yields across locations (5.5, 5.4, and 5.1 t/ha, respectively) than the moderately cold-tolerant check variety BRRI dhan67 (4.4 t/ha and 157 days). Under non-stress conditions, significant genotypic variation was observed for plant height, growth duration, and grain yield at Gazipur, Habiganj, Nikli, and Tahirpur (Table 5).

Plant height ranged from 80-132 cm, while growth duration varied between 128-157 days. Across environments, grain yield exhibited considerable variation, ranging from 4.5 to 9.5 t/ha. The significantly highest average yield was recorded in G3 (7.1 t/ha), followed by G6 (7.0 t/ha) and G9 (6.8 t/ha). The moderately tolerant check variety G14 produced an average yield of 6.3 t/ha while the susceptible check G13 yielded 5.6 t/ha.

Considering both environmental conditions, G3, G6, and G7 demonstrated consistent and superior performance under both cold-stress and non-stress environments, indicating their broad adaptability and yield stability. In contrast, G5 performed well only under cold-stress conditions, suggesting its specific adaptation to low-temperature environments.

**Effect of cold stress on growth and yield attributes compared to non-stress conditions**

Exposure to cold stress at the reproductive stage, various genotypic responses in different growth attributes were observed (Fig. 13). Because Nikli and Tahirpur represented the primary cold-stress conditions, these two locations were also considered for comparison under non-stress conditions. Reductions in plant height varied from -2.43 to 13.61% (average: 5.61%) with minimum or no reduction in plant height was observed with G5 (-2.43%), indicating strong tolerance to cold-induced growth suppression (Fig. 13A).

**Table 5. Genotypic response of 11 selected breeding lines/varieties under non-stress conditions at reproductive stage during Boro 2024-25.**

| Entry ID | Designation | Plant height (cm) |  | Growth duration (days) |  | Yield (t/ha) |  |
|---|---|---|---|---|---|---|---|
|  |  | Average | Range | Average | Range | Average | Range |
| G1 | BR14624-4R-88-Gaz | 101 | 80-118 | 147 | 137-153 | 5.7 | 5.2-6.7 |
| G2 | BR14625-4R-73-Gaz | 102 | 87-111 | 141 | 138-145 | 6.2 | 5.9-6.6 |
| G3 | BR14628-4R-125-Gaz | 107 | 98-119 | 148 | 139-153 | 7.1 | 6.7-9.5 |
| G4 | BR14628-4R-126-Gaz | 108 | 91-128 | 149 | 137-155 | 6.7 | 4.5-8.8 |
| G5 | BR14628-4R-156-Gaz-1 | 110 | 102-119 | 152 | 140-157 | 5.8 | 4.7-7.7 |
| G6 | BR14628-4R-180-Gaz-1 | 112 | 104-127 | 149 | 139-154 | 7.0 | 5.5-9.9 |
| G7 | BR14628-4R-198-Gaz-1 | 110 | 99-126 | 150 | 139-157 | 6.5 | 5.5-8.1 |
| G8 | BR14628-4R-3-Gaz | 112 | 105-122 | 140 | 136-146 | 5.4 | 4.5-6.7 |
| G9 | BR14628-4R-50-Gaz-1 | 105 | 97-118 | 149 | 139-155 | 6.8 | 5.9-8.0 |
| G10 | BR14628-4R-51-Gaz | 99 | 91-110 | 144 | 136-147 | 6.0 | 5.4-6.5 |
| G11 | BR11894-R-R-R-R-169 | 109 | 102-113 | 142 | 129-148 | 6.2 | 5.3-6.6 |
| G12 | BRRI dhan102 | 109 | 104-119 | 150 | 139-153 | 6.4 | 5.1-8.1 |
| G13 | BRRI dhan28 | 103 | 96-111 | 142 | 140-144 | 5.6 | 3.5-6.6 |
| G14 | BRRI dhan67 | 110 | 112-117 | 141 | 129-145 | 6.3 | 5.3-7.1 |
| G15 | TP16199 | 116 | 104-133 | 147 | 139-150 | 5.6 | 4.6-6.2 |
|  | LSD (<0.05) | 6.50 |  | 2 |  | 1.4 |  |
|  | H2B (%) | 0.85 |  | 1 |  | 0.5 |  |
|  | p-value | 4.85E-04 |  | 1.92E-27 |  | 5.54E-06 |  |

D/S: 27/11/2024 (Gazipur); 12/12/2024 (Habiganj); 19/11/2024 (Nikli); 19/11/2024 (Tahirpur)

Susceptible check G13 showed the highest reduction (13.61%) in plant height, whereas genotype G5 showed minimal reduction (-2.43%) Heading delay was observed in the cold-stressed plants, ranging from 2-9 days with a mean value of 4.88 days (Fig. 13B). The minimum delay was exhibited by G4, G7, and G9 (2 days). However, there was a significant reduction in grain yield ranging from -4.33 to 94.41% (average 47.44%). The least reduction was observed with G5 (-4.33%), followed by G7 (22.9%), and G1 (30.8%) (Fig. 13C). The negative yield reduction in G5 resulted from its higher grain yield under cold stress (7.0 t $ha^{-1}$) compared to non-stress environment (5.8 t $ha^{-1}$). Its longer growth duration likely enabled G5 to escape the most damaging cold stress, whereas under non-stress conditions, this extended duration caused G5 to remain in the field after most other entries were harvested. This prolonged exposure increased susceptibility to diseases, insect infestation, and lodging, ultimately reducing its yield under non-stress conditions. The highest grain yield reduction was recorded with susceptible check G13 (94.41%), followed by G2 (83.33%). Under non-stress conditions, yield ranged from 5.36-7.71 (t/ha), while the highest grain yield was produced by G3 (7.71 t/ha), followed by G6 (6.99 t/ha) and G9 (6.77 t/ha) (Fig. 13D). The genotype G5 produced higher grain yield in cold-stress (7.0 t/ha) than non-stress conditions (5.8 t/ha). These may be due to longer growth duration of G5 can escape cold in cold-stress conditions, while disease and infestation was higher in non-stress conditions as all of the plants harvested earlier.

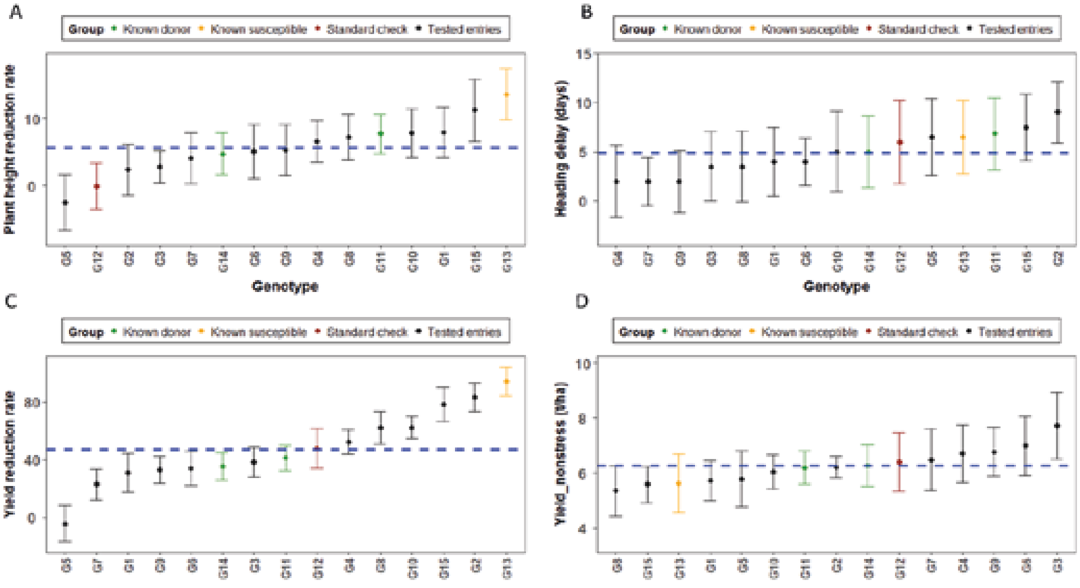


Fig. 13. Reduction rate of (A) plant height, (B) heading delay, and (C) yield compared to non-stress conditions, and (D) yield under non-stress conditions of selected genotypes during Boro 2024-25. Blue horizontal line indicates the mean value of the particular trait. Genotypes G1 = BR14624-4R-88-Gaz, G2 = BR14625-4R-73-Gaz, G3 = BR14628-4R-125-Gaz, G4 = BR14628-4R-126-Gaz, G5 = BR14628-4R-156-Gaz-1, G6 = BR14628-4R-180-Gaz-1, G7 = BR14628-4R-198-Gaz-1, G8 = BR14628-4R-3-Gaz, G9 = BR14628-4R-50-Gaz-1, G10 = BR14628-4R-51-Gaz, G11 = BR11894-R-R-R-R-169, G12 = BRRI dhan102 , G13 = BRRI dhan28, G14 = BRRI dhan67 , and G15 = TP16199. Green whiskers represent tolerant check varieties, orange whiskers represent susceptible check varieties, violet whiskers represent the yield check variety. Data on Fig A, B, C, and D were the average of Nikli and Tahirpur locations.

Panicle degeneration score (PDS), panicle exsertion score (PES), and spikelet fertility score (SFS) are important traits for determining reproductive-stage cold tolerance. The evaluated genotypes exhibited variable responses to PDS and PES under cold stress, ranging from 1.15 (G5) to 5.15 (G8) and 1.00 (G5) to 4 (G8), with mean values of 3.61 and 2.06, respectively (Table 6). Although nearly 70% of the genotypes showed good panicle degeneration and exsertion in the 2nd year under cold stress, SFS varied markedly, ranging from 3.04 to 9.14. The lowest SFS values were observed in G6 (3.04) and G7 (3.54), followed by G5 (4.29) and G3 (4.54), indicating better tolerance to cold stress. In contrast, the highest SFS was recorded in the susceptible check G13 (9.00), followed by G2 (8.04) and G8 (7.54), reflecting greater sensitivity to cold stress during the reproductive stage.

**Table 6. Genotypic response to cold stress in different cold-related traits of selected breeding lines at the reproductive stage during Boro 2024-25.**

| ID | Designation | PDS_CS | PDS_NS | PES_CS | PES_NS | SFS_CS | SFS_NS |
|---|---|---|---|---|---|---|---|
| G1 | BR14624-4R-88-Gaz | 4 | 3 | 3 | 1 | 6 | 3 |
| G2 | BR14625-4R-73-Gaz | 4 | 3 | 3 | 2 | 8 | 4 |
| G3 | BR14628-4R-125-Gaz | 5 | 3 | 2 | 1 | 5 | 3 |
| G4 | BR14628-4R-126-Gaz | 4 | 3 | 1 | 1 | 5 | 3 |
| G5 | BR14628-4R-156-Gaz-1 | 1 | 2 | 1 | 1 | 4 | 3 |
| G6 | BR14628-4R-180-Gaz-1 | 3 | 3 | 2 | 2 | 3 | 3 |
| G7 | BR14628-4R-198-Gaz-1 | 4 | 3 | 2 | 1 | 4 | 3 |
| G8 | BR14628-4R-3-Gaz | 5 | 3 | 4 | 1 | 8 | 3 |
| G9 | BR14628-4R-50-Gaz-1 | 4 | 4 | 2 | 1 | 5 | 3 |
| G10 | BR14628-4R-51-Gaz | 4 | 3 | 3 | 1 | 6 | 3 |
| G11 | BR11894-R-R-R-R-169 | 4 | 2 | 1 | 1 | 5 | 3 |
| G12 | BRRI dhan102 | 3 | 2 | 1 | 1 | 6 | 3 |
| G13 | BRRI dhan28 | 3 | 2 | 3 | 1 | 9 | 4 |
| G14 | BRRI dhan67 | 3 | 2 | 3 | 1 | 5 | 3 |
| G15 | TP16199 | 4 | 2 | 1 | 1 | 7 | 3 |
|  | p-value | 6.11E-09 | 5.69E-13 | 1.13E-12 | 4.08E-02 | 1.19E-06 | 2.75E-03 |
|  | LSD (<0.05) | 0.45 | 0.52 | 0.58 | 0.41 | 0.6 | 0.53 |

PDS: Panicle degeneration score, PES: panicle exsertion score, SFS: Spikelet fertility score, CS: Cold stress, NS: Non-stress. Cold stress data were the mean value of two locations (Nikli and Tahirpur), while non-stress data were the mean value of four locations.

Genotypic performance across Gazipur (1st year), Nikli (2nd year), and Tahirpur (2nd year) environments revealed a clear differentiation in adaptation patterns under cold-stress conditions (Fig. 14). Genotype G6 exhibited moderately higher and stable grain yield with the known donor G11, suggesting broad adaptability and consistent performance across three environments. While G5, followed by G3, G7, and G9, produced higher grain yields in the haor environments- Nikli, and Tahirpur, but had poor performance in Gazipur during the 1st year, indicating that these lines are specifically adapted to *haor* ecosystems where cold stress is more pronounced. Notably, the susceptible check variety G13 consistently produced low yields across three environments, validating its sensitivity to cold stress.

Based on yield performance, the genotypes were ranked with the highest-yielding genotype received rank 1 (S1 Table). Rank-sum values ranged from 22 to 65. Genotype G6 exhibited the lowest rank-sum value (22), indicating superior and stable performance across three tested environments. Genotype G5 had a rank-sum value of 26; although it ranked 14th in Gazipur during the first year, it ranked 1st in Nikli and Tahirpur, demonstrating its specific adaptation to the haor ecosystem. In contrast, G13 showed the highest rank-sum value (65), reflecting consistently poor yield performance across environments.

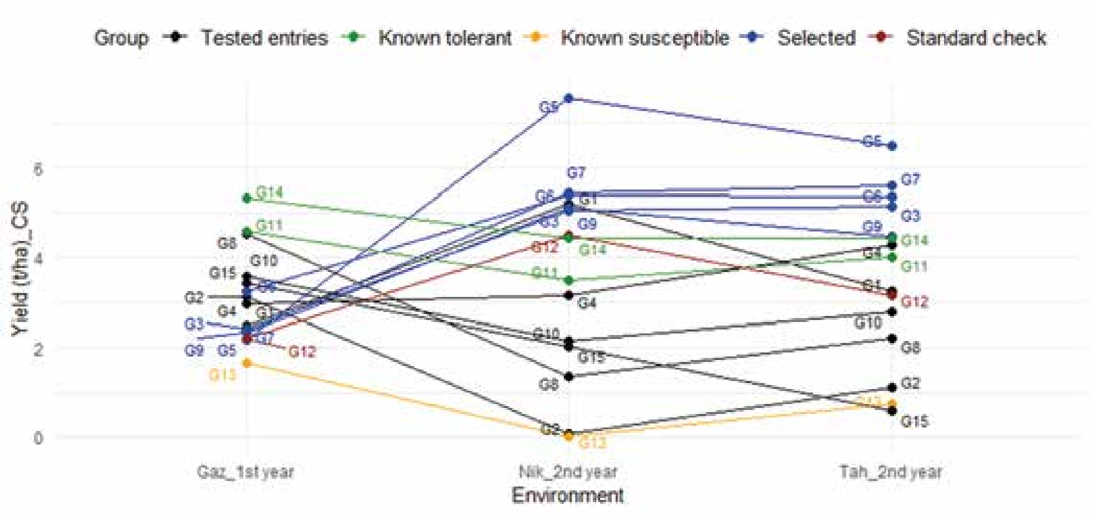


Fig. 14. Genotype × Environment interaction for grain yield (t/ha) of 11 rice genotypes evaluated under cold-stress conditions across three environments during Boro 2023-24 and Boro 2024-25. Black lines indicate tested entries, green lines indicate known tolerant checks, orange lines denote known susceptible checks, blue lines indicate selected genotypes, and dark red lines denote the standard check. Genotypes: G1 = BR14624-4R-88-Gaz, G2 = BR14625-4R-73-Gaz, G3 = BR14628-4R-125-Gaz, G4 = BR14628-4R-126-Gaz, G5 = BR14628-4R-156-Gaz-1, G6 = BR14628-4R-180-Gaz-1, G7 = BR14628-4R-198-Gaz-1, G8 = BR14628-4R-3-Gaz, G9 = BR14628-4R-50-Gaz-1, G10 = BR14628-4R-51-Gaz, G11 = BR11894-R-R-R-R-169, G12 = BRRI dhan102, G13 = BRRI dhan28, G14 = BRRI dhan67, and G15 = TP16199.

### GGE (Genotype and Genotype-by-Environment interaction) analysis for yield

The GGE biplot analysis was carried out to identify the best genotypes in terms of yield under cold-stress and non-stress conditions. Principal components 1 (PC1) and principal components 2 (PC2) captured 92.97% and 7.03% of the variations, respectively (Fig. 15A). Fig 14A characterized the genotypes on the basis of their discriminativeness and representativeness and highlighted that G5 and G3 performed the highest yield under cold-stress and non-stress conditions, respectively. These genotypes being far from the AEC (Average Environment Coordination) axis and higher discriminativeness indicating suitability in a specific environment. On the other hand, G6, G9, and G7 were clustered near the center, indicating average performance across environments.

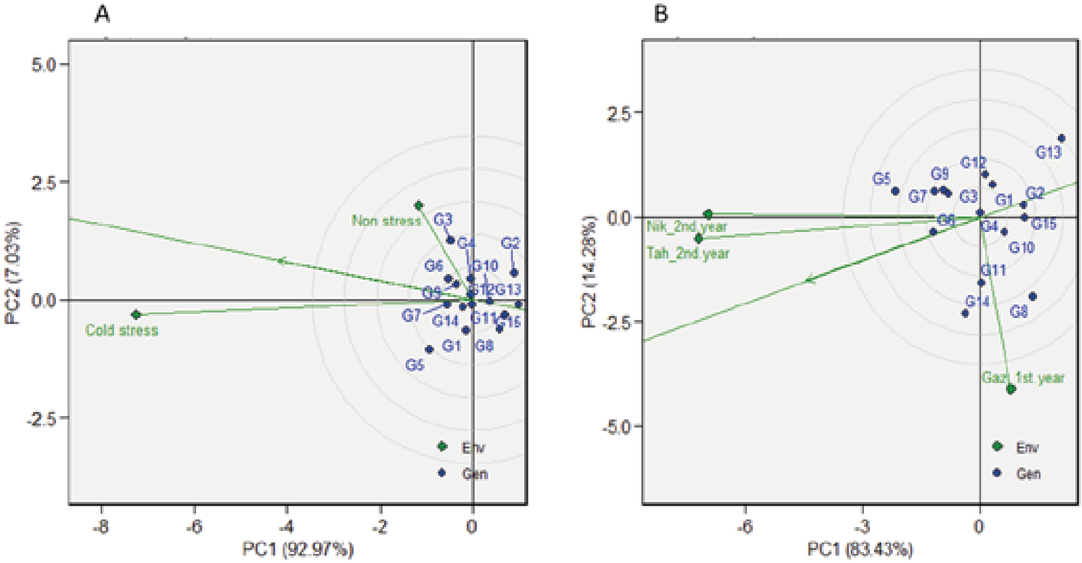


Fig. 15. GGE biplot showing (A) the average performance of 11 rice genotypes under cold-stress and non-stress conditions, and (B) their yield performance across three environments under cold-stress conditions.

Under cold-stress conditions across three environments, PC1 and PC2 explained 97.71% of total variations (Fig. 15B). Genotypes G5, followed by G3, G7, and G9, exhibited superior performance and were specifically adapted to the *haor* environments, indicating their potential suitability for cold-prone ecosystems. By contrast, genotypes G6, G11 and G14 demonstrated consistent and stable performance across all environments, suggesting wider adaptability and potential as reliable contributors to yield stability.

## DISCUSSION

The rapid generation advancement (RGA) method effectively shortened the breeding cycle, but the offseason advancement of segregating populations also introduced several challenges. Environmental fluctuations – including cold spells in the Boro season, and hot and humid conditions in the T. Aman season increased stress on plants and intensified disease and insect pressure, leading to substantial reductions (43.67% to 49%) in population size. Severe sheath rot and sheath blight infestations, also reported by Rahman *et al.* (2019), were major causes of mortality in segregating generations, especially during the wet season. Due to the use of plastic trays with little volume of soil at each cell in the RGA method, root growth is restricted and compacted, resulting in weaker root anchorage and increasing the plant's susceptibility to lodging under strong winds (Rahman *et al.*, 2019). Whereas high temperatures during the dry season also caused panicle sterility, as noted by Beredo *et al.* (2016). These environmental constraints increased the risk of genetic drift and loss of genetic diversity, emphasizing the need to maintain sufficiently large segregating populations and adopt careful selection strategies (Collard *et al.*, 2017). Overall, while RGA accelerates breeding progress, it also demands careful oversight to minimize population loss and safeguard population integrity.

### Evaluation of cold tolerance in rice at the reproductive stage

The optimum temperature for growth and development of rice ranges from approximately 25-35°C. The critical temperature for growth of rice varies across different developmental stages, such as 10°C for germination, and 20°C for the reproductive stage. Beyond these critical thresholds, rice plants suffer from varying levels of physiological stresses (Biswas *et al.*, 2018). Low temperatures of approximately 15-19°C impair microspore development and are responsible for the production of sterile pollen, resulting in lower seed setting and high spikelet sterility. The young microspore stage is the most susceptible stage to cold stress, causing male sterility (Yoshida, 1981), ultimately increasing spikelet sterility and poor grain yield. Cold stress also causes many types of phenotypic damage, such as reduced plant height, delayed heading, incomplete panicle exsertion, and degeneration of spikelets (Ye *et al.*, 2009). Thus, reproductive stage cold tolerance is essential for pollen viability, seed set, and grain filling to obtain high grain yield (Zhang *et al.*, 2014). Traits such as plant height, days to flowering, panicle exsertion, panicle degeneration, and spikelet fertility are used as phenotypic indicators to assess reproductive-stage cold tolerance in rice (Biswas *et al.*, 2020). Under field conditions, early sowing during the Boro season (15-30 October) offers an effective strategy for natural screening, as it aligns the PI to booting stages with prevailing low temperatures (Hossain *et al.*, 2023). This approach has been validated through staggered sowing experiments, such as the study by Samejima *et al.* (2020), who exposed NERICA genotypes to sub-optimal temperatures (<20°C) during the 20-day pre-heading period, underscoring the reliability and applicability of staggered sowing for field-based evaluation of cold tolerance.

Evaluation of 425 rice breeding lines and varieties during Boro 2023-24 under cold-stress revealed that yield per hill was strongly and positively associated with spikelet fertility, and moderately correlated with delayed heading and panicle length, while showing negative associations with PES and SFS, indicated that increased sterility reduces yield (Fig. 5). These results are consistent with physiological evidence that low temperatures during booting and anthesis impair anther development, pollen viability, and carbohydrate metabolism, leading to spikelet sterility and spikelet abortion (Mitchell *et al.*, 2016). Collectively, the findings reinforce that spikelet fertility is the most reliable indicator of reproductive-stage cold tolerance, whereas traits such as PHR and VegS contributed little to yield variation. Therefore, only traits significantly correlated with yield per hill were considered for PC analysis.

PC analysis enabled the identification of genotypes from the 1st year with superior breeding lines with cold tolerance by integrating multiple trait responses. The genotypes highlighted in blue in Fig. 6 were distinguished by lower yield reduction, reduced spikelet fertility percentage, and minimal delay in heading compared to non-stress conditions. They also exhibited low scores for panicle degeneration (PDS), panicle exsertion stress (PES), and spikelet sterility (SFS), which are critical indicators for reproductive stage cold tolerance. Their positioning along the principal components reflected balanced performance across these key cold-tolerance traits, while their clustering away from known susceptible checks and closer alignment with donor profiles further indicated their potential as promising entries. Overall, PCA proved to be an effective multivariate tool for integrating complex trait data and facilitated the identification of genotypes tolerant at the reproductive stage under low-temperature stress. Similar applications of PCA have been reported by Monzon *et al.* (2023), who evaluated 124 rice accessions at germination and seedling stages and showed that cold tolerance can be reliably assessed through ATI (average tolerance index) derived from PCA, underscoring its effectiveness in screening diverse germplasm.

In 2nd year evaluation of 11 genotypes during Boro 2024-25 season across four locations, the daily mean temperature during the critical PI to booting stage remained above 20 °C at Gazipur and Habiganj (Fig. 7A, 8A). This implied that the plants at these sites were not exposed to cold stress, which was reflected in the relatively narrow variation of grain yield observed among the genotypes (Fig. 12A). Despite the absence of cold-stress, all entries exhibited comparatively low grain yield at both locations. This reduction is likely attributable to non-stress factors such as poor soil fertility - particularly in Gazipur, where topsoil was replaced as part of land development with soil from barren fields, which was inherently less productive, as well as increased disease and insect infestation. Thus, these two sites (Gazipur and Habiganj) failed to provide a suitable environment for distinguishing the tolerant genotypes under cold stress conditions and were excluded from further analysis. By contrast, trials conducted at Nikli and Tahirpur experienced cold stress during the reproductive stage (daily mean temperature below 20°C at PI to booting stage), enabling assessment of genotypic responses under stress. These sites were therefore used to evaluate the relative performance of the selected entries under cold-stress compared to non-stress conditions (Fig. 14A-C) and to analyze genotype × environment interactions under cold stress (Fig. 15).

Cold-stress significantly affects yield-attributing traits such as culm length, reduced panicle emergence and panicle length, and grain yield in rice genotypes (Farrell *et al.*, 2006). Genotype G5 showed no reduction of plant height, whereas the susceptible check (G13) suffered from the highest PHR (Fig. 14A). Similar findings have been reported by Zhang *et al.* (2014), who noted that cold stress disrupts cell elongation and internode development, leading to stunted growth in susceptible cultivars. Genotypes G4, G7, and G9 exhibited minimal delays; G5 performed a medium delay in heading, while the highest heading delay was observed in G2. Thus, genotypes with stable heading under stress are advantageous; however, this finding contrasts with the adaptive strategies described by Horai *et al.* (2013), wherein tolerant genotypes sometimes prolong the vegetative phase as an “escape mechanism.” Such delayed heading enables panicle initiation and flowering to occur under relatively favorable thermal conditions, thereby safeguarding spikelet fertility and sustaining grain yield.

The minimum reduction of yield was observed in G5, G7, and G1, while G13 and G2 suffered from extreme yield reductions. Genotypes with lower PDS and PES scores, particularly G5, maintained better panicle development under stress. Spikelet fertility proved to be the most critical determinant of yield stability, with tolerant genotypes (G6, G7, G5, and G3) maintaining relatively high fertility compared to susceptible entries (G13, G2, and G8). These results are consistent with earlier reports by Samejima *et al.* (2020), who identified spikelet fertility as the most reliable indicator of cold tolerance at the reproductive stage. Under non-stress conditions, high-yielding genotypes such as G3, G6, and G9 exhibited high yield potential, and their performance under cold stress remained moderately good. In contrast, G1 showed a relatively lower yield reduction under cold stress but produced comparatively lower yields in non-stress environments. These contrasting responses highlight the necessity of evaluating genotypes across both stress and non-stress conditions. Similar conclusions were drawn by Biswas *et al.* (2020), who reported substantial yield reductions under cold stress at the reproductive stage and emphasized the importance of multi-environment screening to ensure reliable identification of tolerant germplasm.

The multi-environment yield analysis and GGE biplot effectively distinguished genotypic responses to cold stress across *haor* environments. Under cold stress, genotype G5 consistently exhibited the highest yield performance in Habiganj, Nikli, and Tahirpur, followed by G3, G9, and G7, indicating their specific adaptation to cold-prone haor ecosystems. Genotypes G6 and the known donor G11, and G14 demonstrated stable performance across three environments, highlighting their broad adaptability and potential as stable yield contributors under variable conditions (Fig. 14). Under non-stress conditions, G3, G6, and G9 were the top performers, with G3 showing the highest yield. These results were further supported by the GGE biplot, which confirmed

G3 as the highest-yielding genotype under non-stress conditions and G5 as the best performer under cold stress (Fig. 15A). Additionally, G6 and G11 were the most stable performers in terms of yield across environments (Fig. 15B). A similar study by Bala *et al.* (2025) indicated that subsequent seedling growth, reproductive traits, and final yield performance in the field were strongly influenced by genotype × environment interactions, under cold-stress conditions.

## CONCLUSION

Genotypic responses to low-temperature stress varied significantly across environments, with reproductive-stage cold stress adversely affecting both growth and yield-related traits. In the first year of evaluation, RILs and parental genotypes were screened under natural cold-stress conditions, identified 11 entries with comparatively lower reductions in yield and spikelet fertility (SFP). Subsequent multi-location trials of selected 11 genotypes revealed differential adaptation patterns: genotypes G5 (BR14628-4R-156-Gaz-1), G3 (BR14628-4R-125-Gaz), G7 (BR14628-4R-198-Gaz-1), and G9 (BR14628-4R-50-Gaz-1) showed specific adaptation to cold-prone haor environments, demonstrating superior performance under reproductive-stage cold stress. While G6 (BR14628-4R-180-Gaz-1) and known donor genotype G11 (BR11894-R-R-R-R-169) demonstrated broad adaptability and stability across all tested sites. Notably, G3 (BR14628-4R-125-Gaz), G6 (BR14628-4R-180-Gaz-1), and G9 (BR14628-4R-50-Gaz-1) consistently maintained high yields under both stress and non-stress conditions. These cold-tolerant advanced lines represent valuable genetic resources for improving rice adaptation to low-temperature stress. To accelerate genetic improvement while conserving genetic diversity, further efforts could focus on the identification of quantitative trait loci (QTLs) associated with reproductive-stage cold tolerance and integration of marker-assisted selection in genotypes with broad genetic bases across diverse agro-ecological regions.

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*Review*

# Unlocking Durable Sheath Blight Resistance in Rice: The Central Role of GWAS in Genetic Dissection and Trait Enhancement

S M H A Rabbi*

## ABSTRACT

Sheath blight (ShB), caused by *Rhizoctonia solani* AG1-IA, is a globally devastating rice disease resulting in 20–50% yield losses. Its management is challenged by the pathogen's genomic complexity, quantitative host resistance, and trade-offs with agronomic traits. We first discuss the critical foundation of reliable phenotyping, spanning standardized field assays to emerging high-throughput phenomics, which is essential for accurate genetic dissection. This review also synthesizes advances in genetic and molecular dissection of ShB resistance, emphasizing the transformative role of Genome-Wide Association Studies (GWAS). We highlight how GWAS has overcome limitations of traditional QTL mapping, enabling high-resolution discovery of major resistance loci (e.g., *SBRR1*), defense pathways (e.g., ROS-antioxidant systems), and novel candidate genes across diverse germplasm. Integration of GWAS with gene editing (e.g., *dep1*-cys), overexpression strategies (e.g., *OsCSP41b*), and transcription factor networks (e.g., *OsbHLH34, OsBZR1*) reveals pathways to break yield-resistance trade-offs. Future progress hinges on multi-omics-augmented GWAS, epistasis modeling, and deploying genomic selection for durable resistance. This work underscores GWAS as a cornerstone for accelerating ShB-resistant rice breeding.

**Keywords:** *Rhizoctonia solani*, Quantitative Trait Loci (QTLs), Genomic Selection, Haplotype Breeding, Transcription Factors, Disease Resistance, Signaling pathway

## INTRODUCTION

Rice (*Oryza sativa* L.) stands as a cornerstone of global food security, feeding over half of the world's population. Yet, its productivity is perpetually threatened by a multitude of pathogens, among which sheath blight (ShB), caused by the necrotrophic fungus *Rhizoctonia solani* Kühn AG1-IA, ranks as the second most devastating disease after blast. This soil-borne pathogen inflicts staggering yield losses, routinely estimated at 20-50% under conducive conditions, posing a severe threat to rice production systems worldwide (Feng *et al.*, 2025; Thesiya *et al.*, 2025).

Alarmingly, the incidence and severity of ShB are intensifying, driven largely by modern agricultural practices. The widespread adoption of semi-dwarf, high-yielding varieties, coupled with high nitrogen fertilization and increased planting densities, creates dense canopies and humid microclimates that are highly favorable for *R. solani* infection and spread (Molla *et al.*, 2020).

Developing durable genetic resistance to ShB remains an elusive goal and a formidable challenge for rice breeders and pathologists, starkly contrasting the significant progress made against diseases like blast and bacterial blight. This difficulty stems from a confluence of factors inherent to both the pathogen and the host. *R. solani* AG1-IA exhibits high genetic variability (classified into 14 anastomosis groups), possesses an exceptionally broad host

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range (infecting plants from ~32 families), and persists robustly between seasons through long-lived sclerotia in soil and crop debris, complicating management strategies (Molla *et al.*, 2020)

Crucially, resistance in rice is inherently quantitative and polygenic, controlled by numerous small-effect quantitative trait loci (QTLs) rather than single, dominant resistance (R) genes (Li *et al.*, 2022; Molla *et al.*, 2020; Wang *et al.*, 2021). Also, resistant plants often have traits like shorter height, fewer tillers, or earlier maturity, which are not ideal for farming. This makes it hard to tell whether the plant is truly resistant to the disease or just avoiding it because of its shape or growth pattern ( Jia *et al.*, 2012; Li *et al.*, 2022).

Traditional biparental QTL mapping, while valuable, suffers from limited mapping resolution due to the relatively low number of recombination events captured within a narrow genetic base. In this complex landscape, Genome-Wide Association Studies (GWAS) have emerged as a transformative and robust approach for dissecting the genetic architecture of ShB resistance. By leveraging naturally diverse germplasm panels encompassing a wide spectrum of genetic variation, GWAS offers significantly higher resolution for detecting marker-trait associations (MTAs) compared to biparental mapping populations (Rabbi *et al.*, 2021; Rabbi *et al.*, 2021; Yu & Buckler, 2006). This power stems from the historical recombination events captured across diverse accessions. Consequently, GWAS has proven exceptionally effective in identifying novel resistance QTLs, pinpointing causal genes and alleles, and uncovering key defense pathways implicated in the rice-*R. solani* interaction.

Critically, the discoveries facilitated by GWAS directly enable advanced breeding strategies, including haplotype-based breeding, marker-assisted selection (MAS) for pyramiding favorable alleles, and the development of genomic selection (GS) models for complex traits like ShB resistance. Therefore, the primary objective of this review is to synthesize and critically evaluate the substantial progress made in unraveling the genetic and molecular basis of ShB resistance in rice, with a particular emphasis on the pivotal contributions of GWAS. We will:

1. Establish the critical role of robust and scalable phenotyping protocols, from traditional field screens to high-throughput phenomics, as the essential foundation for accurate genetic discovery and germplasm evaluation.
2. Detail how GWAS has overcome limitations of traditional mapping, leading to the discovery of major resistance genes (e.g., *SBRR1*), the elucidation of key defense pathways (e.g., the antioxidant system), and the identification of numerous novel QTLs and promising candidate genes.
3. Highlight how GWAS findings are integrating with and complementing breakthroughs achieved through other cutting-edge approaches, such as targeted gene editing (e.g., *DEP1*), overexpression strategies (e.g., *OsCSP41b, IDD14*), and the characterization of transcription factor networks (e.g., *OsbHLH34, OsBZR1*), all aimed at breaking the resistance-yield trade-off.
4. Discuss the exciting future prospects for GWAS, including enhancing resolution through larger, more diverse panels and improved phenomics, integrating multi-omics data (eQTL, metabolomics), modeling epistatic interactions, exploring non-coding regulatory variation, and directly feeding into genomic selection and precision breeding programs.

By providing a comprehensive overview of these advances, particularly the central role of GWAS, this review aims to illuminate the path forward for developing high-yielding rice cultivars endowed with robust and durable ShB resistance, a critical imperative for safeguarding global rice production.

## Screening Protocols for ShB: From Field to Phenomics

A reliable and repeatable screening protocol is the cornerstone of identifying genetic resistance to ShB. The most common methods involve artificial inoculation at the maximum tillering stage, with the "hill inoculation" technique being a standard in field evaluations (Sharma *et al.*, 1990). This method involves placing inoculum, often mycelial plugs or colonized substrates like typha bits, into the leaf sheath of plants, followed by maintaining high humidity to promote disease development (Bashya *et al.*, 2017; Parveen & Ali, 2018). Disease assessment is typically quantified using the Relative Lesion Height (RLH), which measures the vertical spread of the lesion as a percentage of total plant height, and is scored on a standardized 0-9 scale (IRRI, 1996) as per the Standard Evaluation System (SES) for rice (Kumar *et al.*, 2019).

The strength of these traditional field and greenhouse protocols lies in their direct relevance to agronomic conditions. However, their reliability can be influenced by environmental variability. To address this, controlled-environment methods have been developed to enhance repeatability. Techniques such as the microchamber (Jia *et al.*, 2007), mist-chamber (Liu *et al.*, 2009), and detached sheath inoculation (Venu *et al.*, 2007) provide more uniform conditions by standardizing humidity, temperature, and inoculum pressure (Jia *et al.*, 2013; Park *et al.*, 2008). For instance, the use of liquid-cultured mycelial balls inserted into the sheath and covered with aluminum foil to maintain high humidity has been shown to achieve a 100% infection rate, ensuring consistent and severe disease pressure for discriminating between resistant and susceptible lines (Park *et al.*, 2008). The detached sheath method, conducted in a laboratory setting, offers high throughput and requires minimal space and plant material, making it suitable for rapid preliminary screening. The reliability of these methods is evidenced by their successful use in identifying stable quantitative trait loci (QTLs) and in gene expression studies, underscoring their value for genetic dissection (Jia *et al.*, 2013; Liu *et al.*, 2009).

Looking forward, high-throughput phenotyping technologies are emerging to augment traditional scoring. Hyperspectral imaging, combined with advanced algorithms, has demonstrated remarkable accuracy (over 95%) in automatically detecting ShB scabs at the pixel level, offering a non-destructive, objective, and rapid alternative to visual disease assessment (Zhang *et al.*, 2021). This integration of digital phenomics with established pathological methods promises to further improve the precision, scalability, and repeatability of ShB resistance screening.

## Pathogen Complexity and Infection Challenges

*R. solani* exhibits high genetic variability, classified into 14 anastomosis groups (AGs), with AG1-IA being the primary causal agent of rice ShB (Carling *et al.*, 2002a; Carling *et al.*, 2002b). Its extensive host compatibility, infecting plants from ~32 taxonomic families, and survival between seasons via buoyant sclerotia in soil and stubble significantly complicate control (Molla *et al.*, 2020). Primary inoculum (sclerotia or runner hyphae) initiates infection mainly via infection cushions penetrating the cuticle directly, or less frequently, via lobate appressoria entering stomata (Molla *et al.*, 2013).

## Molecular Defense Mechanisms and Pathogen Countermeasures

Rice activates complex defense signaling pathways upon R. solani infection. Key phytohormones involved are salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), modulating defenses against pathogen lifestyles (Glazebrook, 2005). Although traditionally considered necrotrophic, evidence suggests *R. solani* AG1-IA may exhibit hemibiotrophic traits, blurring defense pathway distinctions (Kouzai *et al.*, 2018). The pathogen deploys an arsenal of virulence factors: validated effectors include cytochrome C oxidase assembly protein (CtaG/cox11), glycosyltransferase GT2, and peptidase inhibitor I9 domains (Anderson *et al.*, 2017; Zheng *et al.*, 2013). Genomic studies predict numerous other candidates (e.g., polygalacturonases like RsPG3, RsPG4, AG1IA_04727; pectin lyases, histone modifiers) (Ghosh *et al.*, 2019; Rao *et al.*, 2019).

Oxalate secretion is a key virulence factor, countered by transgenic rice expressing oxalate oxidase (Molla *et al.*, 2013; Nagarajkumar *et al.*, 2005). A significant immune evasion tactic involves α-1,3-glucan masking of chitin, preventing recognition by plant PRRs; disrupting this mask via α-1,3-glucanase expression enhances resistance (Fujikawa *et al.*, 2012).

## The Quantitative Genetic Architecture of ShB Resistance

Resistance to ShB is inherently polygenic, controlled by numerous small-effect quantitative trait loci (QTLs) rather than single dominant R genes (Wang *et al.*, 2021). Phenotyping is complex, typically involving lesion height (LH), relative lesion height (RLH), disease score (DS), percent disease index (PDI), and area under the disease progress curve (AUDPC) (Aggarwal *et al.*, 2022; Naveenkumar *et al.*, 2023). Resistance often exhibits negative correlations with plant height (PH), tiller number, and maturity time, making it crucial to disentangle true physiological resistance loci from morphological confounders (Jia *et al.*, 2012).

## GWAS: Revolutionizing the Discovery of ShB Resistance Genes

Genome-Wide Association Studies (GWAS) have emerged as a powerful tool for dissecting the complex genetics of ShB resistance, leveraging diverse germplasm to achieve higher resolution than traditional QTL mapping. Key contributions include:

**High-Resolution QTL Identification:** Multiple GWAS have identified significant marker-trait associations (MTAs) for ShB resistance across all 12 rice chromosomes (Aggarwal *et al.*, 2022; Chen *et al.*, 2019; Naveenkumar *et al.*, 2023; Wang *et al.*, 2021). Studies consistently reveal higher inherent resistance in Aus, aromatic, and wild rice (*O. rufipogon, O. nivara*) accessions compared to elite japonica cultivars (Aggarwal *et al.*, 2022; Bhatia *et al.*, 2024; Chen *et al.*, 2019; Goad *et al.*, 2020).

**Germplasm Screening and the Identification of Resistance Sources:** Comprehensive screening of diverse germplasm collections has been a global endeavor to identify sources of ShB resistance, consistently confirming the scarcity of immune genotypes. Evaluations of cultivated varieties, landraces, and wild relatives have identified several sources of partial resistance. For example, international screenings have identified cultivars like Tetep and Jasmine 85 as consistent sources of moderate resistance, which are frequently used in breeding programs (Park et al., 2008). Wild relatives have proven particularly valuable, with accessions of *Oryza rufipogon* (IC336719 and IC336721) and *O. nivara* (IRGC104705, IRGC100898, and IRGC104443) being identified as resistant, exhibiting smaller lesions and reduced fungal penetration structures (Bashya *et al.*, 2017; Prasad & Eizenga, 2008).

In Bangladesh, the Bangladesh Rice Research Institute (BRRI) has been active in screening its genebank accessions to identify promising donors for rice breeding programs. A significant screening effort of 57 rice germplasm accessions identified the local cultivar 'Orgoja' (Acc. No. 5310) as a standout resistant source. Orgoja consistently exhibited the lowest Relative Lesion Height (8.33% - 11.66%) and a minimum SES score of 1 in both field and detached sheath inoculation methods, demonstrating stable resistance across different environments. Another accession, 'Gopal ghosh', was categorized as moderately resistant (Parveen & Ali, 2018). These findings are critical for BRRI's breeding objectives, as Orgoja provides a locally adapted and genetically distinct source of resistance for introgressing ShB resistance into high-yielding BRRI varieties such as BR11, which is highly susceptible. The ongoing research at BRRI thus bridges the gap between traditional germplasm screening and modern genetic improvement, aiming to pyramid resistant QTLs from donors like Orgoja into elite genetic backgrounds to develop durable ShB resistant rice varieties for Bangladeshi farmers.

**Major Gene Discovery - *SBRR1*:** GWAS enabled the landmark discovery of Sheath Blight Resistance Receptor-like Kinase 1 (*SBRR1*) (Feng *et al.*, 2025). They identified a 256-bp insertion in the *SBRR1* promoter (the *SBRR1*-R allele), prevalent in *indica* varieties from ShB-prone regions. This insertion creates a binding site for transcription factor bHLH57, driving high expression. SBRR1-R localizes to the plasma membrane via interaction with SIP1, requires phosphorylation, and activates downstream chitinase genes (*Chit3, Chit4*) to confer significant resistance without yield penalty under disease pressure. This represents the first major gene characterized with high breeding potential identified through GWAS (Table 1).

**Unraveling Defense Pathways - Antioxidant System:** GWAS across rice developmental stages identified 653 associated genes (Wang *et al.*, 2021). Functional validation focused on two key candidate genes: *OsRSR1*, encoding an RPM1-like disease resistance protein, and *OsRLCK5*, encoding a receptor-like cytoplasmic kinase. *OsRSR1* interacts with serine hydroxy-methyltransferase 1 (*OsSHM1*) to modulate ROS burst, while *OsRLCK5* interacts with glutaredoxin (*OsGRX20*), implicating the glutathione-ascorbate (GSH-AsA) antioxidant system as a crucial component of ShB defense (Table 1).

**Novel Loci and Candidate Genes:** GWAS continually identifies novel QTLs and candidate genes. Examples include the identification of 30 MTAs (including 8 novel QTLs) with candidate genes such as *Os05t0566400* (defense-related) (Naveenkumar *et al.*, 2023), and 22 significant SNPs in *O. rufipogon*, with loci on chromosomes 3 and 9 associated with multiple disease traits (Aggarwal *et al.*, 2022). A novel locus, *qShB6* (Chr6: 0.81–4.27 Mb), enriched for LRR-RLKs and transcription factors, was also identified using QTL-seq in a biparental population (Thesiya *et al.*, 2025), consistent with GWAS findings.

### Beyond GWAS: Diverse Genetic Strategies Yield Breakthroughs

While GWAS has been pivotal, other approaches have also identified key resistance genes and strategies:

**Precise Gene Editing (*DEP1*):** Using CRISPR/Cas9 to create a truncated *DEP1* allele (dep1-cys) improved both ShB resistance and yield, avoiding the drawbacks of a full gene knockout. The *dep1* cys variant weakens its interaction with *IDD14*, which enhances PIN1a activity, and strengthens its interaction with IDD10, which suppresses ETR2 expression. Together, these changes reduce disease susceptibility (Zhu *et al.*, 2025) (Table 1).

**Overexpression Strategies:** Overexpression of *OsCSP41b* (chloroplast transcript stabilizer) enhances ShB, drought, and salt tolerance without yield penalty (Zhao *et al.*, 2025). Moderate IDD14 overexpression also boosts resistance without compromising yield (Cui *et al.*, 2022; Sun *et al.*, 2020) (Table 1).

**Transcription Factor Networks:** Recent studies have highlighted the importance of transcription factor (TF) networks in regulating rice resistance to pathogens. Key players include *OsbHLH34*, a positive regulator that enhances ethylene biosynthesis and defense responses by activating *OsERF34*. Plants with *OsbHLH34* knockouts are highly susceptible, while overexpression confers resistance (Zhai *et al.*, 2025) (Table 1). Similarly, *OsBZR1*, a central component of the brassinosteroid (BR) signaling pathway, has been shown to play a positive regulatory role in disease resistance; knockdown mutants exhibit increased susceptibility (Chen *et al.*, 2024) (Table 1). Members of the WRKY family also contribute to resistance modulation. For instance, *WRKY36* negatively regulates resistance by suppressing the sugar transporter *SWEET11* (Gao *et al.*, 2018), while *WRKY53* functions within BR signaling to fine-tune *SWEET2a* expression, also contributing to negative regulation of resistance (Gao *et al.*, 2021) (Table 1).

**Other Genes Controlling the Resistance:** Beyond transcriptional control, several favorable alleles and gene variants have been identified that enable resistance enhancement without compromising yield. The *idd10* mutant enhances ethylene signaling and resistance while maintaining normal yield levels (Li *et al.*, 2025). The *UMP1R2115* allele confers broad-spectrum resistance with no adverse effects on agronomic performance (Hu *et al.*, 2023) (Table 1). Similarly, *AMT1*;1, involved in nitrogen uptake and ethylene signaling, improves resistance while preserving yield stability (Li *et al.*, 2025; Wu *et al.*, 2022) (Table 1). Other genes such as *BGL2* (*β*-glucanase) and *GELP77* (a GDSL lipase) not only bolster resistance but also contribute to improved tillering and yield outcomes (Li *et al.*, 2025; Zhang *et al.*, 2024) (Table 1). GT1, which regulates sugar partitioning and tillering, maintains or slightly enhances resistance without reducing yield (Yang *et al.*, 2024) (Table 1). The gene-edited variant *RBL1Δ12* also increases resistance while sustaining normal growth and yield (Sha *et al.*, 2023). At the *Pigm* locus, *PigmR* provides strong resistance, while *PigmS* supports yield, collectively achieving a resistance–yield balance (Deng *et al.*, 2017). Additional positive regulators include GRF6, which modulates auxin and jasmonate signaling to enhance both yield and bacterial blight resistance (Yuan *et al.*, 2024), and IPA1, which boosts immunity and yield through phosphorylation-mediated mechanisms (Wang *et al.*, 2018). Finally, the *ROD1* allele serves as a dynamic modulator of the defense–growth trade-off, contributing to balanced plant performance under stress (Gao *et al.*, 2021) (Table 1).

## Future Scope and Role of GWAS

GWAS has proven indispensable but its potential is far from exhausted. Key future directions include:

**Enhanced Resolution & Power:** Utilizing larger, more diverse panels (especially under-represented Aus and wild relatives), high-density genotyping (haplotypes, SV calling), and improved, standardized phenotyping (including image-based) will increase power to detect smaller-effect loci and rare alleles (Aggarwal *et al.*, 2022; Bhatia *et al.*, 2024; Mahantesh *et al.*, 2021).

**Multi-Omics Integration:** Combining GWAS with transcriptomics (eQTL), proteomics, metabolomics, and epigenomics will provide deeper mechanistic insights into gene function, regulatory networks (e.g., downstream of *SBRR1, OsbHLH34*), and gene-by-environment interactions (Wang *et al.*, 2021).

**Elucidating Gene Networks & Epistasis:** GWAS data is crucial for modeling epistatic interactions between resistance loci (e.g., how *SBRR1* interacts with antioxidant genes or hormone pathways) and for prioritizing candidates within QTL regions identified by both linkage and association mapping (e.g., *qShB6*) (Thesiya *et al.*, 2025).

**Feeding Genomic Selection (GS) and Breeding:** GWAS findings directly inform the development of robust GS models by identifying predictive markers. Validated genes and alleles (*SBRR1-R, dep1-cys*, favorable *IDD10, UMP1, AMT1;1* alleles) are prime targets for marker-assisted selection (MAS), gene pyramiding, and gene editing in elite backgrounds (Feng *et al.*, 2025; Mahantesh *et al.*, 2021; Zhu *et al.*, 2025).

**Exploring Non-Coding Variation:** GWAS can identify regulatory variants (like the *SBRR1* promoter insertion) controlling gene expression, offering novel targets for manipulation (Feng *et al.*, 2025).

## Table 1. Molecular Determinants of Sheath Blight Resistance in Rice: Genes, Functions, and Mechanisms.

| Gene | Full Name | Function | Reference |
|---|---|---|---|
| ***SBRR1*** | Sheath Blight Resistance Receptor-like Kinase 1 | Plasma membrane-localized receptor-like kinase. The SBRR1-R allele (promoter insertion) drives high expression, activates chitinase genes (*Chit3, Chit4*), conferring significant resistance without yield penalty. | (Feng *et al.*, 2025) |
| ***OsRSR1*** | Rice Starch Regulator 1 | Interacts with serine hydroxymethyltransferase 1 (*OsSHM1*) to modulate the ROS burst, implicating the glutathione-ascorbate (GSH-AsA) antioxidant system in defense. | (Wang *et al.*, 2021) |
| ***OsRLCK5*** | Receptor-Like Cytoplasmic Kinase 5 | Interacts with glutaredoxin (*OsGRX20*), implicating the glutathione-ascorbate (GSH-AsA) antioxidant system in defense. | (Wang *et al.*, 2021) |
| ***DEP1*** | Dense and Erect Panicle 1 | Edited allele (*dep1*-cys) weakens interaction with *IDD14* (enhancing PIN1a activation) and strengthens interaction with *IDD10* (suppressing ETR2 expression), reducing susceptibility and increasing yield. | (Zhu *et al.*, 2025) |
| ***OsCSP41b*** | Chloroplast Stem-Loop Binding Protein of 41kDa b | Chloroplast transcript stabilizer. Overexpression enhances ShB, drought, and salt tolerance without yield penalty. | (Zhao *et al.*, 2025) |
| ***IDD14*** | INDETERMINATE DOMAIN 14 | Transcription factor. Moderate overexpression boosts resistance without compromising yield. | (Sun *et al.*, 2020) |
| ***OsbHLH34*** | Basic Helix-Loop-Helix 34 | Transcription factor. Positive regulator activating *OsERF34* (ethylene biosynthesis/defense); knockouts are susceptible, overexpressors are resistant. | (Zhai *et al.*, 2025) |
| ***OsBZR1*** | BRASSINAZOLE-RESISTANT 1 | Transcription factor central to brassinosteroid (BR) signaling; knockdown increases susceptibility, indicating a positive role in resistance. | (Chen *et al.*, 2024) |
| ***WRKY36*** | WRKY DNA-binding protein 36 | Transcription factor. Suppresses SWEET11, negatively regulating resistance. | (Gao *et al.*, 2018) |
| ***WRKY53*** | WRKY DNA-binding protein 53 | Transcription factor. Via BR signaling, balances SWEET2a expression, negatively regulating resistance. | (Gao *et al.*, 2018) |
| ***IDD10*** | INDETERMINATE DOMAIN 10 | Transcription factor. Mutant (*idd10*) modulates ethylene signaling, increasing resistance without affecting yield. | (Li *et al.*, 2025). |
| ***UMP1*** | Proteasome Maturation Factor UMP1. | Provides strong resistance against a wide range of diseases while maintaining normal growth and yield characteristics. | (Hu *et al.*, 2023) |

| Gene | Full Name | Function | Reference |
|---|---|---|---|
| ***AMT1;1*** | AMMONIUM TRANSPORTER 1;1 | Involved in nitrogen uptake and ethylene signaling. Specific alleles increase resistance and maintain yield stability. | (Li *et al.*, 2025; Wu *et al.*, 2022) |
| ***BGL2*** | β-1,3-GLUCANASE 2 | β-glucanase. Confers increased resistance and promotes tillering/yield. | (Li *et al.*, 2025; Zhang *et al.*, 2024) |
| ***GELP77*** | GDSL LIPASE 77 | GDSL lipase. Promotes JA accumulation, conferring broad-spectrum resistance and increased yield. | (Zhang *et al.*, 2024) |
| ***GT1*** | **GRAIN TILLERING 1** | Affects sugar partitioning and tillering. Can maintain or increase resistance without compromising yield. | (Yang *et al.*, 2024) |
| ***RBL1*** | RESISTANCE TO BLAST1. | The edited allele (*RBL1Δ12*) increases resistance without affecting growth or yield. | (Sha *et al.*, 2023) |
| ***PigmR*** | Pigm Resistant allele | Resistance allele at the *Pigm* locus. Pyramiding with *PigmS* (susceptibility allele) allows sustained blast resistance without yield penalty. | (Deng *et al.*, 2017) |
| ***GRF6*** | GROWTH-REGULATING FACTOR 6 | Modulates auxin (IAA) and jasmonic acid (JA) pathways. Increases yield and bacterial blight resistance. | (Yuan *et al.*, 2024) |
| ***IPA1*** | IDEAL PLANT ARCHITECTURE 1 | Increases immunity and yield via phosphorylation mechanisms. | (Wang *et al.*, 2018) |
| ***ROD1*** | RESISTANCE OF RICE TO DISEASES 1 | Allele dynamically balances defense and growth. | (Gao *et al.*, 2021) |

## CONCLUSION

Research on rice ShB resistance has accelerated rapidly, shifting from a historical scarcity of strong resistance genes to the identification of concrete molecular targets and pathways. This represents a pivotal advance in our capacity to manage the disease. GWAS has played a central role in this transformation, enabling the landmark discovery of *SBRR1*, implicating key defense pathways such as the GSH–AsA antioxidant system, and continually revealing new loci and candidate genes. Its power to dissect polygenic traits has fundamentally reshaped the field.

These GWAS-driven findings are reinforced by diverse genetic approaches. Precise gene editing (e.g., *dep1-cys*), targeted overexpression strategies (*OsCSP41b, IDD14*), and the characterization of major transcription factor networks (*OsbHLH34, OsBZR1*) offer practical routes to enhance resistance, with several studies demonstrating the potential to overcome the long-standing resistance–yield trade-off. Together, these advances create momentum toward more durable and agronomically sound resistance.

Looking ahead, progress will depend on strengthening the pipeline from discovery to deployment. Refining high-throughput digital phenotyping will be essential for generating accurate, scalable datasets that can power advanced GWAS and validate resistance under field conditions. Equally important is the functional validation of priority GWAS-derived genes through knockout, overexpression, and allele-editing studies, supported by multi-omics integration to uncover regulatory networks, epistatic interactions, and non-coding variation underlying ShB resistance. Finally, translating these insights into breeding outcomes will require coordinated use of marker-assisted selection, allele pyramiding, and genomic selection to deliver high-yielding cultivars with robust and durable resistance. Advancing this agenda is critical not only for scientific understanding but also for safeguarding global rice production and ensuring long-term food security under evolving pathogen and climate pressures.

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*Review*

# Evaluation of Progress and Emerging Opportunities for Hybrid Rice in Bangladesh

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## ABSTRACT

Since the initiation hybrid rice research in 1993 in collaboration with International Rice Research Institute (IRRI), Bangladesh Rice Research Institute (BRRI) has made significant progress in developing and releasing hybrid rice varieties adapted to local agro-ecologies. As of 2024, a total of 255 hybrid rice varieties have been officially registered, including 33 developed domestically. This study synthesizes BRRI's achievements in hybrid rice breeding, parental line development, and multilocation performance trials. As documented in 2024, BRRI has released eight hybrid rice varieties five for Boro season, two for T. Aman, and one for T. Aus with a 15–20% yield advantage over modern inbred varieties. Notably, BRRI hybrid dhan3 and BRRI hybrid dhan8 exhibit yield potentials exceeding 10.5 t/ha in the Boro season. BRRI has developed over 20 cytoplasmic male sterile (CMS) lines and 40 restorer lines using germplasm from IRRI, China, India, and indigenous sources. Multi-location trials during T. Aman 2022 season revealed that select hybrids outperformed checks by up to 29% in grain yield. These results underscore the suitability of IRRI-derived CMS lines for Bangladesh's rainfed conditions and highlight BRRI's capacity to generate heterotic hybrids for both irrigated and rainfed ecosystems. Continued investment in locally adapted parental lines and strategic field testing will be critical to scaling hybrid rice adoption and enhancing national food security. Seed production and distribution are driven primarily by private companies, which supply 90 percent of hybrid seed, while public research institutes focus on nucleus seed production and quality control. In 2022-23, BRRI developed hybrid rice variety produce 22.89 ton hybrid seed through contact grower which was distributed to different organizations.

**Keywords:** Yield potential, Seed production, Farmer adoption, Parental lines, Public-private partnership.

## INTRODUCTION

Global food security faces mounting pressure as the world population continues to grow, demanding ever-higher agricultural productivity. Rice, as the staple food for more than half of the global population, plays a central role in this challenge. For every additional billion people, an estimated 100 million tons of paddy rice must be produced annually to meet consumption needs (Bin Rahman and Zhang, 2023). While some countries currently maintain a rice surplus, future projections suggest that climate change, land degradation, and population growth will strain production systems.

Bangladesh, one of the most densely populated countries in the world, exemplifies this urgency. With over 170 million people living within 147,570 km², the country must continually increase rice output to sustain its food supply. Although Bangladesh produced 41.3 million tons of rice in 2022–2023, ensuring a modest surplus, projections indicate that by 2050, the

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nation will need approximately 47 million tons to feed an estimated 210 million people (Biswas and Kabir, 2023; BRRI, 2023). At present, we have a surplus in rice (Kabir *et al.*, 2015). But, in the near future, there will probably be a shortage because of over population and climate change. Every year, 2.2 million people are added to the country's existing population (Streatfield and Karar, 2008). To satisfy these extra millions, we have to produce an additional 0.34 million tons of rice every year. This demand implies a required production increase of nearly 50%, despite shrinking arable land and growing environmental constraints (Ashikari *et al.*, 2005; Srividya *et al.*, 2010).

Hybrid rice technology offers a promising solution to this dilemma. With a yield advantage of 20–30% over conventional inbred varieties (Lin and Yuan, 1980; Siddiq, 1993), hybrid rice has the potential to break the current yield ceiling and support sustainable intensification. Since its introduction in Bangladesh in 1993 through collaboration with the International Rice Research Institute (IRRI), hybrid rice has undergone significant development. However, early adoption was hindered by technical, institutional, and perceptual barriers including limited local expertise, poor seed performance, and skepticism about economic viability.

Despite recent progress in breeding, seed systems, and policy support, hybrid rice still covers only about 12% of the total rice area (DAE, 2022). Moreover, comprehensive insights into its long-term performance, adoption dynamics, and strategic potential remain limited in the literature.

The objective of this paper is to critically review the development, adoption, and future prospects of hybrid rice in Bangladesh, with particular emphasis on its role in ensuring national food security under changing demographic and climatic conditions.

## MATERIALS AND METHODS

This study adopts a qualitative review approach to assess the progress and prospects of hybrid rice in Bangladesh. The methodology involves a comprehensive synthesis of secondary data drawn from peer-reviewed journal articles, government publications, institutional reports, and relevant grey literature published recently.

Publications from the Bangladesh Rice Research Institute (BRRI) and the International Rice Research Institute (IRRI). Reports from the Ministry of Agriculture and the Department of Agricultural Extension (DAE). Data from national surveys and agricultural statistics (e.g., BBS, FAO). Studies by academic researchers and development organizations working on rice innovation and food security. To ensure analytical rigor, the literature was screened for relevance, credibility, and recency. Comparative insights from neighboring countries such as India, China, and Vietnam were also incorporated to contextualize Bangladesh's experience within the broader regional landscape.

This methodology enables a holistic understanding of hybrid rice development, highlighting both empirical findings and strategic implications for future interventions.

## RESULTS AND DISCUSSION

### Progress of hybrid rice in BRRI

The average yield of Boro-season BRRI-released hybrid varieties ranges from 8.0 to 10.5 t/ha (Biswas *et al.*, 2024). BRRI hybrid dhan3 is the most popular variety of the Boro season, followed by BRRI hybrid dhan5. It has tremendous yield potential and had average yield of 9.0 t/ha and the highest yield of more than 11.0 t/ha. The latest BRRI-released hybrid is BRRI hybrid dhan8 for the Boro season, having yield potential of more than 10.5 t/ha coupled with slender grain and growth duration of 145–148 days. It will soon undergo large-scale testing in farmers' fields. The first released BRRI hybrid, BRRI hybrid dhan1, was developed using IRRI CMS line IR58025A. Similarly, Hardinath Hybrid-1 (HH-1) first hybrid rice variety developed and released in Nepal (Subedi *et al.*, 2024). In 2010, BRRI was able to release the first-ever T. Aman hybrid rice variety in Bangladesh. It has yield potential of 6.0–6.5 t/ha with slender grain using the same IRRI CMS line, IR58025A. The most popular

BRRI-released T. Aman hybrid is BRRI hybrid dhan6. It has excellent cooking quality because of high amylose content and good physio-chemical properties and it yields 6.5–7.0 t/ha with 115–120 days' growth duration. It has gained popularity because of its slender grain, good cooking quality, as well as very good seed production ability. It is bred using IRRI CMS line IR79156A. So far, BRRI has been able to breed one T. Aus hybrid (BRRI hybrid dhan7) having yield potential of more than 7 t/ha coupled with very attractive long slender grain with 105–110 days' growth duration. It was released in 2020 and bred using IRRI CMS line IR75608A. So, it is clearly evident that IRRI CMS lines are suitable for the rainfed conditions of Bangladesh.

About 20 potential CMS lines for the dry and wet season with desirable grain type and duration have been developed along with 40 restorer lines (Hasan *et al.*, 2012; Hasan *et al.*, 2015). A specific and goal-oriented work plan has been prepared for developing heterotic rice hybrids for both the irrigated (Boro) and rainfed (T. Aman) ecosystems. Efforts are also being made to develop new sources of CMS lines adapted to Bangladeshi conditions. India has made impressive strides in hybrid rice development. So far, 117 three-line indica hybrids have been released, adapted to various growing conditions and durations (115–150 days), now covering 6.8% of the country's total rice area. Additionally, scientists have developed several indigenous CMS lines using diverse genetic and cytoplasmic sources, which are now key tools in breeding even better hybrid rice varieties (Rout *et al.*, 2020).

## Sources of BRRI hybrid rice parental lines

Bangladesh Rice Research Institute (BRRI) is the leading public-sector organization in Bangladesh with a dedicated mandate for rice research. BRRI is responsible for developing rice varieties, improving cultivation technologies, and supporting national food security through rice research. Informal collaboration in hybrid rice research began between BRRI and IRRI in 1993. The initial work involved testing of F1 hybrids and evaluation of CMS lines and restorer lines from IRRI. Later, BRRI started hybrid rice breeding work to develop hybrid parental lines using germplasm from indigenous sources (BRRI gene bank, conventional breeding program) and international nurseries, mostly from IRRI. Several germplasm lines (A, B, and R) were also supplied by Chinese experts during their consultancy mission under the TCP project funded by FAO in 1997–1998. Those were found to be good CMS sources. Therefore, BRRI used those CMS sources and developed a good number of new CMS lines along with their maintainer lines. IRRI developed the CMS lines IR58025A and IR62829A and these were used to develop locally adapted CMS lines. Several selected local varieties/lines were identified as maintainers and were backcrossed to their respective CMS sources. A large number of high-yielding locally developed elite lines were tested along with some good restorers from IRRI. BRRI also received some Indian germplasm through IRRI. In this situation for promoting hybrid rice cultivation in the country, Bangladesh should develop its own parental lines. Keeping this view in mind, BRRI has developed several A, B, and R lines by using CMS sources from other countries (Table 1).

**Table 1. A, B, R, and local lines used in hybrid rice development in Bangladesh (2002–2021).**

| Sl. no. | Designation | Cyto source | Country of origin | Restorer line | Source |
|---|---|---|---|---|---|
| 1 | Jin23A/B | WA | China | Gui99R | China |
| 2 | Gan 46A/B | Dissi | China | Ajay R | India |
| 3 | II 32A/B | ID | Indonesia | PMSRI-17-4-B-13 | India |
| 4 | IR68886A/B | WA | Philippines | IR52713-2B-8-2B-1-2 | IRRI |
| 5 | IR68888A/B | WA | Philippines | IR65209-3B-6-3-1 | IRRI |
| 6 | IR68897A/B | WA | Philippines | IR65610-38-2-4-2-6-3 | IRRI |
| 7 | IR70960A/B | Gambiaca | Philippines | IR44675R | IRRI |
| 8 | IR75595A/B | Dissi | Philippines | IR71137-328-2-3-3-2R | IRRI |
| 9 | IR75608A/B | Dissi | Philippines | IR69713-3-2-1-3-2R | IRRI |
| 10 | IR77801A/B | Dissi | Philippines | IR69702-91-2-3R | IRRI |
| 11 | IR77805A/B | Dissi | Philippines | IR73885-10-4-3-2-1-6R | IRRI |
| 12 | IR58025A/B | WA | Philippines | IR65482-7-216-1-2R | IRRI |
| 13 | IR79128 /B | WA | Philippines | IR69713-127-2-1-3-2R | IRRI |
| 14 | IR79156A/B | WA | Philippines | BR827R | BRRI |
| 15 | IR80151A/B | WA | Philippines | BR168 R | BRRI |
| 16 | IR80154 A/B | Gambiaca | Philippines | BR736R | BRRI |
| 17 | IR80156 A/B | Kalinga | Philippines | BR6839-41-5-1R | BRRI |
| 18 | BRRI1A/B | WA | Bangladesh | BR7013-62-1-1R | BRRI |
| 19 | BRRI2A/B | WA | Bangladesh | BR7011-37-1-2R | BRRI |
| 20 | BRRI3A/B | WA | Bangladesh | BR6723-1-1-2R | BRRI |
| 21 | BRRI4A/B | WA | Bangladesh | BRRI10R | BRRI |
| 22 | BRRI5A/B | WA | Bangladesh | BRRI11R | BRRI |
| 23 | BRRI6A/B | WA | Bangladesh | BRRI12R | BRRI |
| 24 | BRRI7A/B | WA | Bangladesh | BRRI13R | BRRI |
| 25 | BRRI10A/B | WA | Bangladesh | BRR114R | BRRI |
| 26 | BRRI11A/B | WA | Bangladesh | BRRI15R | BRRI |
| 27 | BRRI13A/B | WA | Bangladesh | BRRI16R | BRRI |
| 28 | BRRI21A/B | WA | Bangladesh | BRRI17R | BRRI |
| 29 | BRRI28A/B | Gambiaca | Bangladesh | BRRI20R | BRRI |
| 30 | BRRI30A/B | WA | Bangladesh | BRRI23R | BRRI |
| 31 | BRRI32A/B | Dissi | Bangladesh | BRRI27R | BRRI |
| 32 | BRRI35A/B | WA | Bangladesh | BRRI31R | BRRI |
| 33 | BRRI41A/B | WA | Bangladesh | BRRI32R | BRRI |
| 34 | BRRI48A/B | WA | Bangladesh | BRRI35R | BRRI |
| 35 | BRRI56A/B | WA | Bangladesh | BRRI37R | BRRI |
| **Local germplasm** | | | | | |
| 1 | Luhagara | | Bangladesh | | BRRI |
| 2 | Malail | | Bangladesh | | BRRI |
| 3 | Binnimuri | | Bangladesh | | BRRI |
| 4 | Sharisha Mota | | Bangladesh | | BRRI |
| 5 | Dongra | | Bangladesh | | BRRI |
| 6 | Dular | | Bangladesh | | BRRI |
| 7 | Kajalsail | | Bangladesh | | BRRI |
| 8 | Kacha Nonia | | Bangladesh | | BRRI |
| 9 | Khato Vajan | | Bangladesh | | BRRI |
| 10 | Sonaroti | | Bangladesh | | BRRI |
| 11 | Jupri | | Bangladesh | | BRRI |
| 12 | Sadamota | | Bangladesh | | BRRI |

## Multi-location trials of promising hybrids

Multilocation trials were conducted to assess the adaptability and yield potential of the identified hybrids. Twenty-one promising hybrids along with three checks were evaluated across five BRRI regional stations during the T. Aman season of 2022 under two sets. In this study, hybrids entries were compared against three checks - BRRI hybrid dhan6, used as the baseline for yield comparison, AZ 7006 and Dhanny Gold used as additional standards.

Results from Set-I showed that BRRI99A/BRRI42R was the top-performing hybrid, which resulted in 29% yield advantage over BRRI hybrid dhan6, 27% over AZ 7006 and 19% over Dhanny Gold. This hybrid also exhibited the highest spikelet fertility (SF), indicating excellent pollination and grain filling capacity. Its grain type was medium-slender (MS) and amylose content was 24%, suitable for non-sticky, fluffy cooked rice. Another strong performer, BRRI97A/BRRI42R, offering a 22% yield advantage over BRRI hybrid dhan6, 20% over AZ 7006, and 13% over Dhanny Gold. It showed good stability across locations and had a spikelet fertility of 80.3%. Similarly, BRRI99A/BRRI43R had a yield advantage of 21%, 19%, and 12% over the three checks, respectively. These three hybrids consistently outperformed the check varieties in grain yield across test locations. They also exhibited favorable agronomic traits, acceptable grain shape and desirable cooking quality (amylose content between 23.6% and 24.2%). Location-wise, Ishwardi and Barishal recorded the highest yields, suggesting that these sites possess favorable agro-ecological conditions for hybrid.

In Set-II, among the ten entries, several hybrids demonstrated significant yield advantages over the standard check varieties. Notably, IR102758A/BRRI43R gave the highest average grain yield, outperforming BRRI hybrid dhan6 by 21%, AZ 7006 by 21%, and Dhanny Gold by 13%. This hybrid also recorded the highest spikelet fertility, reflecting strong reproductive success. It featured a long slender grain type and an amylose content of 24.0%, which aligns well with market demand and consumer preferences. Another performer, IR102758A/BRRI42R showed a 18% yield advantage over both BRRI hybrid dhan6 and AZ 7006, and 10% advantage over Dhanny Gold. IR58025A/BRRI46R also showed promise with desirable spikelet fertility, grain type and amylose content. These findings are consistent with earlier findings by Sarkar (2016), as well as hybrid performance trends observed in multi-location trials conducted in the Philippines (Tabanao *et al.*, 2015) and India (Muralidharan *et al.*, 2020), where hybrid varieties consistently out yielded inbreds under diverse environments.

Overall, hybrids such as BRRI99A/BRRI42R, BRRI97A/BRRI42R, BRRI99A/BRRI43R, IR102758A/BRRI42R, and IR102758A/BRRI43R demonstrated significant yield advantages over the standard checks, along with good grain quality and agronomic traits. These entries hold strong potential for release and wider cultivation in Bangladesh's diverse rice-growing environments.

**Table 2. Results of multilocation yield trials during T. Aman 2022 season (Set-I).**

| Sl. no. | Hybrids | PH (cm) | DTM | Yield (t/ha) | | | | | | SF (%) | GSS | Amy. (%) | Ave. yield advantage over check (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Gaz | Ish | Bari | Ran | Son | Ave. | | | | Ck-1 | Ck-2 | Ck-3 |
| 1 | IR79156A/BRRI46R | 108 | 111 | 7.4 | 7.6 | 5.8 | 7.4 | 5.8 | 6.8 | 76.3 | LS | 24.2 | 8.0 | 6.3 | – |
| 2 | BRRI97A/BRRI53R | 110 | 110 | 7.2 | 7.0 | 8.9 | 6.4 | 5.6 | 7.0 | 78.6 | MS | 23.4 | 11.4 | 9.7 | 3.2 |
| 3 | IR79156A/BRRI53R | 106 | 113 | 5.9 | 7.3 | 7.4 | 6.9 | 6.3 | 6.8 | 76.0 | LS | 23.2 | 8.0 | 6.3 | – |
| 4 | IR105688A/BRRI53R | 104 | 110 | 6.1 | 6.9 | 7.4 | 6.0 | 5.8 | 6.4 | 75.2 | LS | 23.5 | 1.6 | – | – |
| 5 | BRRI97A/BRRI42R | 107 | 114 | 6.8 | 8.7 | 8.4 | 7.7 | 6.7 | 7.7 | 80.3 | MS | 23.6 | 22.2 | 20.3 | 13.2 |
| 6 | BRRI99A/BRRI42R | 111 | 115 | 8.3 | 9.2 | 8.5 | 7.3 | 7.0 | 8.1 | 84.5 | MS | 24.2 | 28.6 | 26.6 | 19.1 |
| 7 | BRRI99A/BRRI37R | 110 | 110 | 6.0 | 7.9 | 6.9 | 7.2 | 6.3 | 6.9 | 77.8 | MS | 23.4 | 9.5 | 7.8 | 1.5 |
| 8 | IR102758A/BRRI36R | 109 | 114 | 6.5 | 7.1 | 6.1 | 6.3 | 5.8 | 6.4 | 75.0 | MS | 23.4 | 1.6 | – | – |
| 9 | BRRI97A/BRRI43R | 107 | 110 | 6.1 | 7.5 | 7.3 | 7.1 | 6.3 | 6.9 | 77.6 | LS | 24.0 | 9.5 | 7.8 | 1.5 |
| 10 | BRRI99A/BRRI43R | 111 | 118 | 7.4 | 8.8 | 7.8 | 7.1 | 7.0 | 7.6 | 79.8 | LS | 23.7 | 20.6 | 18.8 | 11.8 |
| 11 | IR79156A/BRRI43R | 113 | 119 | 6.7 | 8.4 | 7.2 | 7.4 | 6.8 | 7.3 | 78.7 | MS | 23.6 | 15.9 | 14.1 | 7.4 |
| Ck-1 | BRRI hybrid dhan6 | 112 | 116 | 5.8 | 7.3 | 5.8 | 6.4 | 6.2 | 6.3 | 75.0 | LS | 24.0 | | | |
| Ck-2 | AZ 7006 | 116 | 123 | 5.6 | 7.8 | 6.2 | 6.9 | 5.4 | 6.4 | 75.1 | LS | | | | |
| Ck-3 | Dhanny Gold | 118 | 127 | 6.3 | 7.0 | 7.8 | 7.4 | 5.6 | 6.8 | 75.8 | LS | | | | |
| Mean | | 110.1 | 115.0 | 6.6 | 7.8 | 7.3 | 7.0 | 6.2 | 7.0 | 77.6 | | | | | |
| CV (%) | | 3.5 | 4.6 | 11.7 | 9.7 | 13.8 | 7.1 | 8.7 | 7.8 | 3.5 | | | | | |
| LSD (0.05%) | | 2.6 | 3.6 | 0.5 | 0.5 | 0.7 | 0.3 | 0.4 | 0.4 | 1.9 | | | | | |

Note: PH = plant height, DTM = days to maturity, SF = spikelet fertility, GSS = grain size and shape, LS = long slender, MS = medium slender, Amy. = amylose content.

Gaz = Gazipur, Ish = Ishwardi, Bari = Barishal, Ran = Rangpur, Son = Sonagazi.

**Table 3. Results of multilocation yield trials during T. Aman 2022 season (Set-II).**

| Sl. no. | Hybrids | PH (cm) | DTM | Yield (t/ha) |  |  |  |  |  | SF (%) | GSS | Amy. (%) | Ave. yield advantage over check (%) |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Gaz | Ish | Bari | Ran | Son | Ave. |  |  |  | Ck-1 | Ck-2 | Ck-3 |
| 1 | IR58025A/BRRI46R | 111 | 120 | 7.4 | 7.8 | 8.1 | 6.4 | 5.8 | 7.1 | 78.0 | LS | 23.2 | 12.7 | 12.7 | 6.0 |
| 2 | IR102758A/BRRI53R | 110 | 116 | 7.2 | 7.7 | 7.9 | 5.8 | 5.6 | 6.8 | 77.5 | MS | 23.5 | 7.9 | 7.9 | 1.5 |
| 3 | IR79125A/BRRI53R | 114 | 119 | 6.8 | 6.4 | 7.4 | 6.4 | 5.8 | 6.6 | 76.0 | LS | 24.2 | 4.8 | 4.8 | - |
| 4 | IR78369A/BRRI53R | 116 | 123 | 6.1 | 7.5 | 7.4 | 6.2 | 5.8 | 6.6 | 75.6 | MB | 23.5 | 4.8 | 4.8 | - |
| 5 | IR102758A/BRRI42R | 107 | 114 | 6.8 | 8.7 | 7.4 | 6.6 | 6.7 | 7.4 | 75.8 | MS | 23.6 | 17.5 | 17.5 | 10.4 |
| 6 | IR78369A/BRRI42R | 111 | 115 | 6.3 | 7.0 | 7.5 | 6.2 | 5.8 | 6.6 | 79.1 | M | 24.2 | 4.8 | 4.8 | - |
| 7 | IR58025A/BRRI36R | 110 | 118 | 6.0 | 7.9 | 6.9 | 6.0 | 5.8 | 6.5 | 75.1 | LS | 22.4 | 3.2 | 3.2 | - |
| 8 | IR105688A/BRRI43R | 109 | 114 | 6.5 | 7.1 | 6.1 | 6.32 | 5.8 | 6.4 | 75.0 | MS | 23.4 | 1.6 | 1.6 | - |
| 9 | IR102758A/BRRI43R | 112 | 118 | 8.1 | 7.9 | 8.3 | 7.4 | 6.3 | 7.6 | 79.4 | LS | 24.0 | 20.6 | 20.6 | 13.4 |
| 10 | IR58025A/BRRI43R | 111 | 118 | 5.8 | 7.1 | 6.7 | 6.5 | 5.7 | 6.4 | 75.1 | LS | 22.7 | 1.6 | 1.6 | - |
| Ck-1 | BRRI hybrid dhan6 | 112 | 116 | 6.3 | 7.0 | 5.8 | 6.0 | 6.2 | 6.3 | 74.8 | LS | 24.0 |  |  |  |
| Ck-2 | AZ 7006 | 116 | 124 | 5.6 | 7.5 | 6.2 | 6.7 | 5.6 | 6.3 | 77.3 | LS |  |  |  |  |
| Ck-3 | Dhanny Gold | 118 | 128 | 6.3 | 7.6 | 7.0 | 6.8 | 5.8 | 6.7 | 78.0 | LS |  |  |  |  |
| Mean |  | 112.1 | 118.7 | 6.6 | 7.5 | 7.1 | 6.4 | 5.9 | 6.7 | 76.7 |  |  |  |  |  |
| CV (%) |  | 2.8 | 3.5 | 10.6 | 7.6 | 10.8 | 6.5 | 5.3 | 6.1 | 2.1 |  |  |  |  |  |
| LSD (0.05%) |  | 2.2 | 3.0 | 0.5 | 0.4 | 0.6 | 0.3 | 0.2 | 0.3 | 1.2 |  |  |  |  |  |

Note: PH = Plant height, DTM = days to maturity, SF = spikelet fertility, GSS = grain size and shape, LS = long slender, MS = medium slender, MB = medium bold, Amy. (%) = amylose content, Gaz = Gazipur, Ish = Ishwardi, Bari = Barishal, Ran = Rangpur, Son = Sonagazi.

### Evaluation of HRDC hybrids during Boro 2022–2023

Five hybrid rice varieties from the Hybrid Rice Development Consortium (HRDC) were evaluated with five check varieties at BRRI, Gazipur, during Boro 2022-23 season. Among the HRDC entries, IR138766H showed the most promising performance which represents an 8% yield advantage over SL8H and 2.03% over Heera. This hybrid also showed high spikelet fertility, favorable grain weight and growth duration. It demonstrated moderate blast disease resistance, rated 3 on a 1-9 scale (Table 8).

**Table 8. Performance of HRDC hybrids during Boro 2022–2023.**

| Sl. no. | Variety | PH (cm) | SF (%) | TGW (g) | DTM | Yield (t/ha) | Blast Disease reaction on 1–9 scale | Remarks |
|---|---|---|---|---|---|---|---|---|
| 1 | IR138901H | 98 | 77.7 | 22.5 | 144 | 9.40 | 3 | |
| 2 | IR138766H | 99 | 92.6 | 23.8 | 146 | 10.05 | 3 | Good crop |
| 3 | IR139568H | 106 | 80.4 | 23.2 | 153 | 8.80 | 3 | |
| 4 | IR139526H | 113 | 72.2 | 23.1 | 154 | 6.08 | 3 | |
| 5 | IR139010H | 113 | 60.6 | 24.0 | 154 | 7.65 | 3 | |
| 6 | BRRI hybrid dhan5 (Ck-1) | 108 | 81.4 | 30.8 | 148 | 11.07 | 3 | |
| 7 | Tej Gold (Ck-2) | 105 | 78.7 | 23.1 | 145 | 10.63 | 3 | |
| 8 | SL8H (Ck-3) | 96 | 85.0 | 26.2 | 147 | 9.35 | 3 | |
| 9 | Heera (Ck-4) | 98 | 84.3 | 27.0 | 153 | 9.85 | 3 | |
| 10 | BRRI hybrid dhan8 (Ck-5) | 105 | 74.9 | 24.2 | 153 | 10.65 | 3 | |
| | CV (%) | 5.9 | 10.9 | 10.3 | 2.7 | 16.3 | | |
| | LSD (0.5) | 1.57 | 4.39 | 1.27 | 2.16 | 1.66 | | |
| | Heritability | 0.96 | 0.93 | 0.97 | 0.96 | 0.81 | | |

Note: PH = plant height, SF = spikelet fertility, TGW = 1,000-grain weight, DTM = days to maturity.

## CMS seed multiplication

Seed production is a major constraint in hybrid rice cultivation. Four CMS lines (BRRI11A, IR79156A, BRRI74A and BRRI120A) along with their respective maintainers were evaluated during *T. Aman* 2022 season to assess the impact of key components on outcrossing rate (OCR) and seed yield. Seed yields of 470 kg/plot (0.94 t/ha), 315 kg/plot (0.93 t/ha), 10.3 kg/plot (1.03 t/ha), and 7.8 kg/plot (1.30 t/ha) were recorded for BRRI11A, IR79156A, BRRI74A and BRRI120A, respectively. Despite relatively high panicle exertion rates (PER) and OCR (30-33%), seed yield was notably low due to heavy rainfall during the supplementary pollination period. Pollen grain were washout through rainfall. Among these, BRRI120A is the promising candidates for profitable seed production.

**Table 4. CMS seed multiplication of BRRI-released hybrids and promising lines during T. Aman 2022 season.**

| Combinations | Plant height (cm) | | 50% flowering date | | PER (%) | OCR (%) | Yield | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | A line | B line | A line | B line | | | (kg/ plot) | (t/ha) | |
| BRRI11A/B | 90 | 94 | 82 | 80 | 78 | 30 | 470 | 0.94 | Poor seed yield due to rainfall during supplementary pollination |
| IR79156A/B | 93 | 97 | 85 | 83 | 79 | 31 | 315 | 0.93 | |
| BRRI74A/B | 98 | 103 | 80 | 77 | 80 | 33 | 10.3 | 1.03 | |
| BRRI120A/B | 97 | 100 | 79 | 77 | 80 | 32 | 7.8 | 1.30 | |

Note: PER = panicle exertion rate, OCR = outcrossing rate.

During the *Boro* 2022-23 season, efforts were made to produce sufficient quantities of pure CMS seeds for BRRI hybrid dhan2, BRRI hybrid dhan3, BRRI hybrid dhan4, BRRI hybrid dhan5, BRRI hybrid dhan6, and BRRI hybrid dhan8. Seed yields from six CMS lines varied notably. IR79156A and BRRI99A produced the highest yields at 1.99 t/ha and 1.91 t/ha, respectively. Although pollen exertion rates (PER) and outcrossing rates (OCR) were relatively high, seed productivity remained below expectations. This was primarily attributed to elevated temperatures during the flowering period. Excessively hot conditions during pollination and seed set can affect both pollen viability and the receptivity of plant stigmas (Lordon and Zystro, 2024).

**Table 5. CMS multiplication of BRRI hybrid dhan2, BRRI hybrid dhan3, BRRI hybrid dhan4, BRRI hybrid dhan5, BRRI hybrid dhan6, and BRRI hybrid dhan8 during Boro season, 2022–2023.**

| Designation | Plant | | 50% flowering | | PER | OCR | Plot area ($m^2$) | Yield (kg/ plot) | Seed yield (t/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | A line | B line | A line | B line | A line | A line | | | |
| BRRI10A/B | 86 | 88 | 125 | 122 | 78.0 | 38.0 | 1,300 | 58 | 0.45 |
| BRRI11A/B | 95 | 99 | 120 | 118 | 82.3 | 39.9 | 4,000 | 598 | 1.40 |
| IR58025A/B | 84 | 87 | 123 | 120 | 77.3 | 38.0 | 1,500 | 244 | 1.16 |
| BRRI7A/B | 94 | 97 | 120 | 117 | 78.0 | 38.3 | 3,400 | 419 | 1.23 |
| IR79156A/B | 88 | 91 | 123 | 121 | 83.0 | 43.5 | 6,800 | 1,352 | 1.99 |
| BRRI99A/B | 85 | 89 | 126 | 123 | 84.3 | 42.4 | 2,200 | 421 | 1.91 |

### $F_1$ seed production of released hybrids

$F_1$ seed production for seven released BRRI hybrid rice varieties was carried out through contract growers at Ishwardi, Pabna during *Boro* season 2022-2023. Parental lines were sown using appropriate intervals between A and R lines in a two- or three-stagger system to ensure synchronization and effective pollination. The highest seed yield was recorded in BRRI hybrid dhan7, followed by BRRI hybrid dhan6. These results indicate that hybrid seed yields exceeding 2.0 t/ha are achievable on a commercial scale. Lower yields in BRRI hybrid dhan2, BRRI hybrid dhan3 and BRRI hybrid dhan5 were attributed to high temperatures during flowering and hailstorm-induced shattering. Higher seed yield influences the farmers adoption rate in hybrid rice.

**Table 6. $F_1$ seed production of BRRI-developed hybrids through contract growers, Ishwardi, Boro 2022–2023.**

| Sl. no. | Combination | Contract grower | Seed yield (kg) | Area ($m^2$) | Seed yield (t/ha) | Remarks |
|---|---|---|---|---|---|---|
| 01 | BRRI hybrid dhan2 | AUS Bangla Agro | 962 | 10,000 | 0.96 | Less seed due to high temperature at flowering stage and shattering from hail storm |
| 02 | BRRI hybrid dhan3 | | 3,510 | 28,000 | 1.25 | |
| 03 | BRRI hybrid dhan4 | | 1,500 | 10,000 | 1.50 | Good |
| 04 | BRRI hybrid dhan5 | | 2,728 | 28,000 | 0.97 | Less seed due to high temperature at flowering stage and shattering from hail storm |
| 05 | BRRI hybrid dhan6 | | 5,700 | 28,000 | 2.04 | Good |
| 06 | BRRI hybrid dhan7 | | 4,620 | 20,000 | 2.31 | Good |
| 07 | BRRI hybrid dhan8 | | 3,872 | 20,000 | 1.94 | Good |
| | Total | | 22,892 | 144,000 (36 acres) | | |

### Dissemination of hybrid rice technology

Several initiatives were undertaken to support domestic seed production to promote the adoption of BRRI-developed hybrid rice varieties. Hybrid Rice Division distributed a total of 21,606 kg of seed, including 16,750 kg of $F_1$ seed, 3,420 kg of A line, and 1,257 kg of R line seed during *Boro* 2022-23 season. These were provided free of cost to 24 seed companies, 130 farmers, BRRI scientists and staff, BRRI regional stations and extension personnel from DAE (Table 7). This effort aimed to strengthen collaboration with seed producers and accelerate the dissemination of hybrid rice technologies across Bangladesh.

**Table 7. Amount of parental line and hybrid seed supplied to different organizations.**

| Sl. no. | Recipient | Nos. | $F_1$ (kg) | A line (kg) | B line (kg) | R line (kg) |
|---|---|---|---|---|---|---|
| 01 | Seed companies | 24 | 1,250 | 3,290 | – | 1,222 |
| 02 | Farmers | 130 | 1,500 | 130 | – | 35 |
| 03 | BRRI scientists + staff | 19 | 2,000 | – | – | – |
| 04 | BRRI R/S (5) + DAE | 6 | 12,000 | – | – | – |
| Total | | 179 | 16,750 | 3,420 | 0.00 | 1,257 |
| Grand total | | 21,606 | | | | |

## Released hybrid rice varieties in Bangladesh

A total of 254 hybrid rice varieties have been registered so far through the National Seed Board (NSB), including 211 for Boro, 37 for T. Aman, and 6 for T. Aus seasons (Table 9) (Halder, 2025). Of these, 33 hybrids (12.9%) were developed locally, demonstrating Bangladesh's growing capacity in hybrid rice breeding. The majority of registered hybrids originate from China (59.6%), followed by India (26.7%). Although a few domestic mega-varieties have been released, imported varieties from China and India still dominate the market in terms of both yield performance and farmer adoption (Khanh *et al.*, 2021). The country's ability to contribute only 13% of the total registered hybrids highlights its potential for further innovation in this sector.

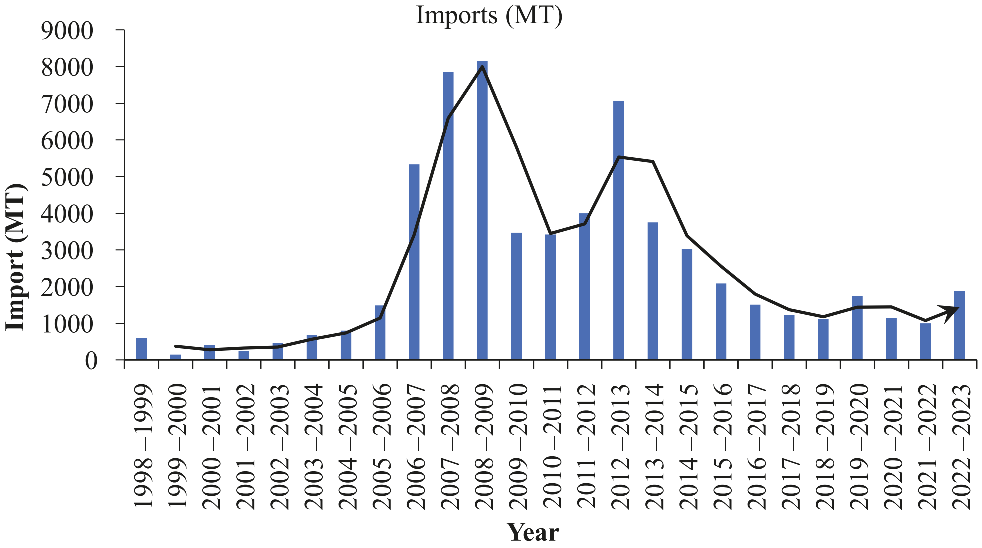
**Table 9. Released hybrid rice varieties in Bangladesh, with their origin.**

| Origin | Number of Varieties | Season |  |  | Country of origin and % share |
|---|---|---|---|---|---|
|  |  | Aus | Aman | Boro |  |
| Bangladesh | 35 | 1 | 12 | 22 | 13.78 |
| China | 153 | 3 | 9 | 141 | 60.24 |
| India | 65 | 2 | 16 | 47 | 25.59 |
| Philippines | 1 | 0 | 0 | 1 | 0.39 |
| Total | 254 | 06 | 37 | 211 | 100 |

(MoA, 2025)

## Imports and local production of hybrid rice seed

During the early years (1998–2004), hybrid rice seed production in Bangladesh was negligible, and the entire demand was met through imports. Local hybrid rice seed production has increased steadily since 2005, reflecting growing domestic capacity. Conversely, import volumes declined gradually after reaching their peak between 2008 and 2010. In recent years, domestic seed production has surpassed 10,000 metric tons, whereas imports have stabilized at relatively low levels. Currently, more than 85% of the total hybrid rice seed demand is met through local production, although the majority of parental lines sourced from abroad (Fig. 1).



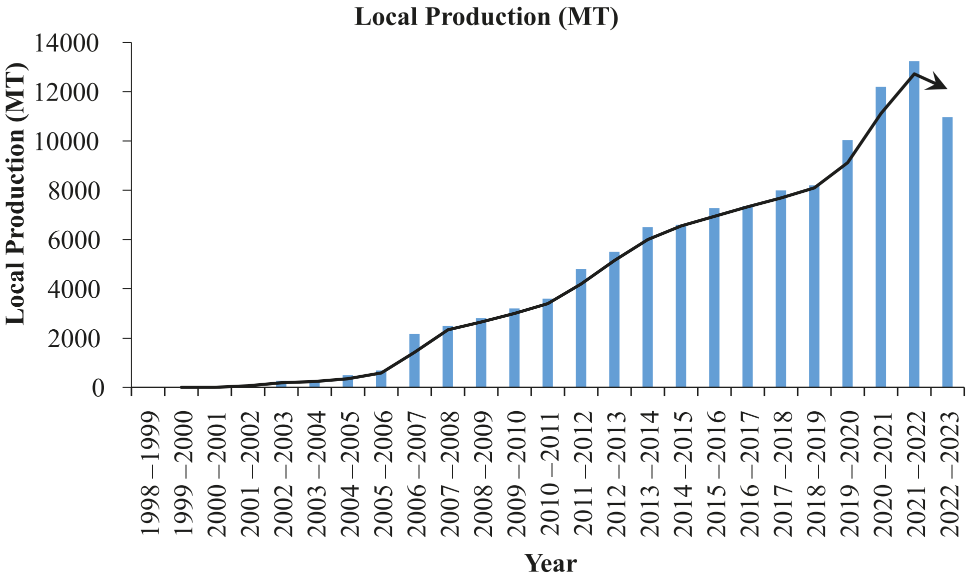


Fig. 1. Year-wise imports and local production of hybrid rice seed from 1998–1999 to 2022–2023 showing in percentage. (MoA, 2024).

**Prospects of hybrid rice in Bangladesh**

Hybrid rice is now a proven technology for Bangladesh and it covers more than 12% of the cultivated area. Most of the hybrids perform well in the dry season (Boro) and monocrop area. Hybrids cover about 25% of the area in the Boro season but about 5% in T. Aus and T. Aman season. It is obviously required for its 15–20% yield advantage and short duration. The government of Bangladesh has taken pragmatic steps to develop and use hybrid rice technology on a large scale by involving public, private, and nongovernment organizations. Research, seed production, and technology transfer agencies in the public, private, and NGO sectors are also interested in exploring the prospects of this technology. The government has allowed some NGOs and private seed companies to introduce and commercialize exotic hybrids for large-scale cultivation by farmers with a view to obtaining immediate benefit from this technology. Special attention has been given to developing hybrid rice varieties within the country and, in this regard, the Ministry of Agriculture (MoA) approved a new hybrid rice project for BRRI titled High-Yielding Hybrid Rice Variety Development through Modernization of Research. The foremost constraint to overcome by researchers is to identify heterotic hybrid combinations that are adaptable under Bangladeshi conditions as well as being able to out yield the most popular commercial varieties by at least 20%. Another hurdle to be overcome is to develop a cheaper seed production package, which is necessary to make this technology commercially viable, along with creating job opportunities for rural dwellers. Zoning of hybrid rice seed production is urgently needed, such as a seed village where only one variety will be produced without any barrier. Cytoplasmic diversity searching is necessary for avoiding sudden disease infestation. Cooking quality needs to be improved coupled with high amylose content and slender grain. As the parental lines of BRRI-developed hybrid rice varieties are readily available, these lines are being provided free of cost to interested persons/companies for small and medium enterprise development, resulting in increasing demand and number of new ventures. Some constraints were identified for prospect of hybrid rice in India (Spielman *et al.*, 2013). Yet, the adoption rate of hybrid rice in Bangladesh still lags behind regional counterparts. Concerns regarding high seed cost, grain acceptability, and yield stability persist, especially among smallholder farmers. Moreover, the lack of comprehensive data on long-term performance, socio-economic impact, and environmental sustainability limits informed decision-making.

**The way forward**

To accelerate the adoption and impact of hybrid rice in Bangladesh, a comprehensive and prioritized strategy is needed:

i) Breeding and Seed Technology
  - Develop stress-tolerant hybrids resilient to drought, flood, and salinity-prone areas.
  - Diversify and stabilize CMS lines with improved grain and cooking quality.
  - Introduce breeder-usable molecular tools to enhance breeding efficiency.
  - Initiate systematic screening for super hybrid rice varieties under Bangladeshi conditions.

ii) Seed Industry and Policy Support
  - Strengthen institutional mechanisms for breeder, foundation, and certified seed production of public hybrids.
  - Establish land-use planning and zoning for hybrid seed villages to ensure genetic purity.
  - Recognize the hybrid rice seed industry as a priority sector in national financial and industrial policy.
  - Provide targeted subsidies and incentives to reduce the high cost of hybrid seed production.
  - The hybrid seed productivity should be enhanced at least up to 2.5-3.0 t/ha to make the availability of hybrid seed at a cheaper rate.

iii) Capacity Building and Knowledge Exchange
  - Enhance scientists' capacity in hybrid rice breeding, seed production, and agronomic management through advanced training.
  - Facilitate exchange of parental lines for both three-line and two-line hybrids.
  - Promote collaborative research, study tours, and higher education (MS, PhD) on hybrid rice within and outside the country.

iv) International and Institutional Collaboration
  - Expand technological cooperation with China, including joint ventures with the China National Hybrid Rice Research and Development Center (CNHRRDC). Foster stronger linkages among public institutes, private companies, and NGOs for seed production and dissemination.

## CONCLUSION

Since the collaboration between BRRI and IRRI in hybrid rice research in 1993, Bangladesh has made significant strides in developing and releasing high-yielding varieties for different agro-ecological conditions of the Bangladesh. BRRI has demonstrated strong national research capacity by developing over 20 CMS, 40 restorer lines and releasing eight hybrid rice varieties that have a yield advantage of 15-20% over traditional inbreeds. However, hybrid rice adoption is still relatively low compared with neighboring countries. Constraints such as high seed cost, grain quality concerns, yield variability, and dependence on imported parental lines hinder the expansion. Strengthening domestic breeding programs, ensuring genetic diversity, and improving hybrid seed production technologies are critical to enhancing local competitiveness.

In order to reduce seed costs and guarantee quality assurance, future success will require consistent investment, efficient public-private cooperation, and enabling policy support. With strategic interventions and wider dissemination, hybrid rice has the potential to significantly improve farmers' livelihoods, increase productivity, and ensure food security of Bangladesh.

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**Conflict of Interests**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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# Harnessing Genetic Diversity by Studying Agro-Morphological Traits for Rice Improvement

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## ABSTRACT

Studying genetic variability and key agro-morphological traits in rice is crucial for breeding because it identifies superior genotypes and reveals how traits are inherited, enabling breeders to select the best parents for developing new, high-yielding varieties. This process provides the essential data needed to plan effective crosses and make targeted improvements in rice crop. This study investigated the genetic variability and key agro-morphological traits of 33 rice genotypes to identify superior lines for future breeding programs. Understanding the genetic potential of these genotypes is crucial for developing high-yielding, resilient rice varieties. We evaluated ten quantitative traits, including days to 50% flowering, plant height, panicle length, and yield per hill. Data were analyzed using analysis of variance (ANOVA), genetic parameters, correlation coefficients, and multivariate techniques such as principal component analysis (PCA) and cluster analysis to determine genetic relationships and trait associations. Significant genetic variation ($P \leq 0.05$) was observed for all traits. The mean performance analysis identified genotypes with superior traits, such as IR146151-B-B-584-44-3(G-30) for the highest plant height (117.17 cm) and yield hill$^{-1}$ (31.71 gm), and IR146164-B-B-543-165-47(G-24) for the longest flag leaf (42.33 cm). High heritability and genetic advance were recorded for flag leaf length and spikelet fertility, indicating strong additive gene action. Yield hill$^{-1}$ showed a significant positive correlation with the number of effective tillers hill$^{-1}$ ($r=0.52$), number of grains panicle$^{-1}$ ($r=0.72$), and spikelet fertility ($r=0.69$). PCA revealed three principal components that explained 70.54% of the total variance. Cluster analysis grouped the genotypes into four distinct clusters, with the largest inter-cluster distance observed between clusters II and IV (5.45), highlighting their high genetic divergence. The study confirmed substantial genetic variability among the rice genotypes, which can be effectively utilized for crop improvement. The identified high-performing genotypes and traits with high heritability are promising for direct selection. Hybridization between genotypes from divergent clusters, particularly clusters II and IV, is recommended to create new genetic combinations and enhance yield potential in future rice breeding programs.

**Keywords:** Rice, Yield-traits, Correlation, Principal component analysis, Cluster analysis

## INTRODUCTION

Rice is a major food for a large portion of the world's population, particularly in Asia, and its civilization and consumption play a substantial part in comprehensive food security and artistic identity. It's a vital source of energy and, to a lower extent, protein, for billions of people (Asma *et al.*, 2023). Rice provides 21% of global mortal per capita energy and 15% of per capita protein (Samal *et al.*, 2022). Although rice protein ranks grandly in nutritive quality among cereals, protein content is modest. Rice also provides minerals, vitamins, and fiber,

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although all constituents except carbohydrates are condensed by milling. It's the easiest source of food and commodity of significance for the people of Bangladesh (Ahmed *et al.*, 2022). It belongs to the family Gramineae. The quantum of rice grown in Asia is about 90 of the world's rice which alone covers the food demand for about 60 population of the world (Haque *et al.*, 2015). Bangladesh is the third-largest rice producer among the 114 rice growing countries of the world. As rice plays a very important part in traditional diets and in the livelihood of people, it earns a special position in numerous nations. Among all food particulars, the significance of rice is supreme. The global population is adding but rice cultivable land is dwindling. In order to meet up the demand, two ways can be effective expanding the rice growing area and adding productivity or both (Hasan *et al.*, 2015). As there's no possibility of adding cultivated area (Horizontal expansion), development of high yielding kinds is the only option to increase the yield of rice (Vertical expansion).

The future approaches in the advance of rice largely depend on the availability of genetic resources and their effective utilization (Sabar *et al.*, 2024). At present, varietal identification for rice are primarily based on morphological and physiological parameters. During varietal development, plant breeders use several techniques to create genetic variation and select within this diversity, ultimately retaining superior plants after final selection (Joshi *et al.*, 2023). Parental genotypes from arbitrary populations are carefully chosen based on the evidence about genetic diversity. Besides genetic diversity, crop yield not only hang on the different yield contributing characters but also on the factors intricate about environment. In case of rice, yield also depends on the higher number of effective tillers hill$^{-1}$, number of filled grains panicle$^{-1}$ and 1000-grain weight (Islam *et al.*, 2013). These traits are also associated among themselves. The extent of genetic variability like as, phenotypic and genotypic variances, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), broad sense heritability, genetic gain (GA) are highly accountable for the genetic enhancement of any crop based on which the breeding methods are found for its additional improvement. The range of variability is also measured by GCV and PCV. Correlation analysis is used to determine the association among the different traits (Faysal *et al.*, 2022). Principal component analysis (PCA) marks out the plant characters that provide the most in generating variation within a group of entries. The above apparatuses are found to be useful for proper description in further breeding program. Plant characterization has been effectively used in searching out appropriate attributes among individual genotype (Prasanna *et al.*, 2024). Elucidation of morphological and genetic characterization and valuation of diversity is very crucial for future varietal improvement. The objectives of the paper are to assess genetic variability and identify superior genotypes among 33 rice genotypes, and to determine trait correlations and genetic parameters to aid in future breeding programs. The study aims to classify genotypes for strategic parent selection and to identify key traits contributing to variation.

## MATERIALS AND METHODS

### Plant materials, exploratory site and design

The experiment was set up at the research Farm of Hybrid Rice Division, Bangladesh Rice Research Institute, Bangladesh during Boro 2024-25 following randomized complete block design (RCBD) with three replications. Thirty three rice genotypes were used as plant materials which were collected from different places of Hybrid Rice Development Consortium (HRDC) (Table 1).

**Table 1. List of used genotypes.**

| Genotypes | Code name | Genotypes | Code name |
|---|---|---|---|
| IR146173-B-B-268-22-45 | G-1 | IR146164-B-B-54-52-41 | G-18 |
| IR146173-B-B-268-68-44 | G-2 | IR146164-B-B-191-15-40 | G-19 |
| IR146173-B-B-268-94-4 | G-3 | IR146164-B-B-191-47-3 | G-20 |
| IR146173-B-B-529-134-4 | G-4 | IR146164-B-B-191-86-4 | G-21 |
| IR146173-B-B-529-178-4 | G-5 | IR146164-B-B-44-122-4 | G-22 |
| IR146173-B-B-527-90-44 | G-6 | IR146164-B-B-543-5-7 | G-23 |
| IR146154-B-B-430-188-46 | G-7 | IR146164-B-B-543-165-47 | G-24 |
| IR146154-B-B-430-209-8 | G-8 | IR146168-B-B-317-122-6 | G-25 |
| IR146154-B-B-141-10-1 | G-9 | IR146168-B-B-15-2-2 | G-26 |
| IR146154-B-B-141-174-42 | G-10 | IR146168-B-B-89-41-4 | G-27 |
| IR146154-B-B-547-168-40 | G-11 | IR146168-B-B-214-81-38 | G-28 |
| IR146154-B-B-793-7-30 | G-12 | IR146151-B-B-439-83-7 | G-29 |
| IR146172-B-B-21-86-5 | G-13 | IR146151-B-B-584-44-3 | G-30 |
| IR146172-B-B-136-81-17 | G-14 | IR146151-B-B-584-82-40 | G-31 |
| IR146172-B-B-236-87-40 | G-15 | IR146151-B-B-1253-16-3 | G-32 |
| IR146172-B-B-596-8-10 | G-16 | IR146151-B-B-1266-13-9 | G-33 |
| IR146164-B-B-54-9-54 | G-17 | | |

The unit plot size was 75cm × 75cm. Twenty-five days old seedlings were used for transplanting @ one seedling hill$^{-1}$ with the space of row to row is 25cm and plant to plant 15 cm. Recommended doses of fertilizers and manures were applied to the soil. Other intercultural operations were done when needed.

### Data Collection

Data on ten quantitative traits such as days to 50% flowering (DFF), days to maturity (DM), plant height (PH), flag leaf length (FLL), number of effective tillers panicle$^{-1}$ (ETH), panicle length (PL), number of grains panicle (NGP), spikelet fertility (SF), thousand grains weight (TGW) and yield hill$^{-1}$ (YH) were recorded from three randomly selected plants of each genotype in each replication. Mean of the three plants for each character were used for statistical analysis.

### Statistical Analysis

The collected data were compiled and analyzed using R software (version 3.4.1). The analysis encompassed one-way ANOVA to evaluate variations among genotypes. To differentiate the means, the LSD test was used at a 5% probability level. In order to identify quantitative variation patterns with the eigen vectors and eigen values principal component analysis (PCA) was also carried out.

### Estimation of Genetic Parameters

Following the procedures described by Johnson *et al.* (1955) and Allard (1960), genetic parameters such as genetic variance, broad-sense heritability ($h^2b$), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), genetic advance (GA), and genetic advance as a percentage of the mean (GA%) were computed. According to the standards put forth by Deshmukh et al. (1986), the phenotypic coefficient of variation and genotypic coefficient of variation estimates were classified as low (20%). Similarly, Johnson et al. (1955) defined GA% as low (20%), and broad sense heritability ($h^2b$) as low (0%-30%), medium (31%-60%), and high (>60%).

### Estimation of Correlation Co-efficient and Cluster Analysis

The phenotypic and genotypic correlation coefficient was estimated using the formula suggested by Miller *et al.* (1958) and path analysis was performed following formula provided by Dewey and Lu (1959), cluster analysis was performed using Ward's method (Ward, 1963).

## RESULTS

### Mean Performance Analysis

The analysis of variance (ANOVA) results showed highly significant ($P \leq .05$) variation for all the traits studied (Table 2). The mean performance of ten measurable traits of rice is documented in Table 3. Among the studied genotypes, the minimum number of days to 50% flowering (95 days) was observed in genotype IR146173-B-B-527-90-44(G-6), IR146154-B-B-141-10-1(G-9), IR146154-B-B-141-174-42(G-10), IR146154-B-B-547-168-40(G-11), IR146154-B-B-793-7-30(G-12), IR146172-B-B-21-86-5(G-13), IR146172-B-B-136-81-17(G-14), IR146172-B-B-236-87-40(G-15), IR146168-B-B-317-122-6(G-5), IR146168-B-B-89-41-4(G-27), IR146168-B-B-214-81-38(G-28), in contrast, the maximum number of days to 50% flowering (109 days) was required for the genotype IR146164-B-B-54-9-54(G-1) (Table 3). The minimum number of days to maturity (126 days) was observed in IR146168-B-B-214-81-38(G-28), whereas, the maximum number of days to maturity (143 days) was recorded for the genotype IR146164-B-B-54-9-54 (G-17). The minimum plant height (87.5 cm) was observed in the genotype IR146154-B-B-430-188-46(G-7). One the other hand, maximum plant height (117.17 cm) was recorded in the IR146151-B-B-584-44-3(G-30). The highest flag leaf length (42.33 cm) was found in IR146164-B-B-543-165-47(G-24). In contrast, the lowest flag leaf length (28.5 cm) was observed in the genotype IR146151-B-B-439-83-7(G-29). The highest number of effective tiller hill$^{-1}$ (10) was observed in the genotype IR146154-B-B-793-7-30(G-12) and IR146151-B-B-1266-13-9(G-33). The minimum panicle length (21.17 cm) found in the genotype IR146172-B-B-136-81-17(G-14). The maximum panicle length (30.33 cm) was recorded in IR146164-B-B-44-122-4 (G-22). In addition, the minimum number of grains panicle$^{-1}$ (96.67) was found in IR146164-B-B-44-122-4 (G-22). The maximum (228.67) was observed in the genotype IR146154-B-B-430-188-46 (G-7). The maximum spikelet fertility (87.83%) was recorded in IR146173-B-B-268-22-45 (G-45) and the minimum (53.86%) was found in IR146154-B-B-547-168-40 (G-11). Among the genotypes, the minimum thousand grains weight (19.62 g) was observed in genotypes IR146154-B-B-430-188-46 (G-7) whereas the maximum (26.68 g) weight was found in the genotypes IR146173-B-B-527-90-44 (G-6). The maximum yield hill$^{-1}$ (31.17 g) was recorded in IR146151-B-B-584-44-3 (G-30), followed by IR146154-B-B-430-188-46 (G-7) (28.99 g) and IR146173-B-B-268-94-4 (G-3) (28.01g). The minimum yield hill$^{-1}$ was recorded in IR146154-B-B-547-168-40 (G-11) as 8.47 g.

### Genetic Parameters Analysis

The characters which showed very high genotypic variance ($\sigma^2g$) and phenotypic variance ($\sigma^2p$) were number of grains panicle$^{-1}$ (712.51 and 1249.48, respectively), followed by spikelet fertility (61.99 and 68.75, respectively) and plant height (56.53 and 59.14, respectively) (Table 4). Co-efficient of variation studies showed that the values of phenotypic coefficient of variation were higher in compare to those of the genotypic coefficient of variation for all the individual traits.

**Table 2. Analysis of variance (mean square) for ten yield and yield attributing traits.**

| Source of variance | df | DFF | DM | PH | ETH | FLL | PL | NGP | **SF** | TGW | **YH** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 20.525 | 28.495 | 0.023 | 1.7449 | 2.031 | 6.3813 | 53.04 | 9.02 | 0.0045 | 6.102 |
| Genotypes | 32 | 63.99*** | 77.044*** | 172.203*** | 5.2085*** | 47.79*** | 14.49*** | 2674.49*** | 192.73***4 | 7.88*** | 99.58*** |
| Error | 64 | 0.369 | 0.349 | 2.609 | 1.0965 | 1.934 | 0.9204 | 536.97 | 6.759 | 0.2464 | 18.397 |

*, ** and *** indicate significant at 5%, 1% and 0.1% level of probability, respectively. Here, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers hill$^{-1}$, PL= Panicle length (cm), NGP= Number of grains panicle$^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (gm) and YH= Yield hill$^{-1}$

**Table 3. Mean performance for ten different morphological traits of rice genotypes grown at BRRI during Boro season of 2024-25.**

| Genotypes | Code name | DFF | DM | PH | FLL | ETH | PL | NGP | SF | TGW | YH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IR146173-B-B-268-22-45 | G-1 | $95^{n}$ | $127^{j}$ | $89.5^{o}$ | $32^{j-n}$ | $5.18^{k}$ | $25.83^{efg}$ | $222.33^{ab}$ | $87.83^{a}$ | $26.41^{a}$ | $26.67^{a-e}$ |
| IR146173-B-B-268-68-44 | G-2 | $99.33^{ij}$ | $132^{h}$ | $102.5^{gh}$ | $30.17^{m-p}$ | $7.17^{c-i}$ | $25^{f-i}$ | $153^{d-k}$ | $79.77^{d-g}$ | $25.27^{b}$ | $22.10^{b-h}$ |
| IR146173-B-B-268-94-4 | G-3 | $99^{j}$ | $133^{g}$ | $107.33^{cd}$ | $30.83^{l-o}$ | $8.67^{abc}$ | $26.5^{ef}$ | $158.67^{d-i}$ | $84.98^{abc}$ | $23.98^{cd}$ | $28.01^{abc}$ |
| IR146173-B-B-529-134-4 | G-4 | $100.33^{gh}$ | $133^{g}$ | $96.17^{k-n}$ | $33^{i-l}$ | $7^{c-j}$ | $25.5^{e-h}$ | $158^{d-j}$ | 76.87f-k | $23.24^{d-g}$ | $19.81^{e-j}$ |
| IR146173-B-B-529-178-4 | G-5 | $100^{hi}$ | $134^{ef}$ | $104.33^{e-h}$ | 32.33i-m | $6.33^{f-k}$ | $28.83a^{bc}$ | $162.67^{c-i}$ | $82.9^{b-e}$ | $22.46^{g-k}$ | $19.13^{f-k}$ |
| IR146173-B-B-527-90-44 | G-6 | $95^{n}$ | $128^{i}$ | $93.67^{n}$ | $32.67^{i-l}$ | $7.3^{b-h}$ | $24h^{i}$ | $165.5^{c-i}$ | $72.74^{klm}$ | 26.68a | $23.59^{b-f}$ |
| IR146154-B-B-430-188-46 | G-7 | $100.33^{gh}$ | $133.33^{fg}$ | $87.5^{o}$ | $28.67^{op}$ | $8.67^{abc}$ | $23.83^{i}$ | $228.67^{a}$ | $74.61^{jkl}$ | $19.62^{p}$ | $28.99^{ab}$ |
| IR146154-B-B-430-209-8 | G-8 | $96^{m}$ | $133^{g}$ | $97.33^{jkl}$ | $30^{nop}$ | $9^{ab}$ | $23.5^{i}$ | $158.83^{d-i}$ | $75.41^{i-l}$ | $21.93^{klm}$ | $23.61^{b-f}$ |
| IR146154-B-B-141-10-1 | G-9 | $95^{n}$ | $127^{j}$ | $95.67^{lmn}$ | $31.33^{k-n}$ | $7.17^{c-i}$ | $25.5^{e-h}$ | $182.33^{c-f}$ | 75.66g-l | $21.78^{klm}$ | $21.663^{c-i}$ |
| IR146154-B-B-141-174-42 | G-10 | $95^{n}$ | $128^{i}$ | $102.33^{h}$ | $39.33^{cd}$ | $7^{c-j}$ | $28.67^{bc}$ | $187.17^{b-e}$ | $80.97^{c-f}$ | $23.37^{def}$ | $24.6^{b-f}$ |
| IR146154-B-B-547-168-40 | G-11 | $95^{n}$ | $128^{i}$ | $95.5^{lmn}$ | $33^{i-l}$ | $6.83^{d-k}$ | $25.67^{efg}$ | $103.5^{lm}$ | $53.86^{q}$ | $22.04^{klm}$ | $8.47^{m}$ |
| IR146154-B-B-793-7-30 | G-12 | $95^{n}$ | $127^{j}$ | $96.33^{klm}$ | $32.5^{i-l}$ | $10.17^{a}$ | $25^{f-i}$ | $137.5^{h-l}$ | $68.96^{mn}$ | $21.93^{klm}$ | $21^{d-i}$ |
| IR146172-B-B-21-86-5 | G-13 | $95^{n}$ | $132^{h}$ | $97.33^{jkl}$ | $33^{i-l}$ | $8.5^{a-d}$ | $25.67^{efg}$ | $147^{f-k}$ | $73.98^{jkl}$ | $23.19^{d-h}$ | $21.41^{c-i}$ |
| IR146172-B-B-136-81-17 | G-14 | $95^{n}$ | $127^{j}$ | $90^{o}$ | $31.67^{j-n}$ | $8^{b-f}$ | $21.17^{j}$ | $147.67^{f-k}$ | $75.71^{g-l}$ | $23.63^{c-f}$ | $21.04^{c-i}$ |
| IR146172-B-B-236-87-40 | G-15 | $95^{n}$ | $128^{i}$ | $112.17^{b}$ | $34.5^{f-i}$ | $5.33^{jk}$ | $28.5^{bcd}$ | $197.17^{abc}$ | $63.57^{p}$ | $23.04^{e-i}$ | $15.38^{h-m}$ |
| IR146172-B-B-596-8-10 | G-16 | $97^{l}$ | $134^{ef}$ | $96.33^{klm}$ | $32.67^{i-l}$ | $5.17^{k}$ | $27^{de}$ | $120.5^{klm}$ | $72.31^{lm}$ | $23.61^{c-f}$ | $10.92^{lm}$ |
| IR146164-B-B-54-9-54 | G-17 | $109^{a}$ | $143^{a}$ | $106^{cdef}$ | $33.33^{h-k}$ | $7.33^{b-h}$ | $25.5^{e-h}$ | $155.83^{d-k}$ | $64.17^{op}$ | $20.39^{op}$ | $15.18^{h-m}$ |
| IR146164-B-B-54-52-41 | G-18 | $108^{b}$ | $141^{b}$ | $105^{d-g}$ | $42^{a}$ | $8.17^{b-e}$ | $28.33^{cd}$ | $180.83^{c-f}$ | $78.04^{f-j}$ | $20.78^{no}$ | $24.27^{b-f}$ |
| IR146164-B-B-191-15-40 | G-19 | $107^{c}$ | $141^{b}$ | $104^{e-h}$ | $39.67^{bcd}$ | $8.67^{abc}$ | $23.83^{i}$ | $140.5^{g-l}$ | $67.85^{no}$ | $22.11^{j-m}$ | $18.78^{f-k}$ |

| Genotypes | Code name | DFF | DM | PH | FLL | ETH | PL | NGP | SF | TGW | YH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IR146164-B-B-191-47-3 | G-20 | $107^{c}$ | $141^{b}$ | $103.67^{fgh}$ | $42.27^{a}$ | $9^{ab}$ | $23.67^{i}$ | $166.67^{c-i}$ | $79.12^{e-i}$ | $21.53^{mn}$ | $24.81^{a-f}$ |
| IR146164-B-B-191-86-4 | G-21 | $107^{c}$ | $141^{b}$ | $105^{d-g}$ | $33.67^{g-j}$ | $7.33^{b-h}$ | $23.67^{i}$ | $138.5^{h-l}$ | $66.07^{nop}$ | $22.51^{g-k}$ | $15.23^{h-m}$ |
| IR146164-B-B-44-122-4 | G-22 | $99^{j}$ | $134^{ef}$ | $110.67^{b}$ | $35.66667^{efg}$ | $5.5^{ijk}$ | $30.33^{a}$ | $148^{f-k}$ | $73.37^{kl}$ | $21.62^{lm}$ | $12.96^{j-m}$ |
| IR146164-B-B-543-5-7 | G-23 | $105^{e}$ | $140^{c}$ | $107.67^{c}$ | $36.33^{ef}$ | $6^{h-k}$ | $26.33^{ef}$ | $177.83^{c-g}$ | $67.75^{nop}$ | $22.89^{f-j}$ | $16.5^{g-l}$ |
| IR146164-B-B-543-165-47 | G-24 | $106^{d}$ | $140^{c}$ | $99.67^{ij}$ | $42.33^{a}$ | $6.5^{e-k}$ | $28.167^{cd}$ | $150.33^{e-k}$ | $65.34^{nop}$ | $24.23^{c}$ | $15.043^{i-m}$ |
| IR146168-B-B-317-122-6 | G-25 | $95^{n}$ | $128^{i}$ | $97^{kl}$ | $34.5^{f-i}$ | $8.5^{a-d}$ | $24.33^{ghi}$ | $152.83^{d-k}$ | $79.71^{d-h}$ | $22.89^{f-j}$ | $23.83^{b-f}$ |
| IR146168-B-B-15-2-2 | G-26 | $102.33^{f}$ | $134.33^{de}$ | $102.17^{hi}$ | 33.17ijk | $7.83^{b-g}$ | $24^{hi}$ | $189^{bcd}$ | $83.4^{bcd}$ | $22.15^{j-m}$ | $26.98^{a-d}$ |
| IR146168-B-B-89-41-4 | G-27 | $95^{n}$ | $127^{j}$ | $106.33^{cde}$ | $33.67^{g-j}$ | $7.67^{b-h}$ | $26.5^{ef}$ | $162.67^{c-i}$ | $77.67^{f-j}$ | $22.18^{j-m}$ | $22.11^{b-h}$ |
| IR146168-B-B-214-81-38 | G-28 | $95^{n}$ | $126^{k}$ | $106.5^{cde}$ | $41.83^{ab}$ | $8.33^{bcd}$ | $24.5^{ghi}$ | $120.83^{j-m}$ | $74.9^{i-l}$ | $25.15^{b}$ | $18.96^{f-k}$ |
| IR146151-B-B-439-83-7 | G-29 | $101^{g}$ | 134ef | $98.67^{jk}$ | $28.5^{p}$ | $6.17^{g-k}$ | $26.5^{ef}$ | $120.67^{klm}$ | $75.51^{h-l}$ | $22.39^{h-l}$ | $12.67^{klm}$ |
| IR146151-B-B-584-44-3 | G-30 | $100^{hi}$ | $134^{ef}$ | $117.17^{a}$ | $37.83^{de}$ | $8.17^{b-e}$ | $29.5^{abc}$ | $188.67^{bcd}$ | $86.54^{ab}$ | $23.74^{cde}$ | $31.71^{a}$ |
| IR146151-B-B-584-82-40 | G-31 | $99^{j}$ | $134^{ef}$ | $117^{a0}$ | $40.5^{abc}$ | $6.33^{f-k}$ | $30^{ab}$ | $169.5^{c-h}$ | $83.23^{b-e}$ | $25.88^{ab}$ | $23.18^{b-g}$ |
| IR146151-B-B-1253-16-3 | G-32 | $98^{k}$ | $133^{g}$ | $88.67^{o}$ | $36.17^{ef}$ | $7^{c-j}$ | $25.5^{efgh}$ | $96.67^{m}$ | $57.76^{q}$ | $22.29^{i-m}$ | $8.75^{m}$ |
| IR146151-B-B-1266-13-9 | G-33 | $101^{g}$ | $135^{d}$ | $93.83^{mn}$ | $35.5^{fgh}$ | $10.17^{a}$ | $28.83^{abc}$ | $129.17^{i-m}$ | $79.87^{d-g}$ | $21.83^{klm}$ | $22.9^{b-g}$ |
| Maximum | | 109 | 143 | 117.17 | 42.33 | 10.17 | 30.33 | 228.67 | 87.83 | 26.68 | 31.71 |
| Minimum | | 95 | 126 | 87.5 | 28.5 | 5.17 | 21.17 | 96.67 | 53.86 | 19.62 | 8.47 |
| SD | | 0.45 | 0.43 | 1.32 | 1.14 | 0.855 | 0.78 | 18.92 | 2.12 | 0.41 | 3.50 |
| LSD Value | | 0.99 | 0.96 | 2.63 | 2.27 | 1.71 | 1.56 | 37.80 | 4.24 | 0.81 | 6.99 |
| CV (%) | | 0.61 | 0.44 | 1.6 | 4.01 | 14.03 | 3.68 | 14.65 | 3.49 | 2.16 | 21.11 |

Different letters in the same column indicated statistically significant differences at 5% level of probability following LSD test. Here, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers hill$^{-1}$, PL= Panicle length (cm), NGP= Number of grains panicle$^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (g) and YH= Yield hill$^{-1}$(g).

**Table 4. Estimation of genetic parameters for yield contributing traits.**

| Traits | Genotypic variance ($\sigma^2_g$) | Phenotypic variance ($\sigma^2_p$) | GCV(%) | PCV(%) | Heritability (%$h^2_b$) | GA | GA (%) |
|---|---|---|---|---|---|---|---|
| DFF | 21.21 | 21.57 | 4.63 | 4.67 | 98.29 | 9.41 | 9.46 |
| DM | 25.57 | 25.91 | 3.8 | 3.82 | 98.65 | 10.35 | 7.78 |
| PH | 56.53 | 59.14 | 7.44 | 7.61 | 95.6 | 15.14 | 14.99 |
| FLL | 15.28 | 17.21 | 11.27 | 11.96 | 88.77 | 7.59 | 21.88 |
| ETH | 1.37 | 2.47 | 15.69 | 21.06 | 55.56 | 1.80 | 24.09 |
| PL | 4.52 | 5.44 | 8.16 | 8.96 | 83.09 | 3.99 | 15.33 |
| NGP | 712.51 | 1249.48 | 16.88 | 22.35 | 57.02 | 41.52 | 26.25 |
| SF | 61.99 | 68.75 | 10.56 | 11.12 | 90.17 | 15.40 | 20.66 |
| TGW | 2.54 | 2.79 | 6.95 | 7.28 | 91.17 | 3.13 | 13.68 |
| YH | 27.06 | 45.46 | 25.61 | 33.2 | 59.53 | 8.27 | 40.71 |

Here, GCV = Genotypic co-efficient of variation, PCV = phenotypic Co-efficient of variation, GA = genetic advance, = GA (%) = Genetic Advance as percent of mean, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers hill$^{-1}$, PL= Panicle length (cm), NGP= Number of grains panicle$^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (g) and YH= Yield hill$^{-1}$(g).

The high phenotypic coefficient of variation and genotypic coefficient of variation were noted for yield hill$^{-1}$ (33.2% and 25.61%, respectively). High phenotypic coefficient of variation and moderate genotypic coefficient of variation were recorded for number of grains panicle$^{-1}$ (22.35 and 16.88%, respectively) and the number of effective tillers hill$^{-1}$ (21.06 and 33.69%, respectively). Moderate phenotypic and genotypic coefficient of variation were recorded for flag leaf length (11.96 and 11.27%, respectively) and spikelet fertility (11.12 and 10.56%, respectively). The low phenotypic and genotypic coefficient of variation were recorded for rest of the traits (Table 3). In general, high broad-sense heritability (>60%) was recorded for maximum studied traits. Importantly, high heritability (>60%) united with high genetic advance as percentage of mean (>20%) was documented for the traits flag leaf length (21.88%) and spikelet fertility (20.66%). Moderate heritability (31-60%%) coupled with high genetic advance as percentage of mean (>20%) was recorded for the traits number of effective tillers hill$^{-1}$ (24.09%), number of grains panicle$^{-1}$ (26.25%) and yield hill$^{-1}$ (40.71%).

## Phenotypic Correlation Co-efficient

Days to 50% flowering exhibited significant positive correlation with days to maturity (0.94**), plant height (0.28**), flag leaf length (0.36**) whereas it showed a significant negative correlation with thousand grains weight (-0.37**) (Table 4). Days to maturity showed significant positive correlation with plant height (0.29**), flag leaf length (0.32**), whereas it showed a significant negative correlation with thousand grain weight (-0.37**). Plant height exhibited significant positive correlation with flag leaf length (0.42**) and panicle length (0.51**) showed positive correlation with flag leaf length (0.31**) and negative with number of effective tillers hill$^{-1}$ (-0.33**). Number of effective tillers hill$^{-1}$ developed significant negative correlation with thousand grains weight (-0.28**) and positive correlation with yield hill$^{-1}$ (0.52**). Number of grains panicle$^{-1}$ exhibited significant positive correlation with spikelet fertility (0.46**) and yield hill$^{-1}$ (0.72**). Spikelet fertility positively correlated with thousand grains weight (0.23**) and yield hill$^{-1}$ (0.69**). Importantly yield hill$^{-1}$ showed significant positive correlation with number of effective tillers hill$^{-1}$ (0.52**), number of grains panicle$^{-1}$ (0.72**) and spikelet fertility (0.69**).

**Principal Component Analysis**

Principal component analysis (PCA) was steered on morphological traits of thirty-three rice genotypes to discover their variability and associations. The analysis revealed three principal components (PCs) that collectively explained 70.54% of the total variance (Table 6). PC1 was the most significant, explaining 26.94% of the variance, followed by PC2 (24.48%) and PC3

**Table 5. Phenotypic correlation co-efficient of yield and yield contributing traits.**

| | DFF | DM | PH | PL | ETH | FLL | NGP | SF | TGW |
|---|---|---|---|---|---|---|---|---|---|
| DM | 0.94** | | | | | | | | |
| PH | 0.28** | 0.29** | | | | | | | |
| PL | 0.03 | 0.10 | 0.51** | | | | | | |
| ETH | 0.05 | 0.02 | -0.13 | -0.33** | | | | | |
| FLL | 0.36** | 0.32** | 0.42** | 0.31** | 0.04 | | | | |
| NGP | 0.02 | -0.03 | 0.10 | 0.09 | -0.06 | -0.03 | | | |
| SF | -0.11 | -0.15 | 0.163 | 0.17 | 0.09 | -0.03 | 0.46** | | |
| TGW | -0.37** | -0.37** | 0.08 | 0.06 | -0.28** | 0.09 | 0.03 | 0.29** | |
| YH | -0.06 | -0.17 | 0.07 | -0.05 | 0.52** | 0.01 | 0.72** | 0.69** | 0.13 |

*, ** and *** indicate significant at 5%, 1% and 0.1% level of probability, respectively. Here, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers hill$^{-1}$, PL= Panicle length (cm), NGP= Number of grains panicle$^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (g) and YH= Yield hill$^{-1}$(g).

**Table 6. Principal components (PCs) for yield and yield-related from PCA with Eigenvectors (loadings) of the first three PCs.**

| Traits | $PC_1$ | $PC_2$ | $PC_3$ |
|---|---|---|---|
| DFF | 0.786 | 0.361 | 0.36 |
| DM | 0.824 | 0.319 | 0.297 |
| PH | 0.362 | 0.627 | -0.388 |
| FLL | 0.241 | 0.447 | -0.63 |
| ETH | -0.133 | 0.084 | 0.779 |
| PL | 0.484 | 0.441 | -0.178 |
| NGP | -0.38 | 0.649 | 0.106 |
| SF | -0.547 | 0.704 | 0.045 |
| TGW | -0.425 | 0.111 | -0.581 |
| YH | -0.577 | 0.701 | 0.395 |
| Eigen value | 2.69 | 2.45 | 1.91 |
| Variance (%) | 26.94 | 24.48 | 19.11 |
| Cumulative variance (%) | 26.94 | 51.42 | 70.53 |

Here, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers hill$^{-1}$, PL= Panicle length (cm), NGP= Number of grains panicle$^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (g) and YH= Yield hill$^{-1}$(g).

(19.11%). Ladings on PC1 indicated strong associations with days to 50% flowering, days to maturity, plant height flag leaf length, and panicle length. days to maturity, plant height flag leaf length, number of effective tillers plan$^{-1}$, panicle length, number of grains panicle$^{-1}$, spikelet fertility, thousand grain weight and yield hill$^{-1}$. PC3 exposed positive correlations with days to 50% flowering, days to maturity, number of effective tillers plan$^{-1}$, number of grains panicle$^{-1}$, spikelet fertility and yield hill$^{-1}$. The eigen values confirmed the importance of PC1 in capturing the most variation, followed by PC2 and PC3.

### Cluster Analysis

The thirty-three rice genotypes were grouped into four distinct clusters under this study based on Euclidean distance following Ward's method (Table 7 & Fig. 1). On the basis of D$^2$-values, the genotypes were grouped into four clusters. The distribution pattern revealed that cluster II was the largest cluster containing 17 genotypes, while cluster I contained only one genotype. But both cluster III and cluster IV contained seven genotypes.

**Table 7. Cluster pattern of thirty-three rice genotypes by Euclidean distance method.**

| Cluster no. | Total no. of genotypes | Genotype (Code name) |
|---|---|---|
| I | 2 | G-30, G-31 |
| II | 17 | G-1, G-2, G-3, G-4, G-6, G-7, G-9, G-10, G-12, G-13, G-14, G-25, G-26, G-27, G-28, G-33 |
| III | 7 | G-17, G-18, G-19, G-20, G-21, G-23, G-24 |
| IV | 7 | G-5, G-11, G-25, G-16, G-22, G-29, G-32 |

### Intra- and -Inter Cluster Distances

According to Fig. 4, cluster II had the largest intra-cluster distance, measuring 3.58. Cluster IV, had the lowest intra-cluster distances (2.65), whereas cluster I and cluster III had intra-cluster distances of 3.43 and 3.06, respectively (Fig. 2). We found that the inter-cluster distance ranged from 4.55 to 5.45. In contrast to the minimum clusters I and II (4.55), followed by clusters II and III (4.70), clusters I and IV (4.86), clusters I and III (4.90), clusters III and IV (5.07) and clusters II and IV (5.45).

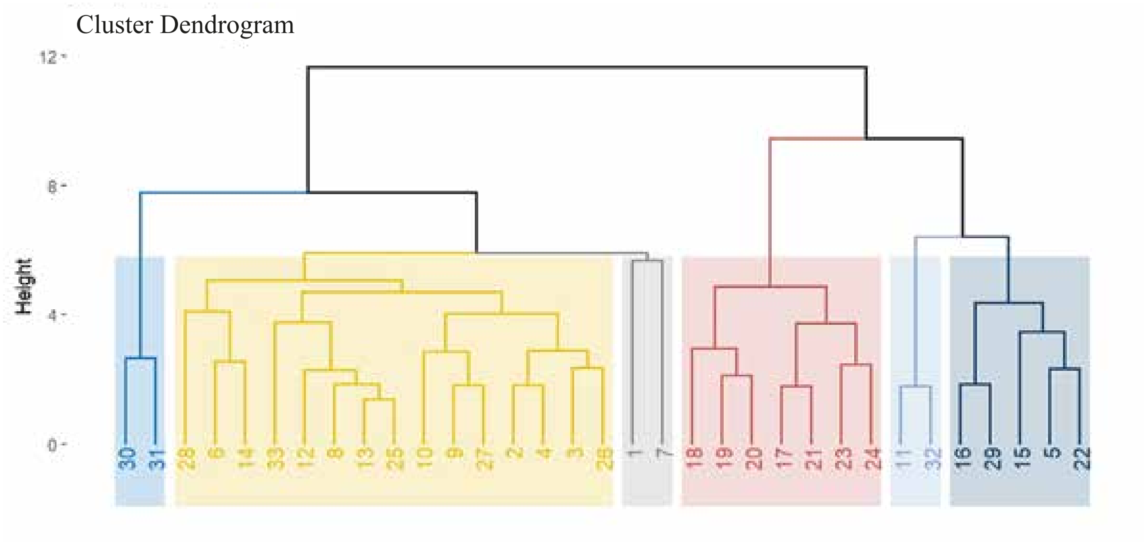

Fig. 1. Hierarchical Wards method dendrogram of thirty three rice genotypes displaying various cluster groups. Legend:1=IR146173-B-B-268-22-45(G-1), 2=IR146173-B-B-268-68-44(G-2), 3=IR146173-B-B-268-94-4(G-3), 4=IR146173-B-B-529-134-4(G-4), 5=IR146173-B-B-529-178-4(G-5), 6=IR146173-B-B-527-90-44(G-6), 7=IR146154-B-B-430-188-46(G-7), 8=IR146154-B-B-430-209-8(G-8), 9=IR146154-B-B-141-10-1(G-9), 10=IR146154-B-B-141-174-42(G-10), 11=IR146154-B-B-547-168-40(G-11), 12=IR146154-B-B-793-7-30(G-12), 13=IR146172-B-B-21-86-5(G-13), 14=IR146172-B-B-136-81-17(G-14), 15=IR146172-B-B-236-87-40(G-15), 16=IR146172-B-B-596-8-10(G-16), 17=IR146164-B-B-54-9-54(G-17), 18=IR146164-B-B-54-52-41(G-18), 19=IR146164-B-B-191-15-40(G-19), 20=IR146164-B-B-191-47-3(G-20), 21=IR146164-B-B-191-86-4(G-21), 22=IR146164-B-B-44-122-4(G-22), 23=IR146164-B-B-543-5-7(G-23), 24=IR146164-B-B-543-165-47(G-24), 25=IR146168-B-B-317-122-6(G-25), 26=IR146168-B-B-15-2-2(G-26), 27=IR146168-B-B-89-41-4(G-27), 28=IR146168-B-B-214-81-38(G-28), 29=IR146151-B-B-439-83-7(G-29), 30=IR146151-B-B-584-44-3(G-30), 31=IR146151-B-B-584-82-40(G-31), 32=IR146151-B-B-1253-16-3(G-32), 33=IR146151-B-B-1266-13-9(G-33).

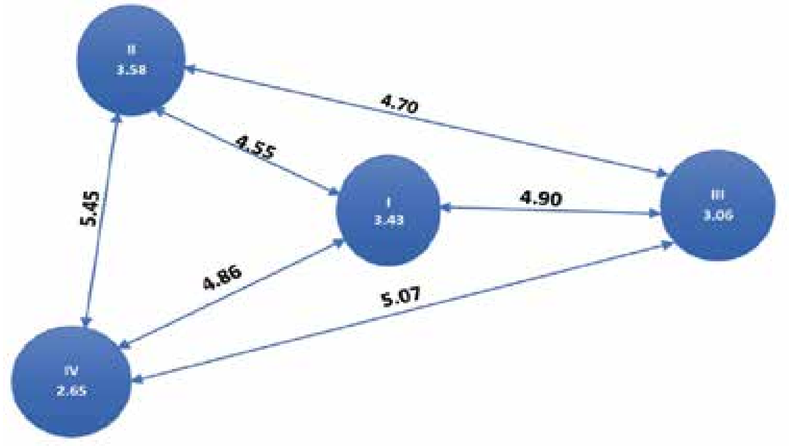

Fig. 2. Cluster diagram showing average intra and inter cluster distance (D=√D2 Values) of the rice genotypes. The values among the lines indicate inter cluster distance and values in the circle indicate intra cluster distance.

## DISCUSSION

This study revealed significant genetic variability among the 33 rice genotypes, indicating substantial potential for genetic improvement. Similar results were also observed by many other researchers for plant height (Sumanth *et al.*, 2017; Haque *et al.*, 2014; Sarkar, 2014); for days to 50% flowering (Saha *et al.*, 2022; Shahriar, 2014); for days to maturity (Patnaik and mohanty, 2006); for number of effective tillers hill$^{-1}$ (Saha, 2018, Hoque, 2013); for grain yield hill$^{-1}$ (Ema *et al.*, 2021; Ranawake *et al.*, 2013). The variations observed in days to 50% flowering and days to maturity suggest differences in flowering and maturity patterns, which are crucial for selecting genotypes suitable for specific agro-climatic conditions. IR146173-B-B-527-90-44(G-6), IR146154-B-B-141-10-1(G-9), IR146154-B-B-141-174-42(G-10), IR146154-B-B-547-168-40(G-11), IR146154-B-B-793-7-30(G-12), IR146172-B-B-21-86-5(G-13), IR146172-B-B-136-81-17(G-14), IR146172-B-B-236-87-40(G-15), IR146168-B-B-317-122-6(G-25), IR146168-B-B-89-41-4(G-27), IR146168-B-B-214-81-38(G-28) exhibited the earliest flowering, likely due to genetic factors favoring early reproductive transitions, while IR146164-B-B-54-9-54(G-17) had the longest duration to first flowering, possibly indicating a late-maturing trait beneficial for prolonged seed filling and yield stability. This variation in flowering time could have significant implications for crop management and the synchronization of planting schedules. When considering days to maturity, the study identified genotype IR146168-B-B-214-81-38(G-28) demonstrating early maturity, while IR146164-B-B-54-9-54 G-17) requiring the longest time for maturity to be harvested (Table 3). Plant architecture, as indicated by plant height and number of tillers plant$^{-1}$, also showed significant diversity among genotypes. Taller plants, such as IR146151-B-B-584-44-3(G-30), IR146151-B-B-584-82-40(G-31), IR146172-B-B-236-87-40(G-15) may offer advantages in terms of light interception and overall productivity, while genotypes with more tillers like, IR146154-B-B-793-7-30(G-12), IR146151-B-B-1266-13-9(G-33), IR146154-B-B-430-209-8(G-8), IR146164-B-B-191-47-3(G-20) could contribute to increased panicle production and yield potential. The flag leaf, the topmost leaf on a rice plant, is decisive for grain yield. It plays a leading role in photosynthesis, providing the majority of carbohydrates for grain filling. Its size, health, and photosynthetic efficiency are closely linked to yield potential. Long flag leaf bearing genotypes like, IR146164-B-B-543-165-47(G-24), IR146164-B-B-191-47-3(G-20), IR146164-B-B-54-52-41(G-18) may offer advantages in terms of chlorophyll interception resulting more carbohydrate production and ultimately higher yield production. Panicle characteristics such as panicle length and number of grains panicle$^{-1}$ are crucial factors influencing yield. Genotypes like IR146164-B-B-44-122-4(G-22) and IR146151-B-B-584-82-40(G-31) exhibited longer panicle, whereas IR146172-B-B-136-81-17(G-14) had shorter panicle. On the other hand, genotypes IR146154-B-B-430-188-46(G-7) and IR146173-B-B-268-22-45(G-1) exhibited maximum number of grains panicle$^{-1}$ whereas IR146151-B-B-1253-16-3(G-32) and IR146154-B-B-547-168-40(G-11) had minimum number of grains panicle$^{-1}$ (Table 3). The study also evaluated thousand grains weight and yield hill$^{-1}$, providing insights into the genetic potential for grain production and overall crop productivity. Genotypes like IR146151-B-B-584-44-3 (G-30) demonstrated superior grains weight and highest yield hill$^{-1}$, highlighting their suitability for commercial cultivation with potential contributions to food security and economic prosperity.

Genetic parameter analysis indicated high genotypic and phenotypic variances for days to 50% flowering, days to maturity, plant height, flag leaf length, number of grains panicle$^{-1}$, spikelet fertility and yield hill$^{-1}$, highlighting their importance in breeding programs. High broad-sense heritability coupled with high genetic advance was recorded for flag leaf length, spikelet fertility and moderate heritability coupled with high genetic advance was recorded for the number of effective tillers hill$^{-1}$, number of grains panicle$^{-1}$ and yield hill$^{-1}$ (Table 3), suggesting that these traits are primarily controlled by additive gene action, making them suitable for direct selection. Similar trend of high heritability with high GA for different traits in rice was also reported earlier (Subbaiah *et al.*, 2011). The higher phenotypic coefficient of variation compared to genotypic coefficient of variation across all traits emphasizes the role of environmental issues in inducing trait expression. This aligns with previous studies highlighting the pivotal role of environmental conditions in modulating plant phenotypes (Dey *et al.*, 2021; Umamaheswar, 2024). However, the small phenotypic coefficient of variation and genotypic coefficient of variation differences for days to 50% flowering, days to maturity, spikelet fertility and thousand grain weight proposed strong genetic control, making them consistent targets for selection (Table 4). Additionally, the assessment of genetic advance provides valuable insights into the potential for improvement through selection in breeding programs. From this study, traits with higher genetic advance, such as flag leaf length, the number of effective tillers hill$^{-1}$, number of grains panicle$^{-1}$, spikelet fertility and yield hill$^{-1}$ indicate greater scope for improvement through breeding programs. Conversely, traits with lower genetic advance, like days to 50% flowering and days to maturity may present challenges for improvement through selection due to limited genetic variability. Lastly, traits like yield hill$^{-1}$ and number of grains panicle$^{-1}$ showed the highest genetic advance as a percentage of the mean, suggesting substantial genetic variability and potential for improvement through selection. Traits having high heritability ($h^2b$) accompanied by high GA might be more advantageous in predicting gain than heritability alone. So the findings of this study might be useful in order to find out the expected traits for crop improvement programmes.

The correlation analysis among various phenotypic traits of rice genotypes revealed intricate relationships that offer valuable insights into the plant's growth and development (Shrestha *et al.*, 2021). Yield hill$^{-1}$ exhibited a strong positive correlation with number of effective tillers hill$^{-1}$, number of grains panicle$^{-1}$ and spikelet fertility emphasizing the importance of these traits in yield enhancement (Table 4). Similar kind of association was also revealed in the works of other researchers (Islam, 2022; Sameera *et al.*, 2016) Days to 50% flowering showed a significant positive correlation with days to maturity, plant height, flag leaf length but had a negative association with thousand grain weight suggesting that early flowering genotypes tend to produce unfilled and partial filled grains (Table 5). This result was in parallel with (Sadimantara *et al.*, 2021). Days to maturity exhibited a negative correlation with thousand grains weight indicating long duration genotypes may produce more tendinous grains than short duration genotypes. Plant height showed positive correlation with panicle length and flag leaf length suggesting long plant may produce long flag leaf that is important for glucose production ultimately yield production. Additionally, the panicle length positively correlated with flag leaf length and developed a negative correlation with number of effective tillers hill$^{-1}$ that indicating flag leaf length may contribute in panicle development and less tiller hill$^{-1}$ may produce long panicle.

Principal component analysis (PCA) is a powerful statistical technique used to uncover patterns in data by reducing the dimensionality of the dataset while retaining most of the original variability. The results revealed several insights into the relationships between these traits and the contribution of each variable to the principal components (PCs). The Eigen values associated with each principal component indicate the amount of variance explained by that component. Principal component analysis (PCA) identified three principal components explaining 70.53% of total variation, with PC1 contributing the most 26.94%, followed by PC2 with 24.48%. Traits like days to 50% flowering, days to maturity, plant height, flag leaf length, panicle length, had high loadings on PC1, reinforcing their role in yield determination (Table 6). These results indicate that PC1 and PC2 are particularly important for capturing the major patterns of variation in the data, while PC3 also contributes significantly to explaining additional aspects of variability. Therefore, the information provided by these principal components can effectively summarize the multidimensional nature of the dataset, facilitating data interpretation and visualization in subsequent analyses (Lin *et al.*, 2025; Shi *et al.*, 2021). This result is similar with SaiVenkat, *et al.*, (2024).

Any effective plant breeding effort must start with appropriate parent selection. Higher genetic gains via selection are predicted for parents with greater genetic variety. Genetic divergence analysis was used in this work to ascertain the genotypes' genetic relationships and choose the best genotypes for a future breeding effort. Four clusters were formed from the genotypes based on the $D^2$-value (Table 7 & Fig. 2). Cluster II had the greatest number of genotypes (17), according to the distribution pattern, while cluster I had the fewest number of genotypes (2), indicating that there is greater diversity among the genotypes in cluster IV than in clusters I, which are more closely related. These results were parallel with the results of (Thang, 2022; Singh *et al.*, 2021; Sathyaraj *et al.*, 2024). The analysis of intra-and inter-cluster distances, as shown in Figure 4, further supported these findings. The maximum inter cluster distance was observed between clusters II and IV (5.45), followed by clusters III and I (5.07), suggesting that genotypes from these clusters are highly divergent. This indicates that hybridization between genotypes from these clusters may result in heterotic progeny with broad genetic variability in the subsequent generations. While cluster II contained the highest number of genotypes, its intra-cluster distance was also the largest, signifying considerable genetic variation within this group. This suggests that genotypes within Cluster II hold strong potential for trait enhancement and breeding improvement in rice cultivars. The intra-cluster distances ranged from 2.65 to 3.58, demonstrating that genotypes within the same cluster share close genetic relationships. This finding is related with previous studies (Pandey, 2019). This insight is particularly valuable for plant breeders in selecting parental lines for crossing programs, ensuring the combination of diverse genetic backgrounds to enhance yield potential and overall genetic improvement. Hybridization between distantly related genotypes is expected to introduce novel variations, contributing to the development of superior high-yielding varieties with enhanced adaptability and resilience.

## CONCLUSION

This study highlighted substantial genetic variation among rice genotypes. Traits such as flag leaf length, number of effective tillers hill$^{-1}$, number of grains panicle$^{-1}$, spikelet fertility and yield hill$^{-1}$ exhibited moderate to high heritability and high genetic advance, making them promising for selection in breeding programs. Yield hill$^{-1}$ showed significant positive correlations with number of effective tillers hill$^{-1}$, number of grains panicle$^{-1}$ and spikelet fertility. Principal component analysis accounted for 70.53% of the total variation, and cluster analysis grouped the 33 genotypes into four clusters, with Cluster II showing the highest diversity. The high-yielding genotypes IR146151-B-B-584-44-3(G-30), IR146154-B-B-430-188-46(G-7) and IR146173-B-B-268-94-4 (G-3) were identified as potential candidates for further breeding. To confirm the stability of high-yielding genotypes, multi-location trials are recommended for varietal development.

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# Mitigation of Greenhouse Gas Emissions Through Different Rice Cultivars During T. Aman and Boro Seasons

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## ABSTRACT

To increase crop yield and improve cropping intensity, several key cropping systems have been practiced in Bangladesh. Nevertheless, greenhouse gas (GHG) emissions from various crops and agricultural fields have primarily been published on a country-by-country basis. In this study, Cool Farm Tool Version 2.0 beta-3 software was used to estimate total GHG emitted from selected rice crops and rice-based cropping patterns. Short-duration rice cultivars exhibited lower methane ($CH_4$) emissions and higher grain yields than long-duration rice cultivars during both the Boro and Aman rice seasons. Among rice cultivars of the T. Aman season, BRRI dhan62, BRRI dhan66, BRRI dhan71, and BRRI dhan75 were relatively more suitable to reduce about 15-25% of $CH_4$ emission, GHG intensity, emission factor of $CH_4$, and subsequent GWP (global warming potential) compared to BRRI dhan49. Short-duration Boro rice cultivars like BRRI dhan28, 58, 68,74 reduced about 8-15% of $CH_4$ emissions compared to long-duration rice cultivars (BRRI dhan29). Spatial variations in rice equivalent yield with a higher amount of net carbon stock were found in Rice-Potato-Rice and Rice-Mustard-Rice cropping patterns in the studied area, Kotiadi and Pakundia, Kishoreganj, Bangladesh. Rice-Mustard-Rice cropping pattern gave a higher net carbon balance and reduced half of the P, K, S, and Zn fertilizers in subsequent Boro season rice cultivation. Additional multi-location/years research trials with recommended cropping pattern and management via GHG measurement are necessary to validate and propose appropriate mitigation measures to reduce GHG emissions and reduce chemical fertilizer use in Bangladesh.

**Keywords:** Cropping pattern, GHG intensity, Emission factor, Methane, Global warming potential

## INTRODUCTION

Bangladesh has deliberately divided into 30 agro-ecological zones (AEZs) (nearly identical ecological and soil characteristics) and 88 sub-zones for its agricultural crop production. These zones have been characterized based on physiography, soil types, nature of seasonal flooding, and agro-climatology. Although different crops are grown across the country, rice plays a dominant role in food security, and rice/rice-based cropping patterns cover almost 75% of the net cropped area (NCA) (Nasim *et al.*, 2017). Rice covers more than 80% of the land area with a steady annual production growth rate of 2.8% during 1981-2007. Recently, rice growth rate has been reported to decline due to poor crop and soil management practices by the farmers and changing climatic conditions, especially changes in rainfall patterns and increasing temperature.

Increased air temperature and associated crop yield losses have been reported in Bangladesh (Maniruzzaman *et al.*, 2018; Amin *et al.*, 2015). According to some estimates, production of wheat may drop 32% by 2050 (IPCC, 2014). If

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temperature rises by 4OC, the potential shortfall in wheat and potato production could be as high as 50% and 70%, respectively (Karim *et al.*, 1996). Temperature increases would also shorten the winter season in Bangladesh. A short winter would adversely affect the vegetative as well as reproductive growth of most of the winter crops and consequently reduce yield (Amin *et al.*, 2015). High temperature, imbalanced fertilizer application, and poor agronomic management are the most important factors for reducing yields of HYVs Aus, Aman, and Boro rice (Haque *et al.*, 2019; Maniruzzaman *et al.*, 2018; Amin *et al.*, 2015), as well as yields of non-rice crops like mustard, potato, wheat, maize, etc.

Many other agricultural management practices are involved for producing better yields, rather than imbalanced fertilization and poor agronomic management only. Researchers have discovered that the GHG emissions from rice paddy fields can be affected by the amount of nitrogenous fertilizer used (Liu *et al.*, 2009; Banger *et al.*, 2012; Pantawat Sampanpanish., 2012; Chena *et al.*, 2024; Walthall *et al.*, 2025). So, balance or rice crop manager (RCM) based fertilizations are important techniques to minimize greenhouse gas emission, better yield, and sustain soil net carbon (C) balance.

Different cropping patterns, fertilizer management, and other cultural management are the main concerns for increasing or decreasing GHG emissions and soil C balance in rice-based cropping of Bangladesh. Especially methane ($CH_4$) emission occurs under anaerobic conditions, while carbon dioxide ($CO_2$) and nitrous oxide ($N_2O$) are emitted under aerobic conditions (Haque *et al.*, 2023a, 2023b). Rice-Fallow-Rice cropping system with only chemical fertilizer application showed a negative carbon balance and also gave a lower yield. Therefore, it is essential to build up knowledge or awareness among the farmers, enabling them to select the most suitable cropping pattern and rice cultivars, which may reduce production cost and GHG emission while increasing productivity and maintaining net soil C balance. We hypothesized that adopting rice-based climate-smart agricultural practices in selected villages of Bangladesh may influence GHG emissions, soil net ecosystem C balance, and rice equivalent yield, which has been evaluated in the present study under different rice cultivars and the most suitable cropping patterns.

## MATERIALS AND METHODS

### Selection of climate-smart upazila and soil characteristics of the area

Pakundia and Kotiadi upazila of Kishoreganj district (24°14-19'45-46.77"N, 90°40-47'37-46.84"E) were selected to conduct field experiments with major cropping patterns following different agricultural managements and estimate GHG emissions and net soil C sequestration during 2015-2018. This region has a complex relief of broad and narrow ridges, inter-ridge depressions, partially filled cut-off channel, and basins. Notable variations exist among soil types, topography, hydrology, and crops grown. Crops suffer due to terminal drought and flash flood both in T. Aman and Boro seasons, especially in low-lying areas. In general, farm holding is small and marginal (0.5-0.7 ha). In selected area, the top soil (0-15cm) texture is silty clay loam with organic matter content: 22.5g $kg^{-1}$, pH-H2O: 7.2, total N: 0. 90 g $kg^{-1}$, and available P: 86.0 mg $kg^{-1}$.

### Crop establishment, Fertilizer application, and Used crop cultivars

In 2015–2018, we carried out ten experiments at each location and year. Only rice crop manager (RCM) based fertilizers for N-P-K-S-Zn were applied at the rates of 140-20-35-6-3 kg $ha^{-1}$, respectively, during Boro season, and 80-25-35 kg $ha^{-1}$, respectively, during T. Aman season. Nitrogen was applied as urea in three equal splits: (1) at the final land preparation before rice transplanting, (2) during the active tillering stage, and (3) one week before the panicle initiation stage. The total P and K were applied as basal fertilizers before rice transplanting by using triple super phosphate and muriate of potash, respectively. In the T. Aman season, 20-25-day-old rice seedlings were transplanted at optimum planting time following proper spacing, water management, and pest control. For this season, the selected short-duration rice varieties were BRRI dhan62, BRRI dhan66, BRRI dhan70, BRRI dhan71, BRRI dhan72, BRRI dhan73, and BRRI dhan75, and the long-duration rice cultivar was BRRI dhan49. Thirty-five to forty-day-old seedlings of BRRI dhan58, BRRI dhan28, BRRI dhan68, and BRRI dhan74 were transplanted in the Boro season. Mustard (BARI Sharisha14)/Potato (Diamant) have been introduced in T. Aman-Fallow- Boro cropping pattern. T. Aman crops were established in July (2015-2017) and harvested in the last week of October (2015-2018). Mustard and potato crops were established in the first week of November (2015-2017). Mustard was harvested in the last week of February (2016-2018) and potato in the first week of February (2016- 2018). Boro rice was established after ~ 5 days of mustard and potato harvest.

**Rice yield**

Grain and straw yields were determined at physiological maturity from 5 m2 areas within each plot following the standard method (Haque *et al.*, 2019). Grain yield was recorded after reducing the moisture content to ca. 14% (wt $wt^{-1}$), and straw weights were expressed on an oven-dry basis (65°C).

**Collection of soil samples and analysis**

Soil samples were collected from the surface layer (0-15 cm) before the start of the experiment and at the end of three years of experimentation. Collected soil samples were then air-dried, ground and sieved by 2mm sieve, as well as analysed for organic C content (Walkley and Black, 1934; Allison, 1965), pH-$H_2O$ (1:2.5), total N (Yoshida *et al.*, 1976), available P (Olsen *et al.* 1982) and exchangeable K by ammonium acetate extraction method (Page *et al.*, 1982). Soil bulk density was determined by the core sampler method (Haque *et al.*, 2015; Black and Hartge,1986).

**Estimation of Greenhouse gas emissions**

Cool Farm Tool Beta-3 (CFT) was used to determine total GHG emission under different cropping systems and expressed as GWP (Haque *et al.*, 2017). In study sites, the major cropping patterns were T. Aman-Mustard-Boro, T. Aman-Potato-Boro, T. Aman-Fallow-Boro, and Jute-T. Aman-Fallow. Input variables and outputs of CFT were as follows:

| Emission factor | Input variables | CFC output |
|---|---|---|
| Fertilizer-induced $N_2O$ | Fertilizer types/application rate $ha^{-1}$/ management practices $ha^{-1}$ | kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Fertilizer production | Fertilizer type/ application rate, production technology | kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Pesticide production | Number of applications | kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Diesel use | Litres used | kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Electricity use | Kwh | Kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Crop residue management | kg/management practice | kg $CO_2$e/ha, kg $CO_2$e/kg product |
| Water management | Litres/management practice | kg $CO_2$e/ha, Kg $CO_2$e/kg product |

**Net soil carbon sequestration**

Total soil organic carbon sequestration (TSOC) was determined as follows:

TSOC sequestration = OC*soil depth*bulk density……….. (i)

**Statistical analysis**

Statistical analyses were performed using the SAS package, version 9.1 (SAS Institute, 2003). A two-way ANOVA was carried out to compare the crops and cropping pattern means. Tukey's test was used for mean comparison, and differences were considered significant at $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Variation of estimated GHG emission across rice cultivars

In the T Aman season, short-duration rice cultivars (100-135 days) BRRI dhan62, BRRI dhan66, BRRI dhan71, BRRI dhan72, and BRRI dhan75 significantly lowered (ranged from 98 to 124 kg $CH_4$ $ha^{-1}$) seasonal $CH_4$ emission compared to long-duration rice cultivar BRRI dhan49 (140-145 days) with 130 kg $CH_4$ $ha^{-1}$ (Table 1). Across short-duration rice cultivars, BRRI dhan62 showed the significantly lowest total $CH_4$ flux (Table 1). Total $CH_4$ flux ranged from 98-124 kg $ha^{-1}$ among short-duration rice cultivars. BRRI dhan62 and BRRI dhan75 reduced about 15-25% total $CH_4$ flux than BRRI dhan49. During Boro season, the long-duration rice cultivar BRRI dhan29 emitted about 156 kg $ha^{-1}$ total $CH_4$ flux. About 133-143 kg $ha^{-1}$ total $CH_4$ was emitted across short-duration rice cultivars. Short-duration rice varieties reduced about 8-15% seasonal $CH_4$ emission compared to long-duration rice cultivars. Among short-duration cultivars, BRRI dhan74 produced the lowest total $CH_4$ flux (133 kg $ha^{-1}$ $season^{-1}$). Long-duration rice cultivars increased about 9-18% $CH_4$ emission due to their longer life cycle and field duration, which were the main sources of producing more $CH_4$ and increased net emission. Previous findings mentioned that different life cycles of rice cultivars are the main factor in increasing or decreasing GHG emissions (Haque *et al.*, 2017; Ding *et al.*, 2022; Zhang *et al.*, 2024). In both seasons, total $N_2O$ emission was significantly lower in all short-duration rice varieties than that in long-duration rice cultivars BRRI dhan49 and BRRI dhan29, while this was the case for Boro season $CO_2$ emission only (Table 1).

**Table 1. Long and short-duration rice cultivars with respective estimated greenhouse gas emissions during T. Aman and Boro season.**

| Variety | T. Aman season |  |  | Variety | Boro season |  |  |
|---|---|---|---|---|---|---|---|
|  | GHG emission (kg $ha^{-1}$) |  |  |  | GHG emission (kg $ha^{-1}$) |  |  |
|  | $CH_4$ | $N_2O$ | $CO_2$ |  | $CH_4$ | $N_2O$ | $CO_2$ |
| BRRI dhan49 | 130.0a | 0.8a | 300.9a | BRRI dhan28 | 143.0b | 1.3a | 493.8b |
| BRRI dhan62 | 97.5c | 0.7a | 285.1a | BRRI dhan29 | 156.0a | 1.4a | 521.7a |
| BRRI dhan66 | 114.4b | 0.7a | 285.1a | BRRI dhan58 | 141.7bc | 1.30a | 490.1b |
| BRRI dhan70 | 123.5ab | 0.7a | 285.1a | BRRI dhan68 | 135.2bc | 1.30a | 490.1b |
| BRRI dhan71 | 117.0ab | 0.7a | 285.1a | BRRI dhan74 | 132.6c | 1.30a | 490.1b |
| BRRI dhan72 | 123.5ab | 0.7a | 285.1a |  |  |  |  |
| BRRI dhan75 | 110.5bc | 0.7a | 300.9a |  |  |  |  |

Here, GCV = Genotypic co-efficient of variation, PCV = phenotypic Co-efficient of variation, GA = genetic advance, = GA (%) = Genetic Advance as percent of mean, DFF = Days to 50% flowering, DM = Days to maturity, PH = Plant height (cm), FLL = Flag leaf length (cm), ETH = Number of effective tillers $hill^{-1}$, PL= Panicle length (cm), NGP= Number of grains $panicle^{-1}$, SF= Spikelet fertility, TGW= Thousand grains weight (g) and YH= Yield $hill^{-1}$(g).

### Grain yield of different rice cultivars in Boro and T. Aman seasons

In the T. Aman season, BRRI dhan49 gave a lower yield than other varieties tested (BRRI dhan70, BRRI dhan71, BRRI dhan72, and BRRI dhan75). Among introduced varieties, BRRI dhan71 and BRRI dhan75 gave higher yield than the old variety BRRI dhan49 (Fig. 1). In the Boro season, BRRI dhan58 and BRRI dhan74 gave higher yield compared to BRRI dhan28.

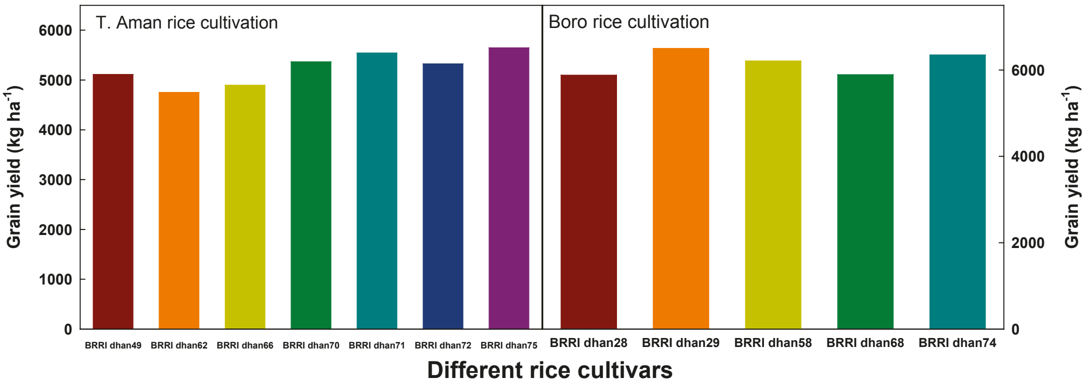

Fig.1. Grain yields of different rice cultivars during T. Aman and Boro rice season

### GWP, GHG intensity, and emission factor of different rice cultivars in T. Aman and Boro seasons

Overall, GWP and emission factor of $CH_4$ in T. Aman season strikingly lowered compared to Boro season rice (Table 2). In the T. Aman season, GWP in short duration rice cultivars was significantly reduced than long long-duration rice variety BRRI dhan49. In this season, also BRRI dhan62 and BRRI dhan75 reduced GWP by 5-23% to that of BRRI dhan70, BRRI dhan71, and BRRI dhan72. Across short-duration rice cultivars, the GHG intensity in BRRI dhan75 was significantly lower than that in BRRI dhan66, BRRI dhan70, and BRRI dhan72. In Boro season, GWP in all short-duration rice cultivars was significantly lower than that in long-duration rice cultivar, BRRI dhan29 (Table 2). Among short-duration cultivars, BRRI dhan74 would be a good fit for the environment because the GHG intensity and emission factor of $CH_4$ in this variety were significantly lower than those in another popular short-duration variety BRRI dhan28. BRRI dhan74 produced more root and above-ground biomass than other varieties, which might have a positive effect on reducing overall GWP. Previous researchers found that some rice cultivars provide more rice root volume, length, radial oxygen loss, exudates, and biomass that might influence reduced $CH_4$ emission (Qi *et al.*, 2024; Qian *et al.*, 2023; Ding *et al.*, 2022; Girkin *et al.*, 2018; Girkin *et al.,* 2020; Girkin & Cooper., 2022).

**Table 2. Global warming potential, GHG intensity, and emission factor of CH4 in long and short-duration rice cultivars during T. Aman and Boro season.**

| Variety | T. Aman season | | | Variety | Boro season | | |
|---|---|---|---|---|---|---|---|
| | GHG emission (kg ha$^{-1}$ ) | | | | GHG emission (kg ha$^{-1}$ ) | | |
| | GWP | GHG intensity | Emission factor of $CH_4$ | | GWP | GHG intensity | Emission factor of $CH_4$ |
| BRRI dhan49 | 4153 | 0.81 | 1.18 | BRRI dhan28 | 4842 | 0.82 | 1.30 |
| BRRI dhan62 | 3201 | 0.67 | 0.98 | BRRI dhan29 | 5261 | 0.81 | |
| BRRI dhan66 | 3674 | 0.75 | 1.14 | BRRI dhan58 | 4802 | 0.77 | 1.28 |
| BRRI dhan70 | 3929 | 0.73 | 1.17 | BRRI dhan68 | 4620 | 0.78 | 1.22 |
| BRRI dhan71 | 3747 | 0.68 | 1.17 | BRRI dhan74 | 4547 | 0.72 | 1.21 |
| BRRI dhan72 | 3929 | 0.74 | 1.17 | | | | |
| BRRI dhan75 | 3580 | 0.63 | 1.13 | | | | |
| $LSD_{0.05}$ | 289 | 0.09 | 0.08 | | 312 | 0.08 | 0.07 |

### Grain yield and GHG emission in different cropping patterns at Pakundia and Kotiadi Upazila

Researchers' management involving climate-smart practices, i.e., introducing Mustard or Potato during the fallow period in double rice cropping, provided almost double annual crop yields compared to the existing Rice-Fallow-Rice cropping pattern. This was also possible via the introduction of short duration improved rice varieties like BRRI dhan62, BRRI dhan66, BRRI dhan71 and BRRI dhan75 which allowed an additional crop instead of leaving the field fallow. In addition, climate-smart or best management practices were followed. The attained rice equivalent yields (REY) ranged from 15-29 t $ha^{-1}$ in Rice-Mustard-Rice and Rice-Potato-Rice cropping patterns, whereas the harvested REY in Rice-Fallow-Rice cropping pattern was only about 11-12 t $ha^{-1}$ (Table 3). Although REY increased due to the inclusion of Mustard and Potato during the fallow period in the studied farmers' fields, T. Aman-Potato-Boro- cropping pattern provided almost twice the REY than T. Aman-Mustard-Boro-Also, T. Aman- Mustard-Boro- T. Aman cropping pattern showed higher REY despite some increase in total GHG emission (707-818 kg $ha^{-1}$) compared to T. Aman-Fallow-Boro cropping system.

**Table 3. Crop yield, rice equivalent yield and GHG intensity of different cropping pattern at Pakundia and Kotiadi Upazila.**

| Location | Cropping pattern | Grain yield (kg $ha^{-1}$) | | | REY | Total GHG (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|
| | | T. Aman | Mustard/Potato | Boro | | |
| Pakundia | T. Aman-Mustard-Boro | 4.76b | 2.01b | 5.66b | 15.44b | 5438b |
| | T. Aman-Potato-Boro | 4.75b | 33.0a | 5.80b | 28.35a | 6129a |
| | T. Aman-Fallow-Boro | 5.11a | - | 6.20a | 11.31c | 4620c |
| Kotiadi | T. Aman-Mustard-Boro | 4.80b | 1.97b | 5.60b | 15.33b | 5224b |
| | T. Aman-Potato-Boro | 4.90b | 34.5a | 5.45b | 28.96a | 5932a |
| | T. Aman-Fallow-Boro | 5.56a | - | 6.30a | 11.86c | 4517c |

### Grain yield of Boro rice influenced by reduced rate of chemical fertilizers in Rice-Mustard-Rice cropping system

Applying full doses of chemical fertilizers for mustard and then half doses of phosphorus (P), potassium (K), sulfur (S), and zinc (Zn) for the subsequent Boro rice can maintain rice grain yield primarily due to the remaining residual effect of these nutrients applied to mustard. In both locations, there was no significant difference in grain yield of Boro rice cultivated with a full dose of PKSZn and a half dose of PKSZn after growing mustard crops, except for Pakundia in 2016-2017 (Table 4). This indicated that a significant portion of these nutrients remains in the soil in a form available to the next crop. Another important nutrient sources come from the decomposition of mustard residues, which further releases nutrients back into the soil, creating a slow-release nutrient source. This approach improves the nutrient use efficiency of the entire cropping system, reducing fertilizer costs and environmental impact without sacrificing yield. This strategy is most effective for reduction of chemical fertilizers during Boro rice cultivation under Rice-Mustard-Rice cropping system.

**Table 4. Grain yield of Boro rice cultivating with reduced rates of chemical PKSZn fertilizers at Pakundia and Kotiadi Upazila, Kishoreganj.**

| Fertilizer management | 2015-2016 | | 2016-2017 | |
|---|---|---|---|---|
| | Location | | | |
| | Pakundia | Kotiadi | Pakundia | Kotiadi |
| | Grain yield | | Grain yield | |
| NPKSZn@140-20-60-6-3kg $ha^{-1}$ | 5.90 | 5.72 | 6.10 | 5.80 |
| 50% reduce rate of PKSZn with full urea-N | 5.60 | 5.63 | 5.75 | 5.65 |
| $LSD_{0.05}$ | 0.32 | 0.21 | 0.20 | 0.19 |

**Net soil C stock under different major cropping systems**

Rice-Jute-Fallow cropping pattern increased about 7-20% net soil C stock compared to Rice-Mustard-Rice and Rice-Potato-Rice cropping systems at both locations (Table 5). Rice-Fallow-Rice cropping system significantly lowered net C stock due to fallow period $CO_2$-C loss. However, when farmers add another crop, like potato or mustard, into the Rice-Fallow-Rice cycle, the soil absorbed more C due to the addition of root biomass, litter, rhizodeposition, and leaf. Therefore, Rice-Mustard-Rice, Rice-Potato-Rice, and Rice-Jute-Fallow cropping patterns supplied more soil organic C (Table 5), which can help to bind soil particles together, form aggregates, and improve water infiltration, aeration, and overall soil structure. All these make the soil more resilient to erosion and compaction, hence facilitate proper root growth as well as increased net soil C budget. Similar results were also reported by Haque *et al.* (2015, 2019, 2020) and Kumar *et al.* (2021).

**Table 5. Soil net C stock influenced by different cropping patterns at Pakundia and Kotiadi, Kishoreganj.**

| Pattern | Pakundia | | | Kotiadi | | |
|---|---|---|---|---|---|---|
| | BD | Net C stock (kg $ha^{-1}$) | Net C balance (kg $ha^{-1}$) | BD | Net C stock (kg $ha^{-1}$) | Net C balance (kg $ha^{-1}$) |
| Rice-Mustard-Rice | 1.35b | 2471a | 581a | 1.34bc | 2472b | 596b |
| Rice-Potato-Rice | 1.38ab | 2381b | 491b | 1.36b | 2407c | 531c |
| Rice-Jute-Fallow | 1.32c | 2495a | 605a | 1.32d | 2515a | 638a |
| Rice-Fallow-Rice | 1.39a | 2189c | 299c | 1.38a | 2215d | 338d |
| Initial | 1.40a | 1890d | - | 1.39a | 1877e | - |

## CONCLUSION

Irrespective of seasons, short-duration rice cultivars reduced about 8-25% CH4 emission compared to long-duration rice cultivars. Spatial variations of rice equivalent yield and net soil C balance were ensured due to the adoption of the most suitable cropping systems with appropriate inputs and management practices. Jute-Rice-Fallow, Rice-Mustard-Rice and Rice-Potato-Rice cropping systems are suitable for increasing net C stock and yield than the Rice-Fallow-Rice cropping pattern. In sum, understanding and implementing cropping patterns that promote a positive C balance are essential strategies for improving soil health, ensuring long-term agricultural productivity and contributing to environmental sustainability.

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# Critical Determinants of Rice Tungro Disease Devastation and Its Vector Population Dynamics in Bangladesh

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## ABSTRACT

Rice Tungro disease caused by Rice Tungro Viruses (Rice Tungro Bacilliform Virus and Rice Tungro Spherical Virus) transmitted by vector Green Leaf Hopper (GLH), *Nephotettix virescens*. Every year tungro disease infection is devastating in several rice growing areas in Cumilla region as well as country-wide. Rice Tungro disease is a major limiting factor of rice production. In Bangladesh, yield loss due to tungro was reported to be as high as 100% under severe conditions. Three crop rice (Rice-Rice-Rice) areas are most vulnerable to Tungro disease infection. Weather parameters such as temperature, rainfall, relative humidity and light trap data of GLH from BRRI Cumilla and GLH data from rice field of Nangalkot Upazila were investigated. The previous tungro disease data were collected from the survey of different Upazila of Cumilla district during 2017 to 2024. In the farmers field condition, GLH data were collected by hand sweeping and light trap from both seedbed to main fields. From the study, intensive rice cultivation (Rice- Rice- Rice), susceptible rice varieties, presence of abundant GLH in the seedbed (20 GLH/hand sweep) as well as viruses with disease symptom, monthly average maximum (>25°C)) and minimum temperature (<17°C) during July to September, and average % relative humidity (70 %) at evening are found the most critical determinants for tungro disease devastation in Cumilla region. The rainfall during January to March and July to September was found very weak relation with GLH population. It is also confirmed and first report that the severe tungro disease infections in the main field come from the virus-infected seedlings of the seedbed for tungro-prone areas in Bangladesh.

**Keywords:** Determinants, Tungro, disease, temperature, rainfall, humidity, green leaf hopper.

## INTRODUCTION

Rice is the main carbohydrate source and main dish for Bangladeshi people as well as different Asian countries. Food security mainly depends on rice security of 160 million people in Bangladesh (Kabir *et al.*, 2016). Change of macroclimate triggers the change of weather factors, which activate the development of numerous biotic and abiotic pressures, which are the bottle-neck for rice production. Tungro disease of rice mainly caused by concurrent infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Along together of RTBV and RTSV are existing in a plant, no distinct symptoms in earlier infection, the later causes minor stunting and slight yellowing of the leaves, and at last it results in mottled leaves, severe growth stunting, and discoloration of the leaves as orange to yellow, which remarkably decreases yield (85% above) (Kumar & Dasgupta, 2020). It is one of the extreme economically vital rice viral diseases in Bangladesh as well as Southeast Asia

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and 38-100% yield loss is carried out due to severe infection of tungro disease of different countries like India, Pakistan, Vietnam, Philippines, Nepal, China, Japan, Srilanka and Indonesia (Gour and Purohit, 2004). Most destructive disease like RTD causes world's annual loss in rice production of about US $1.5 billion and upto 10% rice yield reduction in South and Southeast Asia (Dai and Beachy, 2009).

Tungro disease symptom appears as stunting plant growth, orange yellow color in leaves, tiller number reduction, stunted root growth, hampers of panicle exertion, upper three leaves position in the same node and unfilled black grains. Every year tungro disease infection is devastating in many rice growing areas in Cumilla region specially 3 rice growing areas like Nangalkot, Laksam, Debidwar, Sadar Dakkhin, Lalmai, Burichang, Muradnagar in Bangladesh. Tungro disease is also predominant in different locations of Indian border side areas of Brahmanbaria. Every year hundreds hectare of rice lands infected with tungro disease and huge rice yield loss is occurred. Hundred percent rice yield loss may occur due to tungro disease under severe out-break during early stage of infection (Latif *et al.*, 2013). During T. Aman 2018 season, an eruption of tungro disease caused devastating crop losses in Cumilla region and RTD may cause the 20-100% yield loss of BRRI released high yielding rice varieties. (BRRI, 2018-2019). During T. Aman 2022, 50-100% incidence of RTD and 75-99% yield loss were reported in rice varieties of BRRI dhan71, BRRI dhan75, Binadhan-7, Binadhan-16, Binadhan-17, Binadhan-22. (BRRI, 2022-2023).

In pest management policies, a comprehensive information of the effect of abiotic factors on insect pests is crucial. Different weather parameters and climatic circumstances are recognised to significantly affect the dynamics of insect pests population (Kennedy and Storer, 2000). Information of abiotic factors such as maximum and minimum temperature, day length, daily rainfall and percent relative humidity can be considered as important components in predicting and forecasting the severity of vector population. (Milford and Dugdale, 1990). The dynamics of pest population knowledge is crucial for evolving sustainable crop guard policies and for understanding and forecasting the reply of taxonomic clusters to weather outlines fluctuating on a daily basis, seasonally or as a long-term significance of global climate variation (Denholm *et al.*, 2001). The population dynamics of GLH information in relation to different weather factors under the condition of Cumilla region is not recognised. Very few studies reported the main reasons or factors of tungro disease devastation in Bangladesh condition. Henceforth, considering the importance of tungro vector GLH population dynamics and its correlation with different weather factors, an attempt has been made to study the main critical factors of rice tungro disease prevalence and devastation year after year in Bangladesh.

## METHODOLOGY

### ODK Digital field survey

Digital field survey was conducted using Open Data Kit (ODK) mobile apps. ODK formerly called "Open Data Kit" was founded by Borriello, 2011. ODK is an open-source mobile data collection platform. It enables users to fill out forms offline and send form data to a server when a connection is found. Once on the server, the data can be viewed, downloaded, and acted upon. It supports a wide range of question and answers types and is designed to work well offline. ODK Collect downloads blank forms and submits filled forms to a server. It displays forms as input prompts that can include logic, constraints, and repeating sub-structures. Users work through the prompts and can save their data at any point (Sergio *et al.*, 2020). Disease incidence and severity data were collected by following the Standard Evaluation System (SES, IRRI, 2013), where the disease scores (Disease Score 1: No symptom observed, Score 3: 1-10% height reduction, no distinct yellow to yellow orange leaf discoloration, Score 5: 11-30% height reduction, no distinct yellow to yellow orange leaf discoloration, Score 7: 31-50% height reduction, with distinct yellow to yellow orange leaf discoloration, Score 9: More than 50% height reduction, with distinct yellow to yellow orange leaf discoloration) are included. The scientists of Plant Pathology division was developed a "Rice disease survey" form, where different data pages like plot ID, record GPS from the centre of the plot, Date of survey, season, year, name of the data collector & designation, site name, district, upazila, village, land unit, variety name (inbred, hybrid, local etc.), date of sowing & transplanting, growth stages, name of the major rice diseases with % disease incidence and disease severity of leaf, neck, node blast, tungro, bacterial leaf blight, sheath blight, kresek, seedling blight, bacterial leaf streak, bakanae, brown spot, false smut, sheath rot, leaf scald, yellow dwarf, grain spot and others, farmers name, mobile number, seed source, fungicides application or not, name of fungicides, major cropping pattern, land type, ecosystem, % yield reduction due to major problem, estimated yield at healthy and disease plot (kg/5m$^2$), were included.

### Tungro disease prevalence

Digital field survey was conducted from T. Aman 2017 to Boro 2024-25; eight years and 16 seasons. Percent disease incidence and disease severity data were recorded from different Upazila, namely Adarsha Sadar, Sadar Dakshin, Chandina, Barura, Burichang, Debidwar, laksam and Nangalkot of Cumilla district.

### Data Collection

The dataset used in this study comprises daily weather parameters and the Green Leaf Hopper (GLH) population data. The weather parameters include maximum temperature (Max Temp.), minimum temperature (Min Temp.), rainfall (mm), and % relative humidity (% RH) at 6 am and 12 pm were collected from BRRI regional station, Cumilla. The GLH population was recorded on a daily basis. The data spans a period of time that provides sufficient variability in weather conditions and GLH population fluctuations.

### Green Leaf Hopper data collection

Getting tungro-prone areas of Nangalkot, Laksam and other Upazila in Cumilla region, three villages of Nangalkot were selected for vector GLH population conditions during Aus 2019, Aman 2019 and Boro 2019-20 seasons. Number of Green Leaf Hopper were counted from the seedbed and main field of three-cropped areas of Ossodia, Jorpukuria and Mandra villages in Nangalkot. The GLH population data were presented in twenty complete sweeping with a sweep net.

### Rice varieties

Disease data of different rice varieties of BR16, BR22, BR23, BRRI dhan32, BRRI dhan29, BRRI dhan46, BRRI dhan48, BRRI dhan49, BRRI dhan52, BRRI dhan71, BRRI dhan74, BRRI dhan75, BRRI dhan87, BRRI dhan94, BRRI dhan95, BRRI dhan103, Binadhan-17, Binadhan-26, BRRI hybrid dhan4, Hybrid 7006, Hybrid Balia2, Hybrid Hera-5, Hybrid Micro1, Hybrid oryzae, Hybrid shakti, Hybrid Sonar Bangla were collected which were cultivated by farmers own choice.

### Data Analysis

For showing the spots of tungro disease survey, Google Earth Pro geographic software was used. The mobile apps of ODK survey was installed into a smart phone and downloaded the disease survey form described above. During survey, the ODK apps opening and click "start new form" then click "Rice disease survey" form then give the plot ID then click the "Geo point" and save the data form. After submitting the data from mobile, the data including GPS were colleted from online "KoboToolbox".

### Scatter Plot Visualization of Weather Parameters and GLH Population

### Data Preprocessing

Before conducting the analysis, the dataset underwent cleaning to address missing values, which were imputed using appropriate methods such as mean imputation or forward-fill where necessary. Outliers were identified and handled using standard techniques, such as the interquartile range (IQR) method. All variables were then standardized to ensure comparability across different scales, as the weather parameters are measured in different units (e.g., temperature in °C, rainfall in mm, humidity in %).

### Scatter Plot Visualization

Scatter plots were created to visually explore the relationships between each weather parameter and the GLH population. In this study, scatter plots were generated for the following pairs: Max Temp. vs GLH Population; Min Temp. vs GLH Population; Rainfall (mm) vs GLH Population; RH (%) 6 am vs GLH Population; RH (%) 12 pm vs GLH Population.

### Statistical Analysis

Although the scatter plots primarily served as a visual tool, Pearson's correlation coefficients were also calculated to quantify the strength and direction of the linear relationships observed in the scatter plots.

### Software and Tools

The scatter plots were generated using Matplotlib and Seaborn, both of which are Python libraries that provide flexible plotting capabilities. The data manipulation and preprocessing were performed using the Pandas library, while the correlation analysis was conducted with the scipy library. The visualization process allowed for an intuitive interpretation of the relationships between weather variables and GLH population dynamics.

### Correlation Between Weather Parameters and GLH Population

To explore the relationships between the weather parameters and the GLH population, Pearson's correlation coefficients were computed. Pearson's correlation is a measure of the linear relationship between two continuous variables, with values ranging from -1 to 1. A coefficient of +1 indicates a perfect positive linear relationship, while -1 indicates a perfect negative linear relationship, and 0 signifies no linear relationship.

The correlation coefficients were computed for each pair of variables, including: Temp. Max vs GLH Population; Temp. Min vs GLH Population; Rainfall (mm) vs GLH Population; RH (%) at 6 am vs GLH Population; RH (%) at 12 pm vs GLH Population. A correlogram (heatmap of the correlation matrix) was created using Seaborn and Matplotlib libraries in Python. Values closer to 1 or -1 indicate stronger correlations, while values near 0 suggest weaker relationships. All correlation analyses and visualizations were conducted using the Python programming language. Pandas was used for data manipulation, scipy for statistical tests, and Seaborn and Matplotlib for visualization.

## RESULTS

Tungro disease of rice is mainly transmitted by Green Leaf Hopper (GLH), where the vector as well as virus (RTBV & RTSV) was present then tungro disease may occur. If the vector is present but the virus is absent in a localized area, the disease cannot occur. In this study, we explored the factors which are responsible for tungro disease devastation. The critical factors are described below.

### Base-line survey of tungro disease

Digital field survey was made from Aman 2017-2024 (8 years), and Boro 2018-2025 (8 years), using OKD mobile apps in eight Upazila of Cumilla and locations were presented in Fig. 1. Disease incidence (DI) and disease severity (DS) data were collected from 290 spots in 8 Upazila shown on google earth using Google Earth Pro software (Fig. 2). Percent disease incidence of rice tungro disease was observed almost all the season ranged from 17-100% with DS 3-9 (Fig. 3). During survey, BR16, BRRI dhan52, BRRI dhan75, BRRI dhan74, BRRI dhan87, Binadhan-17, and hybrid rice were found highly infected by tungro disease (Fig. 3).

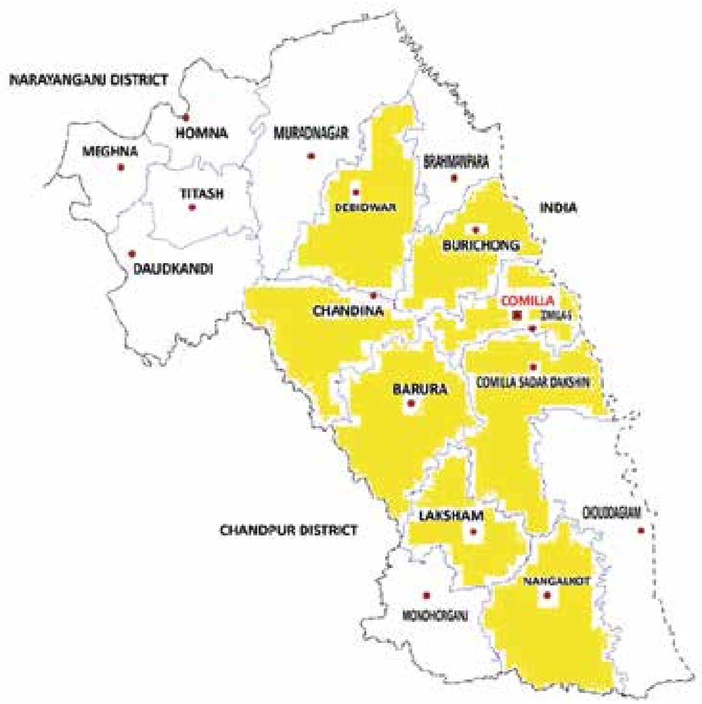


Fig. 1. Yellow color showing the areas of tungro disease prevalence during Aman 2017 to Boro 2024-25 season (8 years 16 seasons survey report).

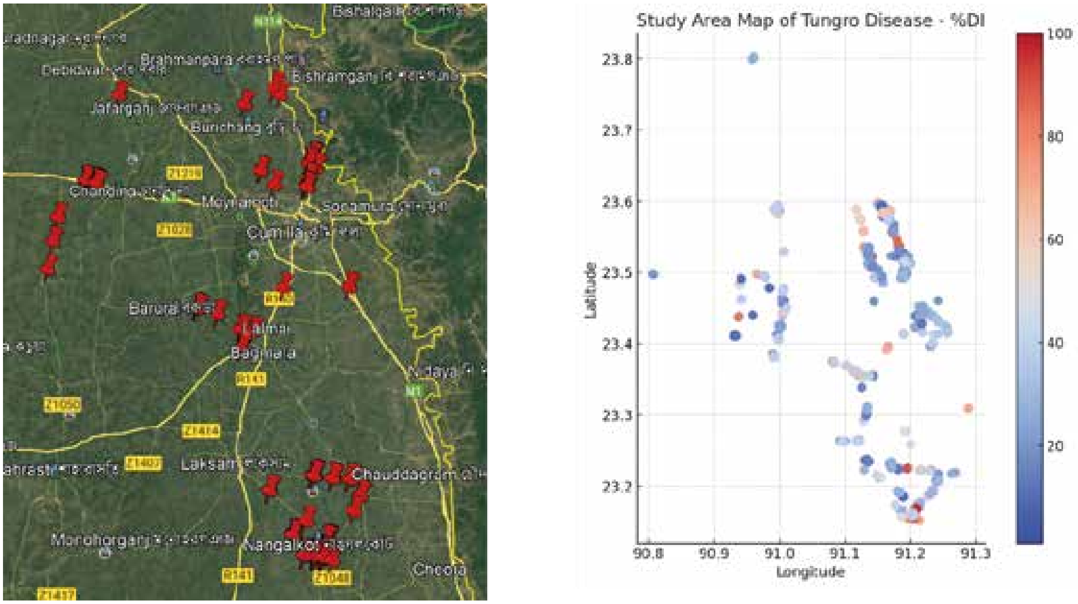


Fig. 2. Google earth pro and Machine Learning Tools showing the tungro disease survey spots (290 field spots) in different Upazila Adarsha Sadar, Sadar Dakshin, Chandina, Barura, Burichang, Debidwar, laksam and Nangalkot of Cumilla district during Aman 2017 to Boro 2024-25 seasons.

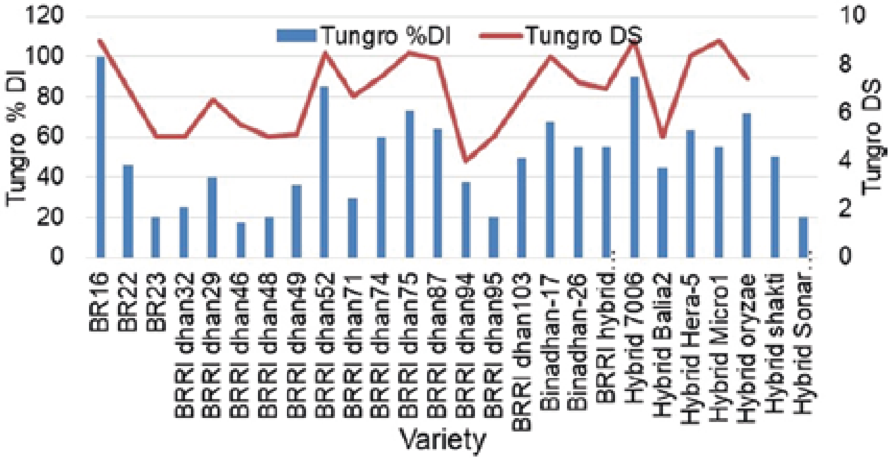


Fig. 3. Tungro disease occurrence during Aman 2017 to Boro 2024-25 season (8 years 16 seasons survey report).

i) **Intensive rice cultivation**

Most of the surveyed and tungro disease infected areas are 3 cropped areas of rice-rice-rice cropping pattern. For this why, tungro disease was observed almost every season in different areas in Cumilla during disease survey, where GLH can get the host year-round (Fig. 3).

**ii) Cultivation of susceptible rice cultivar**

Different rice varieties of BRRI, BINA and private companies Hybrids like BR16, BR22, BR23, BRRI dhan32, BRRI dhan29, BRRI dhan46, BRRI dhan48, BRRI dhan49, BRRI dhan52, BRRI dhan71, BRRI dhan74, BRRI dhan75, BRRI dhan87, BRRI dhan94, BRRI dhan95, BRRI dhan103, Binadhan-17, Binadhan-26, BRRI hybrid dhan4, Hybrid 7006, Hybrid Balia2, Hybrid Hera-5, Hybrid Micro1, Hybrid oryzae, Hybrid shakti, Hybrid Sonar Bangla were found as susceptible rice cultivar during disease survey from 2017-2024 (Fig. 3). During Aus 2018, Aman 2018 and Boro 2018-19, seasons severe tungro disease was observed in some susceptible rice varieties (Table 1) in many rice growing locations of Nangalkot and Laksam (Table 2 & 3).

**Table 1. Tungro disease infection records in different rice varieties in Nangalkot and Laksam, Cumilla during 2018-19 (Rice-Rice-Rice).**

| Aus 2018 | DS | T. Aman 2018 | DS | Boro 2018-19 | DS |
|---|---|---|---|---|---|
| BRRI dhan28 | 7 | BR22 | 7 | BRRI dhan28 | 5 |
| BRRI dhan43 | 7 | BRRI dhan75 | 9 | BRRI dhan29 | 3 |
| BRRI dhan48 | 7 | BRRI dhan49 | 5 | BRRI dhan48 | 7 |
| Hybrid Tia | 9 | Swarna masuri | 9 | BRRI dhan58 | 3 |
| Moyna | 7 | Swarna | 9 | Hybrid 1203 | 3 |
| Chokka | 9 | Pazam | 9 | Jonokraj | 7 |

DS indicate disease severity.

**Table 2. Block-wise tungro disease infection in Nangalkot, Cumilla during 2018-2019.**

| Block | Season | Cultivated area (ha) | Tungro infected area (ha) DS |
|---|---|---|---|
| Ossodia, Kadra, Nurpur, Peria, Sreefolia, kakoirtola, Raykot, Mahini, Jhatiapara, Moukora, Bisnapur, Majhipara, Pourasava, Mokrabpur, Bannogor, Bhuluapara, Hesakhal uttar, Hesakhal Dakkhin, Patowar, Rajapara, Dhalua, Sijiara, Monnara, Doulkhar, Kasipur, Boxgonj, Shuvapur, Ostogram | Aus 2018 | 7550 | 106 (DS 7-9) |
| Ossodia, Bangadda, Kadra, Nurpur, Sreefolia, Raykot, Mahini, Jhatiapara, Moukora, Bisnapur, Majhipara, Pourasava, Mokrabpur, Bannogor, Bhuluapara, Hesakhal Uttar, Hesakhal Dakkhin, Patowar, Addra, Shaktoli, Volaine, Jodda, Rajapara, Manikmura, Dhalua, Sijiara, Monnara, Doulkhar, Kasipur, Boxgonj, Shuvapur, Ostogram | T. Aman 2018 | 12750 | 529 (DS 7-9) |
| Ossodia, Shaktoli, Volaine, Jodda, Nurpur, Kadra, Rajapara, Manikmura, Dhalua, Monnara, Doulkhar, Kasipur, Boxgonj | Boro 2018-19 | 12990 | 190 (DS 5-9) |
| Ossodia, Kadra, Shaktoli, Volaine, Jodda, Rajapara, Manikmura, Dhalua, Monnara, Doulkhar, Kasipur | Boro 2017-18 | 12650 | 130 (DS 5-9) |

**Table 3. Block-wise tungro disease infection in Laksam, Cumilla, 2017-2019.**

| Block | Season | Cultivated area (ha) | Tungro infected area (ha) DS |
|---|---|---|---|
| Pechra, Norpati, Fulgao, Ajgora, Borbam, Charbaria, Pourashova, Bijra, Koitra, Noapara, Chongao | Aus 2018 | 4800 | 90 (DS 5-9) |
| Pechra, Norpati, Fulgao, Ajgora, Borbam, Charbaria, Pourashova, Bijra, Koitra, Noapara, Chongao | T. Aman 2018 | 5100 | 280 (DS 7-9) |
| Pechra, Norpati, Fulgao, Ajgora, Borbam, Charbaria, Pourashova, Bijra, Koitra, Noapara, Chongao | Boro 2018-19 | 9050 | 70 (DS 3-9) |
| Pechra, Norpati, Fulgao, Ajgora, Borbam, Charbaria, Pourashova, Bijra, Koitra, Noapara, Chongao | Boro 2017-18 | 8700 | 20 (DS 5-9) |

### iii) Status of GLH Population in the seedbed and light trap

GLH data collection spots of the villages of Nangalkot, Cumilla were shown in GPS map in Fig. 4. Huge GLH were present in the seedbed upto 180 number per 20 sweeping and the tungro virus is present in these areas, which is most critical factor of tungro disease development (Fig. 5). At BRRI farm, Cumilla, GLH population was counted higher in the light trap during seedbed time from 2018 to 2024 (Fig. 6).

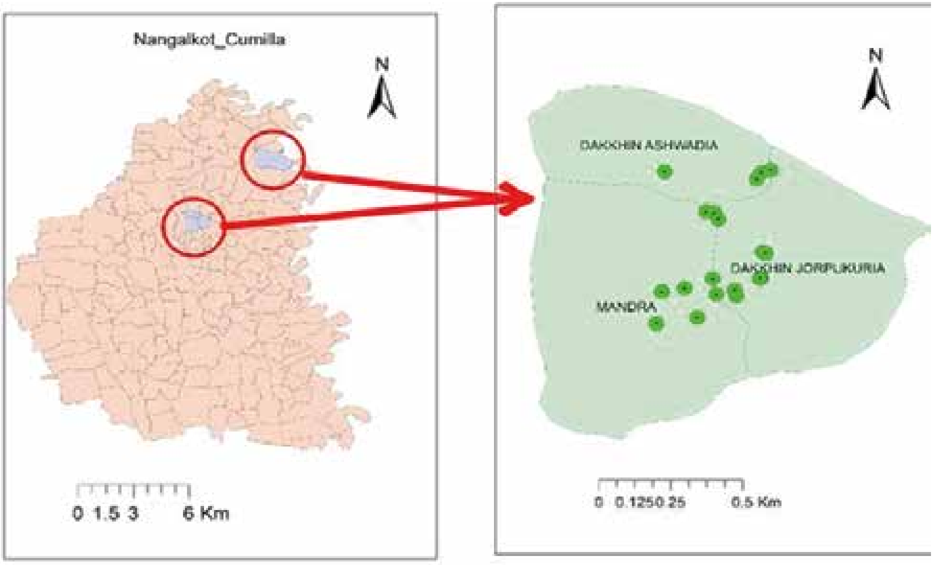


Fig. 4. GPS Location of the seedbed in Nangalkot Upazila in Cumilla during Aus, Aman and Boro season 2019-20.

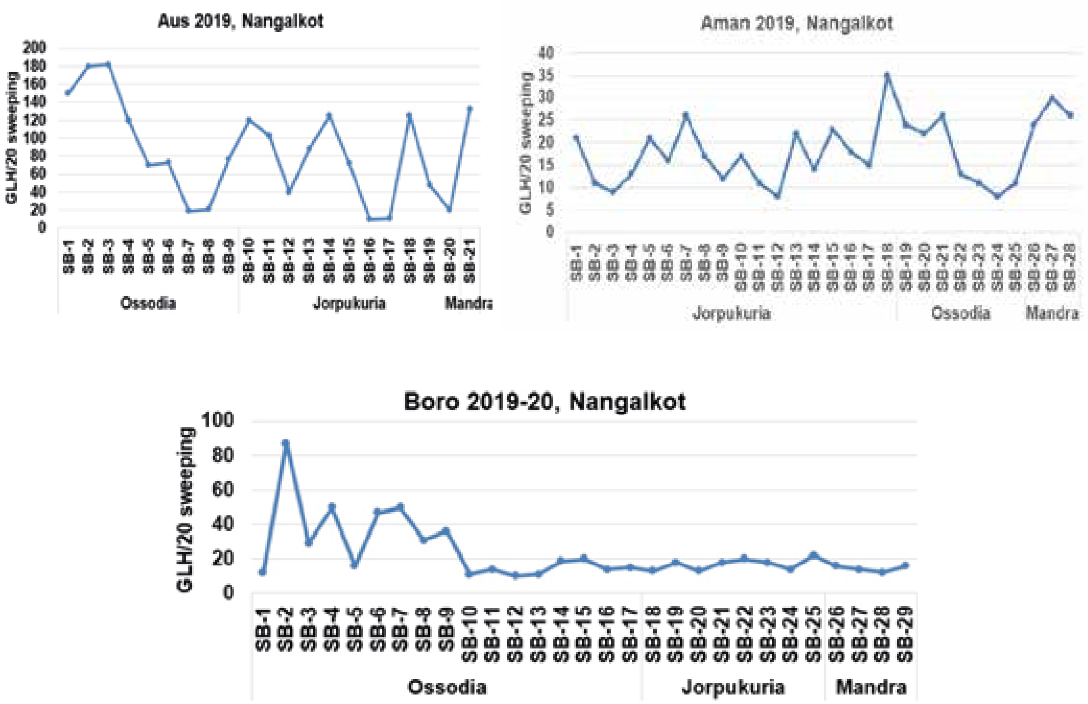


Fig. 5. GLH population collected from the farmers seedbed in Nangalkot Upazila in Cumilla during Aus, Aman, Boro 2019-20. SB= Seedbed.

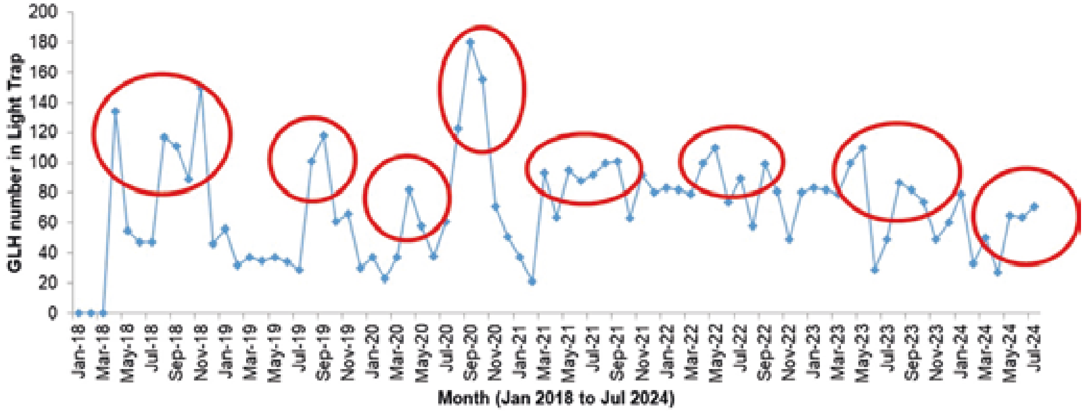


Fig. 6. Month-wise total GLH data collection at BRRI Cumilla during Jan 2018-Jul 2024.

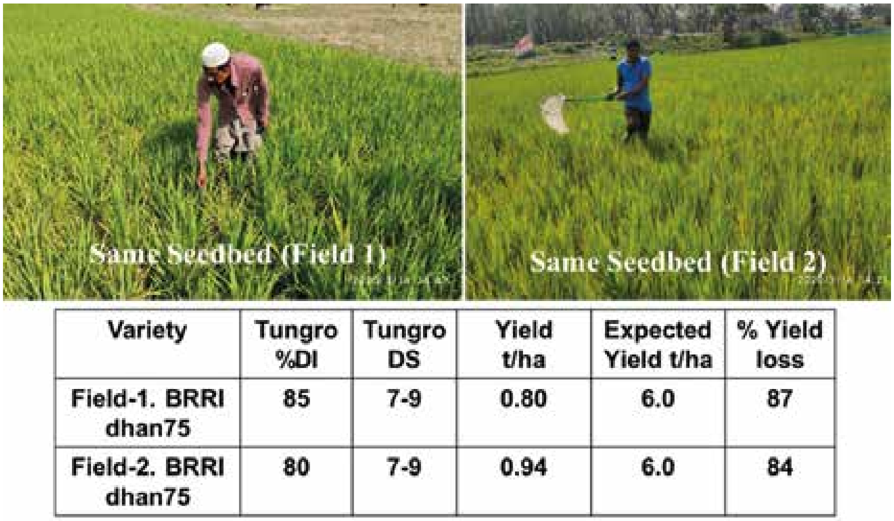
iv) **Seedbed is the main source of tungro devastation**

During Aman 2020 season, we observed that tungro disease symptom was observed even in the seedbed of BRRI dhan75, which is highly susceptible to tungro disease and after transplanting the whole plots were severely infected by tungro disease in at Atakora village, Nangalkot, Cumilla. In the infected plot, 0.56 t/ha yield was obtained and 91% yield was reduced due to tungro devastation (Fig. 7). Another observation that rice seedlings were transplanted in two different places of Kadra village of Nangalkot, Cumilla, during Aman 2020 season by two farmers and tungro disease symptoms were observed same severe as 80-85 % DI with 7-9 DS and the yield was loosed 84-87% (Fig. 8). This is the first report that the tungro disease devastation is depends upon the seedlings infected with viruses in the seedbed by virus infected vector GLH.

Infected Seedlings of BRRI dhan75 Infected rice field of BRRI dhan75

| Variety | Tungro %DI | Tungro DS | Yield t/ha | Expected Yield t/ha | % Yield loss |
|---|---|---|---|---|---|
| BRRI dhan75 | 95 | 9 | 0.56 | 6.0 | 91 |

Fig. 7. Tungro symptom was observed even in the seedbed and yield loosed after transplanting at Atakora in Nangalkot, Cumilla during Aman 2020.



| Variety | Tungro %DI | Tungro DS | Yield t/ha | Expected Yield t/ha | % Yield loss |
|---|---|---|---|---|---|
| Field-1. BRRI dhan75 | 85 | 7-9 | 0.80 | 6.0 | 87 |
| Field-2. BRRI dhan75 | 80 | 7-9 | 0.94 | 6.0 | 84 |

Fig. 8. Tungro symptoms in two different transplanted fields, where the same seedlings are from the same seedbed at Kadra, Nangalkot, Cumilla during Aman 2020 season.

### v) Visualization of Weather Parameters and GLH Population

The scatter plots presented above illustrate the relationships between various weather parameters and the Green Leaf Hopper (GLH) population (Fig. 9). These visualizations provide a preliminary insight into the potential influence of climatic factors on the abundance of GLH over time.

**Temperature (Max and Min):** Both maximum and minimum temperatures exhibit weak, non-linear relationships with the daily GLH population. The scatter plot for Max Temp. shows some fluctuation in the population as the temperature varies (Fig. 9). Similarly, Temp. Min shows variations in GLH numbers with changes in minimum temperature.

**Rainfall:** The scatter plot for Rainfall (mm) does not reveal any strong relationship with the GLH population. The data points are widely dispersed across the plot, suggesting that rainfall, at least in the context of this dataset, does not have a significant impact on the GLH population size (Fig. 9).

**Relative Humidity (6 am and 12 pm):** The scatter plot for RH (%) at 6 am shows a weak inverse relationship with the GLH population, whereas RH (%) at 12 pm displays a slightly stronger positive relationship (Fig. 9 & Fig. 11).

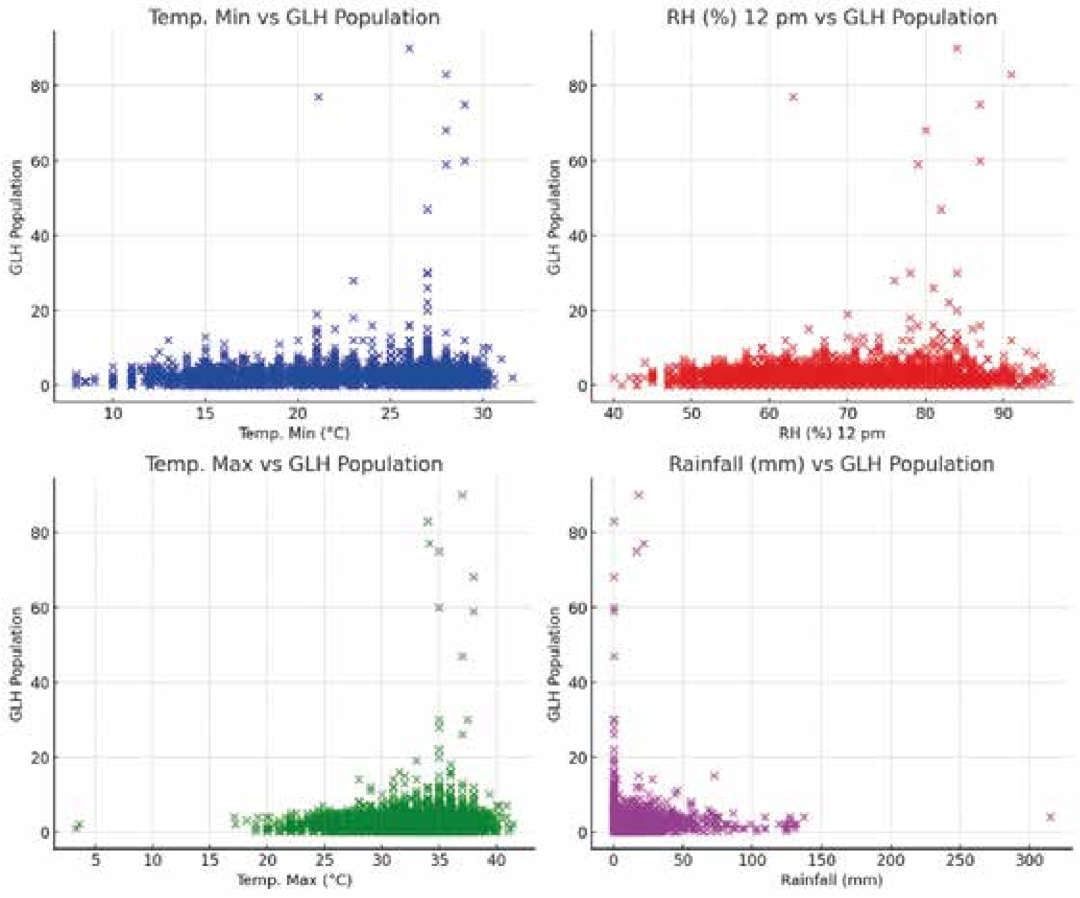


Fig. 9. The scatter plots above visualize the relationships between each weather parameter and the green leaf hopper (GLH) population from Jan 2017 to June 2025.

vi) **Correlation Between Weather Parameters and GLH Population**

The correlogram above illustrates the relationships between the daily and monthly weather parameters and the Green Leaf Hopper (GLH) population from 2017 to 2025, providing insight into how these factors interact (Fig. 10 & Fig. 11). The correlation coefficients, ranging from -1 to 1, indicate the strength and direction of the linear relationships between the variables.

**Temperature (Max and Min):** Both Temp. Max and Temp. Min show a weak positive correlation with the GLH population (ranging from 0.1 to 0.3) (Fig. 10). Maximum month average temperature (>25°C) and monthly average minimum temperature (<17°C) has slightly stronger relations with GLH population during July to September each year (Fig. 11). These correlations suggest that while temperature may play a role in influencing the GLH population at a certain period of time.

**Rainfall:** The correlation between daily rainfall (mm) and the daily GLH population is weak and close to zero (Fig. 10), indicating little to no significant relationship. During January to March and July to September month rainfall (<500 mm) have slightly positively correlate with GLH population (Fig. 11).

**Relative Humidity (6 am and 12 pm)**

The correlation between daily RH (%) at 6 am and the daily GLH population is weakly negative, while RH (%) at 12 pm shows a positive correlation (Fig. 10) and average % RH at 12 pm (70 %) in the month of January to March and October to December have relatively strong correlation with monthly GLH number (Fig. 11). Both values, however, are relatively low, indicating that while relative humidity may have some effect, it is not a strong determinant of GLH population dynamics.

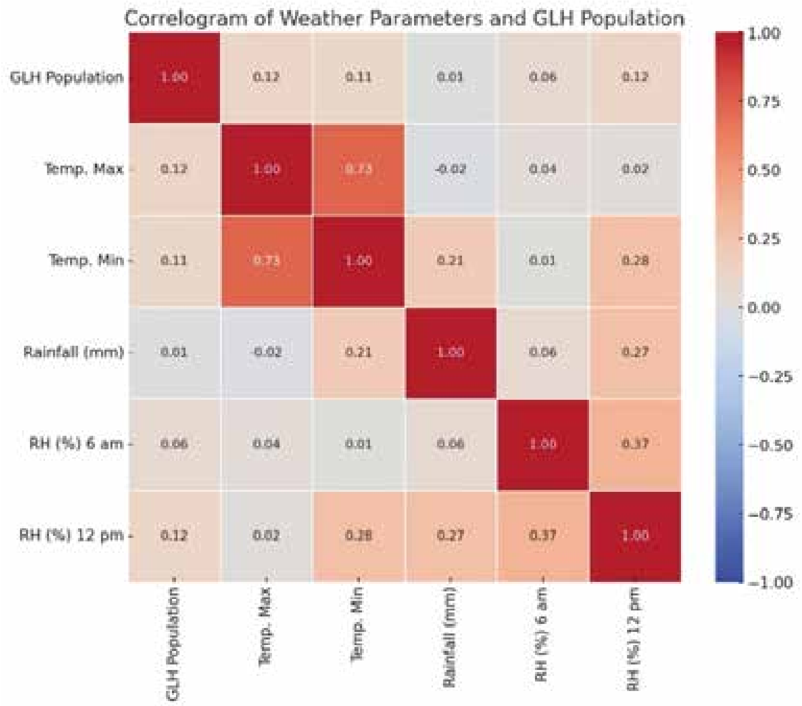


Fig. 10. The correlogram above illustrates the relationships between the weather parameters and the green leaf hopper (GLH) population. The correlation coefficients range from -1 to 1, indicating the strength and direction of the linear relationship between each pair of variables. Positive values indicate a positive correlation, while negative values suggest an inverse relationship. The closer the value is to 1 or -1, the stronger the relationship from Jan 2017 to June 2025.

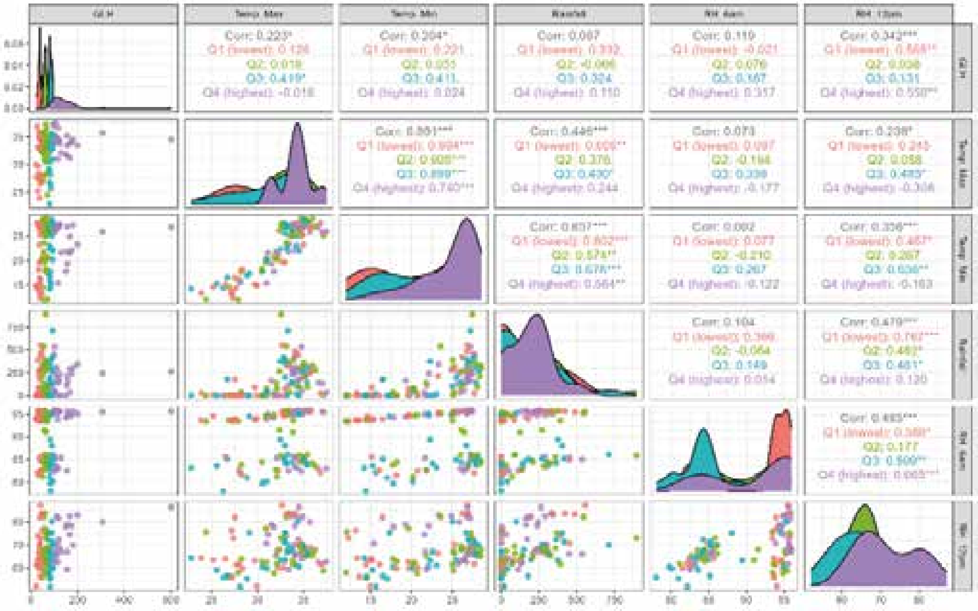


Fig. 11. The correlogram above illustrates the relationships between the monthly weather parameters and the month total green leaf hopper (GLH) population. The correlation coefficients range from -1 to 1, indicating the strength and direction of the linear relationship between each pair of variables. Q1: Jan-Mar, Q2: Apr-Jun, Q3: Jul-Sep and Q4: Oct-Dec each year from 2017 to 2025.

### vii) Feature Importance Analysis of Weather Parameters Influencing GLH Population

The analysis of feature importance reveals the relative influence of each weather parameter on the Green Leaf Hopper (GLH) population. The results, derived from a Random Forest model, highlight the most influential weather parameters in predicting GLH population dynamics.

Temp. Min emerged as the most important factor, accounting for 34.86% of the model's predictive power. This suggests that the minimum temperature plays a significant role in shaping GLH population abundance, with colder or warmer extremes potentially influencing their survival and reproduction rates.

RH (%) at 12 pm was the second most influential parameter, contributing 30.32% to the prediction of GLH population (Fig. 10). Monthly average % RH at evening influence the GLH population increase and slightly stronger correlations was found during first and third quarter of every year.

Temp. Max contributed 16.75% to the model's performance. While it is less influential than Temp. Min, it still plays a role in influencing the daily fluctuations of the GLH population (Fig. 10).

Rainfall (mm) and RH (%) at 6 am were the least influential, contributing 11.91% and 6.16%, respectively. These results suggest that while rainfall and morning humidity levels may have some effect, their role in determining GLH population size is relatively minor compared to temperature and midday humidity.

## DISCUSSION

Out of reported 80 rice diseases, 22 are caused by viruses (Ou, 1985). Rice tungro disease is a widespread in tropical Asia and Asian sub-continent. Some major rice disease distribution mainly depends on location specific climatic condition and other factors. Differences of disease dispersal, environment factors, both microclimate and macroclimate information and the vectors influence on disease progress during the rice crop season.

Yield loss was determined 75-99% of different T. Aman rice varieties due to tungro devastation during T. Aman 2022 season in Debidwar, Cumilla (Nahar *et al.*, 1985; BRRI, 2022-2023). For this why, it is urgent to know the critical factors for tungro disease development.

Different time of planting influences and one of the main causal variables of the appearance of tungro disease. In an asynchronous rice area, there will always be hosts-rice plants, the vector of tungro disease will continue to feed, disperse the virus easily. Both rainfed and irrigated wetland habitats are susceptible to the tungro disease (Sharma *et al.*, 2017). The planting date needs to align with that of other neighbouring farms. Later transplanted rice plants will be more susceptible to tungro disease. The authors suggested that the transplanting period for rice should be varied to reduce the amount of vector populations (Nas & Cortez, 2011). The relationship between the planting of susceptible varieties and asynchronous planting at a given site is critical to expecting tungro incidence (Cabunagan *et al.*, 2001, Holt *et al.*, 1996). They also exhibited the importance of susceptible rice varieties and asynchronism of planting as a vital factor preferring rice tungro devastation. Our study also supports this report that intensive rice cultivation is most vulnerable of tungro disease devastation.

In Bangladesh, the viral disease tungro occur on rice and this disease is a complex disease caused by two virus particles RTSV & RTBV and become severe on rice field during Aus and Aman season (BRRI, 2022-23). The tungro vector, GLH population starts to increase in March, then abruptly decrease in April due to the beginning of monsoon rainfall and wind. Nevertheless, the population rapidly rebuilds to a peak in July. Incidence of tungro disease tracks the GLH population and predominant only during the summer, not during the cool dry winter from October to February when low temperatures suppress the GLH population (BRRI, 1980-81). After 40 years, due to climate change, their findings seem to differ in some cases from our own. We found that tungro disease appears not only in Aus and Aman seasons but also in Boro season. As a result, disease incidence was also appeared in all the seasons and year-round. It is due to increased rice cultivation in Cumilla region. Similar result was obtained from our study that the GLH vector population was obtained year-round and top in July.

Santi *et al.*, (2024) reported that a single vector insect can infect up to 40 seedlings every day in the seedbed. We observed that tungro disease devastation in the main field was due to earlier infection in the seedbed by tungro-infected vectors, resulting in nearly 100% yield loss

Temperature data analysis showed that a weak positive relationship was found between temperature and GLH number and ultimate disease development. These findings are in line with studies indicating that the impact of ambient minimum/maximum temperature can have a mild impact on insect populations, though its effect may be influenced by other environmental and biological factors (Santi *et al.*, 2024). This finding is consistent with previous studies which indicate that temperature can influence insect population dynamics, but the effect may be relatively minor compared to other environmental factors (Madhuri *et al.*, 2017). They reported that during Kharif season, the GLH number was positively correlated with maximum and minimum temperature and negatively correlated with morning and evening relative humidity and rainfall. From our study, it is suggested that both maximum and minimum temperature are necessary for the increasing of GLH population during July to September in every year climatic situation, which is eventually accountable for the spread of tungro disease of rice.

In 1987, the authors reported that the relationship among disease incidence and such climatic factors like minimum temperature, relative humidity, daily rainfall and dew period (Shahjahan *et al.*, 1987). Severe tungro occurrence has been connected with high temperature (25-30 °C) (Kiritani, 1981 and Ou, 1985). The incidence of tungro and other viral diseases like ragged stunt, grassy stunt and transitory orange yellowing in hot and humid regions depends on the temperature requirements of different insect vectors. This result corroborates our present study, where GLH vector population increases due to increase of temperature during July to September, and other months have found a weak relationship.

The scatter plot and correlation for Rainfall have a weak relationship with the GLH population. But our findings suggest that first and third quarter of the year have slightly positive correlations with the number of GLH population. This suggests that rainfall does not substantially influence GLH abundance, aligning with research that suggests other ecological or biological factors may be more influential in shaping GLH populations, rather than rainfall alone (Adiroubane *et al.*, 2007; Kalita *et al.*, 2015; Madhuri *et al.*, 2017).

Our study highlighted the role of midday humidity levels in affecting GLH behavior and distribution. High humidity can create favorable conditions for the survival of GLH population. This finding aligns with literature indicating that GLH number negatively correlated with relative humidity at morning and at evening (Adiroubane *et al.*, 2007; Kalita *et al.*, 2015; Madhuri *et al.*, 2017).

These scatter plots highlighted that while there are some weak correlations between weather parameters and the GLH population, they are not strong enough to conclude that these factors are the primary drivers of GLH dynamics.

## CONCLUSION

In summary, the feature importance analysis indicates that GLH population dynamics is influenced by intensive rice cultivation, susceptible rice cultivar, abundance GLH in the seedbed with presence of virus, monthly average maximum (>25°C)) and minimum (<17°C) temperature during July to September, and average mid-day relative humidity (70 %) are the most influential factors for GLH population advancement. The role of rainfall has very weak relations with GLH population dynamics. These findings align with ecological studies that emphasize the role of different parameters in regulating insect populations, while also suggesting that other environmental factors not captured in this analysis may also be significant contributors to GLH abundance.

## ACKNOWLEDGEMENT

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# Futile Integrated Nutrient Management in Azmiriganj Series for Boro Rice Cultivation

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## ABSTRACT

The experiment was conducted at Bangladesh Rice Research Institute (BRRI) regional station farm, Habiganj, in Boro 2016-17 to observe the effect of integrated nutrient management (INM) on growth, yield, yield parameters and nutrient uptake of BRRI dhan29 in the haor soil. The experimental field belongs to the Azmiriganj series, under the agro-ecological zone ‘Sylhet Basin’ (AEZ-21). The experiment was designed with six treatments, including $T_1$ = Fertilizer control, $T_2$ = Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg $ha^{-1}$, respectively), $T_3$ = Cowdung (CD) @ 2 t $ha^{-1}$ + 50% recommended chemical fertilizer, $T_4$ = CD @ 3 t $ha^{-1}$ + 50% recommended chemical fertilizer, $T_5$ = CD @ 4 t $ha^{-1}$ + 50% recommended chemical fertilizer, and $T_6$ = 120% of recommended chemical fertilizer dose. The experiment was laid out in an RCB design with 3 replications using the test crop BRRI dhan29. Application of CD @ 2, 3 or 4 t $ha^{-1}$ with 50% recommended chemical fertilizer did not exceed the grain yield, yield parameters and nutrient uptake over recommended sole chemical fertilizers. The recommended dose of chemical fertilizer performed better than INM treatments in terms of yield and nutrient uptake in the haor area of the BRRI Habiganj farm.

**Keywords:** Integrated nutrient management, Cowdung, grain yield, nutrient content, nutrient uptake.

## INTRODUCTION

Haor, a bowl-shaped, low-lying single rice ecosystems in north eastern Bangladesh, occupy about 0.68 million hectares (Huda, 2004). There are as many as 373 haors of variable sizes in Bangladesh, mostly cultivated with Boro rice during November – May. Haor soils usually show better rice productivity than that obtained with other soils. Grain sterility due to cold injury and early flash floods are the main climatic hazards in the haor regions. Farmers obtain a good rice yield in the haor with their own fertilizer management, provided there are no climatic difficulties.

Farmers in the haor region usually apply inorganic fertilizers to their Boro rice fields for a long time. The use of inorganic fertilizer in rice cultivation in Bangladesh has been progressively increasing in the haor areas. Expenditure for inorganic fertilizer is high, and thus, identifying appropriate and economically feasible approaches, which are environmentally friendly and healthy, is imperative. The use of inorganic fertilizer to sustain cropping was found to increase yield only for a few years, but in the long-term, it has not been effective and leads to soil degradation (Satyanarayana *et al.*, 2002). On the other hand, continuous application of organic fertilizer alone on the rice field, resulting low yield and low N and K content at the mid-tillering stage of the rice plant. This implies the need for integrated nutrient management for rice production. Therefore, the combined use of organic manures and inorganic fertilizers helps in maintaining yield stability through correction of marginal deficiencies of secondary and micronutrients,

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enhancing efficiency of applied nutrients and providing favorable soil physical conditions (Gill and Walia, 2014). Integrating nutrient management (INM) aims for efficient and judicious use of all the major sources of plant nutrients in an integrated manner (Farouque and Takeya, 2007). When used in combination, interactions occur, and the yield increase is always more than that from the use of equivalent quantities of these nutrient sources alone. Such integrated applications have proved to be complementary and synergistic. The most important methods to establish a relevant INM are: to ensure a balance plant nutrient supply from organic and inorganic sources, and to improve plant nutrient efficiency to optimize crop production and avoid nutrient losses. In this region, farmers seldom use organic manure, and they do not practice balance fertilization. Besides balanced fertilization, integrated nutrient management (INM) practice may be a good option for increasing rice yield. Considering the above context, the study was undertaken to observe the effect of INM practices on the grain yield of the Azmiriganj soil series of the single-cropped Boro area.

## MATERIALS AND METHODS

The experiment was conducted at the BRRI regional station farm, Habiganj, in Boro (dry season rice) 2016-17. The experiment was conducted on Azmiriganj silty clay loam soil, a member of Baniachong group. The experimental field soil had soil pH 4.65, organic carbon 2.27%, total N 0.13%, available P 9.5 mg kg-1 (Bray and Kurtz Method; 0.03N NH4F+0.025 HCl (acidic soils, <pH 6.0) and exchangeable K 0.12 meq/100 g soil. Six different treatment combinations were considered for executing the experiment objectives. Treatments were $T_1$= Fertilizer control, $T_2$= Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg ha$^{-1}$, respectively), $T_3$= Cowdung (CD) @ 2 t ha$^{-1}$ + 50% recommended chemical ferttilizer, $T_4$= CD @ 3 t ha$^{-1}$ + 50% recommended chemical ferttilizer, $T_5$= CD @ 4 t ha$^{-1}$ + 50% recommended chemical ferttilizer, and $T_6$= 120% of recommended chemical fertilizer dose. The experiment was laid out in an RCB design with 3 replications. The tested rice variety was BRRI dhan29. In Boro season, N-P-K-S-Zn @ 130-18-60-13-4 kg ha$^{-1}$, respectively, were used as BRRI recommendation. Well decomposed cowdung was applied at final land preparation in the respective plots with an appropriate dose as sundry basis moisture content (65%) and incorporated in the field properly. All chemical fertilizers except urea were applied as basal during final land preparation. Urea N was applied in equal 3 splits, i.e., one-third at 14 days after transplanting, one-third at the active tillering stage (44 days after transplanting) and one-third at 5 days before the panicle initiation stage of rice. The tested cropping pattern was Boro-Fallow-Fallow using BRRI dhan29 as a test variety. The unit plot size was 5m × 3m. Forty-five-day-old 2-3 seedlings hill$^{-1}$ were transplanted in 20 cm × 20 cm spacing. Irrigation and other management practices were done as per needed. The crop was harvested from 5 m$^2$ area at the center of each plot, and rice grain yield was adjusted to 14% moisture content and straw yield was oven-dry basis. The chemical analysis of plant samples was done in the Soil Science Division's laboratory, BRRI, Gazipur. All data were statistically analyzed by the software STAR.

## RESULTS AND DISCUSSION

### Shoot yield of Boro rice at panicle initiation (PI) stage

The shoot yield at panicle initiation (PI) stage increased significantly with application of chemical fertilizer and organic manure over fertilizer control treatment (Table 1). The highest shoot yield was obtained with the application of 120% chemical fertilizer followed by 100% recommended dose of chemical fertilizer. Statistically similar shoot yield was observed with the application of cowdung @ 2, 3 or 4 t ha$^{-1}$ with 50% recommended chemical fertilizer. The fertilizer control plot gave the significantly lowest shoot yield.

**Nutrient uptake at panicle initiation (PI) stage**

The N, P, and K uptake by rice shoots at the PI stage was significantly influenced by practicing integrated nutrient management in Boro rice (Table 1). The highest N uptake was obtained with 120% chemical fertilizer treatment, followed by 100% chemical fertilizer treatment. Application of 2 or 3 ton CD ha$^{-1}$ with 50% chemical fertilizer showed significantly lower N uptake than 100% recommended chemical fertilizer treatment, and statistically similar N uptake was found with application of 4 t CD ha$^{-1}$ with 50% chemical fertilizer treatment and application of 100% recommended chemical fertilizer treatment. The lowest N uptake was observed in the fertilizer control treatment. The highest P uptake at the PI stage was found in 120% chemical fertilizer treatment, followed by 100% recommended chemical fertilizer treatment and the CD treated plots. Significantly lowest P uptake was found in the control treatment. The K uptake was higher in the application of 4 t CD ha$^{-1}$ with 50% chemical fertilizer treatment, followed by the application of 3 t CD ha$^{-1}$ with 50% chemical fertilizer treatment. But K uptake was statistically insignificant with the application of sole chemical or chemical plus organic matter treated plots. The K uptake was significantly lower in fertilizer control treatment (Table 1). Yamakawa et al. (2004) found that rice plants at the panicle initiation stage contain 2.27% N, 0.40% P and 3.05% K, which certainly endorses the findings of the present study.

**Table 1. Effects of integrated nutrient management on shoot yield and nutrient uptake at panicle initiation stage of BRRI dhan29, BRRI farm, Habiganj, 2016-17.**

| Treatments | Shoot yield (t ha$^{-1}$) | N uptake (kg ha$^{-1}$) | P uptake (kg ha$^{-1}$) | K uptake (kg ha$^{-1}$) |
|---|---|---|---|---|
| $T_1$ | 3.71 | 24.54 | 10.01 | 74.88 |
| $T_2$ | 5.74 | 76.51 | 21.64 | 127.43 |
| $T_3$ | 5.50 | 56.52 | 18.53 | 129.42 |
| $T_4$ | 5.55 | 62.20 | 19.32 | 134.68 |
| $T_5$ | 5.61 | 66.12 | 20.25 | 138.06 |
| $T_6$ | 5.80 | 79.69 | 22.41 | 130.83 |
| $LSD_{0.05}$ | 0.94 | 12.45 | 3.97 | 30.88 |
| CV (%) | 9.80 | 11.20 | 11.70 | 13.90 |

$T_1$=Fertilizer control, $T_2$=Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg ha$^{-1}$, respectively), $T_3$=Cowdung (CD) @ 2 t ha$^{-1}$ + 50% rec. che. fert., $T_4$=CD @ 3 t ha$^{-1}$+ 50% rec. che. fert., $T_5$=CD @ 4 t ha$^{-1}$+ 50% rec. che. fert. and $T_6$=120% of recommended chemical fertilizer dose.

**Yield and yield parameters of Boro rice**

The application of integrated nutrient management (INM) increased tiller and panicle numbers compared to the control plot (Table 2). The application of 120% chemical fertilizers produced the highest numbers of tillers and panicles, followed by 100% chemical fertilizer treatment ($T_2$). Compared to $T_2$, the integrated nutrient management treatments ($T_3$, $T_4$ and $T_5$) produced significantly lower numbers of tillers and panicles.

The grain and straw yield varied significantly with applying only chemical fertilizer and INM for Boro rice (Table 2). The control plot yielded only 5.14 tha$^{-1}$ rice grain. With applying 100% chemical fertilizer, grain yield increased significantly (7.33 tha$^{-1}$), but by applying 120% chemical fertilizer, grain yield decreased significantly (7.05 tha$^{-1}$) than the recommended chemical fertilizer. The INM treatments gave statistically similar grain yield, which were statistically identical with the 120% chemical fertilizer treatment but significantly lower than the recommended chemical fertilizer treatment.

Nitrogen helped in proper filling of seeds, which resulted in higher produced plump seeds and thus the number of grains per panicle.

The straw yield followed a similar trend with INM and chemical fertilizer practices as described above for grain yield.

The percent filled grain and thousand-grain weight were influenced significantly by practicing chemical fertilizer and INM in Boro rice at the haor area (Table 2). The percent filled grain was significantly higher where no fertilizer was used and significantly lower in the 120% chemical fertilizer treatment. The other treatment gave almost a similar filled grain percentage. The thousand-grain weight for all treatments was statistically similar, but a comparatively higher thousand-grain weight was obtained with practicing recommended chemical fertilizer, followed by organic manure treatment.

**Table 2. Effects of integrated nutrient management on yield parameters and grain and straw yield of BRRI dhan29, BRRI farm, Habiganj, 2016-17.**

| Treatments | Tiller $m^{-2}$ | Panicle $m^{-2}$ | GY ($t\ ha^{-1}$) | SY($t\ ha^{-1}$) | Filled grain (%) | 1000 grain wt. (g) |
|---|---|---|---|---|---|---|
| $T_1$ | 241 | 230 | 5.14 | 5.01 | 76.77 | 21.51 |
| $T_2$ | 359 | 333 | 7.33 | 7.21 | 70.71 | 21.77 |
| $T_3$ | 321 | 305 | 7.02 | 6.76 | 70.57 | 21.51 |
| $T_4$ | 326 | 310 | 6.98 | 6.85 | 70.64 | 21.48 |
| $T_5$ | 331 | 314 | 6.92 | 6.89 | 70.48 | 21.45 |
| $T_6$ | 366 | 338 | 7.05 | 7.35 | 62.21 | 21.09 |
| $LSD_{0.05}$ | 29 | 27 | 0.19 | 0.43 | 3.73 | 1.18 |
| CV (%) | 4.9 | 4.9 | 1.6 | 3.6 | 2.9 | 3.0 |

$T_1$=Fertilizer control, $T_2$=Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg $ha^{-1}$, respectively), $T_3$=Cowdung (CD) @ 2 t $ha^{-1}$ + 50% rec. che. fert., $T_4$=CD @ 3 t $ha^{-1}$+ 50% rec. che. fert., $T_5$=CD @ 4 t $ha^{-1}$+ 50% rec. che. fert. and $T_6$=120% of recommended chemical fertilizer dose.

### Nutrient content and nutrient uptake at the maturity stage of Boro rice

The nutrient content in grain and straw varied significantly with practicing the INM approaches (Table 3). The grain with the highest nitrogen (N) content was found in $T_6$ treatment, which used 120% chemical fertilizer, followed by $T_2$ treatment with 100% chemical fertilizer. The other INM treatments gave similar results for grain N content, and the lowest N content was observed in the fertilizer control treatment. A similar result was found for grain P and grain K content of Boro rice of BRRI dhan29 in haor soil. The straw N, P, and K content was also influenced by the application of different doses of chemical fertilizers and INM treatments. In the 120% chemical fertilizer treatment, the straw N content was higher, followed by the 100% chemical fertilizer treatment. The straw N content in organic matter treated plots was almost identical but slightly lower than chemical fertilizers treatment. A similar trend was obtained for straw P and straw K content (Table 3).

**Table 3. Effects of integrated nutrient management on nutrient content in grain and straw of BRRI dhan29, BRRI farm, Habiganj, 2016-17.**

| Treatments | Grain N (%) | Grain P (%) | Grain K (%) | Straw N (%) | Straw P (%) | Straw K (%) |
|---|---|---|---|---|---|---|
| $T_1$ | 0.54 | 0.18 | 0.20 | 0.41 | 0.09 | 1.29 |
| $T_2$ | 0.92 | 0.29 | 0.30 | 0.52 | 0.14 | 1.54 |
| $T_3$ | 0.81 | 0.22 | 0.24 | 0.46 | 0.12 | 1.42 |
| $T_4$ | 0.85 | 0.23 | 0.26 | 0.48 | 0.13 | 1.45 |
| $T_5$ | 0.88 | 0.24 | 0.28 | 0.50 | 0.14 | 1.47 |
| $T_6$ | 1.00 | 0.30 | 0.31 | 0.54 | 0.15 | 1.56 |
| $LSD_{0.05}$ | 0.09 | 0.05 | 0.03 | 0.05 | 0.02 | 0.13 |
| CV (%) | 5.6 | 11.9 | 5.8 | 5.4 | 10.4 | 4.9 |

$T_1$=Fertilizer control, $T_2$=Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg ha$^{-1}$, respectively), $T_3$=Cowdung (CD) @ 2 t ha$^{-1}$ + 50% rec. che. fert., $T_4$=CD @ 3 t ha$^{-1}$+ 50% rec. che. fert., $T_5$=CD @ 4 t ha$^{-1}$+ 50% rec. che. fert. and $T_6$=120% of recommended chemical fertilizer dose.

Nutrient uptake of Boro rice at the maturity stage varied significantly with chemical fertilizer and INM practices (Table 4). Higher N uptake was observed in the $T_6$ treatment with 120% chemical fertilizer, followed by $T_2$ with 100% fertilizer. Among the INM treatments, T5 (4 ton CD per ha with 50% chemical fertilizer) gave significantly higher N uptake than $T_3$ (2 ton CD per ha with 50% chemical fertilizer), but not in $T_4$ (3 ton CD per ha with 50% chemical fertilizer). The $T_3$ and $T_4$ treatments gave similar results for N uptake. Statistically similar result was obtained in the case of P and K uptake at the maturity stage of BRRI dhan29.

**Table 4. Effects of integrated nutrient management on N, P and K uptake at maturity stage of BRRI dhan29, BRRI farm, Habiganj, 2016-17.**

| Treatments | N uptake (kg ha$^{-1}$) | P uptake (kg ha$^{-1}$) | K uptake (kg ha$^{-1}$) |
|---|---|---|---|
| $T_1$ | 48 | 13 | 75 |
| $T_2$ | 104 | 31 | 133 |
| $T_3$ | 88 | 23 | 113 |
| $T_4$ | 92 | 25 | 117 |
| $T_5$ | 95 | 26 | 120 |
| $T_6$ | 111 | 32 | 137 |
| $LSD_{0.05}$ | 5 | 4.9 | 13 |
| CV (%) | 3.2 | 10.7 | 6.3 |

$T_1$=Fertilizer control, $T_2$=Recommended chemical fertilizer dose (N-P-K-S-Zn @ 130-18-60-13-4 kg ha$^{-1}$, respectively), $T_3$=Cowdung (CD) @ 2 t ha$^{-1}$ + 50% rec. che. fert., $T_4$=CD @ 3 t ha$^{-1}$+ 50% rec. che. fert., $T_5$=CD @ 4 t ha$^{-1}$+ 50% rec. che. fert. and $T_6$=120% of recommended chemical fertilizer dose.

Sarwar (2005) reported that yield and different yield components of rice increased significantly with the use of chemical fertilizers alone or in combination with various organic materials. Integrated use of poultry manure @ 2.5 t ha$^{-1}$ with P, K, S, and Zn gave better grain yield than sole use of chemical fertilizer in T. Aman season (Ali *et al.*, 2018). Jisan *et al.* (2014) and Salahuddin *et al.* (2009) reported that the number of grains per panicle increased significantly with increments in the level of nitrogen. Combined application of manures and fertilizers increased the number of grains per panicle (Malika, 2011; Rahman *et al.*, 2007 and Parvez *et al.*, 2008). (Chakraborty *et al.*, 2020) suggested for application poultry manure @ 2.5 t ha$^{-1}$ + 50% prilled urea and full dose of other inorganic fertilizers treatment combination with BRRI dhan29 for increasing yield and improving soil health. Many reports claimed that the INM improved rice yield than the sole chemical fertilizers in Rangpur (Saha *et al.*, 2016); in Dhaka (Naher and Paul, 2017); in coastal soils (Islam *et al.*, 2024). One of the important causes of poor performance of the INM in present experiment is the relatively high organic carbon (2.27%) in the experimental field. The 2.27% organic carbon means 3.91% organic matter compared to the organic matter of 1.25% at Rangpur (Saha *et al.*, 2016), 1.19% at Dhaka (Naher and Paul, 2017), and 2.25% and 3.25% at Satkhira (Islam *et al.*, 2024). Islam *et al.* (2015) and Suresh *et al.* (2013) also reported that a combination of organic and inorganic fertilizer in soil prolongs the availability of essential nutrients, which helps to accumulate greater source and efficient translocation of photosynthates into the sink, as indicated by higher 1000-grain weight. Application of manure and fertilizers increased 1000 grain weight (Rahman *et al.*, 2007 and Parvez *et al.*, 2008). Hossain *et al.* (2010), reported that application of PM @ 2 t ha$^{-1}$with 50% soil test based (STB) chemical fertilizer gave the highest yield and nutrient (N, P, K) uptake by rice. It is well documented that the combined application of organic and inorganic fertilizers in balanced doses not only increases nutrient concentrations in plant cells but also increases crop yields (Rahman *et al.*, 2016).

Integrated nutrient management in the present experiment produced poor growth and yield compared to recommended chemical fertilizers application, which is a clear disagreement with available literature. The soil organic carbon in the present experimental field almost touched the saturation limit of the silt loam soils (Hassink, 1997). Addition of cowdung in such soils would benefit only a little because the level of organic carbon is close to the brink of the saturation point.

More than 5 t ha$^{-1}$ yield in the control plots compared to 2.2 and 3.6 t ha$^{-1}$ in other experimental fields (Saha *et al.*, 2016; Naher and Paul, 2017; Islam *et al.*, 2024) signifies the potentiality of the soil to supply N in the haor soil. Moreover, an increasing dose of cowdung in the present experiment decreased grain yield progressively. The application of 2 t ha$^{-1}$ CD along with 50% of the recommended fertilizer gave 7.02 t ha$^{-1}$, which decreased to 6.98 t ha$^{-1}$ with 3 t ha$^{-1}$ CD. The grain yield further decreased to 6.92 t ha$^{-1}$ when the CD dose was 4 t ha$^{-1}$. Although the yield decline with the increasing dose of CD was not significant, the declining trend is noticeable. Another important finding of the present experiment is the yield in the control plot (5.14 t ha$^{-1}$). Boro rice usually gives almost double the yield of the N-control plot's yield with the appropriate nutrient and other cultural management. Our results show a potentiality of the haor soil of 10 t ha$^{-1}$ yield, but it needs a technology to achieve the target, which is a simple challenge to the BRRI scientists.

## CONCLUSION

Integrated nutrient management, i.e., application of CD @ 2, 3 or 4 t ha$^{-1}$ with 50% chemical fertilizer, did not increase the yield parameters, grain and straw yield and nutrient uptake of BRRI dhan29 in both panicle initiation and maturity stage over recommended chemical fertilizer alone in the haor areas of BRRI Habiganj farm. The 100% recommended dose of chemical fertilizer performed better than INM practices in terms of yield and nutrient uptake in the haor area. It seems the 100% recommended dose of chemical fertilizer is enough to produce maximum grain yield, and no need to apply extra chemical fertilizer, i.e., 120% of the recommended chemical fertilizer.

Furthermore, studies are recommended on the management of inorganic fertilizer to boost the yield level of 10 t ha$^{-1}$ in the haor rice soils of single-cropped areas in the Sylhet region.

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