ISSN 1025-7330

BANGLADESH RICE JOURNAL

VOL. 28 NO. 1 June 2024

BANGLADESH RICE RESEARCH INSTITUTE
GAZIPUR-1701, BANGLADESH

ISSN 1025-7330

BANGLADESH RICE JOURNAL

VOL. 28 NO. 1 June 2024

BANGLADESH RICE JOURNAL

(Bangladesh Rice J.)

ISSN 1025-7330

VOL. 28 **NO. 1** **June 2024**

CONTENTS

Bangladesh Rice J.28(1): 1-16, 2024, doi.org/10.3329/brj.v28i1.86862

Characterizing CRCIL-464 Rice Diversity Panel for Using as Multi-Trait Genetic Resources for Breeding Climate-Resilient Varieties Pertaining to Salinity, Flood, and Cold Stress Tolerance

M S Rahman[1*], A A Shoily[1], J Jannaty[1], A Rahman[2], M S Pervin[1] and K M Iftekharuddaula[2]

ABSTRACT

Rice (*Oryza sativa* L.) is highly vulnerable to abiotic stresses, particularly in climate-sensitive countries such as Bangladesh, where salinity, submergence, and cold pose major threats to sustainable production. To identify genetic resources for climate-resilient breeding, a multi-trait diversity panel of 464 rice genotypes (CRCIL-464), comprising advanced breeding lines, released varieties, and diverse germplasm, was evaluated for tolerance to these stresses. Salinity screening at the seedling stage using hydroponics identified 32 tolerant and 25 moderately tolerant genotypes based on SES scores, with selected lines further evaluated under soil-based reproductive-stage salinity. Three genotypes (BR11715-4R-13, BR11921-4R-100, and BR12567-5R-91) outperformed the tolerant check IR58443 in grain yield, though yield reductions highlighted differential tolerance mechanisms between stages. Submergence screening revealed wide variability in recovery and survivability, with one genotype (IR14T156) showing complete survival (100%), although most entries were highly sensitive. Cold stress assessment at the seedling stage identified 12 genotypes with partial recovery, and two lines (SVIN 297 and IRRI154-Hd9+Pi9) displayed the highest survivability (83.3%). Collectively, the CRCIL-464 panel demonstrated substantial genetic variation across multiple stresses. The identified tolerant lines represent promising candidates for incorporation into BRRI's breeding program to develop multi-stress-tolerant rice varieties, thereby enhancing resilience and securing rice production under climate change.

Keywords: Rice, abiotic stress tolerance, salinity, submergence, climate-resilient breeding, multi-trait screening.

INTRODUCTION

Rice (*Oryza sativa* L.), a member of the Poaceae family, is one of the most important staple foods worldwide, supplying dietary energy for more than half of the global population, particularly in Asia, Africa, and Latin America (Fitzgerald *et al.*, 2009). Its remarkable adaptability across diverse ecosystems-including flooded lowlands, irrigated fields, and upland systems-highlights its central role in global food security (Khush, 2005). Despite its resilience, rice productivity is increasingly threatened by abiotic stresses such as drought, salinity, extreme temperatures, and flooding. These environmental constraints not only reduce grain yield but also deteriorate grain quality, with particularly severe impacts in regions experiencing rapid and unpredictable climate change (Zhu, 2016).

A multi-trait diversity panel is a collection of

[1]Plant Physiology, Division, BRRI, Gazipur and [2]Plant Breeding Division, BRRI, Gazipur
Corresponding author's E-mail: sazzadur.phys@brri.gov.bd (M S Rahman)

rice genetic resources characterized by a wide range of inherited traits. These panels are designed to maximize genetic variation, enabling the study and simultaneous selection of multiple traits within a single population. They provide researchers with valuable insights into the genetic basis of important traits and support efforts to enhance adaptation to environmental challenges. The CRCIL-464 panel was developed to capture a broad spectrum of genetic diversity. Once trait variation and genetic diversity are well understood, breeders can apply strategies such as selection indices to simultaneously select multiple traits and improve breeding populations. In this context, the panel was characterized for three major abiotic stress tolerance traits: salinity, submergence, and cold tolerance.

Salinity poses one of the greatest threats to rice cultivation. Salt accumulation in soils interferes with water and nutrient absorption, resulting in reduced germination, impaired seedling growth, stunted plant development, poor tillering, and lower yields (Munns & Tester, 2008). While improved soil and water management practices have been introduced to mitigate salinity effects (Singh *et al.*, 2018), genetic improvement through the development of salt-tolerant rice varieties remains essential for sustainable crop production. Salinization is the accumulation of water-soluble salts in the soil to a level that impacts on agricultural production. Soil is considered saline if the electrical conductivity of its saturation extract (ECe) is above 4 dSm-1 (USDA-ARS, 2008), which is equivalent to approximately 40mM NaCl and generates an osmotic pressure of approximately 0.2 MPa. This definition of salinity derives from the ECe that significantly reduces the yield of most crops (Munns and Tester, 2008). Loss of arable land via salinization is a major factor undermining the productivity of modern agricultural systems (Galvani, 2007). Salinization of agricultural soils occurs primarily due to agricultural practices, including poor water management, high evaporation, heavy irrigation and previous exposure to sea water (Pitman and Lauchli, 2002).

In Bangladesh, more than 30% of the cultivable land is in the coastal areas. Soil salinity has mainly formed from sea water flooding or capillary rise from shallow ground water close to the coast. Soil Resource Development Institute (SRDI) has temporal and spatial data on regular monitoring of soil and water salinity since 1989 besides reconnaissance survey data of 1973. It was estimated in 1973 and 2009 that the area coverage of soils with different degrees of salinity is about 0.833 and 1.056 million hectares respectively. Total spatial increase of saline area was about ~26% in 2009 over 1973 (SRDI, 2010).

Defining salt tolerance of rice is very difficult because of the complex nature of salt stress and the wide range of plant responses. Rice responses to salinity also vary in different growth stages. Seedling and flowering stages of rice are more likely to be affected by salinity, with reduction in seedling growth and yield. Tolerance during seedling stage seems to correlate poorly with tolerance during reproduction, suggesting different sets of traits are probably involved at each stage (Moradi *et al.*, 2003). Reproductive stage is another developmental stage when rice is sensitive to salinity stress, more specifically this stage is the booting stage (7-10 days before and after booting stage) (Singh and Flowers, 2010). The reproductive stage is crucial as it ultimately determines grain yield, but the importance of the seedling stage cannot be underestimated as it determines crop establishment. There are few studies that address the effects of salinity on yield. Most research has been limited to the seedling or early vegetative stages or only reports parameters such as fresh or dry weight although the ultimate aim has been to increase grain yield with limited resources (Moradi and Ismail, 2007; Cheng *et al.*, 2008; Jain *et al.*, 2008; Zang *et al.*, 2008). Hence, to know the response of the rice plant to salinity as a whole, it is imperative that the effects be observed in all the various stages of its development, that is at early seedling, vegetative and reproductive stages (Gregorio *et al.*, 1997).

Flooding is another major constraint in

rice-growing regions, especially in monsoon-prone areas of South and Southeast Asia. Although rice has greater inherent tolerance to waterlogging compared to other cereals, extended periods of submergence can cause oxygen deprivation, reduce photosynthesis, and delay crop maturity (Bailey-Serres *et al.*, 2010). Breeding progress has been made through the incorporation of the Sub1 gene into high-yielding varieties, conferring enhanced survival under flash flooding (Xu *et al.*, 2006). However, the need for broader submergence tolerance and post-flood recovery traits remains a pressing challenge in breeding programs. Submergence is one of the main obstacles to growing rice, particularly during years and in regions with considerable precipitation. More than sixteen percent of rice fields worldwide are susceptible to flooding. (Mackill *et al.*, 1996., Setter *et al.*, 1997). Rice farming in rainfed lowlands of south and south-east Asia is sometimes hampered by flash floods, which submerge the plants completely in water for roughly two weeks. (Septiningsih *et al.*, 2009).

Cold stress, particularly at the seedling stage, is also a critical barrier to rice establishment in temperate and high-altitude ecosystems. Exposure to temperatures below 20°C adversely affects root development, delays germination, reduces seedling vigor, and lowers survival rates, ultimately resulting in poor plant establishment and yield losses (Sharma *et al.*, 2012). Cold tolerance is particularly important in the northern regions of Bangladesh and other areas where early-season low temperatures limit the success of direct-seeded and transplanted rice. Reduced temperatures effects on rice crops vary depending on the genetic makeup of variety, developmental stage, exposure duration, and cold intensity (Díaz *et al.*, 2006., Sravan *et al.*, 2016). The most sensitive phase of low temperature damage in rice is the booting stage, followed by flowering (Matsuo *et al.*, 1995). Low temperatures during the booting and flowering periods reduce plant growth, significantly reduce spikelet fertility, and increase vulnerability to disease (Zeng *et al.*, 2017). The extent to which fertility is reduced varies by variety and the duration of exposure to cold (Satake *et al*., 1983).

With the global population projected to surpass 9 billion by 2050, rice demand is expected to increase by 70–85% (FAO, 2017; Ray *et al.*, 2013). Meeting this rising demand under climate change requires the development of climate-resilient cultivars that can withstand multiple abiotic stresses. Recent advances in molecular genetics, physiology, and adaptive agronomy provide opportunities to accelerate this process (Zhang *et al.*, 2022). Against this backdrop, the present study was designed to evaluate a diverse panel of 464 rice genotypes (CRCIL-464) for tolerance to salinity, submergence, and cold at the seedling stage. The findings aim to identify promising lines for use in early-generation breeding programs in Bangladesh, thereby contributing to the development of climate-resilient rice varieties capable of sustaining yields, reducing environmental risks, and promoting food security in stress-prone environments.

MATERIALS AND METHODS

A multi-trait rice diversity panel was formed in the Plant Breeding Division of BRRI under the USAID funded project Climate Resilient Cereal Innovation Lab (CRCIL). The panel comprised 464 genotypes including advanced breeding lines of BRRI and IRRI origin, released varieties and important local and exotic germplasm (see List of germplasm for CRCIL-464 multi-trait diversity panel in supplementary file). The following panel was evaluated independently for three different abiotic stresses i.e. salinity, submergence and cold including respective standard tolerant and sensitive checks. The investigations were carried out in Plant Physiology Net house & cold-water tank and submergence tank of the Plant Breeding Division, BRRI, Gazipur, during T. Aman 2024 and Boro 2024-25 season.

Phenotypic characterization for salinity tolerance at seedling stage

Sprouted seeds of the CRCIL-464 panel, along

with tolerant (IR58443) and sensitive (IRRI154) checks including 12 BRRI's salt tolerant HYVs were sown on a nylon net attached to a Styrofoam sheet, floating on Yoshida's full-strength culture solution, as described by Gregorio *et al.* (1997). The experiment followed an RCB design with two replications. Application of salt stress started at 18 days after sowing with a level of EC 6 dS/m and increased by 2 dS/m each day until reaching a maximum of 12 dS/m. The Yoshida solution was replaced weekly throughout the experiment. The pH and electrical conductivity (EC) of the solution were monitored daily and maintained at 5.0 and 12 dS/m, respectively. Two weeks after stress application, progenies were scored through the SES scale (IRRI, 2013) for classification according to their overall tolerance based on leaf damage symptoms (Table 1).

Table 1. The Standard Evaluation System for rice for salinity scoring at seedling stage (IRRI, 2013).

Score	Observation	Remarks
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants drying	Moderately sensitive
9	Almost all plants are dead or drying	Sensitive

Phenotypic characterization for salinity tolerance at reproductive phase

A total of 34 seedling stage salinity tolerant CRCIL-464 genotypes along with 2 checks were evaluated for reproductive stage tolerance under soil-based system. Sprouted seeds were sown in puddled soil placed in perforated plastic pots, which were then placed inside plastic bowls. Each bowl contained six pots, consisting of four test lines along with tolerant and sensitive checks. Seedlings were grown in the perforated pots for 21 days under tap water, submerging the pots to the brim in a net house. Afterwards, the tap water was replaced with saline water at 10 dS/m. The water level was maintained at the soil surface by adding tap water daily. A separate set without salinity served as control. BRRI-recommended cultural practices were followed until maturity. Grain weights and other yield component traits were measured and recorded and analyzed.

Phenotypic characterization for submergence tolerance at vegetative phase

The CRCIL-464 panel germplasm, including checks, were sown in a standard seedbed to raise seedlings. Twenty-one-day-old seedlings from each entry were transplanted into the submergence tank of the Plant Breeding Division. Each entry consisted of 6 seedlings per line, with two lines per entry, arranged randomly. A single seedling was planted per hill with a spacing of 20 cm x 20 cm. Fourteen days after transplanting, the tank was filled with normal tap water to a height of 1 meter above the soil surface, ensuring complete submergence of the plants. To replicate the turbidity of floodwater in Bangladesh, muddy soil was mixed into the tank water daily. Water quality data recorded before and after creating turbidity. Recovery and survivability scores were noted 7 days after recovery.

Phenotypic characterization for cold tolerance at seedling stage

All genotypes of the CRCIL-464 panel, along with three check varieties (BRRI dhan28, BRRI dhan36, and Hbj B-VI), were evaluated for seedling-stage cold tolerance under artificial conditions using cold-water tanks. Seeds were sown in plastic trays (60 cm × 30 cm × 2.5 cm) filled with granular soil and grown until the three-leaf stage. The trays were then placed in cold-water tanks maintained at a constant temperature of 13 °C. After seven days of cold-water treatment, seedlings were scored for leaf discoloration using the SES scale (IRRI, 2013) (Table 2). Recovery and survival were assessed one week after the removal of cold stress.

Table 2. The Standard Evaluation System for rice for cold scoring at seedling stage (IRRI, 2013).

Score	Observation	Remarks
0-1	No damage to leaves, normal leaf color	Strongly tolerant
2-3	Tip of leaves slightly dried, folded and light green	Tolerant
4-5	Some seedlings moderately folded and wilted, 30-50% seedlings dried, pale green to yellowish leaves	Moderately tolerant
6-7	Seedlings severely rolled and dried, reddish-brown leaves	Sensitive
8-9	Most seedlings dead or drying	Highly sensitive

Data analysis

Data from all experiments were organized using Microsoft Excel. Stress response data under three different abiotic stresses were analyzed using RStudio (https://posit.co/download/rstudio-desktop/) and the Statistical Tool for Agricultural Research (STAR) (http://bbi.irri.org). Graphical representations were generated in RStudio.

RESULTS AND DISCUSSION

Bangladesh is widely recognized as one of the country's most vulnerable to climate change, despite its minimal contribution to global emissions. Rice, the country's staple food crop, is particularly sensitive to climatic variability. Consequently, even slight changes in climate increase the uncertainty of rice production, as climate is a major driver of year-to-year fluctuations in productivity. To ensure sustainable rice production, the development of climate-resilient varieties has become a central objective of BRRI's breeding program.

Salinity tolerance at seedling stage

The breeding successes observed for developing existing salt tolerant varieties follows a long-term goal to combine different traits conferring salt tolerance into an elite background. However, the development is not straightforward because tolerance is controlled by multiple loci or QTLs. Grouping of the genotypes based on the inherent physiological mechanism responsible for salinity tolerance, inter-mating of the genotypes with high degree of expression of the contrasting salinity tolerance mechanism and identifying/screening of the recombinants for pooling of the mechanisms is being followed to enhance the level of salt tolerance further.

At the seedling stage salinity tolerance under hydroponics system the tested CRCIL-464 genotypes showed a multi-modal distribution (Fig. 1) revealing the panel was not normal. Out of the 464 genotypes tested, 32 entries with an average SES score ranging from 3.0 to 4.4 were classified as tolerant, while 25 entries with an

average SES score ranging from 4.5 to 5.8 were considered moderately tolerant (Table 3). The remaining genotypes, which had visual scores between 6 and 9, were classified as sensitive to highly sensitive.

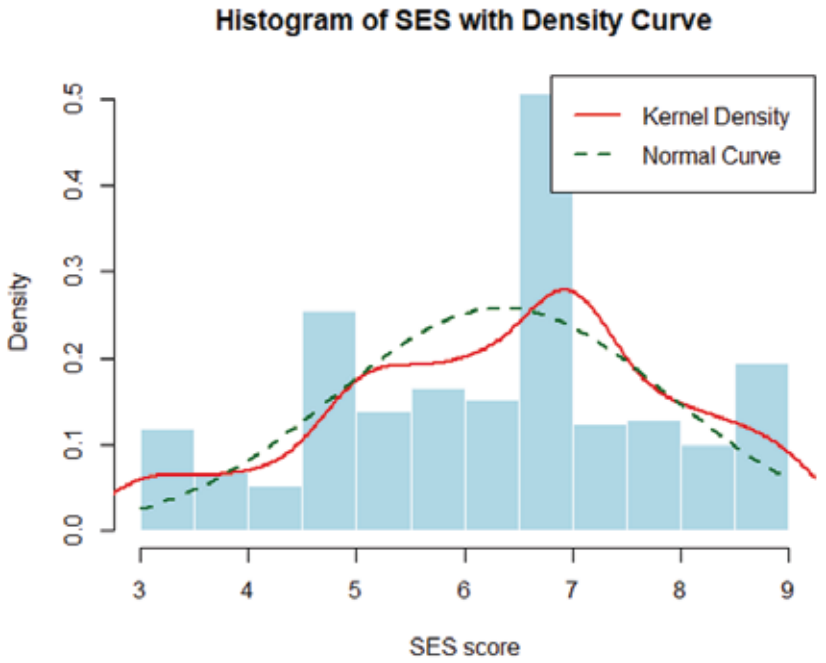


Fig. 1. Histogram of SES with density curve represented distribution of tolerances at seedling stage under salinity stress.

Table 3. List of tolerant and moderately tolerant germplasm identified at seedling stage salinity at 12 dS/m.

SL	Designation	SES	Salinity class	SL	Designation	SES	Salinity class
1	BR10604-5R-58	4.2	**T**	33	BRRI dhan33	4.8	MT
2	BR11715-4R-13	3.6	**T**	34	IR96184-24-1-1-AJY2	4.6	MT
3	BR11607-4R-184	4.4	**T**	35	BR11920-4R-521	4.6	MT
4	BR12459-4R-49	4.0	**T**	36	IR13F478-3	4.6	MT
5	BR12459-4R-3	3.2	**T**	37	BR11921-4R-100	5.0	MT
6	BR12557-5R-80 (835)	3.8	**T**	38	BR11894-R-R-R-R-329	5.0	MT
7	Pi21+Pb1 (34)	4.4	**T**	39	BR12899-4R-169	4.8	MT
8	BR11723-4R-172	4.0	**T**	40	BR13157-4R-174	4.6	MT
9	IR127152-3-22-2-1B	4.4	**T**	41	BR11714-4R-74	4.5	MT
10	BRRI dhan98	4.0	**T**	42	BR10212-4-3-1	4.8	MT

SL	Designation	SES	Salinity class	SL	Designation	SES	Salinity class
11	BR13169-4R-307	3.8	**T**	43	SVIN 352	4.8	MT
12	IR14F550	4.3	**T**	44	BRRI dhan74 (Ck)	5.0	MT
13	BR9536-2-1-17	4.2	**T**	45	IRRI154-Pi9	5.0	MT
14	IR13F458	3.6	**T**	46	BR11940-4R-167	5.0	MT
15	IR13F478	3.2	**T**	47	BR12465-4R-223	5.2	MT
16	Acc. no. 1630	3.0	**T**	48	TP30649	4.5	MT
17	BR10490-1-2-3-87	4.1	**T**	49	BR12274-4R-46	4.8	MT
18	BR11887-5R-368	3.6	**T**	50	BR12423-6R-38	5.0	MT
19	BR12459-4R-103	4.3	**T**	51	BR13171-4R-207	4.6	MT
20	BR12567-5R-91	3.4	**T**	52	IR16F1019	5.0	MT
21	IR16F1148 (Ck)	3.8	**T**	53	Pokkali	5.0	MT
22	BR12459-4R-214	3.5	**T**	54	IR87870-6-1-1-1-1-B	5.0	MT
23	IR14T156	3.8	T	55	BR12096-4R-25	5.5	MT
24	IR100158-B-2-AJYI	3.6	**T**	56	IRRI154-Hd9+Pi9	4.6	MT
25	BR12890-5R-32	4.0	**T**	57	IR64-Pi9 (E)	5.8	MT
26	IR16F1063	3.8	**T**	**T. ck**	**IR58443 (Tolerant)**	**4.12**	T
27	IR4630	4.3	**T**	**S. ck**	**IRRI154 (Sensitive)**	**7.12**	S
28	Acc. no. 234	4.2	**T**				
29	BR13169-4R-227	4.0	**T**				
30	BR11723-4R-322	3.6	**T**				
31	BR12902-4R-257	4.2	**T**				
32	IR 127152-3-22-18-1-B	4.3	T				

Based on the above scoring system the genotypes having average score <4.4 were treated as tolerant (T) and average score 4.5-5.8 were treated as moderately tolerant (MT).

Understanding and manipulating physiological processes related to salt tolerance is crucial for breeding new salt-tolerant rice varieties. The CRCIL-464 diversity core panel demonstrates genetic variation based on SES scores. It is clear from the results that all the materials showed assorted responses to different salt stress conditions. Among these rice genotypes, 32 lines were found to be tolerant, 25 moderately tolerant, and the rest sensitive to highly sensitive. This evaluation helps identify promising rice varieties for breeding salt-tolerant cultivars.

Salinity tolerance at reproductive phase

A total of 35 seedling-stage salinity-tolerant lines from the CRCIL panel, along with two checks, were evaluated for reproductive-stage tolerance under a soil-based system (Gregorio et al., 1997). Among these, three genotypes BR11715-4R-13, BR11921-4R-100, and BR12567-5R-91 produced higher yields than the tolerant check IR58443 (Table 4, Fig. 3). In terms of yield reduction, only BR12557-57-5R-80(835) exhibited less than 50% reduction. The highest-yielding genotype under salinity, BR11715-4R-13, showed a yield reduction of

about 52%, which is close to the threshold for declaring tolerance under stress (Fig. 2). Notably, two genotypes scored 3.8 and 3.6 under seedling-stage salinity stress, suggesting their potential for release and use as whole-growth salinity-tolerant varieties.

Table 4. Phenotypes of selected seedling stage tolerant genotypes at the reproductive phase under salinity stress @10 dS/m.

Genotype	Plant height (cm)	Tiller No.	Filled Grain No.	Spikelet Fertility (%)	Straw Weight (g)	Growth duration (d)
BR13171-4R-125	121.00	13.00	385.00	56.48	26.40	124.00
Acc. no. 1684	123.50	11.00	266.00	38.52	14.15	123.00
BR11303-5R-156	99.50	16.00	211.50	64.35	17.37	120.50
BR11715-4R-13	111.00	13.50	557.00	35.86	26.10	122.50
BR11921-4R-100	89.50	13.50	449.00	52.18	23.81	112.00
BR12459-4R-3	101.00	13.00	458.50	48.19	22.96	125.00
BR12557-57-5R-80(835)	104.50	7.50	324.50	40.76	14.00	124.00
IR17A1211	90.50	9.00	55.57	80.62	7.14	114.00
IR11723-4R-172	92.00	8.50	234.50	59.39	18.51	119.00
IR127152-3-33-2-1B	77.50	12.50	170.00	49.27	14.20	124.00
BRRI dhan98	96.50	11.50	111.00	76.77	24.74	118.00
BR13169-4R-307	105.00	15.00	522.50	31.62	16.81	124.50
BR9536-2-1-17	104.50	12.50	409.50	48.85	20.30	118.00
IR13F458	39.50	4.50	12.50	41.38	5.01	64.50
Acc. no. 1630	106.00	19.50	285.50	68.83	25.68	138.00
BR10490-1-2-3-87	90.00	11.00	256.50	65.58	24.58	125.00
BR11887-5R-368	98.00	10.00	84.50	70.22	23.20	114.50
BR12567-5R-91	89.50	13.00	486.00	26.50	20.52	114.50
IR16F1148	95.00	13.50	165.00	78.89	24.05	117.00
Pi21+Pbi (34)	87.00	12.50	361.00	41.21	16.79	112.00
BRRI dhan67	93.00	16.00	295.50	44.88	20.24	112.00
BRRI dhan97	88.50	16.50	299.00	52.87	24.53	113.00
BRRI dhan99	90.50	16.50	425.50	35.20	24.54	117.00
BR11715-4R-186	126.50	13.50	154.00	77.62	25.23	125.00
IR14T156	106.50	12.50	300.00	52.14	19.15	124.00
BR12459-4R-214	113.00	12.00	461.50	48.77	21.01	126.50
IR100158-B-2-AJY1	107.50	12.50	154.00	71.87	19.36	126.50
Acc. no. 2276	67.50	16.50	396.00	56.37	20.69	112.00
BR12890-5R-32	86.50	16.50	219.00	53.05	22.84	119.50
IR16F1063	47.50	6.50	138.00	33.41	11.16	60.00
Acc. no. 234	131.00	6.00	31.00	45.71	11.17	60.50

Genotype	Plant height (cm)	Tiller No.	Filled Grain No.	Spikelet Fertility (%)	Straw Weight (g)	Growth duration (d)
BR13169-4R-227	94.00	12.50	298.00	52.71	18.48	118.00
BR11723-4R-322	94.00	14.50	276.00	65.56	23.93	124.00
BR12902-4R-257	103.50	12.50	144.34	74.62	17.40	118.00
BR11714-4R-182	98.50	13.00	418.00	22.08	18.53	119.50
IRRI154 (Sen. ck.)	90.29	8.93	146.21	70.05	11.68	124.07
IR58443 (Tol. ck.)	101.50	17.61	450.28	39.68	40.60	121.89
$LSD_{0.05}$	*2.03*	*5.96*	*5.96*	*5.96*		*5.96*
CV (%)	*9.93*	*17.56*	*31.79*	*23.28*		*4.04*

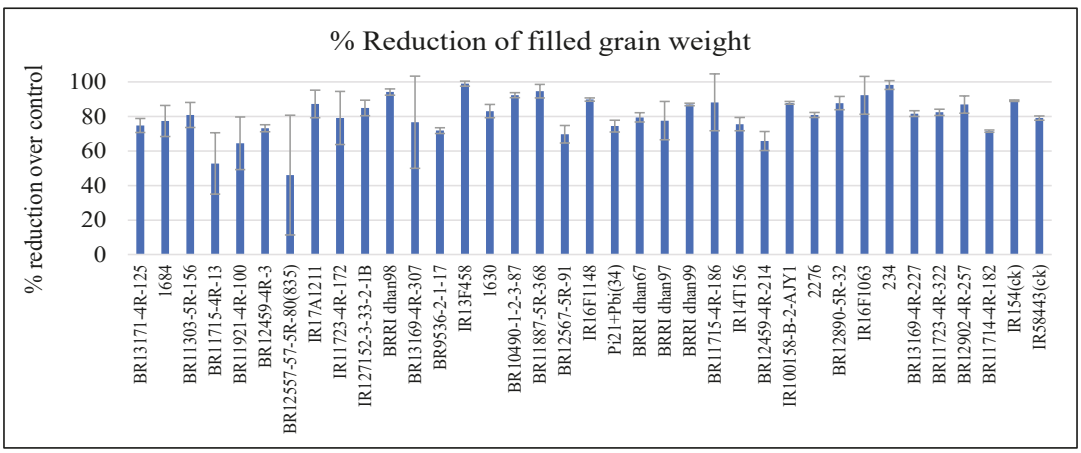


Fig. 2. Percent reduction of filled grain weight under stress condition (salinity 10 dS/m) from 21 DAS to maturity. Error bar indicates standard deviation (*n = 5*).

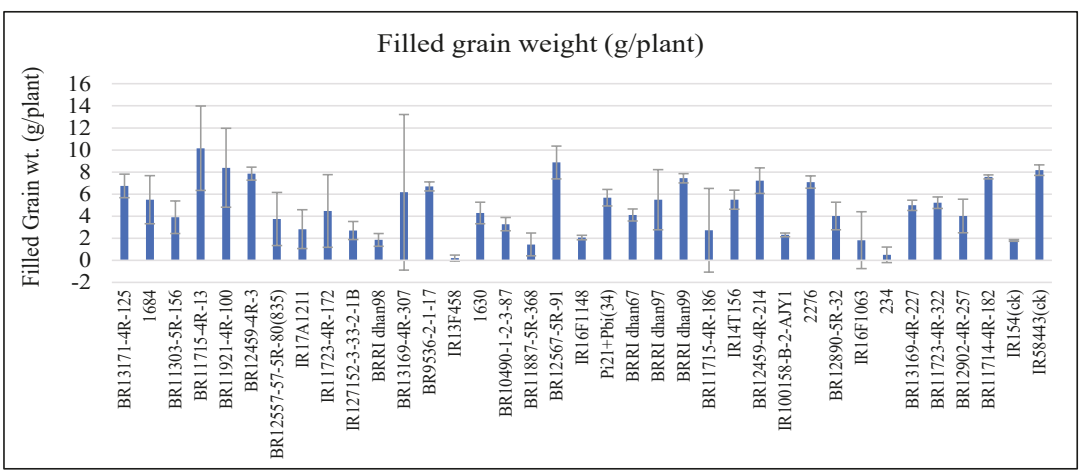


Fig. 3. Filled grain weight under stress condition (salinity 10 dS/m) from 21 DAS to maturity. Error bar indicates standard deviation (*n = 5*).

Submergence tolerance at vegetative phase

The CRCIL-464 panel germplasm, along with standard checks (FR13A and IR42) and four BRRI submergence-tolerant high-yielding varieties (HYVs), were assessed for submergence tolerance at the vegetative stage in the concrete submergence tank at the Plant Breeding Division. Seedlings were raised under normal field conditions in a standard seedbed and transplanted into the concrete tank at 22 days old. Six seedlings per line and two lines per entry were planted using an Augmented Block (RCB) design. Two weeks after transplantation, the plants were completely submerged for 14 days under 1 meter of floodwater. To simulate typical Bangladeshi flood conditions, the water was made turbid daily. Daily measurements of floodwater temperature, pH, dissolved oxygen, and turbidity were taken during the submergence period (Table 6). Seven days after the water receded, plant survivability and recovery scores were recorded. After submergence stress among the CRCIL-464 germplasm, 25 genotypes, including 4 checks, exhibited a survivability range of 8.33% to 100% after recovery, with SES scores ranging from 1 to 9. One genotype, IR14T156, had six planted plants, all of which survived, achieving 100% survivability. Three entries received an SES score of 7, while the remaining scored 9 (Table 5).

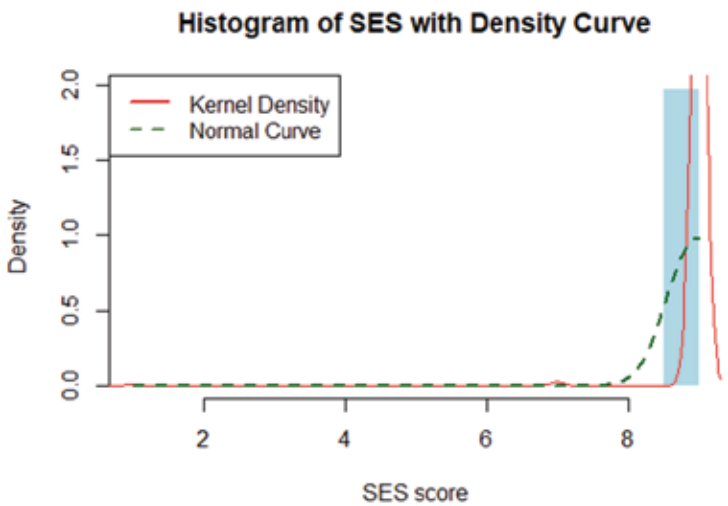


Fig. 4. Histogram of SES with density curve represented distribution of tolerances at seedling stage under submergence stress.

Table 5. Percent survivability (%) with SES of tested germplasm under two weeks of complete submergence.

SL	Entry	Survivability (%) after recovery	SES
1	Capsul	8.33	9
2	BRRI dhan74 (Ck)	8.33	9
3	BR12459-4R-209	8.33	9
4	BR12459-4R-103	8.33	9
5	IR16F1148 (Ck)	50.00	7
6	BR12900-4R-177	16.67	9
7	BR11715-4R-186	8.33	9
8	Acc. no. 2006	60.00	7

SL	Entry	Survivability (%) after recovery	SES
9	Sorsoria	45.45	9
10	Ashful	8.33	9
11	**IR14T156**	**100.00**	**1**
12	BR12905-4R-61	8.33	9
13	BR13169-4R-227	44.44	9
14	BR11894-5R-77	16.67	9
15	IR126952-41-58-26-4-12-12-1	8.33	9
16	BRRI dhan52	25.00	9
17	BRRI dhan108	25.00	9
18	IR16F1019	45.45	9
19	BR12520-5R-75	8.33	9
20	IR126952-28-55-9-9-53-1-6	16.67	9
21	BRRI dhan74 (Ck)	33.33	9
22	BR10211-5-5-7	50.00	7
23	BR11196-5R-83	16.67	9
24	BR11604-4R-84	10.00	9
25	BR11196-5R-445	10.00	9

The results of the submergence screening of the CRCIL-464 panel show significant genetic variability for submergence tolerance among rice genotypes. The distribution for SES was completely skewed towards sensitive indicating less representation of tolerant and moderately tolerant germplasm into the CRCIL-464 panel (Fig. 4). There is a board range of survivability (8.33%–100%), with 100% survival rates for all six seedlings. IR14T156 was the best performer and showed strong resilience in flooding conditions. Most genotypes received SES scores of 9, indicating poor recovery and stress symptoms in post-submergence. Even though certain survivability rates are excellent, the low recovery scores show the importance of post-submergence recovery ability as a breeding trait. Despite the results showing that some genotypes may be able to withstand submergence, they also show the challenge it is to breed for this feature due as survival, recovery, and yield need to be taken into account. Trials should be scaled up, agronomic attributes should be assessed after the flood, and promising lines should be incorporated into crossing or breeding programs with selected elite varieties.

Table 6. Flood water quality data before and after mud mixing in the concrete tank. All data were recorded at 12.00 noon.

	Before mud mixing				After mud mixing			
Day	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)
Day-1 (08/9/2024)	7.6	33.1	6.5	13.0	7.9	33.0	10.1	128.3
Day-2 (09/9/2024)	8.1	33.4	44.8	15.7	8.1	34.0	44.9	100.1
Day-3 (10/9/2024)	8.3	33.5	68.7	14.8	8.2	33.7	62.3	79.9
Day-4 (11/9/2024)	8.3	33.4	57.9	15.5	8.4	33.9	47.9	93.9
Day-5 (12/9/2024)	8.2	34.4	36.5	16.6	8.4	34.7	33.2	323.4
Day-6 (13/9/2024)	8.3	33.4	34.2	57.3	8.5	33.7	28.1	137.3

Day	Before mud mixing				After mud mixing			
	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)
Day-7 (14/9/2024)	*Data not recorded due to rain*							
Day-8 (15/9/2024)	*Data not recorded due to rain*							
Day-9 (16/9/2024)	8.3	28.7	25.6	15.2	8.6	29.9	28.6	69.2
Day-10 (17/9/2024)	8.6	30.7	30.6	6.5	8.7	31.2	28.4	48.6
Day-11 (18/9/2024)	8.7	31.2	30.8	9.1	8.9	32.2	24.3	194.0
Day-12 (19/9/2024)	8.8	32.6	26.2	11.8	8.9	34.1	22.3	182.7
Day-13 (20/9/2024)	8.8	34.1	22.4	15.8	8.9	35.5	17.6	103.3
Day-14 (21/9/2024)	8.8	33.7	19.4	25.9	8.9	34.7	12.3	94.1
Average	8.4	32.7	33.6	18.1	8.5	33.4	30.0	129.6

Note: FNU stands for Formazin Nephelometric Units and also signifies that the instrument is measuring scattered light from the flood water at a 90-degree angle from the incident light. FNU is most often used when referencing the ISO 7027 (European) turbidity method.

Cold tolerance at seedling stage

CRCIL-464 panel germplasm with standard checks BRRI HYVs (4) were evaluated for cold tolerance at the seedling stage. Leaf discoloration scores were assessed 10 days after exposure to cold water treatment, using a 1 to 9 scale. The distribution of leaf discoloration SES follows a multi-modal distribution revealing an uneven inclusion of cold tolerant germplasm (Fig. 5). The recovery ability of rice seedlings was also evaluated two weeks after being removed from the cold-water tank. After cold stress among the CRCIL-464 germplasm, 12 genotypes exhibited a survivability range of 16.7% to 83.3% after recovery, with SES scores ranging from 1 to 9. Two genotypes SVIN 297 and IRRI154-Hd9+Pi9 showed 83.3% survivability which was highest survivability percentage. These genotypes require further evaluation.

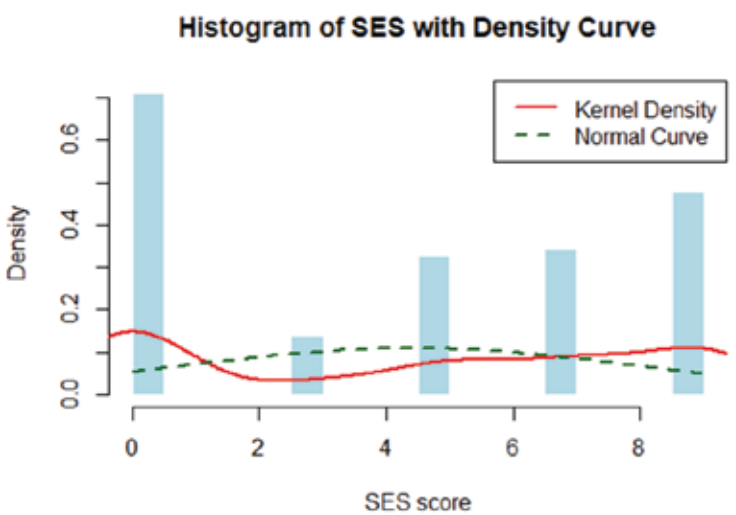


Fig. 5. Histogram of SES with density curve represented distribution of tolerances at seedling stage under cold stress.

Table 7. Effects of cold stress (water temperature 13 ℃ for 10 days) at the seedling stage of CRCIL-464 panel.

SL	Designation	SES Score	Survivability (%)
1	BR10540-4-1-2-4-1	5	50.0
2	**SVIN 297**	**5**	**83.3**
3	BR12557-5R-80 (835)	5	66.7
4	BRRI dhan98	5	33.3
5	BRRI dhan74 (Ck)	3	33.3
6	BR10317-5R-25	3	50.0
7	BRRI dhan92	7	16.7
8	**IRRI154-Hd9+Pi9**	**7**	**83.3**
9	Binashail	7	16.7
10	BRRI dhan81	7	16.7
11	BRRI dhan52	7	16.7
12	BR12902-4R-48	5	50.0
13	**BRRI dhan28 (Ck)**	**9**	**0.00**
14	**BRRI dhan67 (Ck)**	**9**	**0.00**
15	**Hbj-B-VI (Ck)**	**9**	**0.00**

The wide range of SES scores and survival rates represents the genetic diversity present in the CRCIL-464 panel and emphasizes the potential for identifying cold-tolerant lines suitable for breeding programs. The strong performance of SVIN 297 and IRRI154-Hd9+Pi9 recommends their further evaluation under multilocation trials and molecular analysis to confirm the stability of their cold tolerance.

Overall, this study provides promising genetic resources for enhancing cold tolerance in rice, which is particularly critical for direct-seeded rice systems and high-altitude or temperate regions, especially for northern part of Bangladesh where low temperatures at the seedling stage can severely affect crop establishment.

CONCLUSION

The evaluation of the CRCIL-464 multi-trait diversity panel under salinity, submergence, and cold stress revealed substantial genetic variation for abiotic stress tolerance in rice. A set of genotypes demonstrated consistent tolerance at both the seedling and reproductive stages under salinity stress, highlighting their potential for whole-growth-stage salt tolerance. Submergence screening identified a few highly resilient lines, though the overall panel was skewed toward sensitivity, underscoring the need for introgression of stronger submergence tolerance alleles. Cold tolerance assessment identified several promising genotypes with high recovery and survivability, warranting further validation. Collectively, these results provide a valuable genetic resource for breeding climate-resilient rice varieties. The identified tolerant lines offer strong candidates for use in BRRI's breeding pipeline to develop varieties with enhanced resilience to multiple abiotic stresses, thereby supporting sustainable rice production in stress-prone environments such as Bangladesh.

AUTHORS' CONTRIBUTION

MSR and KMI conceived the study and designed the research. AAS, JJ, and MSP assisted in conducting the experiments and contributed to the initial preparation of the manuscript. KMI and AR developed the multi-trait diversity panel and provided financial and technical support. MSR performed the data analysis and led the manuscript writing.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the members of the Plant Breeding Division of BRRI for their contributions to the development of the multi-trait diversity panel CRCIL-464. This research was carried out using the facilities of the Plant Physiology and Plant Breeding Divisions of BRRI. Special thanks are extended to Md. Nazrul Islam and Milon Hossain for their continuous support throughout the study.

FUNDING

This research was supported by the Climate Resilient Cereal Innovation Lab (CRCIL) project, with technical assistance from Kansas State University and funding from the U.S. Department of State.

DECLARATION OF INTERESTS

The authors declare that there are no ethical or financial conflicts of interest.

REFERENCES

Adkins, S. W., Shiraishi, T., & McComb, J. A. (1990). Submergence tolerance of rice: A new glasshouse method for the experimental submergence of plants. *Physiologia Plantarum*, 80(4), 642–646.

Aghaee, A., Moradi, F., Zare-Maivan, H., Zarinkamar, F., Pour-Irandoost, H., & Sharif, P. (2011). Physiological responses of two rice (Oryza sativa L.) genotypes to chilling stress at the seedling stage. *African Journal of Biotechnology*, 10(40), 7617–7621.

Bailey-Serres, J., Fukao, T., Ronald, P., Ismail, A. M., Heuer, S., & Mackill, D. J. (2010). Submergence-tolerant rice: SUB1's journey from landrace to modern cultivar. *Rice*, 3(2–3), 138–147. https://doi.org/10.1007/s12284-010-9048-5

Cheng, H. T., Jiang, H., Xue, D. W., Guo, L. B., Zeng, D. L., Zhang, G. H., & Qian, Q. (2008). Mapping of QTL underlying tolerance to alkali at germination and early seedling stages in rice. *Acta Agronomica Sinica*, 34, 1719–1727.

Da-Cruz, R. P., Milach, S. C. K., & Federizzi, L. C. (2006). Inheritance of rice cold tolerance at the germination stage. *Genetics and Molecular Biology*, 29(2), 314–320.

Díaz, S. H., Morejón, R., Castro, R., & Pérez León, N. (2006). Comportamiento de genotipos de arroz (Oryza sativa L.) seleccionados para tolerancia a bajas temperaturas en siembra temprana de frío. *Cultivos Tropicales*, 27(1), 71–75.

Dolferus, R. (2014). To grow or not to grow: A stressful decision for plants. *Plant Science*, 229, 247–261.

Fischer, R. A., Byerlee, D., & Edmeades, G. O. (2014). *Crop yields and global food security: Will yield increase continue to feed the world?* Australian Centre for International Agricultural Research.

Fitzgerald, M. A., McCouch, S. R., & Hall, R. D. (2009). Not just a grain of rice: The quest for quality. *Trends in Plant Science*, 14(3), 133–139. https://doi.org/10.1016/j.tplants.2008.12.004

Flowers, T. J., & Flowers, S. A. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management*, 78(1–2), 15–24.

Food and Agriculture Organization of the United Nations. (2017). *The future of food and agriculture—Trends and challenges*. FAO.

Food and Agriculture Organization of the United Nations. (2024, November 25). *Bangladesh: Impact of the floods on agricultural livelihoods and food security in the eastern part of the country—DIEM-Impact Report*, *September 2024*. FAO.

Galvani, A. (2007). The challenge of food sufficiency through salt-tolerant crops.

Reviews in Environmental Science and Biotechnology, 6, 3–16.

Gregorio, G. B. (1997). *Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP)* (Doctoral dissertation, University of the Philippines Los Baños).

Haque, S. A. (2006). Salinity problems and crop production in coastal regions of Bangladesh. *Pakistan Journal of Botany*, 38(5), 1359–1365.

Jain, M., Tyagi, A., & Khurana, J. (2008). Constitutive expression of a meiotic recombination protein gene homolog, OsTOP6A1, from rice confers abiotic stress tolerance in transgenic *Arabidopsis* plants. *Plant Cell Reports*, 27, 767–778.

Kamran, M., Parveen, A., Ahmar, S., Malik, Z., Hussain, S., Chattha, M. S., Saleem, M. H., Adil, M., Heidari, P., & Chen, J. T. (2019). An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *International Journal of Molecular Sciences*, 21(1), 148. https://doi.org/10.3390/ijms21010148

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, 59, 1–6. https://doi.org/10.1007/s11103-005-2159-5

Maas, E. V., & Hoffman, G. J. (1977). Crop salt tolerance—Current assessment. *Journal of the Irrigation and Drainage Division*, 103(IR2), 115–134.

Matsuo, T., Kumazawa, K., Ishii, R., Ishihara, K., & Hirata, H. (1995). *Science of the rice plant: Vol. 2. Physiology*. Food and Agriculture Policy Research Centre.

Moradi, F., & Ismail, A. M. (2007). Responses of photosynthesis, chlorophyll fluorescence, and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. *Annals of Botany*, 99(6), 1161–1173.

Moradi, F., Ismail, A. M., Gregorio, G. B., & Egdane, J. A. (2003). Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. *Indian Journal of Plant Physiology*, 8, 105–116.

Munns, R. (2011). Plant adaptations to salt and water stress: Differences and commonalities. In I. Turkan (Ed.), *Advances in botanical research* (Vol. 57, pp. 1–32). Elsevier. https://doi.org/10.1016/B978-0-12-387692-8.00001-1

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nahar, K., Biswas, J. K., Shamsuzzaman, A. M. M., Hasanuzzaman, M., & Barman, H. N. (2009a). Screening of Indica rice (Oryza sativa L.) genotypes against low-temperature stress. *Botany Journal International*, 2, 295–303.

Nahar, K., Hasanuzzaman, M., & Majumder, R. R. (2009b). Effect of low-temperature stress in transplanted Aman rice varieties mediated by different transplanting dates. *Academic Journal of Plant Science*, 2, 132–138.

Najar, Z. A., Sheikh, F. A., Najeeb, S., Shikari, A. B., Ahangar, M. A., Sheikh, G. A., & Wani, S. H. (2018). Genotypic and morphological diversity analysis in high-altitude maize (*Zea mays* L.) inbreds under Himalayan temperate ecologies. *Maydica*, 63, 1–7.

Pitman, M. G., & Läuchli, A. (2002). Global impact of salinity and agricultural ecosystems. In A. Läuchli & U. Lüttge (Eds.), *Salinity: Environment—Plants—Molecules* (pp. 3–20). Kluwer Academic Publishers.

Ram, P. C., Singh, A. K., Singh, B. B., Singh, V. K., Singh, H. P., Setter, T. L., Singh, V. P., & Singh, R. K. (1999). Environmental characterization of floodwater in eastern India: Relevance to submergence tolerance of lowland rice. *Experimental Agriculture*, 35(2), 141–152.

Rashid, A., Sofi, N. R., Shikari, A. B., Khan, G. H., Waza, S. A., Sheikh, F. A., Parray, G. A., Bhat, M. A., Sofi, M., & Hussain, A. (2019). Developing rice hybrids for

temperate conditions using a three-line approach. *Indian Journal of Genetics and Plant Breeding*, 79, 25–33.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PLOS ONE*, 8(6), e66428. https://doi.org/10.1371/journal.pone.0066428

Satake, T., & Koike, S. (1983). Circular dense planting water culture of rice plants for obtaining many uniform panicles of main stems from a pot. *Japanese Journal of Crop Science*, 52(4), 598–600.

Senanayake, R. N. N. H., Herath, H. M. V. G., & Wickramesinghe, I. P. (2017). Phenotypic screening of rice varieties for tolerance to salt stress at seed germination, seedling, and maturity stages. *Tropical Agricultural Research*, 29(1), 90–100.

Septiningsih, E. M., Pamplona, A. M., Sanchez, D. L., Neeraja, C. N., Vergara, G. V., Heuer, S., Ismail, A. M., & Mackill, D. J. (2009). Development of submergence-tolerant rice cultivars: The Sub1 locus and beyond. *Annals of Botany*, 103(2), 151–160.

Singh, R. K., & Flowers, T. J. (2010). Physiology and molecular biology of the effects of salinity on rice. In M. Pessarakli (Ed.), *Handbook of plant and crop stress* (3rd ed., pp. 901–942). Taylor & Francis.

Singh, R., Singh, Y., Xalaxo, S., Verulkar, S. B., Yadav, N., Singh, S., Singh, N., Prasad, K., Kondayya, K., Rao, P. R., et al. (2016). From QTL to variety—Harnessing the benefits of QTLs for drought, flood, and salt tolerance in mega rice varieties of India through a multi-institutional network. *Plant Science*, 242, 278–287. https://doi.org/10.1016/j.plantsci.2015.10.018

Soil Resource Development Institute. (2010). *Saline soils of Bangladesh*. SFSDP Program, Ministry of Agriculture, People's Republic of Bangladesh.

Sravan, T., Jaiswal, H. K., Waza, S. A., & Priyanka, K. (2016). Analysis of variability and character association in indigenous aromatic rice (Oryza sativa L.). *Electronic Journal of Plant Breeding*, 7(1), 159–164.

Suzuki, K., Nagasuga, K., & Okada, M. (2008). The chilling injury induced by high root temperature in the leaves of rice seedlings. *Plant Cell Physiology*, 49(3), 433–442.

Thakur, P., Kumar, S., Malik, J., Berger, J., & Nayyar, H. (2010). Cold stress effects on reproductive development in grain crops: An overview. *Environmental and Experimental Botany*, 67(3), 429–443.

van Genuchten, M. T., & Hoffman, G. J. (1984). Analysis of crop salt tolerance data. In I. Shainberg & J. Shalhevet (Eds.), *Soil salinity under irrigation: Process and management* (pp. 258–271). Springer.

Waza, S. A., & Jaiswal, H. K. (2015). Effect of WA cytoplasm on yield and yield attributes of rice hybrids. *Oryza*, 52(1), 100–104.

Yang, X. L., Wang, B. F., Chen, L., Li, P., & Cao, C. G. (2019). The different influences of drought stress at the flowering stage on rice physiological traits, grain yield, and quality. *Scientific Reports*, 9, 3742. https://doi.org/10.1038/s41598-019-40550-4

Ye, H., Du, H., Tang, N., Li, X., & Xiong, L. (2009). Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. *Plant Molecular Biology*, 71(3), 291–305. https://doi.org/10.1007/s11103-009-9524-8

Zang, J. P., Sun, Y., Wang, Y., Yang, J., Li, F., Zhou, Y. L., Zhu, L. H., Jessica, R., Mohammadhosein, F., Xu, J. L., & Li, Z. K. (2008). Dissection of genetic overlap of salt tolerance QTLs at the seedling and tillering stages using backcross introgression lines in rice. *Science in China Series C: Life Sciences*, 51(7), 583–591.

Zeng, Y., Zhang, Y., Xiang, J., Uphoff, N. T., Pan, X., & Zhu, D. (2017). Effects of low-temperature stress on spikelet-related parameters during anthesis in Indica–Japonica hybrid rice. *Frontiers in Plant Science*, 8, 1350. https://doi.org/10.3389/fpls.2017.01350

Bangladesh Rice J.28(1): 17-39, 2024, doi.org/10.3329/brj.v28i1.87024

Developing Salinity-Tolerant Hybrid Rice Parental Lines through Integrative Breeding and Molecular Approaches

M R Quddus[1,3], M A Rahman[2*], M Hasan[3], M G Rasul[3], M A Mannan[4], M J Hasan[1]

ABSTRACT

Salinity stress is a major abiotic constraint limiting rice production in coastal and irrigated ecosystems, affecting approximately 20% of global agricultural land. Developing salinity-tolerant hybrid rice parental lines is critical for sustaining productivity in salt-affected areas, yet systematic efforts integrating tolerance traits with fertility restoration or maintenance ability remain limited. This study reports the development of 49 new salinity-tolerant hybrid rice parental lines through strategic crossing, Best Linear Unbiased Prediction (BLUP)-based selection, and integrated molecular–phenotypic validation. A total of 286 genetically fixed entries derived from 22 crosses among the parental lines (14 maintainer × maintainer and 8 restorer × restorer), along with 19 elite lines, were evaluated under salinity stress environment. Parental lines with Standard Evaluation Score (SES) values of 3–5 were strategically crossed and advanced through field rapid generation advance (FRGA). BLUP-based selection identified 54 superior genotypes exceeding the population mean plus one standard deviation for yield. Salinity tolerance was further confirmed through screening under 12 dS/m stress at the seedling stage, which identified 10 highly tolerant parental lines (SES 3) and 19 moderately tolerant parental lines (SES 5). Molecular marker analysis using DRRM-Rf3-5 and DRCG-RF4-14 markers, coupled with test cross pollen fertility testing, successfully validated 25 maintainer lines and 24 restorer lines. Ward's D^2 hierarchical clustering classified the selected lines into three distinct clusters representing late-maturing high-yielding, medium-duration tall, and early-maturing high-tillering ideotypes. These newly developed parental lines provide valuable genetic resources for breeding high-yielding, salinity-tolerant hybrid rice varieties suited to salt-affected ecosystems, thereby contributing to sustainable rice production and enhanced food security.

Keywords: salinity tolerance, hybrid rice, parental line development, molecular markers, fertility restoration

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over half the global population, and hybrid rice technology has emerged as a key strategy for enhancing productivity, delivering 15–30% yield advantages over conventional varieties through heterosis exploitation (Rout *et al.*, 2020). However, salinity stress poses a major constraint to rice production, particularly in coastal and irrigated ecosystems where approximately 20% of agricultural lands are affected (Korres *et al.*, 2022). Salinity reduces rice yield through osmotic stress and ionic toxicity, with susceptible varieties experiencing 50–70% yield losses under moderate to severe stress conditions

[1]Hybrid Rice Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh
[2]Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh
[3]Department of Genetics and Plant Breeding, Gazipur Agricultural University, Salna, Gazipur 1706, Bangladesh
[4]Department of Agronomy, Gazipur Agricultural University, Salna, Gazipur 1706, Bangladesh
[*]Corresponding author's E-mail: akhlas08@gmail.com (M A Rahman)

(Sellathdurai *et al.*, 2024). In regions such as South Asia, Southeast Asia, and Africa, expanding salt-affected areas threaten food security and farmer livelihoods, necessitating the development of salinity-tolerant rice varieties, including hybrid rice (Mheni *et al.*, 2024).

Molecular breeding has revolutionized salinity tolerance improvement through the identification and deployment of major quantitative trait loci (QTLs), particularly Saltol on chromosome 1, which harbors the *OsHKT1;5* gene responsible for Na^+ exclusion (Rekha *et al.*, 2022). Marker-assisted backcrossing (MABC) programs have successfully introgressed *Saltol* into elite varieties, yielding significant improvements. For instance, DRR Dhan 58, developed through *Saltol* introgression into Improved Samba Mahsuri, demonstrated a 24% yield advantage in coastal saline areas and was released for cultivation (Rekha *et al.*, 2022). Similarly, the early-maturing variety KKL(R)3 showed robust seedling tolerance and substantial yield gains under saline conditions (Saminadane *et al.*, 2024). Recent meta-QTL analyses have identified 65 consensus genomic regions for salinity tolerance, providing refined targets for marker-assisted selection (Satasiya *et al.*, 2024). Salt tolerance in hybrid rice is governed by complex genetic mechanisms controlling ion homeostasis, particularly Na^+ exclusion and K^+ retention (Thippani, 2017). The major QTL *Saltol* (from Pokkali) on chromosome 1 and the *SKC1* (from Nonabokra) gene regulate Na^+/K^+ balance at seedling and reproductive stages (Yadav *et al.*, 2018). Marker-assisted selection (MAS) employs marker-assisted backcrossing with foreground and background selection using SSR, SNP, and KASP assays to introgress these loci into elite parental lines (Islam *et al.*, 2024). Donor genotypes like FL478, Pokkali, and Nona Bokra provide favorable alleles for lower shoot Na^+ and higher K^+ accumulation (Thippani, 2017). The breeding strategy involves pyramiding multiple tolerance QTLs into cytoplasmic male sterile (CMS), maintainer (B-line) and restorer (R) lines, followed by hybrid development (Niu *et al.*, 2025). Shenyanyou 1 represents a successfully developed salt-tolerant hybrid japonica rice through *SKC1* introgression using KASP markers and high-throughput breeding chips (Niu *et al.*, 2025).

Despite these advances in salinity tolerance breeding, developing hybrid rice parental lines that simultaneously possess salinity tolerance and fertility restoration or maintenance ability remains underexplored. Hybrid rice production depends on well-characterized CMS lines, B-lines, and R-lines carrying *Rf3* and *Rf4* genes (Ponnuswamy *et al.*, 2020). While marker-assisted conversion of partial restorers into complete restorers has been demonstrated (Ponnuswamy *et al.*, 2020), the integration of salinity tolerance QTLs with fertility genes in hybrid parental lines has not been systematically addressed.

In this context, the present study reports the development of 49 new salinity-tolerant hybrid rice parental lines comprising 25 B-lines and 24 R-lines. These lines were developed through strategic crossing of tolerant parents, Best Linear Unbiased Prediction (BLUP)-based selection for yield and agronomic traits, molecular marker validation of *Rf3* and *Rf4* genes, and phenotypic confirmation through pollen fertility testing. These selected parental lines were further characterized using Ward's D^2 clustering and genetic diversity analysis. This integrated breeding approach addresses a critical gap in hybrid rice breeding by providing diverse, salinity-tolerant parental lines specifically designed for developing high-yielding hybrids suitable for salt-affected ecologies, thereby contributing to sustainable rice production and global food security.

MATERIALS AND METHODS

Fourteen B×B cross and eight R×R crosses (Table 1) were done in Aman 2022-23 season following the results of seedling stage screening of 276 parental lines of hybrid rice at the net house of the Plant Physiology division, BRRI. Nineteen elite lines were collected from the salinity breeding team, Plant Breeding division, BRRI, to identify potential restorer and maintainer lines for hybrid rice development.

Table 1. B×B and R×R crosses targeting parental line development with high yield and salinity tolerance.

Cross registration no.	Cross	Female Parent		Male Parent		F_6 Entry evaluated
		SES		SES		
		Env 1	Env 2	Env 1	Env 2	
B×B cross						
HRB 501	BHR95/BHR182	5	5	5	5	12
HRB 502	BHR101/BHR184	5	5	5	4	33
HRB 503	BHR121/BHR187	5	5	3	4	4
HRB 510	BHR15/BHR5	5	5	8	8	13
HRB 511	BHR5/BHR15	8	8	5	5	23
HRB 512	BHR11/BHR22	4	5	3	3	14
HRB 513	BHR22/BHR11	3	3	4	5	31
HRB 516	BHR22/BHR3	3	3	8	8	10
HRB 517	BHR13/BHR8	7	7	5	5	4
HRB 518	BHR8/BHR13	5	5	7	7	5
BBC11	BHR193/BHR164	4	4	5	5	4
BBC12	BHR164/BHR193	5	5	4	4	12
BBC13	BHR182/BHR171	5	5	8	7	8
BBC14	BHR159/BHR182	5	5	5	5	9
R×R cross						
HRR 264	BHR202/BHR365	5	3	4	5	10
HRR 269	BHR323/BHR369	3	5	3	3	8
HRR 271	BHR321/BHR373	5	5	3	5	31
HRR 272	BHR323/BHR373	3	5	3	5	14
RRC3	BHR355/BHR285	3	3	5	5	3
RRC9	EL 224R /BHR371			5	5	12
RRC11	BHR334/ CHA15R	5	5			1
HRR 281	BHR321/BHR374	5	5	6	5	6
Elite lines from Plant Breeding Division, BRRI						19
Total genotypes evaluated						286

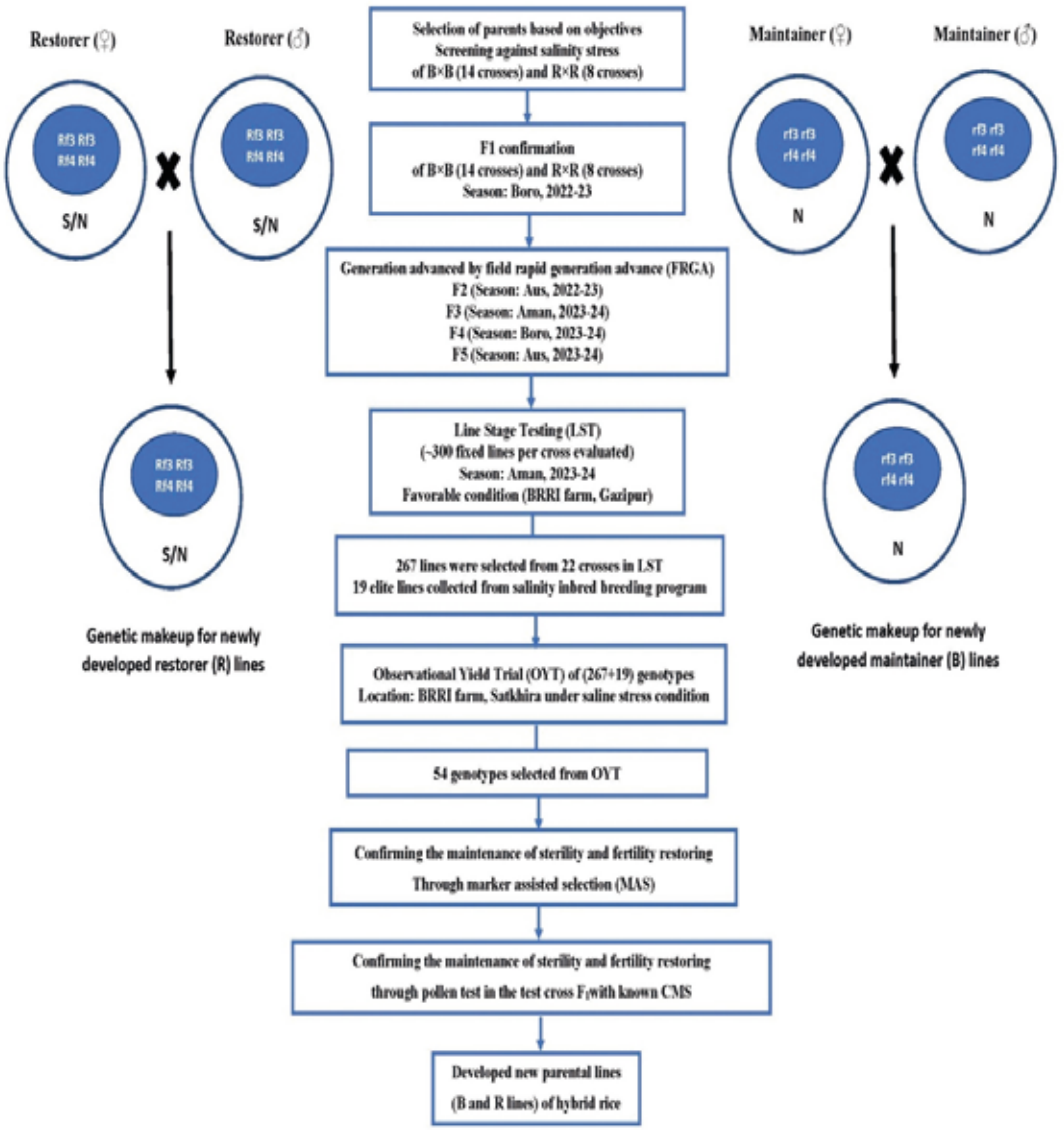
Thirty parental lines (7 BRRI-developed and 23 exotic) were used for crossing and the development of new parental lines. Among these 13 parents contain genes for salinity tolerance (e.g. *Saltol, qSIS1L*) and 7 parents contain genes for both salinity and drought tolerance, 11 parents contain genes for drought tolerance (e.g. *qDTY12.1, qDTY3.1, qDTY3.2*). Parents BHR22 and BHR365 contained both *Saltol* and *qDTY12.1, qDTY3.1, qDTY3.2* genes, conferring a dual tolerance mechanism (Table 2).

Table 2. Genes conferring salinity tolerance in hybrid rice parents utilized for new cross derived B and R line development.

Sl	HRD accession	Genotyping code	Salinity tolerance			Drought tolerance		
			Gene	Allele source	SNP marker	Gene	Allele source	SNP marker
1	BHR5	GS_5_IR77803B_1				*qDTY3.2*	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
2	BHR8	GS_8_IR75595_B_1	*Saltol*	FL478, IR 107321-1-141-3-120	IRRI_SNP0994_SALTOL-AUS (chr1: 11460344)	*qDTY3.2*	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
3	BHR11	GS_11_IR78361B_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)			
4	BHR13	GS_13_IR77811B_1				*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2 (chr12: 17396363)
						qDTY3.2	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
5	BHR22	GS_22_IR79155B_1	*Saltol*	FL478, IR 107321-1-141-3-120	IRRI_SNP0994_SALTOL-AUS (chr1: 11460344)	*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2 (chr12: 17396363)
						qDTY3.1	Apo	IRRI_SNP1021_DTY3-1_2 (chr3: 31008659)
						qDTY3.2	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
6	BHR101	GS_94_BRRI13A_B_1	*qSIS1L*	FL478, Capsule	IRRI_SNP1007_QSES1-2_3 (chr1: 40362958)			
7	BHR159	GS_138_BRRI100B_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)	*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2 (chr12: 17396363)
8	BHR182	GS_157_IR105687B_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)	*qDTY3.2*	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
9	BHR193	GS_168_IR78369B_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)			
10	BHR202	GS_172_BRRI10R_1	*Saltol*	FL478, IR 107321-1-141-3-120	IRRI_SNP0994_SALTOL-AUS (chr1: 11460344)			
11	BHR285	GS_243_BasmatiR_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)			

Sl	HRD accession	Genotyping code	Salinity tolerance			Drought tolerance		
			Gene	Allele source	SNP marker	Gene	Allele source	SNP marker
12	BHR323	GS_262_CHH_67R_1				*qDTY12.1*	Way Rarem,	IRRI_SNP1081_DTY12-1_2(chr12: 17396363)
13	BHR334	GS_273_WinR_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)			
14	BHR355	GS_294_SyngentaR_N_1_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)	*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2(chr12: 17396363)
						qDTY3.1	Apo	IRRI_SNP1021_DTY3-1_2 (chr3: 31008659)
15	BHR365	GS_304_IR76902_C2_1	*Saltol*	Pokkali, Capsule	IRRI_SNP0995_SALTOL-ARO (chr1: 11462124)	*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2(chr12: 17396363)
						qDTY3.1	Apo	IRRI_SNP1021_DTY3-1_2 (chr3: 31008659)
						qDTY3.2	IR64	IRRI_SNP1016_DTY3-2-IR64_1 (chr3: 1271431)
16	BHR371	GS_310_IR86522_12_1				*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2(chr12: 17396363)
17	BHR374	GS_313_IR90928_15_1	*Saltol*	FL478, IR 107321-1-141-3-120	IRRI_SNP0994_SALTOL-AUS (chr1: 11460344)	*qDTY12.1*	Way Rarem, IR64	IRRI_SNP1081_DTY12-1_2(chr12: 17396363)

The flow chart (Fig. 1) illustrates the sequential process of hybrid rice parental line development.



Blue circle indicates nucleus, white space outside the nucleus indicates cytoplasm, Rf3 and Rf4 denote restorer alleles inside nucleus, rf3 and rf4 designate non-restorer i.e., maintainer alleles inside nucleus, S indicates the presence, and N shows the absence of male sterility gene in the cytoplasm.

Fig. 1. Sequential process of hybrid rice parental line development.

The breeding program began with the selection of parents based on breeding objectives, followed by F_1 confirmation of 14 B×B and 8 R×R crosses during Boro 2022–23. Subsequently, F_2 populations from 22 crosses were advanced through the FRGA method in Aus 2022–23, and continued progression through F_3 (Aman 2023–24), F_4 (Boro 2023–24), and F_5 (Aus 2023–24) generations following Rahman et al. (2019) with little modification of collecting and sowing first matured 5-10 seeds per plant that allowed 3 generations advancement per year.

The F_6 generation (LST) was evaluated under favorable conditions at the BRRI Farm, Gazipur during Aman 2023–24. From this evaluation, 267 selected lines, along with 19 elite collected lines, were evaluated in the Observation Yield Trial (OYT) at the BRRI Farm, Satkhira, under salinity stress conditions. Molecular confirmation was carried out to detect the restorer of fertility genes (*Rf3* and *Rf4*) using Marker-Assisted Selection (MAS), followed by pollen fertility tests in test cross F_1 plants with known CMS lines to verify sterility maintenance and fertility restoration. This systematic breeding pipeline ultimately led to the development of new B and R parental lines for hybrid rice improvement.

Salt Stress Application and Monitoring

Salt (NaCl) stress was imposed on 54 selected genotypes along with two checks at the three-leaf stage (14 days after sowing, DAS) by gradually increasing the culture solution's electrical conductivity (EC) of the nutrient solution over a six-day period to a final level of 12 dS/m. The EC was increased stepwise from 6 dS/m to 8 dS/m to 10 dS/m before reaching the final concentration. Iron (Fe) deficiency was prevented by daily adjusting the pH to 5.0 and replacing the entire Yoshida culture solution (Yoshida *et al.*, 1976) every seven days. Five seedlings per genotype were used for SES scoring. After 18 days of exposure to 12 dS/m salinity, the susceptible checks (IRRI 154 and BRRI dhan28) exhibited significant salt stress, with high SES scores of 7 to 9, which validated the severity of the stress condition and prompted the subsequent collection of SES data across all 54 genotypes.

Marker-Assisted Selection

Marker-assisted selection (MAS) was conducted using the DRRM-Rf3-5 marker located on chromosome 1 and the DRCG-RF4-14 marker located on chromosome 10 (Suresh et al., 2012; Ramalingam *et al.*, 2017) to identify potential restorer and maintainer lines of hybrid rice. PCR products were resolved on a 2% agarose gel, and gel images were documented for analysis.

Table 3. Marker details for tracking *Rf3* and *Rf4* genes

Marker	Sequences (5'-3')		Expected PCR amplicon size (bp)	Restorer	Non-restorer
	Forward Primer	Reverse Primer			
DRRM-Rf3-5	GATGGCACAGCTTCAGAACA	CTAATTCTGGGCGAGCAAAG	140/160	160	140
DRCG-RF4-14	GCAATGCTTGTATTCAGCAAA	TCCAGCTGTAAATCCGTCAA	800/885	800	885

Pollen Fertility Assessment

Fifty-four selected genotypes were crossed with the known WA-CMS (Wild Abortive Cytoplasmic Male Sterile) line BRRI97A. The resulting F_1 seeds were harvested and grown in the subsequent season. Pollen grains from the F_1 plants were gently squeezed onto a glass slide and stained with iodine-potassium iodide (I-KI) solution. Pollen exhibiting a deep stain and round morphology was classified as fertile. Observations were made using a ZEISS Axioscope 5 light microscope at 40X magnification.

Data Analysis

Data collected from Satkhira, Bangladesh (a recognized salinity hotspot) were analyzed using an augmented randomized complete block

(RCB) design. The experimental layout incorporated two check varieties, each replicated seven times. Single environment analysis was performed using Plant Breeding Tools software (PBTools, version 1.4, 2014). Best Linear Unbiased Predictors (BLUPs) and broad sense heritability estimates were calculated for each trait and utilized in selecting superior genotypes. Genetic distance estimation and cluster analysis were conducted using Ward D^2 method. DNA band scoring was carried out utilizing AlphaEaseFC 4.0 software (USA).

RESULTS

The study analyzed the development of new hybrid rice parents focusing on salinity tolerance (measured by the SES score, where low score means tolerant) across 22 distinct crosses among the parental lines of hybrid rice and 19 elite lines, resulting in a total of 286 genetically fixed entries and were evaluated. The crosses were grouped into B×B (14 crosses) and R×R (8 crosses). The parental lines exhibited varying degrees of tolerance, ranging from highly tolerant (SES 3) to highly susceptible (SES 8) across two environments (Env 1 and Env 2). For instance, the B×B cross HRB 510 (BHR15/BHR5) paired a relatively tolerant female (SES 5/5) with a highly susceptible male (SES 8/8), while HRB 502 (BHR101/BHR184) involved two tolerant parents (Female SES 5/5, Male SES 5/4). Among all crosses, HRB 502 produced the largest population for selection, with 33 F_6 entries evaluated, followed by the R×R cross HRR 271 (BHR321/BHR373) with 31 F_6 entries.

Out of the 22 crosses among the parental lines, 15 crosses were identified where both parental lines exhibited SES score of 3 to 5 in all environments, signifying that they are the most promising crosses for introgressing high salinity tolerance into new parents. These 15 crosses, comprising 9 B×B crosses and 6 R×R crosses, collectively contributed 250 F_6 entries—a substantial portion of the total evaluated population.

B×B Crosses (9 crosses)

B×B Crosses provided the highest number of individual tolerant progenies. Key examples include HRB 502 (BHR101/BHR184), which had excellent parental SES scores (Female 5/5, Male 5/4) and produced the largest single population of 33 F_6 entries among all F_6 entries, making it a critical source for high-tolerance selections. Other high-contributing crosses like HRB 501 (BHR101/BHR10) also had consistently low SES scores (5/5 for both parents) and contributed 23 entries.

R×R Crosses (6 crosses)

Although fewer in number, R×R Crosses also demonstrated high salinity tolerance. For example, HRR 264 (RRC1/RRC10) showed favorable parental SES scores (5/3 and 4/5) and contributed to 20 F_6 entries, while HRR 270 (RRC11/RRC10) contributed to 23 F_6 entries. The remaining seven crosses involved at least one parent with a highly susceptible SES score of 7 or 8, confirming that the 15 identified crosses are the primary source for the desired trait combination of high yield and high salinity tolerance in the development of new hybrid rice parental lines.

Genotype selection

A total of 286 genotypes were evaluated using an augmented Randomized Complete Block (RCB) design with two checks replicated seven times. The salinity condition of the experimental site ranged from 4.2-7.3 dSm^{-1} (Fig. 2).

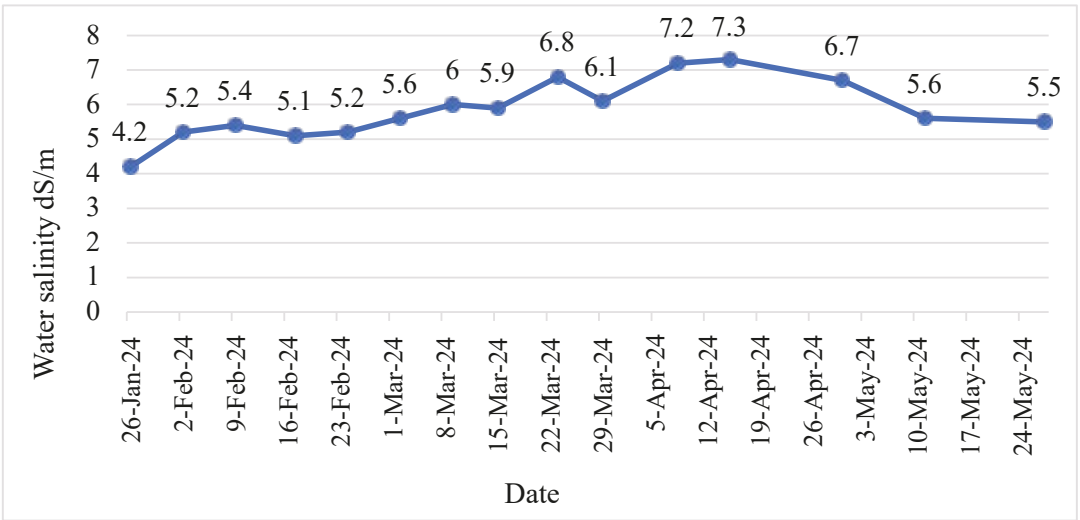


Fig. 2. Salinity stress at the experimental plot in BRRI regional station, Satkhira farm during Boro, 2023-24.

Selection was based on Best Linear Unbiased Predictor (BLUP) values for yield (kg/1.2m2), growth duration (days), plant height (cm), effective tiller number (ET) (Table 4). Genotypes were selected if their yield exceeded both the checks and the population mean plus one standard deviation (μ + 1σ). Only five specific genotypes—BR11716-4R-105, BR11716-4R-120, BR11712-4R-227, BR11715-4R-186, and BR11712-4R-1 achieved a yield greater than the population mean plus two standard deviations (μ + 2σ). In total, 54 genotypes were selected: 27 from BB crosses, 17 from RR crosses, and 10 from collected elite lines.

Table 4. List of selected genotypes with high yield potential.

Entry	Designation	GD*	PH*	ET*	Yield*	Cluster	SES
G1	BR11716-4R-105	150	115	8	1.93	1	5
G2	BR11716-4R-120	161	148	9	1.92	1	5
G3	BR11712-4R-227	159	125	9	1.91	1	5
G4	BR11715-4R-186	159	113	8	1.90	1	5
G5	BR11712-4R-1	153	116	9	1.89	1	5
G6	**HRR271-4R-44**	139	138	8	1.89	2	3
G7	HRB510-4R-51	142	110	13	1.83	3	5
G8	**HRR271-4R-46**	139	147	11	1.83	2	3
G9	BR11723-4R-12	161	115	8	1.83	1	5
G10	BR11723-4R-27	160	117	8	1.83	1	5
G11	**HRB512-4R-108**	132	108	13	1.83	3	3
G12	HRB502-4R-64	137	126	14	1.82	3	5
G13	BBC12-4R-32	144	118	13	1.79	3	5
G14	**HRR269-4R-36**	130	133	11	1.79	2	3
G15	HRR272-4R-16	141	114	14	1.79	3	6
G16	HRR271-4R-45	138	142	11	1.77	2	5
G17	HRB502-4R-132	144	112	13	1.76	3	6

Entry	Designation	GD*	PH*	ET*	Yield*	Cluster	SES
G18	HRB502-4R-236	143	114	19	1.74	3	5
G19	HRB502-4R-35	141	117	16	1.74	3	6
G20	HRB511-4R-27	127	111	11	1.74	3	8
G21	BBC11-4R-35	141	103	11	1.72	3	5
G22	HRB502-4R-227	145	97	14	1.72	3	6
G23	**HRB513-4R-157**	142	117	12	1.72	3	3
G24	BBC14-4R-24	131	99	11	1.71	3	6
G25	**HRB512-4R-129**	136	103	13	1.71	3	3
G26	HRR269-4R-200	144	117	12	1.71	3	6
G27	BR11716-4R-108	161	115	7	1.71	1	3
G28	BBC14-4R-39	132	103	10	1.70	3	6
G29	HRR264-4R-91	129	134	17	1.70	3	7
G30	HRR271-4R-88	141	152	10	1.70	2	5
G31	HRB510-4R-202	134	101	11	1.69	3	8
G32	**HRB503-4R-4**	129	126	14	1.68	3	3
G33	HRR269-4R-15	135	128	9	1.68	3	5
G34	HRR269-4R-23	140	128	17	1.68	3	5
G35	HRR271-4R-63	140	142	15	1.68	3	5
G36	HRR264-4R-44	126	138	16	1.67	3	6
G37	HRR271-4R-32	139	160	10	1.67	2	6
G38	HRB502-4R-216	138	135	14	1.66	3	6
G39	BR11723-4R-172	159	118	7	1.65	1	5
G40	HRB502-4R-52	142	120	13	1.64	3	7
G41	RRC9-4R-51	145	112	12	1.60	3	8
G42	HRR264-4R-8	121	121	14	1.59	3	7
G43	HRB502-4R-68	135	120	10	1.62	3	6
G44	BBC14-4R-49	130	103	10	1.61	3	7
G45	**HRB512-4R-201**	143	114	11	1.64	3	3
G46	**BR11716-4R-102**	160	113	7	1.63	1	3
G47	HRB502-4R-223	135	117	16	1.56	3	6
G48	HRB511-4R-180	135	111	13	1.55	3	9
G49	HRB502-4R-40	139	112	12	1.54	3	6
G50	HRR269-4R-139	132	128	9	1.53	3	6
G51	HRB502-4R-181	127	110	14	1.52	3	6
G52	HRB502-4R-66	132	127	12	1.52	3	6
G53	BBC12-4R-95	139	112	12	1.51	3	5
G54	**RRC9-4R-31**	146	109	10	1.51	3	3
	Population means	136.14	115.16	11.35	1.19		
	SD	9.74	15.08	2.60	0.32		
	LSD (0.05)	9.27	8.92	4.70	0.53		
	Heritability (0.05)	0.90	0.96	0.75	0.80		

*GD: Growth duration (days), PH: Plant height (cm), ET: Effective tiller number, Yield: Rice grain yield in kg per 1.2 sq.m., SD: Standard deviation, LSD (0.05): Least significant difference at 5% level, Heritability (0.05): Heritability in broad sense at 5% level.

Diversity

Genetic distances among the selected 54 genotypes were measured using Euclidean distance. The highest genetic distance was observed between HRB502-4R-181 and BR11716-4R-120 (5.85), followed by HRR264-4R-8 and BR11716-4R-120 (5.56), HRB502-4R-223 and BR11716-4R-120 (5.27), BBC14-4R-49 and BR11716-4R-120 (5.22), HRR264-4R-8 and BR11715-4R-186 (5.14). The lowest genetic distance was observed in BR11723-4R-27 and BR11723-4R-12 (0.17) followed by HRB513-4R-157 and HRR269-4R-200 (0.22), HRB502-4R-40 and BBC12-4R-95 (0.26), BBC14-4R-24 and HRB510-4R-202 (0.37), BR11723-4R-172 and BR11716-4R-102 (0.41). Figure 3 illustrates the frequency distribution of genotype pairs based on pairwise Euclidean distances, which ranged from 0.17 to 5.85, indicating the degree of genetic divergence among the genotypes. The majority of genotype pairs exhibited moderate genetic dissimilarity, with the highest frequency (530 pairs) occurring at a distance class of >2-3. This is followed by 363 pairs at distance >3-4 and 337 pairs at distance >1-2, showing a roughly normal distribution centered around intermediate distances. In contrast, relatively few genotype pairs showed very low (distance 0-1; 61 pairs) or very high (distance 5-6; 11 pairs) divergence. A smaller number of pairs (129) were found at 4-5 distance class (Fig. 3).

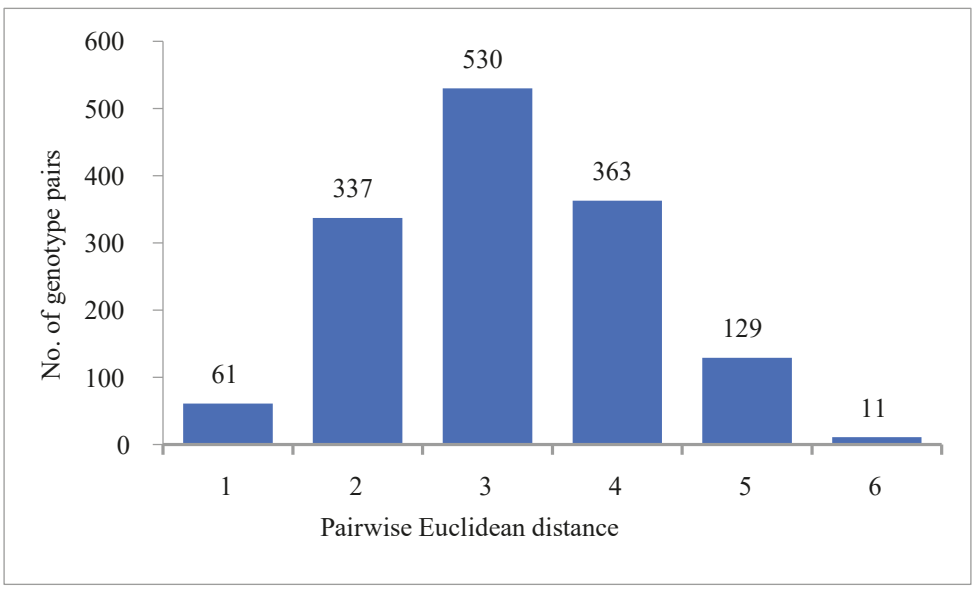


Fig. 3. Frequency distribution of genotype pairs based on pairwise Euclidean distance.

The 54 selected genotypes were then classified into three clusters using Wards D^2 method of clustering (Fig. 4). Clusters 1 and 2 contained potential restorer lines only, whereas cluster 3 contained both restorer and maintainer lines.

Cluster 1 comprised of late-maturing genotypes characterized by the longest growth duration (158.30 days), moderate plant height (119.50 cm), and a moderate number of effective tillers (8.00). Despite having only 10 genotypes, this cluster recorded the highest yield (1.82 kg/1.2 m^2). These results suggest that the late-maturing genotypes in this group were more productive, possibly due to their longer vegetative and reproductive phases, allowing for better assimilate accumulation and grain filling.

Cluster 2 consisted of medium-duration genotypes with an average growth duration of 137.67 days and the tallest plant height (145.33 cm). This group exhibited a moderately high

number of effective tillers (10.17) and achieved a yield of 1.78 kg/1.2 m². With only six genotypes, Cluster 2 represented a small group of tall, medium-duration types that performed moderately well in terms of yield, possibly combining traits of vigor and intermediate maturity (Fig. 4).

Cluster 3 included early-maturing genotypes with the shortest growth duration (136.68 days) and the shortest plant height (116.24 cm). Interestingly, this cluster had the highest number of effective tillers (12.89), yet it recorded the lowest yield (1.67 kg/1.2 m²). Containing 38 genotypes, it was the largest cluster, indicating that early-maturing, short-statured, and highly tillering genotypes were more common but less productive overall.

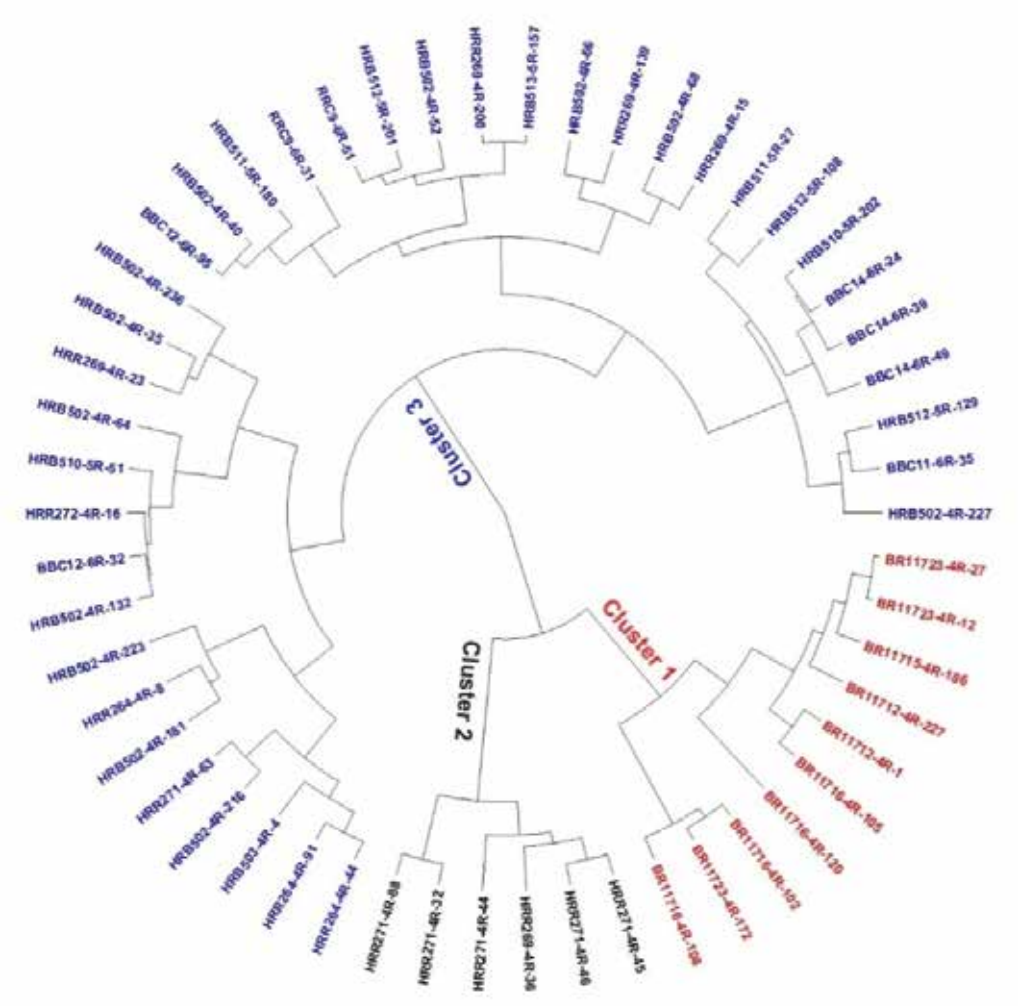


Fig. 4. Hierarchical Clustering using Ward's D^2 method

The intra-cluster distance table, represented by the average silhouette width values, provides an indication of how well each cluster is internally cohesive and distinct from other clusters. Cluster 1 exhibited the highest average silhouette width (0.464), followed closely by Cluster 2 (0.461), and cluster 3 (0.284).

Table 5. Intra and inter-cluster distances depicting the diversity among selected genotypes.

Distance	Intra-Cluster Distance	Inter-Cluster Distance		
	Average Silhouette Width	Euclidean Distance between Scaled Cluster Centers		
Cluster		1	2	3
1	0.464	0	2.88	3.07
2	0.461	2.88	0	2.48
3	0.284	3.07	2.48	0

The inter-cluster distances (Table 5), which shows Euclidean distances between the scaled cluster centers, reflects the degree of genetic divergence among the clusters. The greatest distance (3.07) was observed between Clusters 1 and 3, indicating the widest divergence between these two groups. The distance between Clusters 1 and 2 (2.88) was also substantial, while the smallest distance (2.48) occurred between Clusters 2 and 3, suggesting that these two clusters are relatively closer in multivariate space. Collectively, these results demonstrate that the clusters are reasonably distinct, with Clusters 1 and 3 being the most dissimilar, while Clusters 2 and 3 showing partial similarity or overlap.

Salinity tolerance of the selected lines

Fifty-four rice genotypes along with two susceptible checks (BRRI dhan28 and IR154) were evaluated using RCB design with two replications (Fig. 5). The standard evaluation score (SES) was utilized to select tolerant and susceptible genotypes. Among the selected 54 genotypes, six R lines (HRR271-4R-44, HRR271-4R-46, HRR269-4R-36, BR11716-4R-102, BR11716-4R-108, RRC9-4R-31) and five B lines (HRB512-4R-108, HRB513-4R-157, HRB512-4R-129, HRB503-4R-4, HRB512-4R-201) had SES score of 3 (tolerant). Nineteen genotypes had SES scores of 5 (moderately tolerant) and rest were susceptible having SES scores of 6-9 (Table 4).

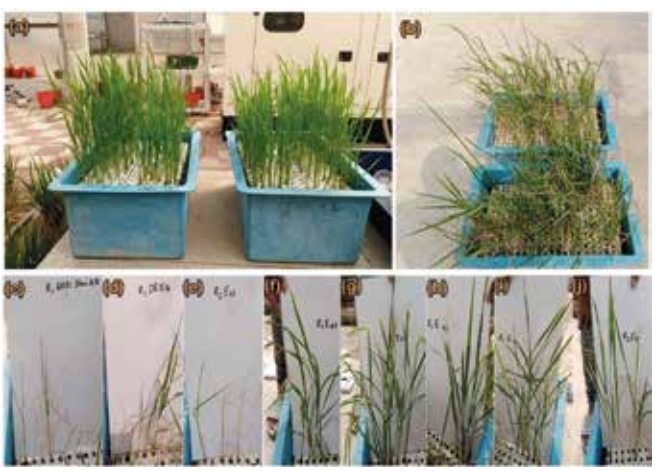


Fig. 5. Comparative Response of 56 Rice Genotypes (including two checks) to Salinity Stress (12 dS/m) at the Seedling Stage. (a) Seedling condition after one week of stress exposure. (b) Seedling condition after three weeks of stress exposure. (c) & (d) Appearance of susceptible checks BRRI dhan28 and IR154 during SES scoring. (e) Example of a susceptible genotype (HRB510-4R-202). (f-i) Examples of tolerant genotypes (HRB512-4R-201, HRR271-4R-44, BR11716-4R-102, and BR11716-4R-108). (j) Example of a moderately tolerant genotype (HRB510-4R-51) based on SES scoring.

Confirmation of Restoring and Maintaining Ability

To identify the restorer and non-restorer alleles of the fertility restorer genes Rf3 and Rf4, the DRRM-Rf3-5 and DRCG-RF4-14 gene-based markers were used, respectively (Table 3, Fig. 6).

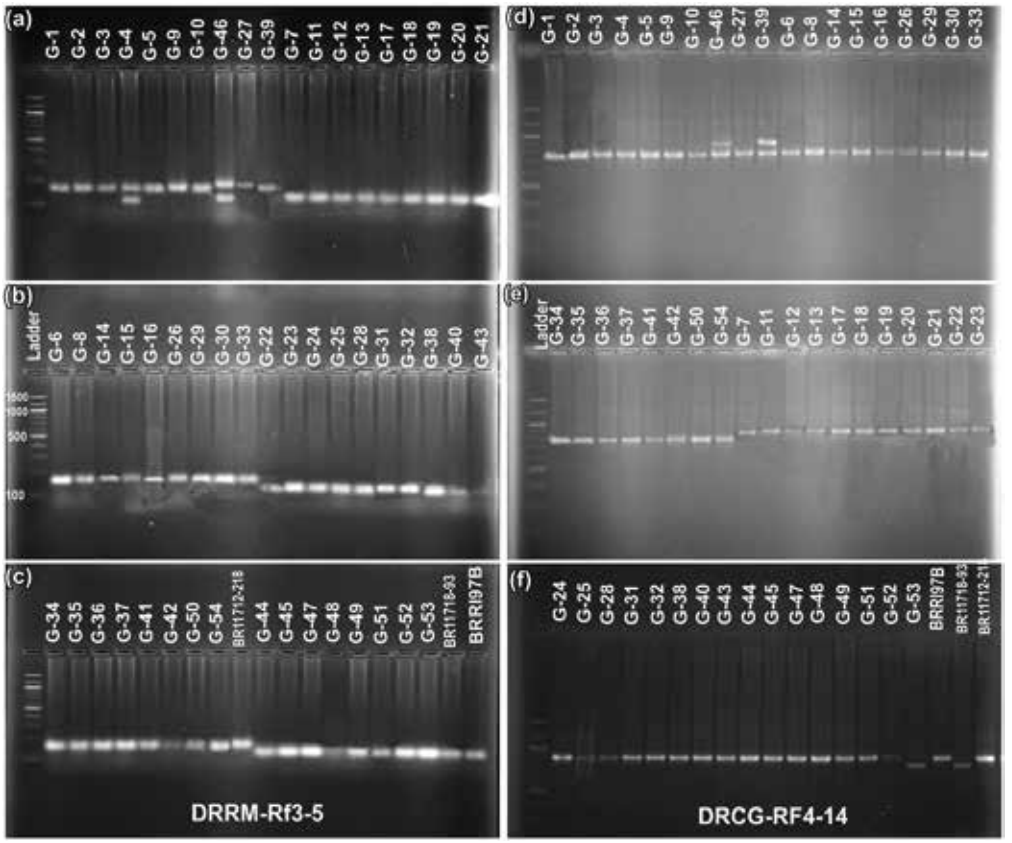


Fig. 6. Marker-aided selection for *Rf3* and *Rf4* genes. (a,b,c): DRRM-Rf3-5 marker showing characteristic bands for G1-G54, BR11712-4R-218, BR11718-4R-93 and BRRI97B (B line for wild abortive cytoplasm of BRRI97A). (d,e,f): DRCG-RF4-14 marker showing characteristic bands for G1-G54, BR11712-4R-218, BR11718-4R-93 and BRRI97B.

The restorer (R line) genotypes showed a homozygous band at 160 bp with the DRRM-Rf3-5 marker and an 800 bp band with the DRCG-RF4-14 marker. In contrast, the maintainer (B line) genotypes produced a homozygous band at 140 bp for the DRRM-Rf3-5 marker and a 885 bp band for the DRCG-RF4-14 marker. Using these markers, 24 genotypes were confirmed as restorer line (R line) and 27 lines as maintainer lines (B line) (Fig. 6). Three selected elite lines BR11715-4R-186 (G4), BR11723-4R-172 (G39), BR11716-4R-102 (G46); and two unselected elite lines BR11712-4R-218, BR11718-4R-93 were discarded due to heterozygosity in the *Rf3* and/or *Rf4* locus.

Pollen Fertility Assessment in test cross F1 Plants

Pollens produced three different staining categories: completely transparent (no stain absorbed), mixture of transparent and black colored pollens and >80% black pollens (Fig. 7). Completely transparent pollens were regarded as sterile, whereas, black colored pollens were classified as fertile.

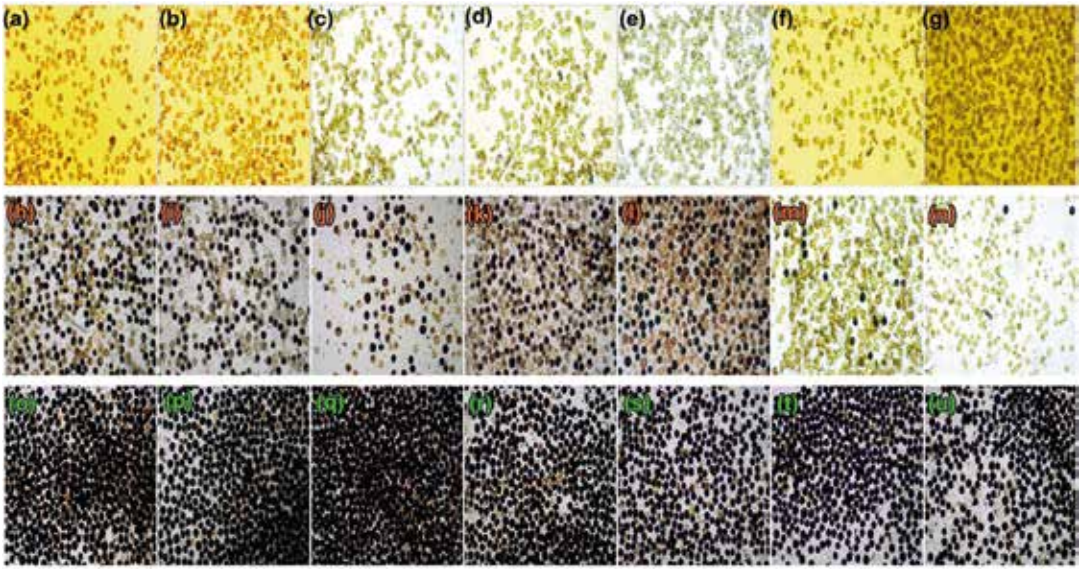


Fig. 7. Pollen test at test cross F_1 progeny (BRRI97A×Elite lines) for Confirmation of restoring and maintaining ability. (a-g): Pollen view of selected new maintainer lines. (h-l): Pollen view of discarded lines due to <80% fertility (BR11715-4R-186 (G4), BR11723-4R-172 (G39), BR11716-4R-102 (G46), BR11712-4R-218, BR11718-4R-93). (m,n): Pollen view of discarded lines due to incomplete sterility of BBC14-4R-49 (G44) and HRB502-4R-181 (G51). (o-u): Pollen view of selected new restorer lines.

Test cross F_1's from the B×B cross derived selected 27 fixed lines, 25 produced 100% sterile pollen (Fig. 7: a-g) and confirmed as new maintainer lines, but two genotypes produced 90-98% sterile pollens (Fig. 7: m,n) and were discarded. Test cross F_1's from the R×R cross derived selected 17 fixed lines produced >80% fertile pollens and selected as new R lines. Test cross F1's from the tested 12 elite lines, 7 produced >80% fertile pollen and identified as new R line, whereas 5 produced <80% fertile pollen and were discarded (Fig. 7: h-l). Though BBC14-4R-49 (G44) and HRB502-4R-181 (G51) showed recessive band for both the used markers but they could not show 100% sterility and showed 90-98% sterility (Fig. 7: m-n).

DISCUSSION

The development of salinity-tolerant hybrid rice parental lines is critical for sustaining rice production in salt-affected areas, which are expanding due to climate change, sea-level rise, and poor irrigation management (Raghavendra *et al.*, 2021). In the present study, 286 genetically fixed entries derived from 22 crosses (14 B×B and 8 R×R) and 19 elite lines were evaluated for salinity tolerance using the Standard Evaluation System (SES) and agronomic performance. The strategic approach of crossing parents with favorable SES scores (3-5) aimed to combine high salinity tolerance with desirable grain yield with agronomic traits and fertility restoration/maintenance ability in the progenies.

Parental Selection Strategy and Cross Performance

The identification of 15 promising crosses (9 B×B and 6 R×R), where both parents exhibited SES scores of 3-5 represents a focused breeding strategy for introgressing salinity tolerance into new hybrid rice parents. This approach aligns with recent marker-assisted breeding programs that have successfully combined salinity tolerance with other desirable traits in parental lines (Niu *et al.*, 2025; Sheng *et al.*, 2023). Among these, cross HRB 502 (BHR101/BHR184) produced the largest F_6

population (33 entries). The varying parental SES scores and F_6 population sizes (ranging from 1 to 33) across the crosses provide a diverse genetic pool for selecting high-yielding and salinity-tolerant fixed lines for use as new hybrid rice parents. The use of tolerant × tolerant crosses is supported by previous studies showing that parental salinity tolerance is a prerequisite for developing stable salt-tolerant hybrids (Raghavendra *et al.*, 2021; Beulah *et al.*, 2023).

The wide variation in F_6 population sizes (1-33 entries) across crosses reflects differences in cross compatibility, seed set, and selection pressure applied during line stage testing (LST) trial. The seven crosses involving at least one highly susceptible parent (SES 7-8) yielded fewer promising selections, confirming that both parents should possess moderate to high tolerance for efficient introgression of this trait. Similar observations have been reported in studies where screening of large germplasm collections identified maintainer and restorer candidates with inherent salinity tolerance for hybrid development (Beulah *et al.*, 2023; Dolo *et al.*, 2016).

BLUP-Based Selection for High-Yielding Genotypes

The application of Best Linear Unbiased Predictor (BLUP) values for selection based on yield, growth duration, plant height, and effective tiller number represents a robust statistical approach for handling the augmented randomized complete block design with unbalanced data. BLUP-based selection has gained prominence in plant breeding due to its ability to account for spatial variation and provide unbiased estimates of genotypic values (Piepho *et al.*, 2008). In this study, the stringent selection threshold of population means plus one standard deviation ($\mu + 1\sigma$) for yield resulted in 54 selected genotypes, while only five exceptional genotypes exceeded $\mu + 2\sigma$. This selection intensity ensures that only superior performers are advanced, maximizing genetic gain while maintaining sufficient genetic diversity for future breeding efforts (Falconer and Mackay, 1996).

The distribution of selected genotypes across breeding populations (27 from B×B crosses, 17 from R×R crosses, and 10 from elite lines) indicates successful recovery of high-performing lines from diverse genetic backgrounds. The elite lines contributed proportionally fewer selections, suggesting that the targeted crosses were more effective in generating superior combinations. This outcome validates the crossing strategy and emphasizes the importance of planned hybridization over relying solely on existing germplasm.

Genetic Diversity and Cluster Analysis

The Euclidean distance analysis revealed substantial genetic diversity among the 54 selected genotypes, with distances ranging from 0.17 to 5.85. The highest genetic distance between HRB502-4R-181 and BR11716-4R-120 (5.85) indicates these lines are highly divergent and could serve as complementary parents in future hybrid combinations to maximize heterosis (Sruthi *et al.*, 2020; Prasanna *et al.*, 2022). Conversely, the lowest distance between BR11723-4R-27 and BR11723-4R-12 (0.17) suggests these lines are closely related, likely derived from the same cross with minimal phenotypic differentiation.

The frequency distribution of pairwise distances showed a roughly normal distribution centered around moderate distances (2-4 units), with the highest frequency (530 pairs) at distance >2-3. This pattern indicates a balanced level of genetic variability within the selected population, which is desirable for maintaining breeding flexibility while ensuring sufficient divergence for heterotic exploitation (Prasanna *et al.*, 2022). The relatively few pairs showing very low (0-1; 61 pairs) or very high (5-6; 11 pairs) divergence further supports the notion that the population represents a well-structured gene pool with moderate diversity.

Clustering Patterns and Agronomic Implications

Ward's D2 hierarchical clustering classified the 54 genotypes into three distinct clusters with

contrasting agronomic characteristics. Cluster 1, comprising late-maturing genotypes (158.30 days), exhibited the highest yield (1.82 kg/1.2 m²) despite having only 10 members and moderate tiller numbers (8.00). This suggests that extended growth duration allows for greater biomass accumulation and enhanced grain filling, consistent with reports that late-maturing varieties often achieve higher yields through prolonged photosynthetic activity and better resource utilization (Yang *et al.*, 2010). These genotypes are particularly valuable for developing high-yielding hybrids in environments where the growing season permits late maturity.

Cluster 2 consisted of medium-duration genotypes (137.67 days) characterized by the tallest plant height (145.33 cm) and moderate yield (1.78 kg/1.2 m²). Tall plant stature in hybrid rice parents can be advantageous for biomass production and lodging resistance when combined with appropriate stem strength traits (Khush *et al.*, 2005). However, excessive height may increase lodging risk under high-input management, necessitating careful evaluation in hybrid combinations.

Cluster 3, the largest group with 38 members, included early-maturing, short-statured genotypes (136.68 days, 116.24 cm) with the highest tiller number (12.89) but the lowest yield (1.67 kg/1.2 m²). This inverse relationship between tiller number and yield suggests a trade-off where high tillering may result in smaller panicles or reduced grain weight per panicle, possibly due to competition for assimilates among tillers (Huang *et al.*, 2011). Previous studies have demonstrated that while effective tiller number is positively correlated with yield, excessive tillering can lead to unproductive tillers and reduced individual panicle productivity (Huang *et al.*, 2011). This cluster represents genotypes suitable for breeding programs targeting early maturity and adaptation to short-season environments, though yield improvement would require selection for larger panicle size or grain weight.

Cluster Validation and Inter-Cluster Divergence

The silhouette width analysis confirmed the clustering structure, with Clusters 1 and 2 exhibiting higher average silhouette widths (0.464 and 0.461, respectively) compared to Cluster 3 (0.284). High silhouette values indicate that genotypes within these clusters are well-grouped and distinct from other clusters, reflecting strong internal cohesion (Rousseeuw, 1987). The lower silhouette width of Cluster 3 suggests greater internal variability and potential overlap with other clusters, which may be attributed to its larger size and broader genetic composition.

The inter-cluster distance analysis revealed that Clusters 1 and 3 were the most divergent (distance 3.07), indicating substantial phenotypic differentiation between late-maturing, high-yielding types and early-maturing, high-tillering types. This divergence provides opportunities for heterotic crosses between these groups to exploit complementary traits (Sruthi *et al*., 2020). The smallest inter-cluster distance between Clusters 2 and 3 (2.48) suggests some phenotypic similarity, possibly in maturity duration, which could limit heterotic potential in crosses between these groups.

Salinity Tolerance Evaluation of Selected Lines

The evaluation of 54 selected genotypes under 12 dS/m salinity stress at the seedling stage identified 11 highly tolerant lines (SES 3): six restorer lines and five maintainer lines. This represents a 20.4% success rate in recovering highly tolerant lines from the selected population, which is consistent with the expected segregation patterns when crossing moderately tolerant parents (Beulah *et al.*, 2023; Singh and Flowers, 2010). An additional 19 genotypes exhibited moderate tolerance (SES 5), providing a broader pool of genetic material for further improvement through recurrent selection or marker-assisted backcrossing.

The visual differences in seedling response to salinity stress (Fig. 5) clearly distinguished

tolerant genotypes (minimal leaf damage, continued growth) from susceptible ones (severe leaf scorching, stunted growth), validating the SES scoring system as an effective phenotyping tool. The susceptible checks BRRI dhan28 and IR154 displayed severe stress symptoms, confirming the effectiveness of the screening protocol. The SES-based screening approach has been widely used in rice breeding programs and has proven reliable for identifying salinity-tolerant donors and breeding lines (Raghavendra *et al.*, 2021; Beulah *et al.*, 2023; Dolo *et al.*, 2016).

The recovery of both highly tolerant and moderately tolerant lines from the breeding populations confirms the effectiveness of the crossing strategy. The tolerant lines identified in this study can serve as new parental lines for hybrid rice breeding programs targeting salt-affected ecologies, while moderately tolerant lines can be used as donors in marker-assisted backcrossing programs to further enhance salinity tolerance (Niu *et al.*, 2025; Thomson *et al.*, 2010).

Molecular Confirmation of Fertility Restoration and Maintenance Ability

The use of gene-based markers DRRM-*Rf3*-5 (for *Rf3*) and DRCG-RF4-14 (for *Rf4*) successfully differentiated restorer lines from maintainer lines at the molecular level. The *Rf3* gene on chromosome 1 and *Rf4* gene on chromosome 10 are the major fertility restorer genes in rice, encoding pentatricopeptide repeat (PPR) proteins that restore pollen fertility in cytoplasmic male sterile (CMS) lines (Huang *et al.*, 2015). The clear banding patterns observed-160 bp and 800 bp for restorer lines versus 140 bp and 885 bp for maintainer lines-enabled efficient classification of 24 genotypes as restorers and 27 as maintainers.

Molecular markers for *Rf* genes have been extensively validated and are routinely used in hybrid rice breeding programs to accelerate parental line development (Huang *et al.*, 2015; Kumar *et al.*, 2017; Tang *et al.*, 2014). Studies have reported marker efficiencies of 80-90% for SSR and gene-based markers linked to *Rf3* and *Rf4*, making them valuable tools for preliminary screening of breeding materials (Kumar *et al.*, 2017; Sheeba *et al.*, 2009). However, the efficiency of these markers is not 100%, necessitating phenotypic confirmation through test cross evaluation (Tang *et al.*, 2014; Nagaraju *et al.*, 2023).

Pollen Fertility Testing and Phenotypic Validation

The test cross evaluation using I-KI staining provided phenotypic confirmation of fertility restoration and maintenance ability. Among the 27 B×B-derived lines tested, 25 produced 100% sterile pollen in test cross F1s, confirming their maintainer status, while two genotypes (BBC14-4R-49 and HRB502-4R-181) showed only 90-98% sterility and were discarded. This discordance between molecular marker prediction and phenotypic expression highlights the limitation of relying solely on molecular markers for parental line classification (Tang *et al.*, 2014; Nagaraju *et al.*, 2023).

Several factors may contribute to incomplete sterility in putative maintainer lines despite showing recessive alleles for both *Rf3* and *Rf4*. These include: (1) environmental effects on pollen fertility expression (Li and Yang, 2007); (2) allelic variation at the *Rf* loci that affects restoration efficiency (Huang *et al.*, 2009); and (3) possible errors in marker scoring or DNA quality issues. Similar observations have been reported in other studies where marker-based predictions did not perfectly align with phenotypic fertility restoration, emphasizing the need for combined molecular and phenotypic selection (Tang *et al.*, 2014; Nagaraju *et al.*, 2023; Kumar *et al.*, 2016).

All 17 R×R-derived lines and 7 out of 12 tested elite lines produced >80% fertile pollen in test cross F1s, confirming their restorer status. The threshold of >80% pollen fertility is commonly used in hybrid rice breeding to classify effective restorers when both *Rf3* and *Rf4* are present in homozygous dominant condition (Kumar *et al.*, 2017). The five elite lines that failed to meet this threshold were appropriately discarded, demonstrating the value of phenotypic

validation in eliminating false positives from molecular screening. Among the selected 54 genotypes, six R lines (HRR271-4R-44, HRR271-4R-46, HRR269-4R-36, BR11716-4R-102, BR11716-4R-108, RRC9-4R-31) have SES 3 score (tolerant) and confirmed as new restorer lines except BR11716-4R-102. Five B lines HRB512-4R-108, HRB513-4R-157, HRB512-4R-129, HRB503-4R-4, HRB512-4R-201 have SES 3 score (tolerant) and confirmed as new maintainer (B) lines.

Implications for Hybrid Rice Breeding

The successful development of 25 new maintainer lines (5 maintainer having SES 3) and 24 new restorer lines (5 restorer having SES 3) with combined salinity tolerance and desirable agronomic traits represents a significant achievement for hybrid rice breeding programs targeting salt-affected areas. These parental lines can be immediately utilized in hybrid combination trials to develop salt-tolerant hybrids with improved yield potential. The diversity analysis revealed sufficient genetic divergence among the selected lines to enable heterotic grouping and systematic hybrid development.

The integration of phenotypic screening (SES scoring, yield evaluation), molecular marker analysis (*Rf* gene markers), and statistical genetics (BLUP, clustering) in this study exemplifies a comprehensive and efficient approach to parental line development. This multi-faceted strategy maximizes selection efficiency while ensuring that only lines with confirmed genetic and phenotypic attributes are advanced (Niu *et al.*, 2025; Collard and Mackill, 2008). Future research work should focus on: (1) evaluating the combining ability of these parental lines through line × tester analysis; (2) assessing salinity tolerance at reproductive stage in addition to seedling stage; (3) incorporating additional trait-linked molecular markers for other important traits such as grain quality, disease resistance, and yield-related QTLs; and (4) conducting multi-location trials to assess stability and adaptability of salinity tolerance and yield performance across diverse saline environments.

CONCLUSION

This study successfully developed 49 new salinity-tolerant hybrid rice parental lines (25 maintainers and 24 restorers) through strategic crossing of tolerant donor parents, BLUP-based selection, and combined molecular-phenotypic validation. The newly developed parental lines need to be genotyped to identify which mechanisms and underlying genes/QTLs introgressed into the developed lines and provided tolerances. The identified lines exhibited diverse agronomic characteristics, grouped into three distinct clusters representing different growth duration-yield contributing ideotypes. The integration of SES-based salinity screening with molecular marker analysis for Rf genes proved effective, though phenotypic validation through test cross pollen fertility testing is crucial for confirming parental line classification. Overall, these newly developed parental lines provide valuable genetic resources for breeding salinity-tolerant hybrid rice varieties suited to salt-affected ecologies.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

This study was supported by a national project of Bangladesh "High-yielding hybrid rice variety development through modernization of research," funded by Ministry of Agriculture (224344400), Bangladesh.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding authors upon reasonable request.

ACKNOWLEDGMENTS

We extend our sincere gratitude to the Bangladesh Rice Research Institute (BRRI) and Gazipur Agricultural University (GAU), Bangladesh. Special thanks to Dr. Salma Pervin (Head, Plant Physiology Division, BRRI) and Tuhin Halder (Scientist, Plant Physiology Division, BRRI) for generously providing the essential screening facility used in this research.

REFERENCES

Beulah, P., Manasa, Y., Patel, N., Prasad, A. S. H., Senguttuvel, P., Kemparaju, K. B., Gireesh, C., Subrahmanyam, D., Sundaram, R. M., Madhav, M. S., & Rani, M. G. (2023). Evaluation of salinity-tolerant backcrossed inbred lines (BILs) for fertility restoration using molecular markers. *Journal of Experimental Agriculture International*, 45(5), 1–13. https://doi.org/10.58297/rqvq6630

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 557–572. https://doi.org/10.1098/rstb.2007.2170

Dolo, E., Raghavendra, P., Anantha, M. S., Swamy, B. P. M., Dwivedi, J. L., & Serraj, R. (2016). Identification of salinity tolerant parental lines for hybrid rice breeding. *Oryza*, 53(1), 89–94.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Longman Group Ltd.

Huang, M., Zou, Y. B., Jiang, P., Xia, B., Feng, Y. H., Cheng, Z. B., & Mo, Y. W. (2011). Relationship between grain yield and yield components in super hybrid rice. *Agricultural Sciences in China*, 10(10), 1537–1544. https://doi.org/10.1016/S1671-2927(11)60149-1

Huang, W., Yu, C., Hu, J., Wang, L., Dan, Z., Zhou, W., He, C., Zeng, Z. Y., Yao, G., Qi, J., Zhang, Z., & Zhu, R. (2015). Pentatricopeptide-repeat family protein RF6 functions with hexokinase 6 to rescue rice cytoplasmic male sterility. *Proceedings of the National Academy of Sciences*, 112(48), 14984–14989. https://doi.org/10.1073/pnas.1511748112

Huang, X., Feng, Q., Qian, Q., Zhao, Q., Wang, L., Wang, A., Guan, J., Fan, D., Weng, Q., Huang, T., Dong, G., Sang, T., & Han, B. (2009). High-throughput genotyping by whole-genome resequencing. *Genome Research*, 19(6), 1068–1076. https://doi.org/10.1101/gr.089516.108

International Rice Research Institute. (2014). PBTools v1.4 [Computer software]. Biometrics and Breeding Informatics, PBGB Division, International Rice Research Institute.

Islam, M. M., Begum, S. A., & Khanom, M. S. R. (2024). Genetic enhancement of rice for salt-affected areas of Bangladesh. In Genetic enhancement of rice for salt-affected areas (pp. 10). Springer. https://doi.org/10.1007/978-981-99-3830-8_10

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, 59(1), 1–6. https://doi.org/10.1007/s11103-005-2159-5

Korres, N. E., Norsworthy, J. K., Tehranchian, P., Gitsopoulos, T. K., Loka, D. A., Oosterhuis, D. M., Gealy, D. R., Moss, S. R., Burgos, N. R., Miller, M. R., & Palhano, M. (2022). Salinity effects on rice, rice weeds, and strategies to secure crop productivity and effective weed control: A review. *Agronomy for Sustainable Development*, 42(3), 56. https://doi.org/10.1007/s13593-022-00794-4

Kumar, A., Bhowmick, P. K., Singh, V. J., Sharma, S., Shivapriya, Ellur, R. K., Nagarajan, M., Singh, A. K., & Vinod. (2017). Marker-assisted identification of restorer gene(s) in iso-cytoplasmic restorer lines of WA cytoplasm in rice and assessment of their fertility restoration potential across environments. *Physiology and Molecular Biology of Plants*, 23(4), 891–909. https://doi.org/10.1007/s12298-017-0464-5

Kumar, V. A., Balachiranjeevi, C. H., Naik, S. B., Rambabu, R., Rekha, G., Harika, G., Hajira, S. K., Pranathi, K., Anila, M., Kousik, M. B. V. N., Kale, R. R., Pranav, D., Dipti, S., Yugandhar, P., Dilip, T., Aravind Kumar, J., Aruna, J., Sravani, D., Mahadevaswamy, H. K., … Sundaram, R. M. (2016). Development of gene-pyramid lines of the elite restorer line, RPHR-1005 possessing durable bacterial blight and blast resistance. *Frontiers*

in Plant Science, 7, 1195. https://doi.org/10.3389/fpls. 2016.01195

Li, S., & Yang, D. (2007). Characterization of a novel fertility restorer gene for CMS-WA in rice. *Acta Agronomica Sinica*, 33(10), 1611–1617.

Mheni, N., Kilasi, N. L., Quiloy, F. A., Dimoso, N., Mgonja, M., Mzengeza, T., Mtambo, M., Makame, M., Kilima, M., Ngailo, S., & Mkuya, M. (2024). Breeding rice for salinity tolerance and salt-affected soils in Africa: A review. *Cogent Food & Agriculture*, 10(1), 2327666. https://doi.org/10.1080/23311932.2024.2327666

Nagaraju, P., Beulah, P., Manasa, Y., Prasad, A. S. H., Senguttuvel, P., Kemparaju, K. B., Gireesh, C., Subrahmanyam, D., Sundaram, R. M., Madhav, M. S., & Rani, M. G. (2023). Evaluation of improved drought-tolerant parental lines of KMR3R for fertility restoration by molecular analysis. *Journal of Experimental Agriculture International*, 45(6), 1–11. https://doi.org/10.58297/puqw2548

Niu, F., Zhang, A., Cheng, C., Li, Z., Xu, Y., Zhao, X., Wang, Y., & Zhou, L. (2025). Molecular marker-assisted breeding of high-quality and salt-tolerant hybrid japonica rice combination Shenyanyou 1. *Agronomy*, 15(8), 2006. https://doi.org/10.3390/agronomy15082006

Piepho, H. P., Möhring, J., Melchinger, A. E., & Büchse, A. (2008). BLUP for phenotypic selection in plant breeding and variety testing. *Euphytica*, 161(1–2), 209–228. https://doi.org/10.1007/s10681-007-9449-8

Ponnuswamy, R., Singh, A. K., Raman, M. S., Nagarajan, M., Reddy, J. N., Patra, B. C., Ram, T., Sharma, R. P., Mohapatra, T., & Singh, N. K. (2020). Conversion of partial restorer Swarna into restorer by transferring fertility restorer Rf gene(s) through marker assisted back cross breeding (MABB) in rice. *Scientific Reports*, 10(1), 1524. https://doi.org/ 10.1038/s41598-020-58019-1

Prasanna, G. S. R., Eswari, K. B., Senguttuvel, P., Gireesh, C., Anantha, M. S., Rao, P. R., Brajendra, P., Suneetha, K., Rao, L. V. S., Kemparaju, K. B., Viraktamath, B. C., & Rani, M. G. (2022). Studies on genetic divergence and screening of parental lines for Rf3 and Rf4 genes through molecular markers in hybrid rice (Oryza sativa L.). *Electronic Journal of Plant Breeding*, 13(4), 1259–1268. https://doi.org/10.37992/2022.1304.154

Raghavendra, P., Kumar, B. M. D., Shashikumara, P., Gowda, D. K. S., Shankar, A. G., Kundur, P. J., Joshi, N., Desai, S., Gowda, T. K. S., & Ramkumar, G. (2021). Marker-assisted identification of novel genetic lines for salinity tolerance and their categorization for utilization in development of hybrid rice (Oryza sativa L.). *Cereal Research Communications*, 49, 253–262. https://doi.org/10.1007/s42976-020-00104-2

Rahman, M. A., Quddus, M., Jahan, N., Rahman, M. A., Sarker, M. R. A., Hossain, H., & Iftekharuddaula, K. M. (2019). Field rapid generation advance: An effective technique for industrial scale rice breeding program. *The Experiment*, 47(2), 2659–2670.

Ramalingam, J., Savitha, P., Alagarasan, G., Saraswathi, R., & Chandrababu, R. (2017). Functional marker assisted improvement of stable cytoplasmic male sterile lines of rice for bacterial blight resistance. *Frontiers in Plant Science*, 8, 1131. https://doi.org/10.3389/fpls.2017.01131

Rekha, G., Kumar, V. A., Gokulan, C. G., Shobha Rani, N., Viraktamath, B. C., Anantha, M. S., Jagadeeshwar, R., Rao, P. R., Rao, L. V. S., Prasad, A. S. H., Kemparaju, K. B., Madhav, M. S., & Sundaram, R. M. (2022). DRR Dhan 58, a seedling stage salinity tolerant NIL of Improved Samba Mahsuri shows superior performance in multi-location trials. *Rice*, 15(1), 44. https://doi.org/10.1186/s12284-022-00591-3

Rousseeuw, P. J. (1987). Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *Journal of Computational and Applied Mathematics*, 20, 53–65. https://doi.org/10.1016/0377-0427(87)901

25-7

Rout, D., Jena, D., Singh, V., Rani, M. G., & Mohapatra, T. (2020). Hybrid rice research: Current status and prospects. *In Recent advances in rice research* (pp. 1–33). IntechOpen. https://doi.org/10.5772/intechopen.93668

Saminadane, T., Geddam, S., Krishnaswamy, P., Ramasamy, S., Ramanathan, V., Raveendran, M., Pillai, M. P. A., Muthurajan, R., & Subramaniam, S. (2024). Development of early maturing salt-tolerant rice variety KKL(R)3 using a combination of conventional and molecular breeding approaches. *Frontiers in Genetics*, 14, 1332691. https://doi.org/10.3389/fgene.2023.1332691

Satasiya, P., Patel, S., Patel, R., Parmar, D. J., Patel, M., Sasidharan, N., Kumar, S., & Patel, A. I. (2024). Meta-analysis of identified genomic regions and candidate genes underlying salinity tolerance in rice (Oryza sativa L.). *Scientific Reports*, 14(1), 5508. https://doi.org/10.1038/s41598-024-54764-9

Sellathdurai, D., Burman, D., Mandal, U. K., Maji, B., Sarangi, S. K., Sharma, P. C., & Digar, S. (2024). Crop improvement strategies for addressing waterlogging and salinity in coastal ecosystems. *Journal of Soil Salinity and Water Quality*, 16(2), 83–99. https://doi.org/10.56093/jsswq.v16i2.157117

Sheeba, N. K., Viraktamath, B. C., Sivaramakrishnan, S., Gangashetti, M. G., Khera, P., Sundaram, R. M., Sharma, R. P., Tokas, J., Neeraja, C. N., & Balachandran, S. M. (2009). Validation of molecular markers linked to fertility restorer gene(s) for WA-CMS lines of rice. *Euphytica*, 167(2), 217–227. https://doi.org/10.1007/s10681-009-9880-5

Sheng, X., Ai, Z., Tan, Y., Zheng, Y., Zhang, S., Tang, W., & Liang, G. (2023). Novel salinity-tolerant third-generation hybrid rice developed via CRISPR/Cas9-mediated gene editing. *International Journal of Molecular Sciences*, 24(9), 8025. https://doi.org/10.3390/ijms24098025

Singh, R. K., & Flowers, T. J. (2010). Physiology and molecular biology of the effects of salinity on rice. In M. Pessarakli (Ed.), *Handbook of plant and crop stress* (3rd ed., pp. 899–939). CRC Press.

Sruthi, K., Divya, B., Senguttuvel, P., Jaldhani, V., Beulah, P., Nagaraju, P., Manasa, Y., Prasad, A. S. H., Brajendra, P., Gireesh, C., Anantha, M. S., Suneetha, K., Rao, P. R., Rao, L. V. S., Sundaram, R. M., Kemparaju, K. B., Rani, M. G., & Viraktamath, B. C. (2020). Evaluation of genetic diversity of parental lines for development of heterotic groups in hybrid rice (*Oryza sativa* L.). *Journal of Plant Biochemistry and Biotechnology*, 29, 10–23. https://doi.org/10.1007/s13562-019-00529-9

Suresh, P. B., Srikanth, B., Kishore, V. H., Rao, I. S., Vemireddy, L. R., Dharika, N., Rao, K. S., Sundaram, R. M., Laha, G. S., Madhav, M. S., Jagadeeshwar, R., Sharma, R. P., Prasad, M. S., Balachandran, S. M., Prasad, A. S. H., Viraktamath, B. C., Sarma, N. P., & Neeraja, C. N. (2012). Fine mapping of Rf3 and Rf4 fertility restorer loci of WA-CMS of rice (Oryza sativa L.) and validation of the developed marker system for identification of restorer lines. *Euphytica*, 187(3), 421–435. https://doi.org/10.1007/s10681-012-0737-6

Tang, H., Luo, D., Zhou, D., Zhang, Q., Tian, D., Zheng, X., Chen, L., & Liu, Y. (2014). The rice restorer Rf4 for wild-abortive cytoplasmic male sterility encodes a mitochondrial-localized PPR protein that functions in reduction of WA352 transcripts. *Molecular Plant*, 7(9), 1497–1500. https://doi.org/10.1093/mp/ssu047

Thippani, S. (2017). Genetic analysis of hybrid rice (*Oryza sativa* L.) and introgression of Saltol QTL into maintainer line (APMS-6B) [Doctoral dissertation, Professor Jayashankar Telangana State Agricultural University]. Krishikosh. https://krishikosh.egranth.ac.in/server/api/

core/bitstreams/34e4cb23-2a42-43b6-8da1-6083d315563a/content

Thomson, M. J., de Ocampo, M., Egdane, J., Rahman, M. A., Sajise, A. G., Adorada, D. L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z. I., Singh, R. K., Gregorio, G. B., & Ismail, A. M. (2010). Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice, 3(2–3), 148–160. https://doi.org/10.1007/s12284-010-9053-8

Yang, J., & Zhang, J. (2010). Crop management techniques to enhance harvest index in rice. Journal of Experimental Botany, 61(12), 3177–3189. https://doi.org/10.1093/jxb/erq112

Yadav, V., Pratap, N., Achar, D., Chaurasia, A. K., & Singh, A. K. (2018). Study of simple sequence repeat (SSR) polymorphism for Saltol QTL targeted at chromosome 1 in elite CMS IR 58025B of paddy and FL478. *Journal of Pharmacognosy and Phytochemistry*, 7(1), 2519–2522.

Yoshida, S., Forno, D. A., & Cock, J. H. (1976). Laboratory manual for physiological studies of rice. International Rice Research Institute (IRRI).

Bangladesh Rice J.28(1): 41-56, 2024, doi.org/10.3329/brj.v28i1.87012

Genetic Diversity of Agro-morphological Traits and Blast Disease Reaction in Elite Rice Genotypes for Hybrid Breeding

L F Lipi[1, 3*], M A Rahman[2], U Sarker[3], M Hasan[3] and M M Hossain[4]

ABSTRACT

Global rice production faces enormous challenges from climate-induced abiotic and biotic stresses, with rice blast disease caused by the fungus *Pyricularia oryzae* posing a major threat to yield and food security. Developing and deploying rice cultivars with resistance to blast disease remains the most effective and sustainable approach to mitigate this constraint. To identify potential sources of resistance, a study was conducted during 2022–2023 focusing on the development of hybrid rice parental lines. Out of 43 genotypes evaluated, 18 exhibited resistance to a virulent blast isolate, while the remaining 25 showed susceptible reactions. Agro-morphological traits such as plant height, panicle length, days to maturity, number of effective tillers, total spikelets per panicle, filled grains per panicle, spikelet fertility percentage, grain yield per hill, and notably the blast disease severity score, were key contributors to phenotypic variability among these genotypes. Based on these traits, the genotypes were categorized into five distinct clusters. Principal component analysis revealed that the first three components explained 67.45% of the total variation. Genotypes G22 (IR126055-46-3-2-B), G13 (BHR362- 8-6-5-26-9), G11 (BHR360- 86-41-7-26-1), and G10 (BHR359-11-30-7-2) from cluster III exhibited high yield potential, whereas genotypes G42 (IR127278-114-3-3-2), G38 (BHR383-53-8-13-1-1), and G43 (BR9390-6-2-2-1) from cluster IV showed notable blast resistance, underscoring their value for hybrid rice development programs. To employ these traits, genotypes from the aforementioned clusters could be made testcross with promising CMS (Cytoplasmic Male Sterile) lines that would offer a good scope to explore the source of high yielding and blast resistance elite restorer or maintainer lines. These findings offer valuable insights for rice breeders aiming to develop high yielding and blast-resistant hybrid varieties tailored to the agro-ecological conditions of Bangladesh.

Keywords: Genetic diversity, cluster, PCA, blast disease

INTRODUCTION

Rice serves as the primary food source for over half of the world's population, contributing significantly to daily energy and protein intake; hence, ensuring its sustainable production is vital for global food security (Saha *et al.*, 2021). To address the challenges posed by population growth, climate change, and limited arable land, hybrid rice has emerged as a key technological innovation. Hybrid rice consistently demonstrates a yield advantage of 15-20% over conventional varieties (Virmani *et al.*, 2001; Yuan, 2017). However, rice production faces

[1]Hybrid Rice Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh
[2]Plant Breeding Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh
[3]Department of Genetics and Plant Breeding, Faculty of Agriculture, Gazipur Agricultural University (GAU), Gazipur-1706, Bangladesh
[4]Department of Plant Pathology, Faculty of Agriculture, Gazipur Agricultural University (GAU), Gazipur-1706, Bangladesh
[*]Corresponding author's E-mail: ferdousi.lipi@yahoo.com (L F Lipi)

significant challenges due to climate-related disruptions, which often trigger a combination of abiotic and biotic stresses. Among the biotic stresses, rice blast disease caused by the fungus *Pyricularia oryzae* is one of the most devastating diseases, leading to major yield reductions and threatening global rice production (Salleh *et al.*, 2022; Yadav *et al.*, 2017). This disease severely affects aromatic and hybrid rice varieties, resulting in substantial crop losses (Ye *et al.*, 2025; Nalley *et al.*, 2016). The development of rice hybrids is a key strategy for overcoming the yield plateau, as hybrid rice often demonstrates superior performance due to heterosis (hybrid vigor) (Nivedha *et al.*, 2024). For an effective hybrid breeding program, it is essential to first identify and select genetically distinct parental genotypes. Crossing these lines must be aimed at maximizing desirable traits in the offspring (Nivedha *et al.*, 2024). Consequently, a thorough evaluation of these elite genotypes is crucial, particularly by assessing their disease resistance alongside numerous yield and yield-related characteristics. Assessing rice germplasm for desirable traits is a complex endeavor, as key agronomic features like grain yield, plant height, and tiller number are polygenic and strongly influenced by environmental interactions (Wang *et al.*, 2024; Deepika *et al.*, 2021). Similarly, blast resistance is governed by multiple genes whose effectiveness may vary in durability and expression (Debnath *et al.*, 2018; Yadav *et al.*, 2017).

Multivariate statistical tools, including Principal Component Analysis (PCA) and cluster analysis, play an important role in breeding programs. These methods help in evaluating trait variability, identifying key contributors to genetic divergence, and grouping genotypes based on overall similarity (Deepika *et al.*, 2021; Nivedha *et al.*, 2024). Integrating these approaches enables breeders to efficiently select elite genotypes that are both high-yielding and blast-resistant, thereby improving the precision of parental selection (Salleh *et al.*, 2022).

Although chemical treatments and antibiotics are available to manage blast disease, they pose risks to human health and the environment. In contrast, the use of resistance (R) genes offers a safer and more sustainable alternative, providing broad-spectrum and long-lasting protection. Identifying resistant sources through systematic screening is a critical step in breeding programs aimed at developing durable blast-resistant varieties. A genetically diverse gene pool is essential for initiating such programs (Sivaranjani *et al.*, 2010; Nihad *et al.*, 2020). Genetic diversity analysis evaluates the genetic distance among the selected genotypes and shows the relative contribution of evaluated traits towards the total divergence (Falconer, 1960). Several researchers have used agro-morphological traits for diversity analysis and characterization of Bangladesh rice germplasm accessions (Siddique *et al.*, 2011; Banik *et al.*, 2012; Khalequzzaman *et al.*, 2012; Baktiar *et al.*, 2013; Siddique *et al.*, 2013; Islam *et al.*, 2014; Ahmed *et al.*, 2015a, 2015b; Kulsum *et al.*, 2015; Akter *et al.*, 2016; Biswash *et al.*, 2016; Siddique *et al.*, 2016a; Akter *et al.*, 2017; Islam *et al.*, 2017; Akter *et al.*, 2018; Islam *et al.*, 2018; Siddique *et al.*, 2018; Islam *et al.*, 2019; Muti *et al.*, 2020; Khalequzzaman *et al.*, 2022a; Khalequzzaman *et al.*, 2023). Hence, this study aims to evaluate elite rice genotypes to identify promising sources of blast resistance and to initiate testcross program for the identification of restorer and maintainer lines for developing blast resistant heterotic hybrid rice varieties.

MATERIALS AND METHODS

The experimental materials consisted of 43 elite rice (*Oryza sativa* L.) genotypes. Nine advanced breeding lines- IR126055-46-3-2-B, IR126066-85-5-2-1, IR126069-48-3-2-2, IR126076-67-3-2-3-4, IR126037-59-3-2-3-4, IR126072-83-3-3-1, IR126076-122-1-1-2, IR127278-152-1-3-1 and IR127278-114-3-3-2 originated from the Hybrid Rice Development Consortium (HRDC), International Rice Research Institute (IRRI), Philippines. All advanced materials were obtained from the Hybrid Rice Division, Bangladesh Rice Research Institute (BRRI), Gazipur. The

genotypes code, genotypes/ advanced lines name and blast disease reactions are provided in Table 1.

Evaluation of leaf blast disease resistance

To assess blast disease reaction, the genotypes were grown from October to December 2022 in a randomized complete block design (RCBD) with three replications at the Blast Nursery of Plant Pathology Division, BRRI, Gazipur. A sowing frame was used to place 5–7 seeds per genotype per grid. The highly susceptible check variety US2 was planted along the borders of each bed to serve as a disease severity benchmark. Artificial inoculation was performed using a spore suspension of the virulent isolate U23-i7-k177-z06-ta423 of *Pyricularia oryzae* (Khan *et al.*, 2016). At the 4–5 leaf stage (21 days after sowing), seedlings were sprayed with a spore solution adjusted to 3–8 × 10^4 spores/mL. Meteorological data for the experimental period (October to December, 2022) were obtained from the meteorological station of the Bangladesh Rice Research Institute (BRRI), Gazipur. Monthly average air temperatures for October, November, and December were 29.2°C, 25.3°C, and 21.9°C, respectively. Relative humidity ranged from 62.1% to 74.0%. Rainfall was recorded only in October (9 mm), with no precipitation in November and December. To promote infection, nursery beds were covered with polythene sheets and misted using a sprinkler system to maintain humidity levels above 80% for six consecutive days. Disease scoring was initiated once the susceptible check (US2) showed severe blast symptoms. Leaf blast disease severity was recorded from three seedlings per genotype using the 0–5 JIRCAS rating scale (Hayashi and Fukuta, 2009). A score of 0 indicated no visible symptoms, while 5 represented spindle-shaped lesions exceeding 3 mm in diameter with lesion coalescence. scores between 0 and 2 were considered as resistant, whereas scores from 3 to 5 were categorized as susceptible.

Evaluation of agro-morphological traits

The 43 genotypes were transplanted for agro-morphological assessment during the Boro 2023–24 season at the research farm of Hybrid Rice Division, Gazipur. The field experiment followed an RCBD layout with three replications. Thirty-day-old seedlings were transplanted at a spacing of 20 × 20 cm between rows and 15 × 15 cm between plants, using one seedling per hill. Fertilization included Urea, TSP, MP, Gypsum, and $ZnSO_4$ were applied at the rates of 270:130:120:70:10 kg/ha, respectively. All fertilizers except urea were applied during final land preparation. Urea was top-dressed into three equal splits: at 15 days after transplanting (DAT), 30 DAT (tillering stage), and 45 DAT (just before panicle initiation). Standard agronomic practices recommended by BRRI for irrigated rice were strictly followed. Eight agro-morphological traits were recorded during field evaluation, based on observations from ten randomly selected plants for each genotype. The data were recorded of the following characters: Days to maturity (DM), Plant height (PH), Effective tillers per hill (ETPH), Panicle length (PL), Filled grains per panicle (FGPP), Total spikelets per panicle (TSPP), Spikelet fertility (SF %) and Grain yield per hill (GYPH).

Statistical analysis

Correlations, principal component and cluster analyses were done through several programme packages using R studio. The clustering was performed using the Ward's method, and correlation analysis was conducted through Pearson's test using R programming language. For data visualization and multivariate analysis, the R packages ggplot2, FactoMineR, Factoextra, and ggrepel were employed, while clustering procedures were further supported by Factoextra and cluster packages. To illustrate phenotypic correlation matrices, a heatmap was generated using the metan package in R Studio. Descriptive statistics for the nine evaluated traits were generated using the Statistical Tool for Agricultural Research (STAR, version 2.0.1; IRRI, Philippines).

Table 1. List of the materials used in the study with leaf blast reactions.

Code	Genotype	DR	Code	Genotype	DR
G1	BHR350-4-12-3-1-2	S	G23	IR126066-85-5-2-1	S
G2	BHR351-50-22-3-2-2	S	G24	IR126069-48-3-2-2	S
G3	BHR352-22-4-7-2	R	G25	IR126076-67-3-2-3-4	R
G4	BHR353-39-21-4-3	S	G26	IR126037-59-3-2 -3-4	S
G5	BHR354-55-27-9-2	R	G27	BHR372-8-11-1-1-1	S
G6	BHR355-5-26-7-3	R	G28	BHR373-8-65-22-29-10	S
G7	BHR356-13-20-3-4	S	G29	BHR374-90-9-2-8-15	S
G8	BHR357-19-29-4-3	S	G30	BHR375-6-5-26-21-2	S
G9	BHR358-12-11-7-2	S	G31	BHR376-B-6-52-6-10	S
G10	BHR359-11-30-7-2	S	G32	BHR377-60-81-9-34-2	S
G11	BHR360- 86-41-7-26-1	S	G33	BHR378-12-13-1-1-1	R
G12	BHR361-8-65-2-6-4	R	G34	BHR379-6-15-10-1-1	R
G13	BHR362- 8-6-5-26-9	R	G35	BHR380-26-1-8-1-1-2	R
G14	BHR363-86-52-6-4-7	S	G36	BHR381-66-11-9-1-1	S
G15	BHR364-86-5-26-14	S	G37	BHR382-50-3-3-1-1	S
G16	BHR365-76-90-2-11	R	G38	BHR383-53-8-13-1-1	R
G17	BHR366-126-4-2-2	R	G39	IR126072-83-3-3-1	R
G18	BHR367-18-2-1-14-1	S	G40	IR126076-122-1-1-2	S
G19	BHR368-5-B-16-2	S	G41	IR127278-152-1-3-1	R
G20	BHR369-6-55-16-1	S	G42	IR127278-114-3-3-2	R
G21	BHR370-51-8-1-3	R	G43	BR9390-6-2-2-1	R
G22	IR126055-46-3-2-B	R			

Legend: HRD= Hybrid Rice Division, BRRI= Bangladesh Rice Research Institute, HRDC= Hybrid Rice Development Consortium, IRRI= International Rice Research Institute, DR= Disease Reaction, R=Resistance, and S=Susceptible.

RESULTS

Reaction of genotypes to blast isolates

Following screening, 18 genotypes showed resistant reactions and 25 genotypes showed susceptible reactions (score 3-5) to the blast isolate (Table 1).

Pearson's correlation coefficient

Correlation analysis revealed the relationships among the studied traits to take decision in designing an effective breeding strategy. As shown in Fig. 1, grain yield per hill (GYPH) was significantly and positively correlated with spikelet fertility (SF%) (0.52***) and effective tillers per hill (ETPH) (0.31*). Total spikelets per panicle (TSPP) displayed a highly significant and positive correlation with filled grains per panicle (FGPP) (0.90***). Panicle length (PL) showed a strong positive correlation with and plant height (PH) (0.84***) and days to maturity (DM) (0.33*). FGPP had non-significant positive correlation with GYPH (0.14). PH had significant but negative correlation with SF (-0.34*).

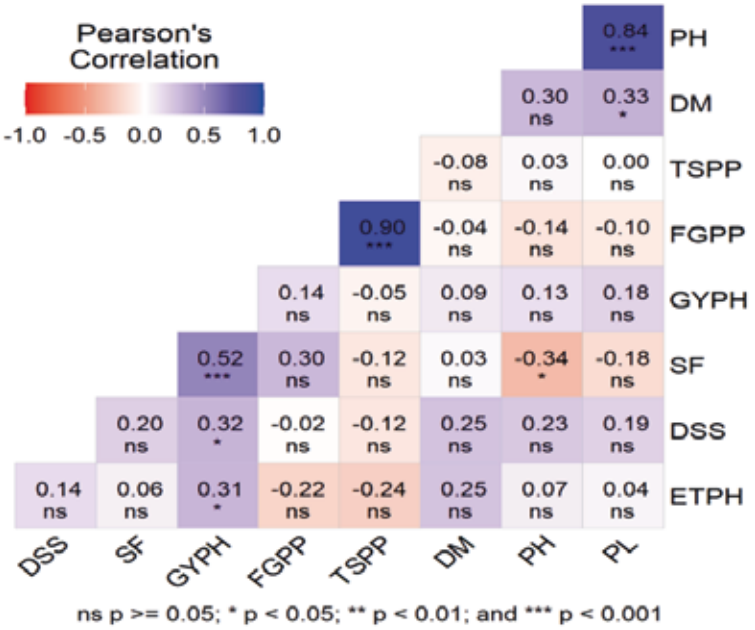


Fig. 1. Pearson's correlation analysis of the studied traits.

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a widely used multivariate statistical method for distinguishing genotypes based on trait variation. Fig. 2 and 3 illustrate the eigenvalues and the proportion of total phenotypic variance accounted for by each principal component among elite rice genotypes. Out of nine components, only three exhibited eigenvalues greater than 1, collectively explaining approximately 67.45% of the total trait variability (Fig. 2). The first principal component (PC1) accounted for the largest share of variance at 26.59%, followed by PC2 with 20.90%, and PC3 with 19.96% (Fig. 3).

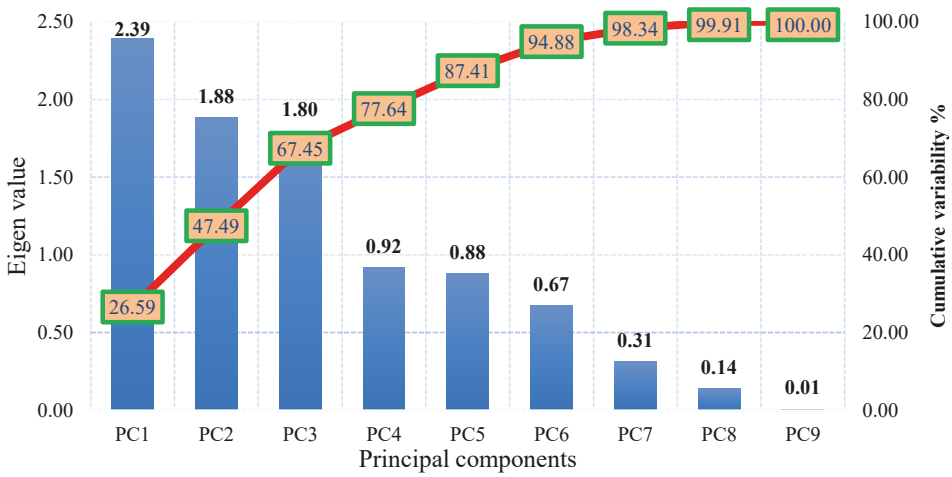


Fig. 2. Principal component analysis of elite rice genotypes showing eigenvalues and their contribution.

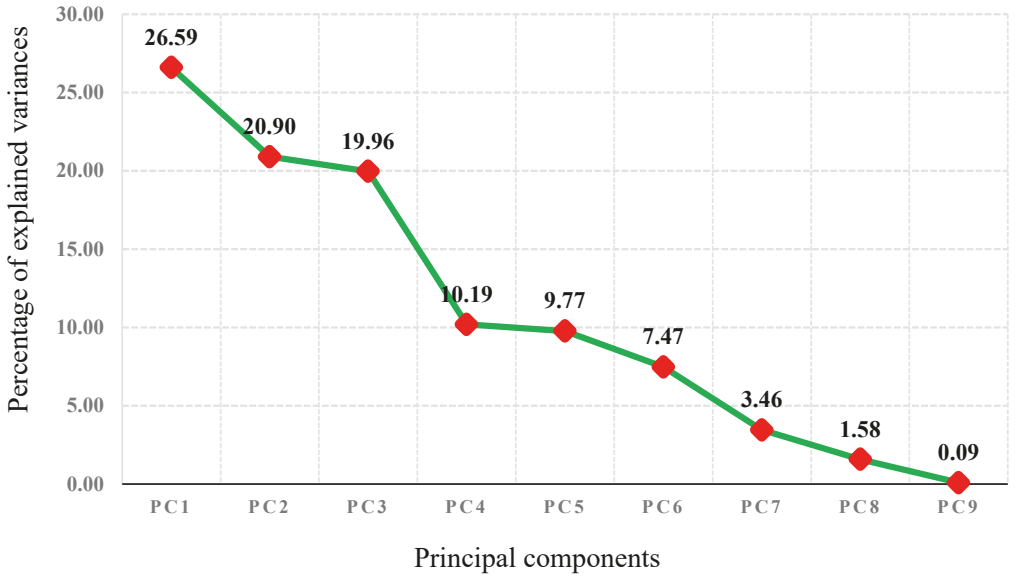


Fig. 3. Contribution of principal components to total explained variance in phenotypic diversity of elite rice genotypes to total variance.

Analysis of principal components and their corresponding vector loadings (Table 2) revealed that PC1 was strongly influenced by panicle length (0.75), plant height (0.74), days to maturity (0.54), effective tillers per hill (0.40), and grain yield per hill (0.23), all contributing positively. PC2 showed positive associations with total spikelets per panicle (0.82), filled grains per panicle (0.77), panicle length (0.50), plant height (0.49), days to maturity (0.22), and grain yield per hill (0.20). PC3 was primarily influenced by spikelet fertility percentage (0.85), grain yield per hill (0.78), effective tillers per hill (0.40), and filled grains per panicle (0.24).

Table 2. Contribution of variability of the first three principal components.

Variable	PC1	PC2	PC3
Days to maturity (days)	0.54	0.22	0.18
Plant height (cm)	0.74	0.49	-0.27
Effective tillers per hill (count)	0.40	-0.21	0.40
Panicle length (cm)	0.75	0.50	-0.15
Filled grains per panicle (count)	-0.57	0.77	0.24
Total spikelets per panicle (count)	-0.49	0.82	-0.12
Spikelet fertility percentage (percent %)	-0.21	-0.01	0.85
Grain yield per hill (g)	0.23	0.20	0.78
Disease severity score (ordinal values)	0.42	0.06	0.31
% of explained variances	26.59	20.90	19.96
Cumulative % of variances	26.59	47.49	67.45

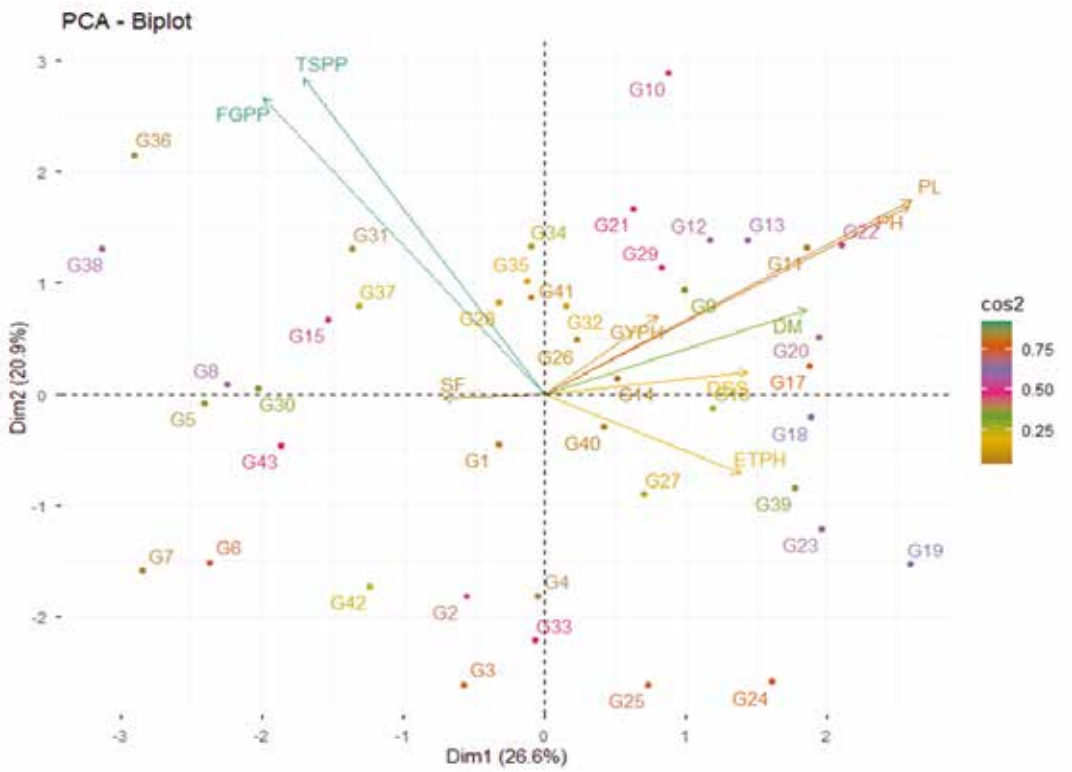


Fig. 4. The Biplot of 43 elite genotypes. The arrows show the contribution of the traits. Cos2 is the quality representation of both individuals and variables. (DM: days to maturity, PH: plant height (cm), ETPH: effective tillers per hill, PL: panicle length, FGPP: filled grain per panicle, TSPP: total spikelets per panicle, SF%: spikelet fertility percentage, GYPH: grain yield per hill (g) and DSS: Disease Severity Score).

A two-dimensional PCA biplot (Figure 4) was generated using principal component scores to visualize the extent and direction of trait contributions across components. The first dimension (Dim1) accounted for 26.6% of the total variation, while the second dimension (Dim2) explained 20.9%. Traits were grouped into two major components: Dim1 was primarily linked to GYPH, PL, and PH. These traits had long vectors and high cos² values, indicating strong influence. The Dim2 captured variation associated with TSPP and FGPP, which also showed strong contributions. Traits like SF, ETPH, and disease severity score (DSS) had shorter vectors and lower squared cosine values, indicating minimal representation in these two dimensions. Genotypes G22, G13, G11, G8, G12, G29, G20, G17, and G18 were positioned close to the grain yield vector, suggesting potential for yield-related traits. Genotypes G36, G37, G38, and G15 showed strong associations with TSPP and FGPP. The vector for DSS points towards the positive Dim1 and slightly positive Dim2 direction. Genotypes located opposite to the DSS vector (arrow) in the PCA biplot especially those farthest from it are likely to have low disease severity scores and are ideal candidates for blast resistance. In relation to the DSS vector, the genotypes G7, G6, G42, G2, G3, G38, G43 and G5 are the promising candidates for blast resistance and could be prioritized for breeding programs. Notably, genotypes G22, G13, G11, and G10 emerged as top performers for overall yield, while G10

showed strong expression of traits aligned with Dim2.

Trait Characterization

Descriptive statistics outlining the genetic variation among traits are presented in Table 3. Days to maturity among the genotypes ranged from 132 days (G38) to 162 days (G37). Plant height varied between 84 cm (G6) and 127 cm (G22). The number of effective tillers ranged from 5 (G38) to 10 (G23, G24, G33, G40). Panicle length extended from 19 cm (G7) to 27 cm (G10–G13, G17). Filled grains per panicle ranged between 95 (G19) and 255 (G36), while total spikelet's per panicle varied from 115 (G19) to 294 (G36). Spikelet fertility percentages spanned 66% (G42) to 97% (G7). Grain yield per hill ranged from 15 g (G30) to 41 g (G5). Disease severity scores varied from 0 (G5, G7, G10, G19, G26, G30, G36, G38, G42) to 5 (G14, G16).

Table 3. Descriptive statistics for nine agro-morphological traits of 43 rice genotypes.

SL No.	Variable	Mean	Min	Max	SD	SE
1	Days to maturity (days)	149.51	132	162	7.07	0.82
2	Plant height (cm)	104.88	84	127	10.29	1.49
3	Effective tillers per hill (count)	7.79	5	10	1.25	0.18
4	Panicle length (cm)	23.28	19	27	2.5	0.35
5	Filled grains per panicle (count)	169.77	95	255	35.04	5.47
6	Total spikelets per panicle (count)	200.98	115	294	41.18	6.96
7	Spikelet fertility percentage (percent %)	84.88	66	97	7.59	1.23
8	Grain yield per hill (g)	27.91	15	41	6.47	0.94
9	Disease severity score (ordinal values)	1.81	0	5	1.5	0.24

Cluster analysis

Based on multivariate analysis of nine studied traits, the 43 genotypes were classified into five separate clusters (Table 4 and Fig. 5). Cluster III encompassed the largest number of genotypes (11). Clusters I and IV each comprised 10 genotypes. Clusters II and V were the smallest, with six genotypes in each.

Table 4. Arrangement of 43 genotypes in different clusters.

Name of the cluster	Number of genotypes	Name of the genotypes code
I	10	G1, G4, G14, G17, G18, G27, G29, G32, G35, G40
II	6	G2, G3, G5, G6, G7, G8
III	11	G9, G10, G11, G12, G13, G17, G20, G21, G22, G26, G34
IV	10	G15, G28, G30, G31, G36, G37, G38, G41, G42, G43
V	6	G19, G23, G24, G25, G33, G39

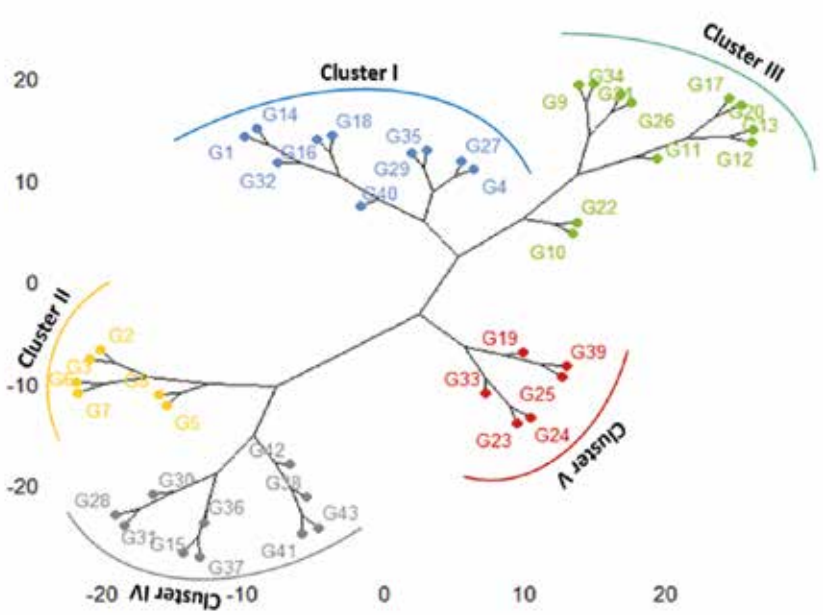


Fig. 5. Illustration of an un-rooted phylogenic tree among 43 rice genotypes based on Euclidean distance.

The mean performance of nine characters across five distinct clusters is presented in table 5. Notable differences were observed among clusters for most traits. Cluster V recorded the highest averages for days to maturity (154.17 days) and effective tillers per hill (9.00). Cluster IV showed the greatest mean values for filled grains per panicle (196.3) and total spikelets per panicle (241.2), along with the lowest disease severity (0.7), indicating strong resistant to blast. Cluster III exhibited the highest averages for panicle length (26.27 cm) and grain yield per hill (33.36). Spikelet fertility percentage peaked in Cluster II (94.50%). Meanwhile, Cluster I had the lowest average plant height (105.6 cm).

Table 5. Cluster means value of nine characters in the studied materials.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to maturity (days)	150.20	142.50	152.73	146.70	154.17
Plant height (cm)	105.6	89.83	115	101.9	105.17
Effective tillers per hill (count)	7.7	7.83	7.91	7	9
Panicle length (cm)	23.2	19.83	26.27	22.1	23.33
Filled grains per panicle (count)	164	175	178.27	196.3	114.33
Total spikelets per panicle (count)	195.6	185.5	208.64	241.2	144.33
Spikelet fertility (%)	83.7	94.5	87.18	81.2	79.17
Grain yield per hill (g)	28	32.83	33.36	20.6	25
Disease severity score (ordinal values)	3.8	1.17	1.82	0.7	1

Table 6 presents both intra- and inter-cluster distances among the five identified clusters. The intra-cluster distances varied from 2.69 to 3.77, with Cluster III showing the lowest internal variation and Cluster V exhibiting the highest variation. Regarding inter-cluster relationships, the highest distance was observed between Cluster I and Cluster V ($D^2 = 5.09$), indicating

substantial genetic divergence. This was followed by distances between Cluster I and IV ($D^2 = 4.90$), Cluster III and IV ($D^2 = 4.77$), and Cluster IV and V ($D^2 = 4.65$). The smallest inter-cluster distance was recorded between Cluster II and Cluster III ($D^2 = 3.55$). These results highlight considerable diversity among the clusters, which is beneficial for selecting genetically distinct genotypes in hybrid rice breeding programs.

Table 6. Intra- (Italic) and Inter- cluster divergence of 43 rice genotypes.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	*3.01*	4.13	4.4	4.90	5.09
Cluster II		*2.96*	3.55	4.27	4.38
Cluster III			*2.69*	4.77	4.64
Cluster IV				*2.72*	4.65
Cluster V					*3.77*

DISCUSSION

Grain yield in rice is a complex quantitative trait influenced by numerous factors. These include indirect contributors such as plant height, growth duration, effective tiller number, panicle length, and seed setting percentage as well as direct factors such as panicle number, grains per panicle, filled grains and thousand-grain weight (Sakamoto & Matsuoka, 2008; Huang *et al.*, 2011). Due to this complexity, direct selection for yield traits can be challenging and time-intensive. In contrast, selecting traits that are strongly correlated with yield offers a more efficient and practical approach (Ahmadikhah *et al.*, 2008). The variation among the evaluated genotypes reflected a distinct interaction between blast resistance and genotypes. Some genotypes demonstrated strong resistance, while others showed susceptible reactions. These contrasting reactions emphasize that blast resistance varies across genotypes and is governed by both resistance genes and responsiveness to pathogen pressure. Such findings are valuable for breeding programs, as they help identify genotypes with more consistent resistance under pathogen pressure, thereby supporting the development of durable blast resistant hybrid cultivars.

Principal Component Analysis (PCA) revealed three components with eigenvalues of 2.39, 1.88, and 1.80, accounting for 26.59%, 20.90%, and 19.96% of the total variance, respectively. These components are essential in identifying key traits for selecting superior genotypes from diverse breeding populations, as supported by findings from Majid *et al.* (2013) and Akhtar *et al.* (2022). Understanding the relationships among yield components is essential for effective genotype selection in breeding programs. Correlation analysis plays a vital role in pinpointing traits that significantly impact yield, guiding breeders in prioritizing traits for improvement (Eriksson *et al.*, 2018). Correlation analysis revealed a significant positive association between SF (%) and GYPH implies that the higher the fertility rate, the higher the grain yield. Bist *et al.* (2025) and Bhadru *et al.* (2012) stated that the lower the sterility rate, the higher the grain yield. A significant positive correlation between grain yield and number of effective tillers per hill was stated by Islam *et al.* (2025) and Gupta *et al*. (2023), Debsharma *et al.* (2020) and Kohnaki *et al.,* (2013). FGPP was positively correlated with GYPH. The positive connection of GY with FGPP was reported by Perween *et al.,* (2020) and Kohnaki *et al.,* (2013). These results indicate that selection for these traits could effectively enhance grain yield in the evaluated rice genotypes. Integrating these traits into breeding will speed up the development of high-yielding rice cultivars to meet national food demand. The number of FGPP had a highly significant and positive correlation with TSPP was reported by Ara *et al.,* (2023). A highly significant and positive correlation between PL

and PH also stated by Galib *et al.* (2025). Similarly, the findings of positive correlation between PL and DM corroborate with Bist *et al.,* (2025). The FGPP was not significantly correlated with GYPH; similar to the results of Li *et al.,* (2019).

PCA and cluster analysis are effective tools for assessing variability in quantitative traits and identifying high-performing genotypes (Sawarkar *et al.*, 2025). PCA helps reduce data dimensionality while retaining maximum variability, allowing genotypes to be ranked based on component scores (Shoba *et al.*, 2019). Similar patterns of variation were reported by Kumar *et al.* (2021), who found 31.848% and 19.553% variation in PC1 and PC2, respectively, using 119 rice breeding lines. PCA-based clustering revealed substantial genetic diversity and trait associations, which are crucial for targeted breeding efforts. The PCA biplot (Fig. 4) highlighted key traits driving variability and genotype differentiation, consistent with studies by Chiquet *et al.* (2018) and Saha *et al.,* (2022). Traits near the origin contributed little to variance, and clustered genotypes indicated strong phenotypic associations (Kose *et al.*, 2018). Notably, our analysis indicated a robust association among GYPH and a cluster of agronomic traits, including PL, PH, ETPH, DM and DSS as evidenced by the acute angles by their respective dimension vectors. The association between GYPH and PH suggests a compact plant architecture with shorter stature and more tillers or panicles, which can reduce lodging risk and improve yield stability (Hairmansis *et al.*, 2010; Avakyan & Dzhamirze, 2018). Similarly, the angle between GYPH and DM implies that longer growth periods may enhance yield through increased biomass and grain filling (Al-Karaki, 2012). Genotypes G22, G13, G11, G8, G12, G29, G20, G17, and G18 were closely aligned with the GYPH vector, indicating their potential as high-yielding candidates for direct selection or use in breeding programs. Rao *et al.* (2021) and Prakriti *et al.* (2017) documented variability in rice germplasm, with panicle length ranging from 16–33.9 cm and 21.7–29.6 cm, respectively. In a more recent study, Sultana *et al.* (2025) reported trait ranges in T. Aman rice, including effective tillers per hill (10.03–13.05), panicle length (21.82–23.0 cm), sterile spikelets per panicle (12.65–13.05), and grains per panicle (123.53–141.33).

Cluster analysis grouped the 43 genotypes into five distinct clusters. This classification revealed substantial genetic diversity among the genotypes, particularly in traits related to yield and disease resistance. The genotypes demonstrated a wide range of variability, with several unique entries showing potential for use in breeding programs. Different authors reported that genetic diversity among different rice genotypes has been classified into distinct cluster groups. For instance, Girma *et al.* (2018) grouped 64 genotypes into six clusters using seven yield-related traits. Bekis *et al.* (2021) classified 30 lowland rice genotypes into five clusters based on 17 morphological and yield traits, while Worede *et al.* (2014) divided 24 upland rice genotypes into two clusters using 17 morpho-agronomic traits. The phylogenetic tree further illustrated the genetic relationships and diversity among the cultivars. Such clustering is instrumental for breeders in selecting genetically distant parents for developing hybrids with enhanced yield potential and resistance to blast disease. The insights gained from this study contribute to breeding strategies aimed at meeting the evolving demands of agriculture and consumers.

The cluster mean value indicated that neither any cluster contained the genotypes with all desirable traits which could be selected and utilized directly in the crossing program of hybrid rice breeding. Thus, hybridization between different clusters is needed to develop desirable genotypes. The analysis of cluster distance revealed that the inter-cluster distances were higher than the intra-cluster distance indicating wider genetic diversity among the genotypes of different groups. The above result indicated that the genotypes included in the cluster I and cluster V were more diverse than the genotypes of others cluster. Therefore, genotype selection for hybridization from the

cluster I and V may give the desirable heterosis for heterotic rice hybrids. The genotypes under cluster IV having lower DSS values. So, the genotypes of this cluster could be used as a male parent of testcross program for identifying blast resistant maintainer or restorer lines. These results are in agreement with those of Khan *et al.* (2014) and Latif *et al.,* (2011). Genotypes of distantly located clusters were suggested to use in hybridization programs for obtaining a wide spectrum of variation among the segregates as suggested by Yadav *et al.* (2011) and Latif *et al.,* (2011). The present study provided important information for blast resistance at the seedling stage, but it may not fully represent responses at reproductive stages. Similarly, the morphological traits are influenced by environmental factors; therefore, assessment of morphological traits in a single environment potentially confounds only genetic effects. So, phenotypic screening with molecular markers at both the seedling and reproductive stages alongside multi-location or multi-season trials would facilitate the identification of stable and broad-spectrum resistance.

CONCLUSION

Multivariate analyses of nine quantitative traits were performed to determine the genetic diversity of 43 genotypes, which were grouped into five different clusters. The 67.45% variation was explained by the first three primary axes. Cluster III and IV represent high yielding and disease resistant cluster, respectively. So, testcrosses could be made of the aforementioned clusters with promising CMS line that would offer a good scope to identify blast-resistant and high yielding elite restorer or maintainer lines. The study revealed that blast resistance varied among genotypes, reflecting genetic differences in resistance genes and pathogen responsiveness. Future research should focus on validating these findings across diverse environments and pathogen populations, as well as integrating molecular markers (SSR or SNP) to identify genes for blast resistance (*R*-genes), fertility restoration (*Rf*), wide compatibility (WC), identifying yield QTLs and heterotic loci for greater heterosis and introducing them into the background of hybrid parental genotypes to accelerate the rate of heterosis for hybrid rice breeding program.

ACKNOWLEDGMENTS

The authors are thankful to the Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh for providing facilities for phenotypic screening of leaf blast of rice at the blast nursery field.

FUNDING

This study was supported by a national project of Bangladesh "High-yielding hybrid rice variety development through modernization of research," funded by Ministry of Agriculture (224344400), Bangladesh.

AUTHOR'S CONTRIBUTIONS

M. Akhlasur Rahman (M.A.R) and Umakanta Sarker (US): Conceptualization, designing the experiment, validation, resources, monitoring of the experiments, writing-manuscript draft, review and editing. Laila Ferdousi Lipi (LFL): Methodology, validation, experimentation, data curation, data analysis, data interpretation, writing first manuscript draft, review and editing. Mohammad Mehfuz Hasan Saikat (MMHS) and Md. Motaher Hossain (MMH): review and editing.

REFERENCES

Ahmadikhah, A. (2018). Selection effects in rice as assessed by genetic analysis in segregating populations. *Plant Breeding and Seed Science*, 77, 69-77.

Ahmed, M. S., Parveen, S., Baktiar, M. H. K., & Siddique, M. A. (2015a). Study of qualitative agro-morphological characters of Dhaliboro rice germplasm. *Eco-friendly Agricultural Journal*, 8(8), 91–96.

Ahmed, M. S., Parveen, S., Islam, M. Z., & Siddique, M. A. (2015b). Characterization of Dhaliboro rice (*Oryza sativa* L.) germplasm of Bangladesh for morpho-physicochemical traits. *International Journal of Bio-Research*, 19(1), 1–11.

Akter, N., Begum, H., Islam, M. Z., Siddique, M. A., & Khalequzzaman, M. (2018). Genetic diversity in Aus rice (*Oryza sativa* L.) genotypes of Bangladesh. *Bangladesh Journal of Agricultural Research*, 43(2), 253–266.

Akter, N., Islam, M. Z., Bhuiyan, A., Siddique, M. A., & Khalequzzaman, M. (2017). Distinctness of 45 irrigated rice (*Oryza sativa* L.) landraces of Bangladesh through agro-morphological traits. *Eco-friendly Agricultural Journal*, 10(7), 100–107.

Akter, N., Islam, M. Z., Siddique, M. A., Chakrabarty, T., Khalequzzaman, M., & Chowdhury, M. A. Z. (2016). Genetic diversity of Boro rice (*Oryza sativa* L.) landraces of Bangladesh. *Bangladesh Journal of Plant Breeding and Genetics*, 29(2), 33–40.

Akhtar, R., Iqbal, A., & Dasgupta, T. (2022). Genetic diversity analysis of aromatic rice (*Oryza sativa* L.) germplasm based on agro-morphological characterization. *Oryza*, 59(2), 141-149.

Al-Karaki, G. N. (2012). Phenological development-yield relationships in durum wheat cultivars under late-season high-temperature stress in a semiarid environment. *International scholarly research notices*, 2012(1), 456856.

Ara, A., Nihad, S. A. I.., Rashid, M. M.., Akter, A., Uddin, A. B. M. A., Ansari, T. H.., & Latif, M. A. (2023). Genetic diversity of INGER rice genotypes based on morphological charac-ters and bacterial blight resistance. *Bangladesh Rice Journal*, 26(1), 17–31. https://doi.org/10.3329/brj.v26i1.65876

Avakyan, E. R., & Dzhamirze, R. R. (2018). Rice lodging resistance. *RUDN Journal of Agronomy and Animal Industries*, 13(4), 366-372.

Baktiar, M. H. K., Samad, M. A., Khalequzzaman, M., Sharma, N., & Siddique, M. A. (2013). Morphological characterization of local Aus rice germplasm (*Oryza sativa* L.). *Eco-friendly Agricultural Journal*, 6(10), 238–242.

Banik, S., Islam, M. Z., Haque, M. A., Siddique, M. A., Mallik, A., & Siddiquee, M. A. (2012). Physico-chemical properties based genetic diversity of T. Aman rice genotypes. *Eco-friendly Agricultural Journal*, 5(7), 69–72.

Bekis, D., Mohammed, H., & Belay, B. (2021). Genetic divergence and cluster analysis for yield and yield contributing traits in lowland rice (*Oryza sativa* L.) genotypes at Fogera, Northwestern Ethiopia. *International Journal of Advanced Research in Biological Sciences*, 8(5), 1-11.

Bhadru, D., Reddy, D. L., & Ramesha, M. S. (2012). Correlation and path analysis of yield and yield components in hybrid rice (*Oryza sativa* L.). *Agricultural Science Digest-A Research Journal*, 32(3), 199-203.

Bist, D. R., Chapagaee, P., Rawal, R., Bist, G., & Bohara, R. (2025). Multivariate analysis of yield and phenotypic diversity in rainfed rice (*Oryza sativa* L.) landraces from Gokuleshwor, Baitadi, Nepal. *Discover Agriculture*, 3(1), 167.

Biswash, M. R., Sharmin, M., Rahman, N. M. F., Farhat, T., & Siddique, M. A. (2016). Genetic diversity in modern T. Aman rice varieties of Bangladesh (*Oryza sativa* L.). *Sains Malaysiana*, 45(5), 709–716.

Chiquet, J., Mariadassou, M., & Robin, S. (2018). Variational inference for probabilistic Poisson PCA. *Annals of Applied Statistics*, 12(4), 2674–2698.

Debnath, A., Bhutia, K. L., & Sumpi, H. (2018). Mapping and deployment of blast resistance gene in rice—A work in progress. *International Journal of Current Microbiology and Applied Sciences*, 7, 2073-2094.

Debsharma, S. K., Disha, R. F., Ahmed, M. M. E., Khatun, M., Ibrahim, M., & Aditya, T. L. (2020). Assessment of genetic variability and correlation of yield components of elite rice genotypes (*Oryza sativa* L.). *Bangladesh Rice Journal*, 24(1), 21-29.

Deepika, K., Lavuri, K., Rathod, S., Yeshala, C. M., Jukanti, A. K., Reddy, S. N., & Badri, J. (2021). Multivariate analysis of

geographically diverse rice germplasm for genetic improvement of yield, dormancy and shattering-related traits. *Plant Genetic Resources*, 19(2), 144-152.

Eriksson, D., Akoroda, M., Azmach, G., Labuschagne, M., Mahungu, N., & Ortiz, R. (2018). Measuring the impact of plant breeding on sub-Saharan African staple crops. *Outlook on Agriculture*, 47(3), 163-180.

Girma, B. T., Kitil, M. A., Banje, D. G., Biru, H. M., & Serbessa, T. B. (2018). Genetic variability study of yield and yield related traits in rice (*Oryza sativa* L.) genotypes. *Advances in Crop Science and Technology*, 6(4), 381.

Gupta, R., Debsharma, S. K., Jahan, N., & Rani, M. H. (2023). Genetic Variability Analysis for Yield Contributing Traits in Rice (*Oryza sativa* L.) in hilly areas of Bangladesh. *Bangladesh Rice Journa*l, 27(2), 51–65. https://doi.org/10.3329/ brj.v27i2.77556

Hairmansis, A., Kustianto, B., & Suwarno, S. (2010). Correlation analysis of agronomic characters and grain yield of rice for tidal swamp areas. *Indonesian Journal of Agricultural Science*, 11(1), 11-15.

Huang, X., Zhao, Y., Wei, X., Li, C., Wang, S., Zhao, Q., & Xu, C. (2011). Genome-wide association study of 14 agronomic traits in rice landraces. *Nature Genetics*, 44(1), 23-30.

Islam, R., Hossain, M. S., Aktar, S., Sharma, D. C., Islam, S., & Kayess, M. O. (2025). Genetic and multivariate analysis of some local and advanced selected Aman rice genotypes under Bangladesh condition. *Discover Plants*, 2(1), 271.

Islam, M. Z., Akter, N., Chakrabarty, T., Bhuiya, A., Siddique, M. A., & Khalequzzaman, M. (2018). Agro-morphological characterization and genetic diversity of similar named aromatic rice (*Oryza sativa* L.) landraces of Bangladesh. Bangladesh *Rice Journal*, 22(1), 45–56.

Islam, M. Z., Khalequzzaman, M., Siddique, M. A., Akter, N., Ahmed, M. S., & Chowdhury, M. A. Z. (2017). Phenotypic characterization of jhum rice (*Oryza sativa* L.) germplasm collected from Rangamati district in Bangladesh. *Bangladesh Rice Journal*, 21(1), 47–57.

Islam, M. Z., Siddique, M. A., Akter, N., Prince, M. F. R. K., Islam, M. R., Anisuzzaman, M., & Mian, M. A. K. (2019). Morpho-molecular divergence of restorer lines for hybrid rice (*Oryza sativa* L.) development. *Cereal Research Communications*, 47(3), 531–540.

Islam, M. Z., Siddique, M. A., Rashid, E. S. M. H., Ahmed, M. S., & Khalequzzaman, M. (2014). Genetic diversity in Sadajira rice (*Oryza sativa* L.) germplasm. *The Agriculturists*, 12(1), 26–32.

Khalequzzaman, M., Chakrabarty, T., Islam, M. Z., Rashid, E. S. M. H., Prince, M. F. R. K., & Siddique, M. A. (2022). Assessment of mean performance, variability, traits association and path coefficient in transplanted Aman rice (Oryza sativa L.) landraces. *Asian Journal of Biology*, 16(4), 27–39.

Khalequzzaman, M., Chakrabarty, T., Islam, M. Z., Rashid, E. S. M. H., Prince, M. F. R. K., & Siddique, M. A. (2023). Deciphering genetic variability, traits association, correlation and path coefficient in selected Boro rice (*Oryza sativa* L.) landraces. *Asian Journal of Biology*, 19(2), 33–45.

Khalequzzaman, M., Siddique, M. A., & Bashar, M. K. (2012). Rice genetic resources conservation and utilization in Bangladesh. In *Genetic Resources and Seed Division* (pp. 50–60). Gazipur, Bangladesh.

Khan, M. A., Ali, M. A., Monsur, M. A., Kawasaki-Tanaka, A., Hayashi, N., Yanagihara, S., & Fukuta, Y. (2016). Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Bangladesh. *Plant Disease*, 100(10), 2025-2033.

Kohnaki, M. E., Kiani, G., & Nematzadeh, G. (2013). Relationship between morphological traits in rice restorer lines at F3 generation using multivariate analysis. *International Journal of Advanced*

Biological and Biomedical Research, 1(6): 572-577. 10.26655/ijabbr.2017.9.1

Kose, A., Onder, O., Bılır, O., & Kosar, F. (2018). Application of multivariate statistical analysis for breeding strategies of spring safflower (*Carthamus tinctorius* L.). *Turkish Journal of Field Crops*, 23(1), 12-19.

Kulsum, M. U., Hasan, M. J., Siddique, M. A., Begum, F., & Akond, Z. (2015). Genetic diversity in selected rice genotypes (*Oryza sativa* L.). *Bangladesh Journal of Agriculture*, 38–40, 63–72.

Latif, M., Rahman, M., Kabir, M., Ali, M., Islam, M., & Rafii, M. (2011). Genetic diversity analyzed by quantitative traits among rice (*Oryza sativa* L.) genotypes resistant to blast. *African Journal of Microbiology Research*, 5, 4383-4391. https://doi.org/10.5897/ajmr11.492.

Li, R., Li, M., Ashraf, U., Liu, S., & Zhang, J. (2019). Exploring the relationships between yield and yield-related traits for rice varieties released in China from 1978 to 2017. *Frontiers in plant science*, 10, 543. https://doi.org/10.3389/fpls.2019.00543.

Mazid, M. S., Rafii, M. Y., Hanafi, M. M., Rahim, H. A., Shabanimofrad, M., & Latif, M. A. (2013). Agro-morphological characterization and assessment of variability, heritability, genetic advance and divergence in bacterial blight resistant rice genotypes. *South African Journal of Botany*, 86, 15-22.

Muti, S. A. A., Hoque, M. I., Islam, M. M., Siddique, M. A., & Islam, M. S. (2020). Morpho-molecular characterization and screening of rice (*Oryza sativa* L.) genotypes for salinity tolerance at seedling stage. *SAARC* Journal of Agriculture, 18(2), 1–15.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A., & Thoma, G. (2016). Economic and environmental impact of rice blast pathogen (*Magnaporthe oryzae*) alleviation in the United States. *PloS one*, 11(12), e0167295.

Nihad, S. A. I., Ara, A., Rashid, M. M., Hasan, M. A. I., Khan, M. A. I., & Latif, M. A. (2020). Genetic divergence of rice genotypes revealed by bacterial blight disease and morphological traits. *Bangladesh Rice Journal*, 24(1), 73-84.

Nivedha, R., Manonmani, S., Kalaimagal, T., Raveendran, M., & Kavitha, S. (2024). Assessing the genetic diversity of parents for developing hybrids through morphological and molecular markers in rice (*Oryza sativa* L.). *Rice*, 17(1), 17.

Perween, S., Kumar, A., Singh, S. P., Satyendra, Kumar, M., & Ravi Ranjan Kumar, R.R. (2020). Genetic Variability Parameters for Yield and Yield Related Traits in Rice (*Oryza sativa* L.) under Irrigated and Drought Stress Condition. *International Journal of Current Microbiology and Applied Science*, 9(2), 1137-1143. doi: https://doi.org/10.20546/ijcmas.2020.902.133

Prakriti, M., Bhandarkar, S., Shrivas, Y., Nair, S. K., Ojha, G. C., & Pachauri, A. K. (2017). Assessment of genetic variability for quantitative and qualitative traits in Rice Germplasm Accessions (*Oryza sativa* L.). *Bulletin of Environment, Pharmacology and Life Science*, Special issue (1), 76-83.

Rao, N. N. D., Roja, V., Tushara, M., Rao, V. S., & Rao, V. S. (2021). Characterization and diversity analysis of rice germplasm. In *Biological Forum–An International Journal*, 13(3a), 170-179.

Saha, I., Durand-Morat, A., Nalley, L. L., Alam, M. J., & Nayga, R. (2021). Rice quality and its impacts on food security and sustainability in Bangladesh. *PLoS One*, 16(12), e0261118.

Saha, S. R., Ferdausi, A., Hassan, L., Haque, M. A., Begum, S. N., Yasmin, F., & Akram, W. (2022). Rice landraces from haor areas of Bangladesh possess greater genetic diversity as revealed by morpho-molecular approaches along with grain quality traits. *Cogent Food & Agriculture*, 8(1), 2075130.

Sakamoto, T., & Matsuoka, M. (2008). Identifying and exploiting grain yield genes in rice. *Current opinion in plant biology*, 11(2), 209-214.

Salleh, S. B., Rafii, M. Y., Ismail, M. R., Ramli, A., Chukwu, S. C., Yusuff, O., & Hasan, N.

A. (2022). Genotype-by-environment interaction effects on blast disease severity and genetic diversity of advanced blast-resistant rice lines based on quantitative traits. *Frontiers in Agronomy*, 4, 990397.

Sawarkar, A., Das, S., & Saha, S. (2025). Genetic Cause and Effect Interrelationships for Yield and Yield Components of Mungbean [*Vigna radiata* (L.) Wilczek]. *Legume Research-An International Journal*, 1, 9.

Shoba, D., Vijayan, R., Robin, S., Manivannan, N., Iyanar, K., Arunachalam, P., & Geetha, S. (2019). Assessment of genetic diversity in aromatic rice (*Oryza sativa* L.) germplasm using PCA and cluster analysis. Electronic *Journal of Plant Breeding*, 10(3), 1095-1104.

Siddique, M. A., Islam, M. Z., Khalequzzaman, M., & Ahmed, M. S. (2011). Genetic diversity in rice (*Oryza sativa* L.) landraces of hilly areas in Bangladesh. *Bangladesh Journal of Plant Breeding and Genetics*, 24(2), 25–30.

Siddique, M. A., Khalequzzaman, M., Islam, M. Z., Ahmed, M. S., & Rashid, E. S. M. H. (2013). Genetic diversity in local Boro rice (*Oryza sativa* L.) genotypes of Bangladesh. *Bangladesh Journal of Plant Breeding and Genetics*, 26(1), 19–24.

Siddique, M. A., Khalequzzaman, M., Islam, M. Z., Baktiar, M. H. K., & Ahmed, M. S. (2016). Genetic diversity assessment of quantitative traits in landraces of Aus rice. *Bangladesh Journal of Plant Breeding and Genetics*, 29(2), 17–24.

Siddique, M. A., Rashid, E. S. M. H., Khalequzzaman, M., Islam, M. Z., Ahmed, M. S., & Baktiar, M. H. K. (2010). Genetic diversity of local rainfed rice (*Oryza sativa* L.). *Bangladesh Journal of Plant Breeding and Genetics*, 23(2), 41–46.

Siddique, M. A., Islam, M. Z., Khalequzzaman, M., & Bhuiya, A. (2018). Agro-morphological characterization of geographical indication rice cultivars grown in Bangladesh. *The Experiment*, 45(4), 2598–2610.

Sivaranjani, R., Reddy, R. S., & Reddy, K. R. (2010). Assessment of genetic diversity in Indian rice germplasm (*Oryza sativa* L.) using SSR markers. *Journal of Genetics*, 92(3), 545–557. https://www.ias.ac.in/article/fulltext/jgen/092/03/0545-0557

Sultana, N., Das, B., Akhter, M. S., Sultana, N., & Rahman, M. A. (2025). Comparative Performance of Transplanted Aman Rice Varieties under Raised Bed and Conventional Cultivation Methods in Bangladesh. *Asian Journal of Agricultural and Horticultural Research*, 12(3), 123-136.

Virmani, S. S., Mao, C. X., & Hardy, B. (2001). *Hybrid rice: Seeds of innovation*. Los Baños, Philippines: International Rice Research Institute.

Wang, C., Fa, X., Meng, Q., Zhang, Y., Wang, W., Zhu, K., & Zhang, H. (2024). Comparison of agronomic and physiological characteristics for rice varieties differing in water use efficiency under alternate wetting and drying irrigation. *Agronomy*, 14(9), 1986.

Worede, F., Sreewongchai, T., Phumichai, C., & Sripichitt, P. (2014). Multivariate analysis of genetic diversity among some rice genotypes using morpho-agronomic traits. *Journal of plant sciences*, 9(1), 14.

Yadav, M. K., S, A., Ngangkham, U., Shubudhi, H. N., Bag, M. K., Adak, T., & Jena, M. (2017). Use of molecular markers in identification and characterization of resistance to rice blast in India. *PloS one*, 12(4), e0176236.

Yadav, V. K., Yuvraj Singh, Y. S., Soni, S. K., & Yadav, A. K. (2011). Genetic divergence analysis in salt tolerance rice (*Oryza sativa* L.) genotypes. *Plant Archives*, 11 (2), 593-595.

Yuan, L. P. (2017). Progress in super-hybrid rice breeding. *The Crop Journal*, 5(2), 100–104. https://doi.org/10.1016/ j.cj.2017.02.001

Bangladesh Rice J.28(1): 57-68, 2024, doi.org/10.3329/brj.v28i1.86746

Diversity of Pathogenicity Among Rice Blast Fungus (*Pyricularia oryzae*) Isolates in Bangladesh

M S Mian[1*], T H Ansari[1], M A T Mia[1], I H Mian[2] and I R Choi[3]

ABSTRACT

A study was undertaken to determine the pathogenic and the genetic diversity of *Pyricularia oryzae* isolates causing blast disease of rice in Bangladesh. To find out pathogenic diversity of *P. oryzae*, 100 blast pathogen isolates were collected from five agro ecological zones (AEZ) abbreviated as AEZ23, AEZ19, AEZ13, AEZ28 and AEZ2 of Bangladesh. They were tested for their pathogenicity against 26 rice (*Oryza sativa* L.) differential varieties (DVs) having 23 resistance genes designated as *Pish, Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi12(t), Pi19*, and *Pi20*(t). The virulence analysis showed that four genes, *Pish, Pi9, Piz* and *Pita-2* revealed a wide spectrum of moderate resistance to those isolates. The isolates were categorized into 94 races on the basis of the reaction patterns against rice differential varieties harboring twenty-three resistance genes and one susceptible variety, Lijiangxintuanheigu (LTH) by latest designation system. The findings demonstrate the existence of a wide variation in blast pathogens in Bangladesh. The average virulence of isolates from individual AEZ reveals that the maximum virulent isolates occur in AEZ2 (67%) followed by AEZ19 (63%) and AEZ28 (55%). Moreover, 100 *Pyricularia oryzae* isolates were grouped into five clusters viz. I, II, III, IV and V based on the results of the pathogenicity on 26 differential varieties including LTH on the basis of principal component analysis. Cluster I comprised of 18 isolates, Cluster II contained maximum of 31 isolates and cluster III contained 16 isolates. Twenty three isolates were placed in cluster IV and 12 isolates belonged to cluster V. The average virulence frequency of five Cluster I, II, III, IV and V showed 50%, 63%, 57%, 59% and 56% respectively. The findings of the present study reveal that the monogenic lines selected as differential varieties and the representative 25 blast pathogen isolates may be used to characterize the resistance of rice varieties.

Keywords: Rice blast fungus, Diversity of pathogenicity, *Pyrcularia oryzae*

INTRODUCTION

About half of the world's population relies on rice to achieve their daily caloric requirements (Wennberg, 2014). According to severity and incidence, 32 rice diseases have been identified in Bangladesh to far, blast (Nihad *et al.*, 2022), bacterial blight (Latif *et al.*, 2024a; Nihad *et al.*, 2021), sheath blight (Latif *et al.*, 2022), tungro (Nihad *et al.*, 2021) and false smut (Nessa *et al.*, 2015) considered as catastrophic disease throughout the nation. Rice blast caused by the fungal pathogen *Pyricularia oryzae* (telemorph: *Magnaporthe grisea* (Hebert) Barr.) is one of the most important diseases of rice in worldwide (Latif *et al.*, 2024b; Nihad *et al.*, 2022; Zeigler *et al.*, 1994). The use of resistant varieties is the most practical and economical method to control

[1]Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh
[2]Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur-1706, Bangladesh
[3]International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines
[*]Corresponding author's E-mail: salimspml@yahoo.com (M S Mian)

blast disease. But availability of effective blast resistant variety is limited due to breakdown of resistance by blast pathogen races virulent against the resistance (Koizumi, 2007). The dynamic interaction between host resistance and fungus virulence in the rice blast pathosystem can be explained by the gene-for-gene theory that every resistance gene in the host is corresponding with avirulence gene in the pathogen (Flor, 1971, Silue *et al.,* 1992). With the basis of the gene-for-gene theory, differential varieties and lines with various resistance genes against rice blast fungus have been developed to monitor the blast pathogen population structure and predict the emergence of new races *P. oryzae*.

The diversity of pathogenicity of blast pathogen isolates has been examined in many countries including Bangladesh, Indonesia, and China using differential varieties and lines. Over 100 blast resistance genes have been identified globally (Shahriar *et al.*, 2020; Yadav *et al.*, 2019), with *Pi9* and *Pb1* being the most prevalent. The *Pb1* gene, effective against a broad range of blast pathogen races, has demonstrated sustained resistance against panicle blast for over 35 years in Korea (Inoue *et al.*, 2017; Lee *et al.*, 2015b). Notably, *Pi9* and *Pb1* have also been shown to be effective against blast pathogen races in Bangladesh (Khan *et al.*, 2016; Nihad *et al.*, 2022). Noda *et al.* (1999) identified 12 blast pathogen races among 129 isolates collected from the Mekong River Delta area in Vietnam using 12 Japanese differential varieties carrying one of the resistance genes against blast pathogen (Yamada *et al.*, 1976; Kiyosawa, 1981; Kiyosawa *et al.*, 1984). Mekwatanakarn *et al.*, (2000) classified 527 blast pathogen isolates from Thailand into 175 races using differential near-isogeneic lines (NILs) developed from cultivars CO 39 and Lijiangxintuanheigu (LTH). Thinlay *et al.*, (2000) collected 110 isolates in Bhutan, and classified them into 53 races on the basis of the reactions of CO 39-based differential NILs. Chen *et al.*, (2001) collected 792 isolates in China, and classified them into 344 races using differential NILs derived from CO 39 and LTH. The pathogenicity of 119 blast pathogen isolates collected from the Philippines were categorized into 70 races (Telebanco-Yanoria *et al.*, 2008) using 18 Japanese differential varieties (Yamada *et al.*, 1976; Kiyosawa 1981, Kiyosawa *et al.*, 1984). These studies indicated that the variations in pathogenicity of blast fungus can be manifested by examining the reactions of differential varieties and lines to the pathogens.

In Bangladesh, blast disease severely affects rice production, especially that in rainfed lowland (July to December) and irrigated land (November to May) ecosystems (Nihad *et al.*, 2022; Islam *et al.*, 2001). In this study we examined the reactions of 25 monogenic lines carrying different resistance genes to *P. oryzae isolates* occurring in Bangladesh to find out variation in pathogenicity of the *P. oryzae isolates*, and to identify dominant races and resistance genes potentially effective to blast disease in Bangladesh.

MATERIALS AND METHODS

Differential lines for blast fungus isolates. The 25 differential monogenenic lines derived from LTH were used to evaluate the pathogenicity of blast fungus isolates (Tsunematsu *et al.*, 2000). Each of the 25 monogenic lines contains one of the 23 resistance genes, *Pish, Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi12(t), Pi19,* or *Pi20*(t) (Table 1). LTH and a local variety, Nizersail of our native environmental check, were also included as susceptible controls in the evaluation.

Collection and isolation of blast pathogen isolates. Rice leaves, nodes and panicles infected with blast fungus were collected from 5 agro ecological zones (AEZs) of Northern (AEZ2), Barisal (AEZ13), Comilla (AEZ19), Chittagong (AEZ23) and Gazipur regions (AEZ28) of Bangladesh during Aman (80 isolates) and Boro (20 isolates) 2010 to 2012. The samples were cut into small pieces, and surface was sterilized in 0.001% $HgCl_2$. The sterilized samples were placed on moist filter paper in Petri dishes, and incubated for two days under near UV light (12 hrs. under light and 12 hrs. in dark) for sporulation. Conidia developed on the colony were transferred into Petri dishes containing 0.03% water agar medium containing streptomycin sulfate (40 mg/l). After 3 days of incubation, the individual colony was transferred to prune agar medium, and allowed to grow for 3-4 days at 28°C. Sterilized rice straw was put on the culture to allow the mycelium to grow on the straw. The straws were colonized with mycelium of *P. oryzae* were put under near UV light for sporulation as before. Conidia developed on the straw pieces were suspended in 5 ml sterilized distilled water, and poured into Petri dishes containing 20 ml liquid water agar medium. After two days of incubation, germinated conidium along with growing hyphae were transferred individually to prune agar medium in test tube slants and allowed to grow. Each of the single spore pure culture was noted as an isolate.

Preservation of blast isolates: Sterilized filter paper disks colonized with pure culture of blast isolates were made air dry for seven to ten days. Then colonized filter papers were preserved in -20°C under dry condition with silica gel at Seed Pathology and Molecular Lab.

Preparation of blast fungus inoculum. Filter paper disks colonized with isolates were transfer to prune agar plates and allowed to grow at about 25°C for 5 days. The blocks of mycelium (4 mm diameter) were transferred to oat meal agar plates. The oat meal culture was allowed to grow on the surface of the medium for 7 days at 25°C for sporulation. The crushing of mycelia was done with tooth brush, conidia produced were scraped from the surface of the culture, and suspended in sterilized distilled water. The spore concentration was measured and adjusted to 1×10^5 spores/ml. Exactly 50 ml of spore suspension of each isolate was used for inoculation of rice seedlings.

Evaluation for reactions of rice to blast fungus. Ten to twelve sprouted seeds/lines of the 25 monogenic lines, LTH, and Nizersail were sown in continuous lines in plastic trays (60 cm × 30 cm) filled with sandy loam soil. Inoculation of blast pathogen was done as described in Bonman *et al.*, (1986) and Hayashi *et al.*, (2009). About 21-25 days old seedlings were inoculated by spraying with 50 ml spore suspension of individual isolate using a hand sprayer in the afternoon at 5- 6 pm. All inoculated seedlings were incubated for 24 hours immediate after inoculation by covering polythene. The relative humidity and temperature of the net house were maintained to 70 to 80% and 25±1ºC, respectively for disease development. Severity of blast disease developed on inoculated rice plants was evaluated as described in Mackill and Bonman (1992) and Hayashi *et al.*, (2009) at 7 to 8 days after inoculation.

Races of blast fungus. The pathogenic races of blast isolates were classified following the international designation system as detailed in Hayashi and Fukuta (2009) and Gilmorour (1973). The 25 differential monogenic lines and LTH were divided into five groups of U (4 lines and LTH), i (3 lines), k (7 lines), z (4 lines) and ta (7 lines) depending on the loci of resistance genes present in the respective lines (Hayashi and Fukuta, 2009). The monogenic lines and LTH within a group were further divided into subgroups of one to three lines. A number code of 1, 2, or 4 was assigned to each line within a subgroup. The codes (1, 2, or 4) assigned to the individual lines within a subgroup were added up if the lines were susceptible to a blast isolate to define the race number of the isolates. For

example, the race number of a blast fungus isolate causing a susceptible reaction in all the monogenic lines and LTH is U73-i7-k177-z17-ta773, while that of an isolate causing a resistance reaction in all the lines and LTH is U00-i0-k000-z00-ta000.

Data Analysis. A hierarchical clustering analysis for blast fungus isolates was done by the method by Ward (1963) using JMP 7.0.2 software (SAS Institute, Inc., Cary, NC, USA) based on the degree of infection also referred to the severity of infection of blast pathogen isolates to the 25 monogenic lines and LTH. Diversity of blast isolates within an AEZ was calculated based on their reactions to the groups of monogenic lines and LTH by the method of Simpson (1949). The diversity index varies from 0 to 1, where 0 represents no diversity and 1 for maximum diversity. Non-hierarchical clustering of blast fungus isolates based on their reactions to the monogenic lines was carried out by the GENSTAT 5.5 program (Rahman *et al.*, 2010). Distances (Mahalanobis distance) among the clusters of blast fungus isolates were obtained as a result of a canonical variate analysis using of the GENSTAT 5.5 program (Rahman *et al.*, 2010).

RESULTS AND DISCUSSION

Virulence spectrum of blast fungus isolates from Bangladesh

Frequencies of virulence of blast fungus isolates collected from different AEZs to the 25 differential monogenic lines was evaluated by inoculating the isolates to the lines and susceptible control plants. A large proportion (≥ 60%) of the isolates collected from AEZ23 were able to induce a susceptible (compatible) reaction in 14 monogenic lines carrying one of the resistance genes *Pib, Piz-5(Pi2(t)), Pit, Pia, Pik-s, Pik-m, Pik-h, Pik, Pik-p, Pi7(t), Piz-t, Pi12(t), Pita*, or *Pi20*(t) (Fig. 1A). In contrast, the proportions of isolates from AEZ23 showing a resistance (incompatible) reaction were only 20% or less in four lines for *Pita-2, Pish, Pi9* and *Piz* (Fig. 1A).

The proportions of the isolates from AEZ19 that caused a susceptible reaction were 60% or higher in 17 lines carrying one of the resistance genes *Pik-m, Piz-5(Pi2 (t)), Pi1, Pit, Pi5(t), Pi19, Pii, Piz-t, Pia, Pik-h, Pib, Pi7(t), Pi12(t), Pi20, Pik, Pik-p*, or *Pik-s* (Fig. 1B). Especially, all the isolates collected from AEZ19 showed a susceptible reaction in the line with *Pik-s*. The proportions of the isolates from AEZ19 caused a resistance reaction in three lines carrying *Piz* or *Pita-2* (Fig. 1B) were less than 20%.

The proportions of the isolates from AEZ13 showing a susceptible reaction were 60% or higher in ten lines carrying one of the resistance genes *Pi5(t), Pik, Piz-5(Pi2 (t)), Piz-t, Pi20, Pi12(t), Pit, Pib, Pia,* or *Pik-s* (Fig. 1C). The proportions of the isolates from AEZ13 causing a susceptible reaction in three lines carrying one of the resistance genes *Pita-2, Pi9* or *Pish* were less than 20% (Fig. 1C).

The proportions of the isolates from AEZ28 causing a susceptible reaction were 50% or less in 8 lines carrying one of the resistance genes *Piz, Pita-2, Pish, Pi9, Pit-2, Pita=Pi4(t), Pii,* or *Pi3(t)* (Fig. 1D). Especially 20% or less of the isolates from AEZ28 were able to cause a susceptible reaction in three lines for *Piz, Pita-2* and *Pish*.

The proportions of the isolates from AEZ2 causing a susceptible reaction were less than 40% in only five lines carrying one of the resistance genes *Pish, Piz, Pita-2,* or *Pi9* (Fig. 1E). The rest of the lines were found to show a susceptible reaction with more than 60% of the isolates from AEZ2 (Fig. 1E). These results indicate that the isolates from AEZ2 are more virulent than those from other AEZs.

When the virulence frequencies of the blast fungus isolates from the individual AEZs are combined, the proportions of the blast fungus isolates from five AEZs causing a susceptible reaction were less than 20% in the lines carrying *Pish, Pita-2* and *Piz*, and the proportions were between 20% and 60% in the lines carrying *Pi9, Pita-2, Pita=Pi4(t), Pi3(t)* and *Pi1* (Fig. 1F). The results suggested that rice cultivars carrying resistance genes such as *Pish, Pita-2, Pi9,* and *Piz* may serve as promising sources of resistance to blast in Bangladesh. Effectiveness of *Pish*,

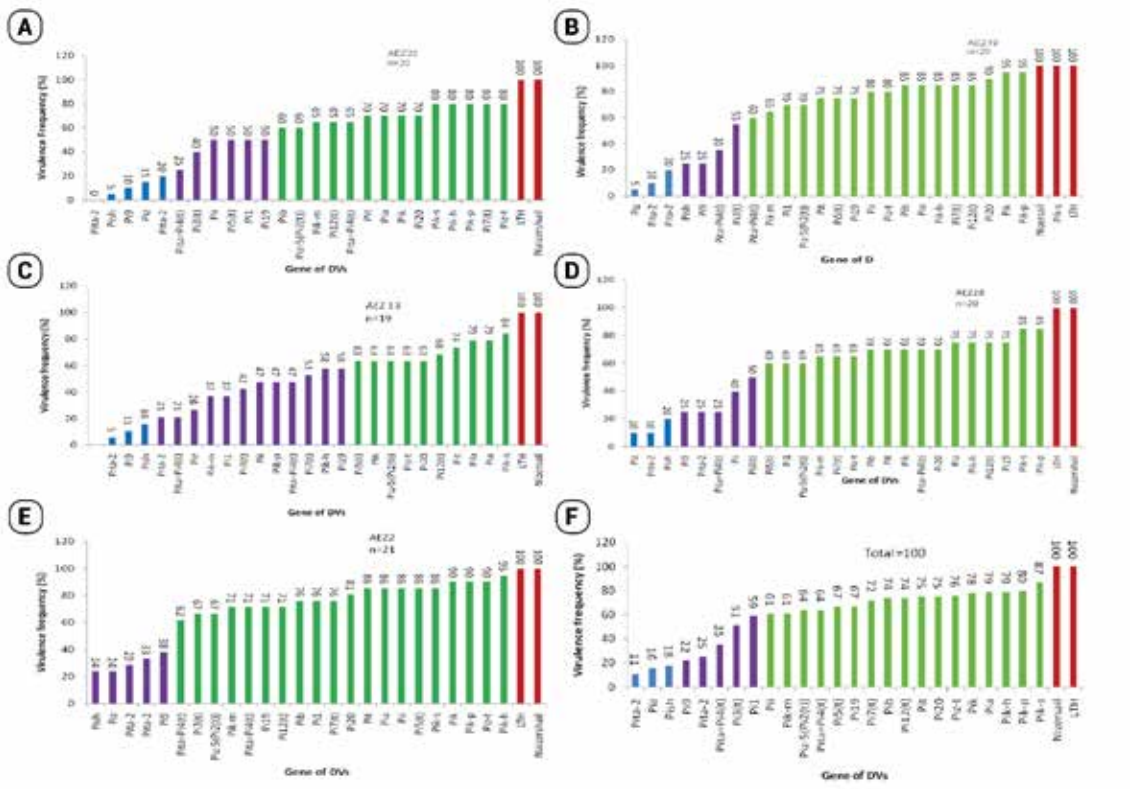


Fig. 1: Virulence frequencies of blast fungus isolates collected (Aman and Boro season) from A) AEZ23, B) AEZ19, C) AEZ13, D) AEZ28, and E) AEZ2, and F) all five AEZs combined against differential monogenic lines and susceptible checks.

Pita-2, Pi9 and *Piz* as a resistance gene against blast fungus were also documented in studies using blast fungus isolates in other countries. Mithrasena *et al.*, (2012) reported that rice plants carrying *Pish* or *Pi9* were resistant to a majority of blast pathogen isolates collected in Sri Lanka. Telebanco-Yanoria *et al.*, (2008) showed that the resistance genes *Pish, Piz-5*, and *Piz* showed a wide spectrum of moderate resistance to blast fungus isolates in Philippines. Koide *et al.*, (2011) also reported that a monogenic line carrying *Pi9* was resistant to all standard differential blast isolates in Philippines. Du *et al.*, (2007) reported that a rice plant carrying *Piz* showed effective resistance to 179 blast isolates in Vietnam. Sathya *et al.* (2013) reported that rice plants carrying four resistant genes *Pish, Piz, Pi9* and *Pita-2* showed broad spectra of resistance to Cambodian blast fungus isolates.

Races of blast isolates from Bangladesh

Based on the reactions of LTH and the four U group monogenic lines carrying *Pish, Pia, Pib,* or *Pit,* the 100 blast isolates were classified into ten pathotypes. The U63 type that can cause susceptible reactions in LTH and lines carrying *Pia, Pib,* or *Pit* was most predominant. The U63 type represented 41% of the total population and found in all the five zones (Supplementary Table 1).

Table 1. Characteristics of international standard differential monogenic lines derived from Lijiangxintuanheigu by Hayashi *et al.*, (2009).

Differential monogenic line	Resistance gene in monogenic lines		
	Gene name	Chromosome	Locus/group
IRBLsh-B	*Pish*	1	*Pish*/U
IRBLb-B	*Pib*	2	*Pib*/U
IRBLt-K59	*Pit*	1	*Pit*/U
IRBLa-A	*Pia*	11	*Pia*/U
IRBLi-F5	*Pii*	9	*Pii* / i
IRBL3-CP4	*Pi3*	9	*Pii* / i
IRBL5-M	*Pi5(t)*	9	*Pii* / i
IRBLks-F5	*Pik-s*	11	*Pik* / k
IRBLkm-Ts	*Pik-m*	11	*Pik* / k
IRBL1-CL	*Pi1*	11	*Pik* / k
IRBLkh-K3[LT]	*Pik-h*	11	*Pik* / k
IRBLk-Ka[LT]	*Pik*	11	*Pik* / k
IRBLkp-K60	*Pik-p*	11	*Pik* / k
IRBL7-M	*Pi7(t)*	11	*Piz* / z
IRBL9-W	*Pi9*	6	*Piz* / z
IRBLz-Fu	*Piz*	6	*Piz* / z
IRBLz5-CA	*Piz-5=Pi2(t)*	6	*Piz* / z
IRBLzt-T	*Piz-t*	6	*Piz* / z
IRBLta2-Pi	*Pita-2*	12	*Pita* / ta
IRBLta2-Re	*Pita-2*	12	*Pita* / ta
IRBL12-M	*Pi12(t)*	12	*Pita* / ta
IRBLta-K1	*Pita=Pi4(t)*	12	*Pita* / ta
IRBLta-CP1	*Pita=Pi4(t)*	12	*Pita* / ta
IRBL19-A	*Pi19*	12	*Pita* / ta
IRBL20-IR24	*Pi20(t)*	12	*Pita* / ta

The diversity indexes of the pathotypes determined by the U group lines in the respective AEZs were between 0.71 and 0.81.

Table 2. Pathogenic races of 100 blast fungus isolates collected in Bangladesh.

Clusters	Number of members	Races
I	18	U21-i7-k174-z07-ta623, U61-i1-k122-z06-ta400, U43-i1-k117-z00-ta423 U43-i1-k157-z14-ta021, U41-i0-k135-z02-ta022, U21-i4-k073-z06-ta622 U63-i7-k157-z06-ta423, U73-i7-k157-z14-ta733, U61-i0-k141-z07-ta403 U63-i7-k157-z06-ta423 , U63-i5-k122-z06-ta403, U63-i7-k177-z07-ta632 U63-i7-k135-z17-ta411, U63-i4-k176-z12-ta431, U63-i3-k167-z16-ta132 U21-i6-k157-z04-ta423,U63-i7-k177-z16-ta721, U43-i7-k177-z06-ta333,
II	31	U21-i0-k046-z04-ta032, U61-i6-k157-z06-ta403, U63-i7-k177-z06-ta603 U63-i7-k167-z06-ta423, U63-i7-k167-z07-ta733, U03-i1-k100-z00-ta002 U73-i5-k167-z16-ta433, U23-i1-k173-z06-ta003, U43-i7-k137-z14-ta433, U23-i4-k152-z04-ta003, U63-i4-k020-z02-ta622U63-i7-k166-z04-ta633, U23-i5-k122-z04-ta022, U41-i0-k040-z00-ta401,U73-i0-k177-z16-ta623, U73-i7-k167-z16-ta533, U03-i7-k127-z12-ta403,U73-i7-k177-z06-ta633, U01-i0-k042-z04-ta400, U61-i0-k040-z05-ta410 ,U63-i5-k175-z16-ta432, U63-i7-k177-z00-ta723, U23-i7-k177-z06-ta021,U43-i5-k173-z07-ta002, U43-i7-k137-z10-ta732, U63-i7-k167-z17-ta403,U73-i5-k177-z16-ta423, U63-i7-k177-z15-ta633, U73-i7-k177-z17-ta433,U21-i4-k143-z06-ta433, U43-i5-k053-z04-ta203,
III	16	U43-i0-k146-z06-ta400, U73-i4-k147-z04-ta422, U41-i6-k167-z00-ta400 U33-i5-k173-z00-ta623, U43-i0-k147-z00-ta422, U03-i3-k141-z02-ta421 U01-i0-k104-z04-ta000, U63-i1-k101-z02-ta403, U63-i0-k104-z06-ta000 U63-i6-k177-z04-ta423, U01-i0-k173-z06-ta203, U03-i0-k042-z00-ta021 U41-i6-k100-z10-ta502, U73-i3-k173-z06-ta423, U63-i5-k177-z06-ta633 U63-i5-k177-z06-ta633
IV	23	U61-i0-k057-z05-ta033, U03-i0-k020-z06-ta402,U43-i7-k177-z00-ta402, U63-i7-k167-z00-ta422, U03-i0-k146-z04-ta400, U63-i7-k157-z16-ta033, U03-i7-k177-z06-ta433,U63-i5-k157-z04-ta433, U63-i1-k157-z16-ta422, U63-i3-k177-z14-ta423,U63-i6-k105-z07-ta421, U73-i1-k143-z11-ta423, U23-i4-k114-z06-ta403,U73-i7-k177-z04-ta733, U63-i0-k057-z00-ta022, U43-i2-k127-z07-ta421, U41-i5-k173-z07-ta422, U73-i7-k147-z06-ta713, U63-i7-k177-z04-ta033, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433, U63-i7-k177-z06-ta433
V	12	U03-i7-k175-z07-ta623, U63-i7-k173-z06-ta413, U63-i0-k157-z06-ta211 U63-i1-k177-z06-ta021, U61-i4-k127-z02-ta400, U63-i1-k157-z06-ta403 U63-i4-k141-z06-ta002, U01-i6-k042-z00-ta000, U73-i7-k077-z02-ta020 U63-i6-k157-z02-ta422, U63-i0-k114-z04-ta413, U73-i7-k077-z02-ta020

Based on the reactions to the three i group monogenic lines carrying *Pii, Pi3,* or *Pi5(t)*, the 100 isolates were classified into seven pathotypes (Supplementary Table 2). The i7 type that can cause susceptible reactions to all I group lines was most dominant, and represented 36 isolates. Especially 13 isolates among 21 from AEZ2 were the i7 type, and thus the diversity index among the isolates from AEZ21 (0.57) were notably lower than the indexes of isolates from other AEZs (0.71 to 0.81) (Supplementary Table 2). The i0 type that can cause resistance reactions in all the i group lines was not found among the isolates from AEZ19, suggesting that rice plants carrying the i locus resistance genes are vulnerable to the isolates from AEZ19.

Based on the reaction to the seven k group monogenic lines for *Pik-s, Pik-m, Pi1, Pik-h, Pik, Pik-p*, or *Pi7(t)*, the 100 isolates were classified into 31 pathotypes (Supplementary Table 3).

Table 3. Pathogenic races selected for the standard differential blast fungus isolates in Bangladesh.

Blast Isolates				Differential variety and susceptible control																										
				U					i			k							z				ta							
				IRBLsh-B	IRBLb-B	IRBLt-K59	USV, LTH	IRBLa-A	IRBLi-F5	IRBL3-CP4	IRBL5-M	IRBLks-F5	IRBLkm-Ts	IRBL1-CL	IRBLkh-K3	IRBLk-Ka	IRBLkp-K60	IRBL7-M	IRBL9-W	IRBLz-Fu	IRBLz5-CA-	IRBLzt-T	IRBLta2-Pi	IRBLta-Re	IRBL12-M	IRBLta-K1	IRBLta-CP1	IRBL19-A	IRBL20-		
Sl. No.	Isolate Code	Name of the race	Virulent frequency (%)	Resistant gene harboring in the genetic background																										
				Pish	Pib	Pit	None	Pia	Pii	Pi3(t)	Pi5(t)	Pik-s	Pik-m	Pi1	Pik-h	Pik	Pik-p	Pi7(t)	Pi9(t)	Piz	Piz-5(Pi2(t))	Piz-t	Pita-2	Pita-2	Pi12(t)	Pita=Pi4(t)	Pita=Pi4(t)	Pi19(t)	Pi20(t)		
1	BS-97	U63-i7-k177-z07-ta632	85	R	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	s	s	s	R	s	s	s	s	R	s	
2	NS-109	U43-i7-k137-z10-ta732	73	R	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	R	R	R	s	s	s	s	s	R	s	
3	CS-135	U63-i3-k177-z14-ta423	69	R	s	R	s	s	s	s	R	s	s	s	s	s	s	s	s	R	R	s	R	R	s	R	s	s	s	
4	CS-84	U41-i6-k167-z00-ta400	46	R	s	s	s	R	R	s	s	s	R	s	s	s	s	s	R	R	R	R	R	R	s	R	R	R	R	
5	GS-14	U63-i6-k157-z02-ta422	62	R	s	s	s	s	R	s	s	s	s	R	s	s	s	s	R	R	s	R	R	R	s	R	s	R	s	
6	CS-124	U73-i5-k167-z16-ta433	81	s	s	s	s	s	s	R	s	s	R	s	s	s	s	s	s	R	s	s	R	R	s	s	s	s	s	
7	GS-98	U73-i3-k173-z06-ta423	77	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	R	R	s	s	R	s	s	R	s	s	s	
8	BS-98	U73-i3-k173-z06-ta423	62	s	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	R	s	R	R	R	R	R	s	R	R	
9	CS-142	U73-i7-k157-z14-ta733	92	s	s	s	s	s	s	s	s	s	R	s	s	s	s	s	s	R	s	s	s	s	s	s	s	s	s	
10	CS-136	U63-i7-k157-z06-ta423	73	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	R	R	s	s	R	R	s	R	s	s	s	
11	BS-30	U63-i6-k105-z07-ta421	58	R	s	s	s	s	R	s	s	s	R	R	R	s	R	s	R	s	s	s	R	R	s	R	s	s	R	
12	BS-88	U63-i5-k122-z06-ta403	54	R	s	s	s	s	s	R	s	s	R	s	R	R	s	R	R	R	s	s	R	R	s	R	R	s	s	
13	HS-4	U03-i7-k175-z07-ta623	73	R	R	R	s	s	s	s	s	s	s	s	s	s	R	s	R	s	s	s	R	s	s	R	s	s	s	
14	HS-53	U63-i7-k173-z06-ta413	73	R	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	s	s	R	R	s	s	R	s	s	
15	NS-112	U63-i7-k177-z06-ta433	81	R	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	R	s	s	s	s	s	
16	GS-129	U73-i7-k177-z06-ta633	88	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	s	s	s	s	s	s	
17	BS-141	U73-i7-k177-z04-ta733	88	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	s	s	s	s	s	s	s	s	
18	NS-106	U63-i7-k177-z00-ta723	80	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	R	R	s	s	s	R	s	s	s	
19	NS-117	U73-i7-k177-z17-ta433	92	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	s	s	s	
20	CS-76	U63-i7-k177-z06-ta433	84	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	R	R	s	s	R	R	s	s	s	s	s	
21	HS-101	U63-i7-k157-z16-ta033	77	R	s	s	s	s	s	s	s	s	s	R	s	s	s	s	s	R	s	s	R	R	R	s	s	s	s	
22	GS-79	U41-i5-k173-z07-ta422	69	R	s	s	s	s	s	R	s	s	s	s	s	s	s	R	R	s	s	s	R	R	s	R	s	R	s	
23	NS-103	U73-i7-k147-z06-ta713	81	s	s	s	s	s	s	s	s	s	R	R	s	s	s	s	R	R	s	s	s	s	s	s	R	s	s	
24	CS-133	U63-i1-k157-z16-ta422	65	R	s	s	s	s	s	R	R	s	s	R	s	s	s	s	s	R	s	s	R	R	s	R	s	R	s	
25	GS-13	U63-i6-k177-z04-ta423	65	R	s	s	s	s	R	s	s	s	s	s	s	s	s	s	R	R	R	s	R	R	s	R	s	s	R	

The k177 type that can cause susceptible reactions to all the k group lines was most dominant, and represented 24% of the isolates. The proportion of the k177 type was especially high among the isolates from AEZ2, representing 52% of the isolates, and thus the diversity index of the isolates from AEZ2 (0.70) was notably lower than the indexes of the isolates from other AEZs (0.79 to 0.91) (Supplementary Table 2).

Based on the reactions of the four z group lines for *Pi9, Piz, Piz-5,* or *Piz-t*, the 100 isolates were classified into13 pathotypes (Supplementary Table 4). The z06 type that shows incompatible (resistance) reactions in the lines for *Pi9* and *Piz* was most dominant, representing 33% of the isolates. Especially, the z06 type represented 45% of the isolates from AEZ19, which showed the lowest diversity index (Supplementary Table 2) The isolates of the z00 type that can cause

susceptible reactions in all the z group lines represented 12% of the isolates, and found in all the five AEZs.

Based on the reactions of the seven ta group lines for *Pita-2, Pi12(t), Pita, Pi19(t),* or *Pi20(t)*, the 100 isolates were classified into 38 pathotypes (Supplementary Table 5). The ta403 type showing incompatible reactions in the lines for *Pita-2* and *Pita* and ta423 types showing incompatible reactions in the lines for *Pita-2* and one of the lines for Pita were the major types, each representing 9% of the isolates.

When the reactions to all the 25 lines were considered together, the 100 blast isolates from the five AEZs were represented by 94 pathogenic races that were classified into five clusters (Table 2 and Fig. 2). Four isolates were classified as the same race U63-i7-k177-z06-ta433 in cluster IV. Each of three races U63-i5-k177-z06-ta633 in cluster III, U63-i7-k157-z06-ta423 in cluster I, or U73-i7-k077-z02-ta020 in cluster V represented two different isolates. The rest of the races represented single isolates. Cluster II was the largest one comprised of 33 isolates followed by cluster IV comprised of 23 isolates. A canonical variate analysis showed that the inter-cluster distance was maximum between clusters I and V, and was minimum between clusters III and IV (Fig. 2). The highest average virulence frequency (63%) was observed in Cluster II and cluster I showed lowest virulence frequency (50%). The virulence frequency of cluster III, IV and V showed 57%, 59% and 56% respectively.

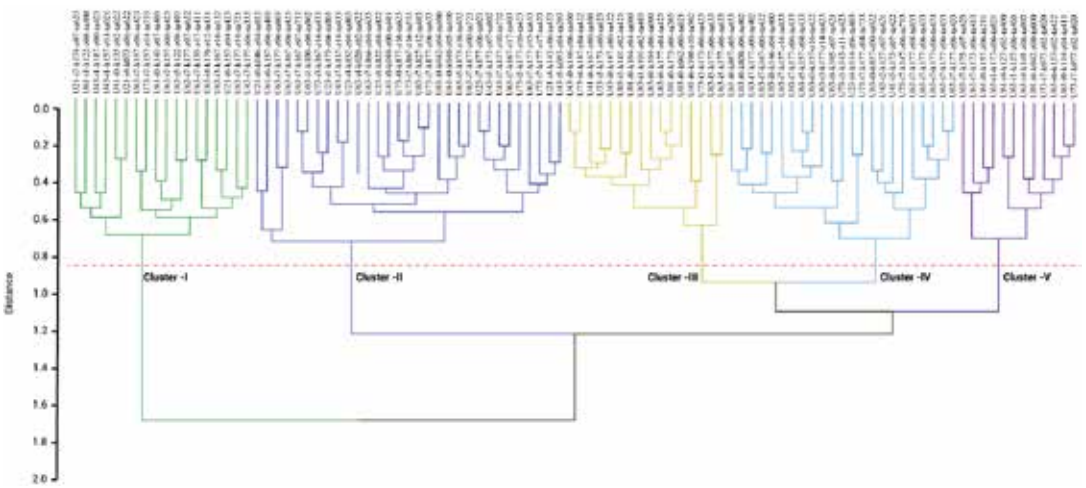


Fig. 2. Dendrogram showing Pathogenic races of 100 blast fungus isolates collected in Bangladesh.

Standard differential blast isolates in Bangladesh

As the result of our evaluation of blast fungus isolates for the reactions to 25 differential monogenic lines, twenty-five isolates were selected as the standard differential blast fungus isolates in Bangladesh based on their virulence to differentiate 23 resistance genes in the 25 monogenic lines, revive capacity and sporulation potentiality (Table 3). A standard set of differential blast fungus isolates has been used to characterize the resistance of rice breeding lines and varieties in Philippines (Telebanco-Yanoria *et al.*, 2008; Koide *et al.*, 2011). We expect the standard differential blast fungus isolates can also be used to characterize the resistance spectra of new rice varieties, and to examine pathogenicity of blast causing fungus isolates from other AEZs of Bangladesh.

CONCLUSION

Based on the reaction patterns against 25 LTH-derived differential monogenic lines carrying one of the 23 resistance genes, 100 isolates from five AEZs in Bangladesh were classified into 94 pathogenic races. Races designated by pathotypes of U63, i0, i7, k157, k177, z00, z04, z06, ta403, ta423, and ta433 were found as the dominant types, among which races designated by pathotypes of U63, i7, k177, z00, z04, z06 and ta423 were commonly found in all the five AEZs of Bangladesh. The results indicated that any rice varieties relying on *Pib, Pit, Pia, Pii, Pi3, Pi5(t), Pik, Pik-h, Pik-m, Pi1, Pik-p, Pik-s, Pi7(t), Piz-t, Piz-5, Pi12(t), Pita(2), Pi19(t)*, and *Pi20(t)* might be at the risk of blast fungus infection in Bangladesh, especially in areas such as AEZ2 and AEZ19 where such races were found to present at a high frequency. In contrast, for a number of isolates incompatible to lines carrying *Pish, Pi9, Piz, Pita-2,* and *Pita* were also found among the 100 isolates examined, suggesting these genes are promising as the sources of robust resistance in Bangladesh. These genes can be used in breeding program for development of blast resistant variety. The standard set of 25 blast fungus isolates selected would be a useful tool in developing rice varieties, resistant to blast disease in Bangladesh.

REFERENCES

Bonman, J. M., Vergel De Dios, T. I., & Khin, M. M. (1986). Physiological specialization of *Pyricularia oryzae* in the Philippines. *Plant Dis*, 70, 767–769.

Chen, H. L., Chen, B. T., Zhang, D. P., Xie, Y. F., & Zhang, Q. (2001). Pathotypes of *Pyricularia grisea* in rice fields of central and southern China. *Plant Dis*, 85.

Du, P. V., Loan, L. C., & Sang, N. D. (2007). Blast research in the Mekong River Delata of Vietnam. *Japan International Research Center for Agricultural Sciences*, 53, 53–63.

Flor, H. H. (1971). Current status of gene-for-gene concept. Adv. Genet. 8: 29–54.

Gilmour, J. (1973). Octal notation for designating physiological races of plant pathogens. Nature: 242 p. 620.

Hayashi, N., & Fukuta, Y. (2009). Proposal for a new international system of differentiating races of blast (*Pyricularia oryzae* Cavara) by using LTH monogenic lines in rice (*Oryza sativa* L.). *Japan International Research Center for Agricultural Sciences*, (63), 11–15.

Hayashi, N., Kobayashi, N., Vera Cruz, C. M., & Fukuta, Y. (2009). Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. *Japan International Research Center for Agricultural Sciences*, 63, 17–33.

Inoue, H., Nakamura, M., Mizubayashi, T., Takahashi, A., Sugano, S., Fukuoka, S., & Hayashi, N. (2017). Panicle blast 1 (Pb1) resistance is dependent on at least four QTLs in the rice genome. *Rice (New York, N.Y.)*, 10(1), 36. doi:10.1186/s12284-017-0175-0

Islam, K. M. H., Islam, M. K., Rahman, A. J. M. M., Jalaluddin, M., & Mia, M. A. T. (2001). Screening of some rice varieties and induced mutants against blast (*Pyricularia grisea*). *Bangladesh Journal of Plant Pathology*, 17, 49–53.

Khan, M. A. I., Ali, M. A., Monsur, M. A., Kawasaki-Tanaka, A., Hayashi, N., Yanagihara, S., … Fukuta, Y. (2016). Diversity and distribution of rice blast (*Pyricularia oryzae* Cavara) races in Bangladesh. *Plant Disease*, 100(10), 2025–2033. doi:10.1094/PDIS-12-15-1486-RE

Kiyosawa, S. (1984). Establishment of differential varieties for pathogenicity test of rice blast fungus. *Rice Genet. Newsl*, 1, 95–96.

Kiyosawa, S., Ikehashi, H., Kato, H., & Ling, Z. Z. (1981). Pathogenicity tests of Philippine isolates of blast fungus using two sets of rice varieties. *Jpn. J. Breed,* 31.

Kobayashi, N., Telebanco-Yanoria, M. J., Tsunematsu, H., Kato, H., Imbe, T., & Fukuta, Y. (2007). Development of new sets of international standard differential

varieties for blast resistance in rice (*Oryza sativa* L.). *JARQ*, 41, 31–37.

Koide, Y., Telebanco-Yanoria, M. J., Pen, F. D., Kobayashi, N., Fukuta, Y., & Kobayashi, N. (2011). Characterization of Rice Blast Isolates by the Differential System and their Application for Mapping a Resistance Gene. *J Phytopathol*, 1(9), 85–93.

Latif, M. A., Rahman, L., Islam, N., Kayess, M. O., Rashid, M. M., Hasan, M. A.-I., & Khan, M. A. I. (2024). Pathotypic diversity of *Xanthomonas oryzae* pv. oryzae, and stringent evaluation of resistance lines of Rice in Bangladesh. *European Journal of Plant Pathology*. doi:10.1007/s10658-024-02900-6

Koizumi, S. (2007). Durability of resistance to rice blast disease. *JIRCAS Working Report*, 53, 1–10.

Latif, M. A., Nihad, S. A. I., Hasan, R., Rahman, L., Anik, T. R., Manidas, A. C., & Bhuiyan, R. (2024). Pyramiding of multiple resistant genes of blast and bacterial blight diseases in the background of rice (*Oryza sativa*) mega variety BRRI dhan29. *Current Plant Biology*, 40(100400), 100400. doi:10.1016/j.cpb.2024.100400

Latif, M. A., Nihad, S. A. I., Mian, M. S., Akter, S., Khan, M. A. I., & Ali, M. A. (2022). Interaction among sheath diseases complex of rice and ribosomal DNA analysis for the differentiation of *Rhizoctonia solani, R. oryzae* and *R. oryzae-sativae. Plant Stress*, 5(100100), 100100. doi:10.1016/j.stress.2022.100100

Latif, M. A., Rahman, L., Islam, N., Kayess, M. O., Rashid, M. M., Hasan, M. A.-I., & Khan, M. A. I. (2024). Pathotypic diversity of *Xanthomonas oryzae* pv. *oryzae*, and stringent evaluation of resistance lines of Rice in Bangladesh. *European Journal of Plant Pathology*. doi:10.1007/s10658-024-02900-6

Le, M. T., Arie, T., & Teraoka, T. (2010). Population dynamics and pathogenic races of rice blast fungus, *Magnaporthe oryzae* in the Mekong Delta in Vietnam. *J Gen Plant Pathol*, 76, 177–182.

Lee, J.-H., Lee, J.-Y., Yoon, Y.-N., Kim, S.-Y., Hur, Y.-J., Yeo, U.-S., … Cho, J.-H. (2015). Enhancement of panicle blast resistance in Korean rice cultivar 'saeilmi' by marker assisted backcross breeding. *Plant Breeding and Biotechnology*, 3(1), 1–10. doi:10.9787/pbb.2015.3.1.001

Mackill, D. J., and Bonman, J. M. (1992). Inheritance of blast resistance in near-isogenic lines of rice. *Phytopathology*. 82: 746-749.

Mekwatanakarn, P., Kositratana, W., Levy, M., & Zeigler, R. S. (2000). Pathotype and avirulence gene diversity of *Pyricularia grisea* in Thailand as determined by rice lines near-isogenic for major resistance genes. *Plant Dis*, 84(1), 60–70.

Mithrasena, Y. J. P. K., Wijesundera, W. S. S., Wijesundera, R. L. C., Wimalasir, D. C., & Priyanthi, R. P. N. (2012). Pathogenic and Genetic Diversity of *Magneporthe oryzae* Populations from Sri Lanka. *Rice Science*, 19(3), 241–246.

Nessa, B., Salam, M. U., Haque, A. M., Biswas, J. K., Macleod, W. J., Ali, M. A., & Galloway. (2015). FLYER: A simple yet robust model for estimating yield loss from rice false smut disease (*Ustilaginoidea virens*). *American Journal of Agricultural and Biological Sciences*, 10(1).

Nihad, S. A. I., Ara, A., Rashid, M. M., Hasan, M. A. I., Khan, M. A. I., & Latif, M. A. (2021). Genetic divergence of rice genotypes revealed by bacterial blight disease and morphological traits. *Bangladesh Rice Journal*, 24(1), 73–84. doi:10.3329/brj.v24i1.53241

Nihad, S. A. I., Hasan, M. K., Kabir, A., Hasan, M. A.-I., Bhuiyan, M. R., Yusop, M. R., & Latif, M. A. (2022). Linkage of SSR markers with rice blast resistance and development of partial resistant advanced lines of rice (*Oryza sativa*) through marker-assisted selection. *Physiology and Molecular Biology of Plants: An International Journal of Functional Plant Biology*, 28(1), 153–169. doi:10.1007/s12298-022-01141-3

Noda, T., Hayashi, N., Du, P.V. and Dinh, H. D. E. L. V. (1999). Distribution of pathogenic races of rice blast fungus in Vietnam. Ann. Phytopath. Soc.Jpn. 65: 526-530.

Rahman, M. M., Ansari, A., & Rashid, M. M. (2010). Diversity analysis in rice using GENSTAT and SPSS programs. *The Agriculturists*, 8(2), 14–21.

Sathya, K. S., Koga, I., Ung, T., Sathya, K., Kobayashi, N., Yadana, H., Fukuta, Y. (2013). Pathogenicity of rice blast (Magnaporthe oryzae B. Couch) isolates from Cambodia. Annual meeting for Blast research Network for Stable Rice Production under the JIRCAS research project. *Japan International Research Center for Agricultural Sciences*, 72–82.

Shahriar, S. A., Imtiaz, A. A., Hossain, M. B., Husna, A., & Eaty, M. N. K. (2020). Review: Rice Blast Disease. *Annual Research & Review in Biology*, 50–64. doi:10.9734/arrb/2020/v35i130180

Sharma, T. R., Shanker, P., Singh, B. K., & Jana, T. K. (2005). Molecular mapping of rice blast resistance gene Pi-kh in the rice variety Tetep. Tetep. *J Plant Biochem Biotechnol*, 14, 127–133.

Silue, D., Notteghem, J. L., & Tharreau, D. (1992). Evidence for a gene for gene relationship in the *Oryza sativa-Magnaporthe grisea pathosystem*. *Phytopathology*, 82, 577–580.

Simpson, E. H. (1949). Measurement of diversity. Nature : 163: 688.

Telebanco-Yanoria, M. J., Imbe, T., Kato, H., Tsunematsu, H., Ebron, L., Vera Cruz, C. M., Fukuta, Y. (2008). A set of standard differential blast isolates (*Magnaporthe grisea* (Hebert) Barr. from the Philippines for rice. (*Oryza sativa* L.) *Resistance. JARQ*, 42(1), 23–34.

Telebanco-Yanoria, M. J., Koide, Y., Fukuta, Y., Imbe, T., Kato, H., Tsunematsu, H., & Kobayashi, N. (2010). Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. *Breed. Sci*, 60, 629–638.

Thinlay, X., Zeigler, R. S., & Finckh, M. R. (2000). Pathogenic variability of *Pyricularia grisea* from the high- and mid-elevation zones of Bhutan. *Phytopathology*, 90, 621–628.

Tsunematsu, H., Yanoria, M. J. T., Ebron, L. A., Hayashi, N., Ando, I., Kato, L., Khush, G. S. (2000). Development of monogenic lines for rice blast resistance. *Breed. Sci*, 50, 229–234.

Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, 58(301), 236–244.

Yadav, M. K., Aravindan, S., Ngangkham, U., Raghu, S., Prabhukarthikeyan, S. R., Keerthana, U., Rath, P. C. (2019). Blast resistance in Indian rice landraces: Genetic dissection by gene specific markers. *PloS One*, 14(1), e0211061. doi: 10.1371/journal.pone.0211061

Yamada, M., Kiyosawa, S., Yamaguchi, T., Hirano, T., Kobayashi, T., Kushibuchi, K., & Watanabe, S. (1976). Proposal of a new method of differentiating races of *Pyricularia oryzae* Cavara in Japan. *Ann. Phytopathol. Soc. Jpn*, 42, 216–219.

Zeigler, R. S., Cuoc, L. X., Scott, R. P., Bernardo, M. A., Chen, D. H., Valent, B., & Nelson, R. J. (1995). The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. *Phytopathology*, 85, 443–451.

Zeigler, R. S., Thome, J., Nelson, R., Levy, M., & Correa-Victoria, F. (1994). Lineage exclusion: A proposal for linking blast population analysis to resistance breeding. In R. Blast Disease (Ed.), *CABI/IRRI* (pp. 267–292). Wallingford, Oxon, UK.

Bangladesh Rice J.28(1): 69-79, 2024, doi.org/10.3329/brj.v28i1.86812

Evaluation of Yield Stability and Adaptability of Boro Rice (*Oryza sativa* L.) Genotypes in Bangladesh Using AMMI and GGE Biplot Analyses

H Khatun[1], T Saha[2], P L Biswas[3], M R B H Pranto[1], S Mia[1], S Maniruzzaman[3], M A syed[3], M H Rashid[1], R N Eva[4], M T I Chowdhury[5], K M Ifterkharuddaula[3], M R Islam[6] and M A I Khan[1,*]

ABSTRACT

Genotype × environment (G × E) interaction is a critical consideration in rice breeding, as it determines the adaptability and stability of genotypes across diverse agroecological zones. This study aimed to explore the most suitable and stable Boro rice genotypes for Bangladesh. In this study, seven rice genotypes were evaluated in Boro season across twelve locations in Bangladesh during the 2024 Boro season to investigate genotype-environment interaction (GEI) and yield stability performance. The experiment utilized three replications of a completely randomized block design. The analyses were performed through the Additive Main Effects and Multiplicative Interaction (AMMI) and Genotype plus Genotype × Environment (GGE) biplot models. The AMMI analysis revealed that environment accounted for the largest proportion of total variation (51.9%), followed by G × E interaction (16.7%) and genotype (3.7%) The AMMI identified genotypes G2 and G6 as stable, high-yielding genotypes. The GGE biplot analysis accounted for 78.4% of the total G × E interaction, with identifying G2 and G6 as elite genotypes for broad adaptation with high yield potential. Both the AMMI and GGE analyses consistently identified genotypes G2 and G6 as stable and emerge as elite genotypes for broad adaptation with high yield potential, while G1 and G5 show strong performance in specific environmental sets. The combined use of AMMI and GGE analyses effectively captured both yield stability and adaptability patterns, supporting that G2 was broadly adapted, high-yielding entries suitable for rice development program in Bangladesh.

Keywords: Rice, Boro, GxE, GGE biplot, AMMI, Stability, Adaptability

INTRODUCTION

Rice (*Oryza sativa* L.) serves as the primary staple food for a large portion of the global population, contributing nearly 20% of the world's dietary energy supply. Over the past three decades, Bangladesh has achieved remarkable progress in rice production and is widely recognized as a nation of rice growers and consumers (Fukagawa & Ziska, 2019; Hashim *et al.*, 2021; Mainuddin *et al.*, 2021)

To meet the increasing food demand of its growing population, Bangladesh must enhance genotypic adaptability, adopt improved

[1]Bangladesh Rice Research Institute, Regional Station, Barishal;
[2]Bangladesh Rice Research Institute, Regional Station, Cumilla;
[3]Bangladesh Rice Research Institute, Gazipur;
[4]Bangladesh Rice Research Institute, Satellite Station, Patuakhali;
[5]Department of Botany, National University, Gazipur;
[6]Director Research, Bangladesh Rice Research Institute, Gazipur
[*]Corresponding author's E-mail: ashikjp@gmail.com (M A I Khan)

agricultural technologies, and strengthen water management practices (Rahman *et al.*, 2023). The adaptability of rice genotypes to diverse and changing environments plays a pivotal role in ensuring yield stability across different locations and years. Moreover, the development and deployment of high-yielding genotypes are fundamental to sustaining the country's food security through enhanced productivity and yield consistency (Penna *et al.*, 2023).

Rice is cultivated in Bangladesh throughout the year under three distinct seasons Aus, Aman, and Boro. Among them, Boro rice is one of the major contributors to national rice production. Despite having a favorable subtropical climate for Boro cultivation, the average yield in Bangladesh remains relatively low compared to other Asian countries such as Indonesia and Malaysia. Variations in Boro rice yield are influenced by multiple factors, including genotypic differences, regional and seasonal conditions, inefficient nutrient management, pest and disease incidence, and various abiotic stresses such as drought, flooding, salinity, temperature extremes, and poor soil fertility. These factors collectively contribute to yield reduction and instability across environments in Bangladesh (Akter *et al.*, 2023).

When a genotype is evaluated under different growing conditions, its performance often varies significantly, a phenomenon referred to as genotype × environment (G×E) interaction (Allard & Bradshaw, 1964; Kang, 2004). Genotype × environment (G×E) interaction analysis provides a framework to assess how genotypes respond to variations in environmental conditions. Among various statistical tools, the Additive Main Effects and Multiplicative Interaction (AMMI) model and the Genotype plus Genotype × Environment (GGE) biplot are widely applied to study G×E interactions. These two statistical analyses (AMMI and GGE) were adopted in multi-environment (MET) two-way data matrices (Zhang *et al.*, 2016). In recent years, GE interaction has been only taken into account in AMMI analyze, but genotype effects were ignored while evaluating genotypes comprehensively. On the other hand, GGE biplot model has been considered as highly effective method to identify genotype difference and evaluation test environment (Ding *et al.*, 2007). Increasingly, crop breeders have used GGE biplot widely for evaluating mean performance, stability of cultivars (Kang *et al.*, 2006) and discrimination of test sites (Dimitrios *et al.*, 2008). By using both models, rice breeders can get robust and interpretable results for making decisions to select stable genotypes for rice breeding program or identifying a location-specific variety/genotype for rice production. The AMMI model combines analysis of variance for main effects with principal component analysis for interaction effects, while GGE biplot uses environment-centered principal components to graphically display genotype performance across environments. Despite methodological differences, both approaches provide comparable insights, allowing breeders to identify stable genotypes and environment groupings with minimal crossover interactions (Gouch & Zobel, 1996; Mohan *et al.*, 2023). However, limited studies have jointly applied both AMMI and GGE models to Boro rice genotypes across Bangladesh's major agrological zones, leaving uncertainty about adaptability under different stress conditions. Therefore, G×E analysis has become a standard component of plant breeding programs (Gupta *et al.*, 2022). Many studies in rice have explored various concepts and applications of G×E interaction analysis. (Poli *et al.*, 2018; Hashim *et al.*, 2021; Panda *et al.*, 2023; Anshori *et al.*, 2024; Ghazy *et al.*, 2024).

Grain yield is a complex quantitative trait influenced by both genetic and environmental factors. A desirable genotype should not only provide high yield but also perform consistently across diverse agroecological environments. Assessing genotype × environment (G×E) interaction is therefore critical in plant breeding to determine the adaptability and stability of improved lines before their commercial release (Dewi *et al.*, 2014).

The present study applied both AMMI and GGE

biplot analyses to evaluate the adaptability and stability of seven rice genotypes for grain yield across twelve locations in Bangladesh. The objectives were to identify high-yielding and stable genotypes, and determine potential mega-environments, and identify ideal genotypes and environments suitable for rice production areas in Bangladesh.

MATERIALS AND METHODS

Test Genotypes and Locations

The trial was conducted during the 2024–25 Boro (dry) season across twelve locations in Bangladesh, representing distinct agroecological zones (Table 1). These included Barishal (tidal submergence area), Satkhira (salinity-affected area), Rangpur (cold-prone areas), and Habiganj (Haoar area) Gazipur, Kushtia, Rajshahi, Sonagazi, Cumilla, Gopalganj, Bhanga, and Sirajganj (favorable areas). In this study, five advanced rice genotypes from BRRI Regional Station, Barishal and two check varieties were evaluated (Table 1).

Experimental Design and Crop Management

The experiment was conducted across multiple environments following a randomized complete block design (RCBD) with three replications. Within each environment, seven genotypes were randomized independently. In addition to individual environment analyses, data from all environments were pooled across environments to perform a combined analysis of variance (ANOVA) for assessing genotype × environment interactions and stability performance.

Forty-day-old seedlings were transplanted into 10.8 m² plots, each consisting of 10 rows with 27 hills per row, at a spacing of 20 × 20 cm with one seedling per hill. Fertilizers were applied at 260:100:120:110:11 kg ha^{-1} of urea, TSP, MP, gypsum, and $ZnSO_4$, respectively. Nitrogen was top-dressed in three splits: 15 days after transplanting (DAT), 30 DAT, and five days before panicle initiation, while the remaining fertilizers were applied during final land preparation. Standard agronomic and crop protection practices were followed according to BRRI guidelines (BRRI, 2010). Grain yield was determined from the harvest of the entire plot, as no border rows were excluded, and the yield was adjusted to 14% moisture content.

In addition to grain yield, ancillary agronomic traits such as days to 50% flowering, plant height, growth duration, number of effective tillers per hill, and panicle number per hill (pAcp) were also recorded during the trial. However, only grain yield data were used for the present genotype × environment (G×E) analysis.

Data Collection and Statistical Analysis

Grain yield data were subjected to combined analysis of variance (ANOVA) across environments to partition the effects of genotype (G), environment (E), and their interaction (G×E). AMMI and GGE biplot analyses were performed using R software (version 4.4.2) and PBTools (version 1.3, IRRI). In the model genotypes were treated as both fixed and random and environments were treated as fixed, and replications nested within environment as random effects. Data normality was not checked before analysis.

The Grain mean yield was analyzed using STAR 2.0.1 version. In the AMMI approach, main effects were first analyzed using ANOVA, followed by principal component analysis (PCA) of the residuals to interpret G×E interaction patterns. In parallel, the GGE biplot method was employed to visualize both genotype main effects (G) and G×E interactions (GE) in a single framework, facilitating the identification of high-yielding and stable genotypes as well as the delineation of mega-environments (Hashim *et al.*, 2021).

Table 1. Description of Rice Genotypes examined and corresponding location codes.

Genotype code	Genotype Name	Location code	Location Name
G1	BRBaNGR 324-1	E1	Barishal
G2	BRBaNGR 1256-1	E2	Bhanga
G3	BRBaNGR350-2	E3	Cumilla
G4	BRBaNGR736-1	E4	Gazipur
G5	BRBaNGR994-1	E5	Gopalganj
G6	BRRI dhan74(Ck)	E6	Habiganj
G7	BRRI dhan102(Ck)	E7	Kustia
		E8	Rajshahi
		E9	Rangpur
		E10	Satkhira
		E11	Sirajgonj
		E12	Sonagazi

RESULTS AND DISCUSSION

Mean Grain Yield Performance

The Table 2 presents the grain yield performance (t/ha) of seven rice genotypes (five advanced breeding lines and two checks) evaluated across twelve environments (E1–E12) in Bangladesh. Considerable variation in yield performance was observed among genotypes and across locations, indicating strong genotype × environment (G×E) interaction.

Table 2. Mean Performance of Grain Yield Across Environments.

Genotype	Yield ($t ha^{-1}$)												Ave.mean
Location	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	
G1	6.45 a	8.32 a	7.93a	5.37a	8.84 a	6.56 a	6.92a	7.97a	7.34c	8.17a	5.21ab	9.49a	7.38
G2	5.66 ab	7.81 a	7.14 ab	5.16a	8.17 ab	5.55 a	6.28ab	7.34a	7.09c	7.45a	5.08ab	6.82b	6.63
G3	5.95ab	8.01 a	7.20 a	4.72 a	6.90 b	6.18 a	5.11b	8.18a	7.02c	7.02a	6.38a	6.17b	6.57
G4	6.26ab	8.34 a	5.04 c	5.34a	8.63 a	5.83 a	6.25ab	7.31a	9.09ab	7.74a	4.67b	5.92b	6.71
G5	6.00 ab	8.60 a	5.80 bc	5.68 a	9.02 a	6.23 a	7.00a	7.64a	9.63a	7.90a	5.23ab	7.13b	7.16
G6	5.97 ab	8.05 a	6.95 ab	5.79 a	8.27 a	5.87 a	6.17ab	7.06a	6.93c	7.87a	5.17ab	7.07b	6.77
G7	5.07 b	8.14 a	7.11 ab	5.21 a	8.44a	5.213a	7.26a	8.04a	7.76bc	8.33a	6.14a	6.54b	6.94
Location Mean	5.91	8.19	6.74	5.32	8.33	5.92	6.43	7.65	7.84	7.79	5.41	7.03	
LSD at 0.05													0.6
CV (%)													8.45
Heritability(H2)													0.72

Means with the same letter are not significantly different

Across environments, the mean yield ranged from 6.63 t/ha to 7.38t/ha. G1 showed the highest yield among all genotypes across environments. On the other hand, G2 performed moderate to high yields in several environments (7.81 t/ha in E2; 8.17 t/ha in E5). G5 exhibited good performance across most locations, with the highest yields recorded in E5 (9.02 t/ha) and E9 (9.63 t/ha). Among the tested lines, G1 and G5 demonstrated better performance compared to checks. There is no significant difference among the G2 with checks and it showed almost similar performance to checks. Therefore, it is important to observe G×E interaction

underscores the importance of multi-environment testing in selecting widely adapted and stable rice genotypes for Bangladesh's diverse agro-ecologies.

ANOVA and AMMI Analysis of Variance

The combined use of AMMI and GGE biplot analyses in this study offered a robust evaluation of genotype × environment (G×E) interaction among seven rice genotypes (G1–G7) across twelve environments (E1–E12). This dual-method approach, commonly applied in crop studies reaffirmed its value in dissecting patterns of stability and adaptability (Mohan *et al.*, 2023).

The AMMI analysis of variance (Table 3) showed that environment (E) was the largest source of variation, accounting for 51.9% of the total sum of squares (TSS) and was highly significant ($p < 0.001$). This indicates that yield performance of rice genotypes was strongly influenced by environmental differences, which is typical in multi-location trials.

Table 3. ANOVA and AMMI analysis of variance for grain yield across 12 environments.

Source of variation	Df	Sum Square	Mean Square	F value	Pr(>F)	Proportion (%)	Accumulated (%)	%TSS
Environment(E)	11	268.938	24.4489	50.88	6.71E-14			51.9
Replication(R)	24	11.533	0.4806	1.53	6.54E-02			2.2
Genotype(G)	6	19.237	3.2062	10.23	2.02E-09			3.7
(G×E)	66	86.643	1.3128	4.19	3.77E-13			16.7
PC1	16	45.423	2.8389	9.06	0.00E+00	52.4	52.4	8.8
PC2	14	23.196	1.6569	5.29	0.00E+00	26.8	79.2	4.5
PC3	12	12.073	1.0061	3.21	4.00E-04	13.9	93.1	2.3
PC4	10	4.663	0.4663	1.49	1.49E-01	5.4	98.5	0.9
PC5	8	0.756	0.0945	0.30	9.65E-01	0.9	99.4	0.15
PC6	6	0.532	0.0887	0.28	9.46E-01	0.6	100.0	0.1
Residuals	144	45.111	0.3133					8.7
Total	317	518.105	1.6344					100

The %TSS indicates the proportion of total sum of squares explained by each source of variation. IPCA = Interaction Principal Component Axis. DF = degree of freedom, G x E = genotype by environment interaction, SS = sum of squares, %TSS = percent total sum of squares.

The genotypic effect (G) contributed only 3.7% of TSS, although it was highly significant, suggesting the presence of inherent but relatively small genetic variation for yield among the tested entries. In contrast, the genotype × environment interaction (GEI) explained 16.7% of TSS and was highly significant, demonstrating that genotypes responded inconsistently across environments, thus justifying the need for stability and adaptability analysis.

Partitioning of GEI into interaction principal component axes (IPCAs) showed that PC1, PC2, and PC3 together captured 93.1% of GEI variation, with PC1 alone contributing 52.4%. The significance of the first three IPCAs suggests that they are sufficient to describe the major crossover interactions among genotypes and environments. Higher-order IPCAs (PC4–PC6) explained only minor, non-significant portions of the interaction and were therefore less relevant. Residual variation accounted for 8.7% of TSS, which is acceptable for field trials.

These findings are consistent with previous studies in rice and other cereals, where the environment typically explains the largest proportion of yield variation, followed by GEI and a smaller contribution from genotypes. For example, Gauch & Zobel (1996) reported that environmental effects usually exceed 50% of TSS in AMMI models, while Purchase *et al.*

(2000) in maize and Yan & Tinker (2006) in wheat observed similar patterns. In rice, Huang et al. (2021) also reported that GEI accounted for 15–20% of yield variation, highlighting the importance of stability analysis in cultivar evaluation. Thus, these results confirm that environmental heterogeneity dominates rice yield performance, but genotype and GEI effects are critical for identifying stable, widely adapted varieties.

Genotype Performance and Stability

AMMI biplot for Yield Mean

The AMMI biplot (Fig. 1) explained a substantial proportion of the total variation, with PC1 and PC2 accounting for 52.4% and 26.8%, respectively. Together, these two components captured more than 79.2% of the genotype × environment (G×E) interaction, indicating a reliable representation of yield stability patterns which is a level of explanatory power consistent with effective G×E interpretation (Gauch, 2006; Akter *et al.*, 2014; Hasina *et al*., 2015). In this biplot, genotypes and environments close to the origin are considered more stable, while those further away exhibit greater interaction with the environment. Genotypes G2 and G6 were located near the origin, suggesting their yield performance was stable across environments. Conversely, genotypes G1, G3, and G4 were positioned farther from the origin, displayed strong interaction and greater sensitivity to environmental changes. G5 show high yield potential, but their strong G×E interaction suggests they are suitable only for specific environments (not broadly adaptable). A similar type of findings has been reported in the literature on rice crops by Hasina *et al.*, (2015); Bose *et al.*, (2014), and Akter *et al.*, (2014).

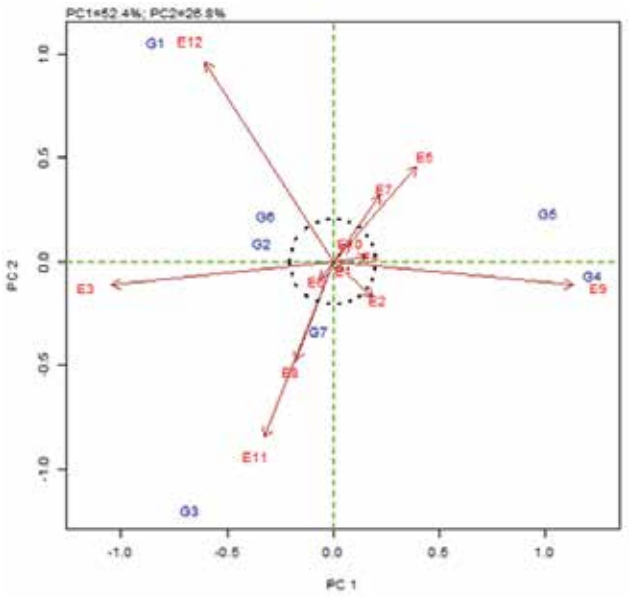


Fig. 1. AMMI biplot for Yield Mean.

Among environments, E12 and E3 were highly interactive, showing greater capacity to discriminate among genotypes, while E5 and E6 also contributed to G×E interaction. In contrast, environments clustered near the center, such as E2 and E7, were less interactive, reflecting more uniform genotypic responses. These results suggest that E12 and E3 are critical sites for identifying environment-specific adaptation, while E2 and E7 are more useful for testing general adaptability.

Ranking of Genotypes based on Yield and Stability performance

The GGE genotype view (Fig. 2) highlighted performance differences. Genotypes G2 and G6 were placed in the positive direction of the average environment axis (AEA), indicating good yield potential across environments. G7 was close to the AEA origin, suggesting stable though moderate performance. In contrast, G3 appeared at the upper extreme of the plot and G4 at the opposite lower side, indicating poor yield potential and strong interaction effects. Genotypes close to the ideal genotype center (represented by concentric circles) are considered more desirable. Here, G2 and G6 were closest, identifying them as high-yielding and broadly adapted entries.

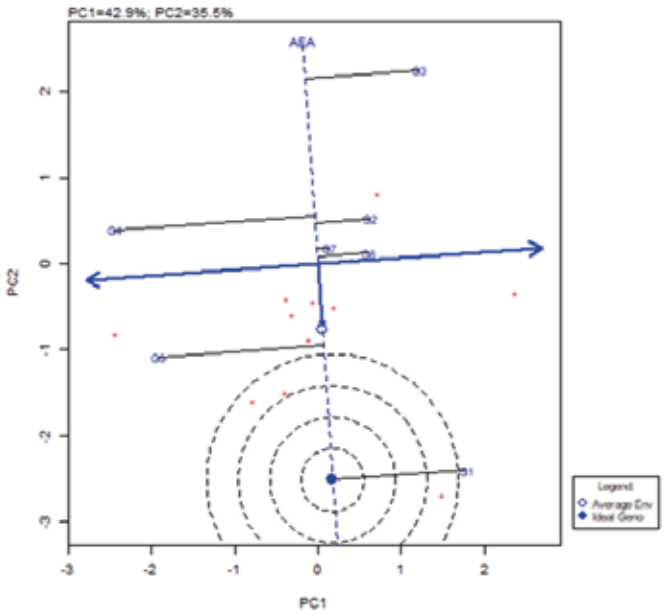


Fig. 2. GGE biplot genotype view showing the ranking of genotypes for both yield and stability performance over environment.

The estimation of genotype yield and stability (Fig. 2) was carried out using the average environment coordinates (AEC) method (Yan *et al.*, 2007). The average environment is defined by the average values of PC1 and PC2 for all environments and it is presented with a circle. The average ordinate environment (AOE) defined by the line which is perpendicular to the AEA (average environment axis) line and passes through the origin. This line divides the genotypes into those with higher yield than average and into those lower yield than average. The stability of genotypes is determined by their distance from the AE abscissa. Genotypes positioned closer to the abscissa are more stable than those farther away. In this study, the greatest stability in the high yielding group had genotypes G2 and G6, while the most unstable of all was G7. Although G1 showed the highest mean yield but it is unstable across the environment. Based on the ranking of the genotypes for both yield and stability performance were G2 and G6.

The results confirm that the AMMI and GGE models provide complementary insights AMMI quantifies stability, while GGE effectively visualizes adaptability and mega-environment. The identification of G2 and G6 as stable performers and G1 as a specifically adapted high-yielding genotypes for specific environment which is potential for rice breeding program advancement in Bangladesh.

Polygon view of GGE biplot analysis

The "which-won-where" view of the GGE biplot has been widely recognized as an effective visual tool for mega-environment analysis (Yan *et al.*, 2007). In this approach, a polygon is formed by connecting the vertex genotypes, with rays drawn from the origin of the biplot to each side of the polygon, thereby dividing the biplot into different sectors. Each sector represents a potential mega-environment, and the vertex genotypes located furthest from the origin are considered either the best or the poorest performers within those environments (Yan and Kang, 2003).

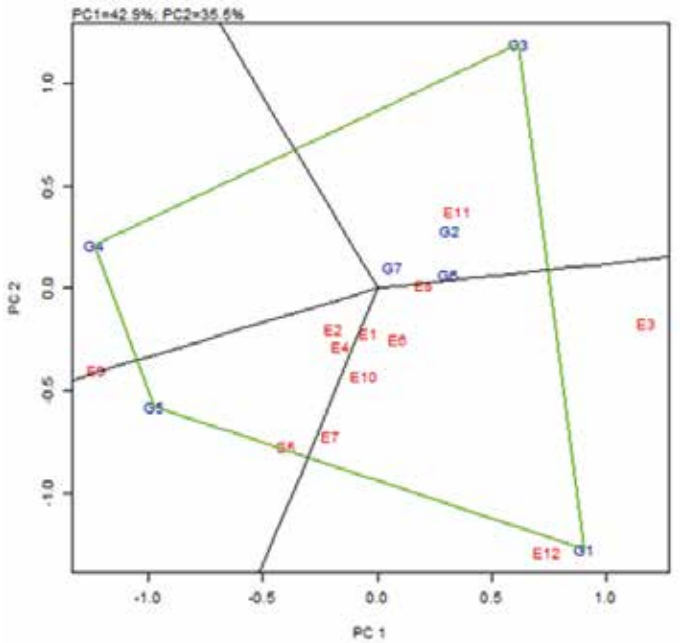


Fig. 3. The "which-won-where" view of the GGE biplot.

In this study, PC1 (42.9%) and PC2 (35.5%) together captured a large share of the total variation, indicating the GGE model effectively represents GEI (Fig. 3). Genotypes with positive PC1 scores were associated with higher yield performance, whereas those with negative scores were considered lower yielding, in agreement with the findings of Kaya et al. (2006). Similarly, the PC2 axis was indicative of genotype stability, where values closer to zero reflected stable performance across environments.

The polygon view identified G1, G3, G4, and G5 as vertex genotypes (Fig. 3), suggesting that these were either highly responsive or extremely poor performers across the test environments. For instance, G4 consistently showed poor performance, as they were positioned far from most environments, confirming their low adaptability and yield potential across sites. Conversely, environments such as E11, which clustered with genotypes G2, G6, and G7, were associated with relatively higher and stable yield performance, as reflected by their near-zero PC2 scores. Genotypes G1, G3, and G5 showed superiority in specific environments, indicating potential for targeted adaptation.

Overall, the analysis demonstrated the existence of distinct environmental groupings, with certain genotypes performing consistently well in specific environments while others exhibited poor adaptability. The clustering of environments and identification of vertex genotypes align with earlier findings that GGE

genotypes align with earlier findings that GGE biplot analysis not only reveals mega-environment structures but also aids in selecting genotypes with wide or specific adaptability (Karimizadeh *et al.*, 2013). These results are highly relevant for targeted rice improvement, as they provide breeders with valuable insights into which genotypes are broadly stable, and which are best suited for specific ecological niches.

CONLUSION

This study demonstrated that environmental factors exert the strongest influence on rice grain yield, accounting for more than half of the observed variation. However, the significant genotype × environment interaction highlighted the importance of stability and adaptability testing in breeding programs. The AMMI analysis identified G2 and G6 as the most stable and high-yielding genotypes, while GGE biplot analysis further confirmed their wide adaptability across test environments. Conversely, G1, G3, and G5 were highly interactive and suggesting limited adaptability. Among environments, E12 and E3 were identified as key discriminative sites for evaluating genotype adaptability. The combined application of AMMI and GGE models thus proved effective in identifying genotypes with both wide and specific adaptation. These findings provide valuable insights for rice breeders aiming to select high-performing, stable genotypes suitable for multi-environment cultivation in Bangladesh.

ACKNOWLEDGEMENT

The authors are grateful to all concerned scientists of BRRI Headquarter and Regional Stations and BRRI Management team for this research.

REFERENCES

Akter, A., Hassan, J. M., Kulsum, M. U., Islam, M. R., Hossain, K., & Rahman, M. M. (2014). AMMI biplot analysis for stability of grain yield in hybrid rice (*Oryza sativa* L.). *Journal of Rice Research*, 2, 126. https://doi.org/10.4172/jrr.1000126

Akter, F., Ahmed, A., Sumiya, N. N., & Alam, N. (2023). Assessing the impacts of pre-monsoon extreme weather events and associated factors on rice yield: A study on Tahirpur Upazila of Sunamganj District, Bangladesh. *The Dhaka University Journal of Earth and Environmental Sciences*, 11(1), 111–130.

Allard, R. W., & Bradshaw, A. D. (1964). Implications of genotype–environment interactions in applied plant breeding. *Crop Science*, 4(5), 503–508.

Anshori, M. F., Musa, Y., Farid, M., Jayadi, M., Padjung, R., Kaimuddin, K., Huang, Y. C., Casimero, M., Bogayong, I., Suwarno, W. B., Sembiring, H., Purwoko, B. S., Nur, A., Wahyuni, W., Wasonga, D. O., & Seleiman, M. F. (2024). A comprehensive multivariate approach for G×E interaction analysis in early maturing rice varieties. *Frontiers in Plant Science*, 15, 1462981. https://doi.org/10.3389/fpls.2024.1462981

Bangladesh Rice Research Institute (BRRI). (2010). *Adhunik dhaner chash* (15th ed., pp. 20–50). Bangladesh Rice Research Institute.

Bose, L. K., Jambhulkar, N. N., & Singh, O. N. (2014). Additive main effects and multiplicative interaction (AMMI) analysis of grain yield stability in early duration rice. *Indian Journal of Agricultural Sciences*, 83(4), 366–371.

Dewi, A. K., Chozin, M. A., Triwidodo, H., & Aswidinnoor, H. (2014). Genotype × environment interaction and stability analysis in lowland rice promising genotypes. *International Journal of Agronomy and Agricultural Research*, 5, 74–84.

Dimitrios, B., Christos, G., Jesus, R., & Eva, B. (2008). Separation of cotton cultivar testing sites based on representativeness and discriminating ability using GGE biplots. *Agronomy Journal*, 100, 1230–1236.

Ding, M., Tier, B., & Yan, W. K. (2007). Application of GGE biplot analysis to evaluate genotype (G), environment (E),

and G×E interaction on P. radiata: Case study. In *Australasian Forest Genetics Conference*, April 11–14, 2007, The Old Woolstore, Hobart, Tasmania, Australia.

Fukagawa, N. K., & Ziska, L. H. (2019). Rice: Importance for global nutrition. J*ournal of Nutritional Science and Vitaminology*, 65(Suppl.), S2–S3.

Gauch, H. G. (2006). Statistical analysis of yield trials by AMMI and GGE. *Crop Science*, 46(4), 1488–1500.

Gauch, H. G., & Zobel, R. W. (1996). AMMI analysis of yield trials. In M. S. Kang & H. G. Gauch (Eds.), *Genotype-by-environment interaction* (pp. 85–122). CRC Press.

Ghazy, M. I., Abdelrahman, M., El-Agoury, R. Y., El-Hefnawy, T. M., El-Naem, S. A., Daher, E. M., et al. (2024). Exploring genetics by environment interactions in some rice genotypes across varied environmental conditions. *Plants*, 13, Article 1.

Gupta, V., Kumar, M., Singh, V., Chaudhary, L., Yashveer, S., Sheoran, R., Dalal, M. S., Nain, A., Lamba, K., & Gangadharaiah, N. (2022). Genotype by environment interaction analysis for grain yield of wheat (*Triticum aestivum* L.) genotypes. *Agriculture*, 12(7), 1002.

Hashim, N., Rafii, M. Y., Oladosu, Y., Ismail, M. R., Ramli, A., Arolu, F., & Chukwu, S. (2021). Integrating multivariate and univariate statistical models to investigate genotype–environment interaction of advanced fragrant rice genotypes under rainfed condition. *Sustainability*, 13(8), 4555. https://doi.org/10.3390/su13084555

Hasina, K., Islam, R., Anisuzzaman, M., Ahmed, H.U., Haque, M. (2015). GGE bipot analysis of genotype x environment interaction in rice (*Oryza sativa* L.) genotypes in Bangladesh. *Scientia Agriculturae*, 12(1), 34-39. DOI: 10.15192/PSCP.SA.2015.12.1.3439

Huang, X., Jang, S., Kim, B., Piao, Z., Redoña, E., & Koh, H.-J. (2021). Evaluating genotype × environment interactions of yield traits and adaptability in rice cultivars grown under temperate, subtropical, and tropical environments. *Agriculture*, 11(6), 558. https://doi.org/10.3390/agriculture11060558

International Rice Research Institute (IRRI). (2013). *Climate change and rice: Abiotic stress and adaptation*. IRRI.

Kang, M. S. (2004). *Quantitative genetics, genomics, and plant breeding*. CABI Publishing.

Kang, M. S., Aggarwal, V. D., & Chirwa, R. M. (2006). Adaptability and stability of bean cultivars as determined via yield stability statistic and GGE biplot analysis. *Journal of Crop Improvement*, 15, 97–120.

Karimizadeh, R., Mohammadi, M., & Sabaghnia, N. (2013). GGE biplot analysis of yield stability in multi-environment trials of lentil genotypes under rainfed condition. *Notulae Scientia Biologicae*, 5(2), 256–262. https://doi.org/10.15835/nsb529018

Kaya, Y., Palta, C., & Taner, S. (2006). AMMI analysis of yield performance in bread wheat genotypes across environments. *Turkish Journal of Agriculture and Forestry*, 30(5), 325–337.

Khatun, H., Islam, R., Anisuzzaman, M., Ahmed, H. U., & Haque, M. (2015). GGE biplot analysis of genotype × environment interaction in rice (*Oryza sativa* L.) genotypes in Bangladesh. *Scientific Agriculture*, 12(1), 34–39.

Mainuddin, M., Alam, M. M., Maniruzzaman, M., et al. (2021). Yield, profitability, and prospects of irrigated Boro rice cultivation in the North-West region of Bangladesh. *PLoS ONE*, 16(4), e0250897.

Mohan, Y. C., Krishna, L., Srinivas, B., Rukmini, K., Sreedhar, S. S., Prasad, K. S., & Kishore, N. S. (2023). Stability analysis of short duration rice genotypes in Telangana using AMMI and GGE biplot models. *Environnent Conservation Journal*, 24, 243–252.

Panda, S., Nk, R., Pavani, S. L., Ganesan, S., Singh, P. K., Sah, R. P., Padmakumar, V., Subudhi, H., Mahender, A., Anandan, A., &

Ali, J. (2023). Multi-environment evaluation of rice genotypes: Impact of weather and culm biochemical parameters against sheath blight infection. *Frontiers in Plant Science*, 14, 1280321.

PBTools. (2014). *PBTools version 1.3*. Biometrics and Breeding Informatics, Plant Breeding Division, International Rice Research Institute. http://bbi.irri.org/

Penna, S., Bidabadi, S. S., & Jain, S. M. (2023). Mutation breeding to promote sustainable agriculture and food security in the era of climate change. In *Mutation breeding for sustainable food production and climate resilience* (pp. 1–23). Springer Nature.

Poli, Y., Balakrishnan, D., Desiraju, S., Panigrahy, M., Voleti, S. R., Mangrauthia, S. K., & Neelamraju, S. (2018). Genotype × environment interactions of Nagina22 rice mutants for yield traits under low phosphorus, water-limited, and normal irrigated conditions. *Scientific Reports*, 8(1), 15530.

Purchase, J. L., Hatting, H., & Van Deventer, C. S. (2000). Genotype × environment interaction of winter wheat in South Africa: II. Stability analysis of yield performance. *South African Journal of Plant and Soil*, 17, 101–110.

R Core Team. (2024). *R: A language and environment for statistical computing* (Version 4.4.2).

Rahman, N. M. F., Malik, W. A., Kabir, M. S., et al. (2023). 50 years of rice breeding in Bangladesh: Genetic yield trends. *Theoretical and Applied Genetics*, 136(1).

STAR. (2014). *STAR version 2.0.1*. Biometrics and Breeding Informatics, Plant Breeding Division, International Rice Research Institute. http://bbi.irri.org/

Wang, S. (2025). Advancing stress-resilient rice: Mechanisms, genes, and molecular breeding approaches. *Agriculture*, 15(7).

Yan, W., & Kang, M. S. (2003). *GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists*. CRC Press.

Yan, W., & Rajcan, I. (2002). Biplot analysis of test sites and trait relations of soybean in Ontario. *Crop Science*, 42(1), 11–20.

Yan, W., & Tinker, N. A. (2006). Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, 86(3), 623–645.

Yan, W., Cornelius, P. L., Crossa, J., & Hunt, L. A. (2007). Two types of GGE biplots for analyzing multi-environment trial data. *Crop Science*, 41(3), 656–663.

Yan, W., Hunt, L. A., Sheng, Q., & Szlavnics, Z. (2000). Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science*, 40, 597–605.

Zhang, P. P., Hui, S., Liu, Y., Qu, Y., Su, W., & Zheng, D. F. (2016). GGE biplot analysis of yield stability and test location representativeness in proso millet (*Panicum miliaceum* L.) genotypes. *Journal of Integrative Agriculture*, 15(6), 1218–1224.

Bangladesh Rice J.28(1): 81-90, 2024, doi.org/10.3329/brj.v28i1.86887

Optimum Nitrogen Dose and its Use Efficiency of a Bacterial Blight Disease Resistant Rice ALART Material in Chhiata Clay Loam Soil

F Rahman[1, *], ATM S Hossain[2] and M R Islam[3]

ABSTRACT

Given the widespread deficiency of nitrogen in Bangladesh, its optimum dose of advanced breeding lines needs to be determined before releasing them as a variety. A field experiment was conducted with advanced line BR8938-19-4-3-1-1-P2-HR3 (BBRAL) using BRRI dhan28 as a susceptible check. Both the genotypes received six nitrogen doses – 0 to 200 kg N ha^{-1} with an interval of 40 kg ha^{-1} from urea in a split-plot design. Both the tested genotypes received flat standard doses of P, K, S, and Zn. A quadratic regression model was used to determine the optimum N requirement. Quadratic equations $\hat{y} = 3.46 + 0.04N - 0.00013N^2$, ($R^2 = 0.95$, $p = 0.006$) for the bacterial blight resistant line BR 8938-19-4-3-1-1-P2-HR3, and $\hat{y} = 3.73 + 0.04N - 0.00012N^2$, ($R^2 = 0.99$, $p = 0.006$) for the check variety BRRI dhan28 explained the relationship for N rates and estimated grain yield. The calculated economic optimum N dose using the quadratic regression model for BBDRL (Bacterial Blight Disease Resistant Rice) and the susceptible check BRRI dhan28 was 153 and 162 $kgNha^{-1}$, respectively. The application of 153 and 162 kg N ha^{-1} predicted the estimated yield of 6.81 and 7.21 for the advanced line and BRRI dhan28, respectively. Nutrient use efficiencies, such as agronomic use efficiency, apparent recovery of applied N, internal use efficiency and nitrogen harvest index of the tested advanced line, were similar to those of BRRI dhan28.

Keywords: Bacterial Blight line, grain yield, N response curve, N requirement, N efficiencies.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop for more than 3 billion people in the world and 135 million people in Bangladesh. The demand for rice is predicted to rise from 439 million tons (mt) in 2010 to 496 mt by 2020, 553 mt by 2035, and 623 mt by 2050 (Timsina *et al.*, 2021). The yield of rice largely depends upon the nutritional status of the soil and the availability of nutrients from chemical fertilizers. Increase in rice production depends on improved varieties coupled with judicious fertilizer use, and appropriate irrigation management (Gairhe *et al.*, 2018) and other cultural practices. Among the major nutrient elements, nitrogen (N) is the most limiting nutrient for rice crop growth and yield, and it is required in compared to other nutrients (Djaman *et al.*, 2018). N is universally deficient in almost all the agricultural soils and cropping systems of the world, so it is essential to use external nitrogen inputs (N fertilizers) to produce the crops for satisfying the ever-increasing demands of human populations (Mohan *et al.*, 2015). Globally, farmers using around 108 million metric tons of nitrogenous fertilizer each year (FAO, 2024). Nitrogen is the most yield-limiting and widely applied nutrient in Bangladeshi rice fields in all rice growing seasons, as rice genotypes exhibit a stronger response to applied N than other major nutrients. Nitrogen

[1]Senior Scientific Officer, Soil Science Division, BRRI
[2]Principal Scientific Officer, Soil Science Division, BRRI.
[3]Chief Scientific Officer and Head, Soil Science Division, BRRI.
[*]Corresponding author's E-mail: fahmida72@gmail.com (F Rahman)

management is essential in growing rice, and N fertilization can largely improve rice productivity and profitability. Rice crop generally requires 20 kg N for each ton of grain yield under optimum conditions (Attanandana *et al.*, 2010). The N requirement of rice varies with the rice genotypes (Saleque *et al.*, 2005; Jing *et al.*, 2008; Hirzel *et al.*, 2011). Biomass production of irrigated rice is mainly driven by the supply of N. Even the demand of the rice plant for other macro-nutrients are also depends on N supply. While increased N application may increase grain yield in rice, it often increases plant N uptake and consequently grain N concentration (Wood *et al.*, 2021). While increased grain protein concentration (GPC) may be advantageous nutritionally and also for some grain quality such as head rice yield, it often affects overall palatability (Meng *et al.*, 2024). Conventionally, to fulfil this large requirement of N, farmers completely depend on chemical nitrogen.

Excess use of N in crop input can cause imbalance of ecosystem functions and services (Hutchinson *et al.*, 2003; Meena *et al.*, 2014), exacerbates soil degradation and environmental pollution (Ladha *et al.*, 2016; Islam *et al.*, 2022; Yu *et al.*, 2018) and even soil acidification (Guo *et al.*, 2010, Schroder *et al.*, 2011).

Recently, BRRI dhan28 lost its popularity because of bacterial leaf blight. The invention of BBRAL is a breakthrough in Bangladesh to substitute BRRI dhan28. It is empirical to understand the N fertilizer requirement along with the N use efficiency of the advanced disease-resistant line compared to popular variety BRRI dhan28.

MATERIALS AND METHODS

Experimental location

In Boro 2020-21, the experiment was conducted at the Bangladesh Rice Research Institute (BRRI) farm, Gazipur. The climate of the experimental location is subtropical in nature and experiences periodic southwestern monsoon with an average annual rainfall of 2000 mm. The 80% of the rainfall occurs from mid-June to the end of September. The lowest mean temperature (15°C) prevails in January and the highest (30°C) in May. The soil of the experimental site belongs to Chhiata clay loam, a member of fine, hyperthermic Vertic Endoaquepts. The soil of the experimental field had a clay loam texture and a pH of 6.70. The other nutrient status was as follows: organic carbon 1.18%, total N 0.13%, exchangeable K 0.12 meq/100g soil, available S 14.0 mg kg^{-1} and available Zn (DTPA extraction) 1.5 mg kg^{-1}.

Experimental design and treatments

The experiment was conducted in a split-plot design with three replications, where N fertilizer doses were assigned in main-plot and rice genotypes in sub-plot. The individual main-plot size was 7 m x 3 m. Six N levels with the application rates of 0, 40, 80, 120, 160 and 200 kgha-1 were evaluated with PVT-DRR (BB) line BR8938-19-4-3-1-1-P2-HR3 and BRRI dhan28 as susceptible check. All plots had received a blanket dose of chemical fertilizer P-K-S-Zn @ 20-60-10-2 $kgha^{-1}$, respectively. All fertilizers except urea were applied as basal at final land preparation. Urea was applied into three equal splits in with first top-dressing on 15 days after transplanting (DAT), second one on 30 DAT and the rest one on 5 days before panicle initiation (PI) stage. Thirty-five-day-old seedlings of each rice genotypes were transplanted on 30 December 2020. Irrigation, weeding and other cultural management practices were done equally as per needed. At maturity, 1st the check variety BRRI dhan28 was harvested on 17 April 2021 and the PVT-DRR (BB) line BR8938-19-4-3-1-1-P2-HR3 was harvested on 24 April.

Data collection analysis

Plant height was recorded at maturity from 3 hills per plot from the soil surface to the tip of the tallest panicle of each hill. Number of tillers and panicles per m2 were counted from 16 hills in each plot. Number of filled and unfilled grains per panicle were counted from five panicles in each plot. Panicle length (cm) was measured from the panicle neck to the apex of the panicle from five panicles. Grain yield was calculated

from the area of 5 m^2 at 15 cm above ground level, however, 16 hills from each plot were harvested at the ground level for straw yield. The grain yield was recorded at 14% moisture content and straw yield as oven dry basis. Plant samples were analyzed for N content and N uptake. The grain and straw samples were analyzed for their N content by the micro-Kjeldahl method (Nelson & Sommers, 1973) and the crop N uptake was calculated from dry biomass (grain + straw) weight and N concentrations (Sarkar *et al.*, 2016).

Optimum and economic dose of N

Differentiation of the quadratic equation between derived from the relationship between N and grain yield derived the optimum and economic optimum dose of N (Gomez and Gomez, 1984) as follows:

$Y = a + bN + cN2$…….....(1)

Differentiating the above equation with respect to N,

$$\frac{dY}{dN} = b + 2cN$$

The optimum dose of N to maximize the yield would be at the level of N when

$$\frac{dY}{dx} = 0$$

or,

$$b + 2cN = 0$$

$$Ny = -\frac{b}{2c} \text{ …….....……(2)}$$

Economic optimum dose,

$$N_P = \frac{1}{2c}\left(\frac{P_f}{P_y} - b\right) \text{ ……..…….(3)}$$

where, P_f is price of fertilizer N (USD per kg) and Py is price of grain (USD/t).

Agronomic N use efficiency (ANUE)

$$ANUE = \frac{(Y_N - Y_0)}{N_F} \text{ ……(4)}$$

where,

ANUE = agronomic N use efficiency (kg grain/kg N)

Y_N = yield of N-applied plots

Y_0 = yield of N control plots

N_F = applied N (kg/ha) from N fertilizer

Apparent recovery efficiency (ARE)

$$ARE\ (\%) = \frac{(U_N - U_0)\times 100}{N_F} \text{ ……..……(5)}$$

where,

ARE = apparent recovery efficiency (%)

U_N = total nitrogen uptake (kg) from N-applied plots

U_0 = total nitrogen uptake (kg) from N control plots

N_F = applied N (kg/ha) from N fertilizer

Internal N use efficiency (INE)

$INE(t/kg) = YN - Y0 - UN - UO$ ..... (6)

Partial factor productivity: Partial factor productivity refers to the yield of rice per kg of applied nutrient.

Nitrogen harvest index (NHI)

$$NHI = \frac{NU_G}{(NU_G + NU_S)} \text{ …..……(7)}$$

where,

NHI = Nitrogen harvest index

NU_G = Nitrogen uptake by grain

NU_S = Nitrogen uptake by straw

Statistical analysis

The analyses were carried out using the STAR (Statistical Tool for Agricultural Research) software version 2.0.1 and MS Excel.

RESULTS AND DISCUSSION

Grain yield

Nitrogen fertilization significantly increased grain yield of both the BBRAL (Bacterial Blight Disease Resistant Rice) and BRRI dhan28 in Boro season (Fig. 1). With native soil N fertility, BBRAL and BRRI dhan28 yielded 3.26 and 3.64 t ha^{-1}, which increased with the application of N and continued increase progressively with the increased doses of N fertilizer. However, the rate of yield increase was slower with the higher doses of N. The grain yield of rice reached to a plateau at the N level of 160 kg ha^{-1} and showed

a declination with the highest N dose of 200 kg ha^{-1}.

Similar trend was observed for straw yield (Data not shown). The improved growth attributes, viz. plant height and dry-matter production, might be responsible for improved yield attributes. The straw yield of both varieties in Boro season significantly responded to the applied N rates. The straw yield of both varieties increased with the increase of N rates and was highest 7.16 tha^{-1} for V1 and 7.36 tha^{-1} for V2 with 200 kg Nha^{-1} which was statistically identical to 160 kg Nha^{-1}.

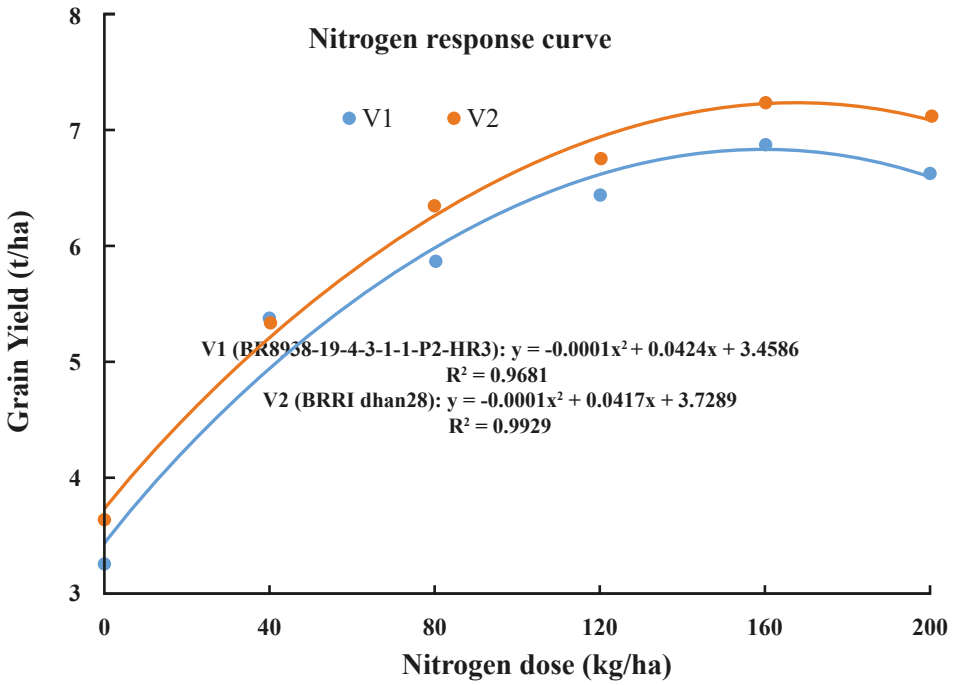


Fig. 1. Nitrogen response curve of PVT-DRR (BB) and BRRI dhan28 rice genotypes in Boro 2020-21, BRRI, Gazipur.

Grain yield showed a significant quadratic response to the N fertilizer application (Fig. 1). The quadratic response curve for the BBRAL and BRRI dhan28 as shown (Fig. 1) had significant coefficient of determination (R^2), intercepts, and coefficients both linear and quadratic terms for both genotypes (Table 1). The intercept for the BBRAL was 3.46±0.28 compared to 3.73±0.14 for BRRI dhan28. The p value of the intercepts was 0.0011 for BBRAL and 0.0001 for BRRI dhan28, respectively. BRRI dhan28 showed significantly greater intercept than that of BBRAL. The *'b'* coefficient for both genotypes was 0.04 with significant p value, indicates that the coefficient was not significantly different between two genotypes. The *'c'* coefficients for both the genotypes were statistically similar and very low, -0.00013 for BBRAL and for BRRI dhan28 it was -0.00012. The regression equation signifies that there was varietal difference in grain yield but both genotypes responded similarly with receiving N fertilizer application. There was insignificant genotype×N interaction on grain yield of rice.

Solving the equation (2) the calculated maximum dose of N for BBRAL was 158 kg Nha^{-1} and that for BRRI dhan28 was 167 kg ha^{-1}. According to the equation (3), the economic optimum dose of N was 153 kg ha^{-1} and 162 kg ha^{-1}, respectively. Tayefe *et al.* (2014) and Moro *et al.* (2015) reported similar result the grain yield to N fertilization. Pamela *et al.* (2009) illustrated that grain yield showed a significant quadratic response to N fertilization. Khatun *et al.* (2014) reported economic optimum dose of N for BRRI dhan28 and BRRI dhan29 as 156 and 158 kg ha^{-1}, respectively. Soil, varietal, location, and yearly differences in N requirement for rice may vary substantially (Islam *et al.*, 2015; Bhuiyan *et al.*, 2017). Bhuiyan *et al.* (2017) reported economic optimum dose of N for BRRI dhan29 as 166 and 155 kg ha^{-1}, in two consecutive years. Islam *et al.*, (2015) obtained economic optimum dose N for BRRI dhan29 as 145 kg ha^{-1} in the first year and 200 kg ha^{-1} in the second year in the same field. The large variation in N dose may be attributed to the variation in climatic elements, mainly high night temperature during the growing season.

In comparison of two rice genotypes, BRRI dhan28 responds better than the PVT-DRR (BB) line BR8938-19-4-3-1-1-P2-HR3 in the same N doses.

Table 1. Parameters of the regression equation between applied N and grain yield of two rice genotypes.

	BBRAL	*t-stat*	*p-value*	BRRI dhan28	*t-stat*	*p-value*
Intercept	3.46±0.28	12.45	0.0011	3.73±0.14	27.52	0.0001
N	0.04±0.01	6.49	0.0074	0.04±0.00	13.10	0.0010
N^2	-0.00013	-4.27	0.0235	-0.00012	-8.16	0.004
R^2	0.97		0.006	0.99		0.006

Nitrogen content and uptake

The application of fertilizer significantly influenced nitrogen content and uptake in grain and straw in both varieties (Table 2). Varietal difference in nitrogen concentration appeared significant. The tested advanced line had consistently greater grain N concentration at all the levels of applied N except at the highest rate. At N-control plot, BBRAL had grain and straw N concentration of 0.78 and 0.42% compared 0.69 and 0.43% in BRRI dhan28. The nitrogen concentration in grain and straw increased gradually with increasing the N doses in both the genotypes. The highest N concentration (1.04% and 1.05%) was noted with the highest level of N application (200 kg ha^{-1}) similar trend was observed for straw N content. Contrary to the grain N, straw N concentration was slightly

higher in BRRI dhan28 than BBRAL at all levels of N applications. It was found that total N uptake by rice plant increased with increased N rates up to a certain level (160 kg Nha^{-1}), then it decreased. The trend of N uptake by straw and grain followed the similar trend of N concentration. Varietal difference in uptake was different from that in concentration trend. At 0 to 120 kg ha^{-1} level of N application, BBRAL had slightly higher grain N uptake than that of BRRI dhan28. On the contrary, 160 and 200 kg ha^{-1} doses of N application BRRI dhan28 had higher grain N uptake than that of BBRAL. Varietal difference in straw N uptake was slightly different from that of straw N uptake. At 40 kg ha^{-1} N application, straw N uptake in both the genotypes was the same 24.84 kg ha^{-1}, but in all other N doses BRRI dhan28 had greater straw N uptake than that of BBRAL. BRRI dhan28 had greater total N uptake than that of BBRAL except at in 40 and 80 kg ha^{-1} levels of N application. Yesuf and Balcha, (2014) also reported that N uptake increases sharply with an increase in the application of N doses up to a certain level, and further increases in the N dose the N uptake remaining static.

Table 2. Nitrogen response in the N content and N uptake of PVT-DRR (BB) and BRRI dhan28 rice genotypes in Boro 2020-21, BRRI, Gazipur.

N ($kgha^{-1}$)	N content (%)		N uptake ($kgha^{-1}$)		Total N uptake ($kgha^{-1}$)
	Grain	Straw	Grain	Straw	
	BBRAL (BR8938-19-4-3-1-1-P2-HR3)				
0	0.78	0.42	25.43	14.87	40
40	0.81	0.45	43.50	24.84	68
80	0.91	0.47	53.33	28.25	81
120	0.97	0.50	62.37	33.25	95
160	1.01	0.52	69.39	36.24	106
200	1.04	0.54	68.85	38.66	108
	BRRI dhan28				
0	0.69	0.43	25.12	16.00	41
40	0.75	0.46	40.05	24.84	65
80	0.78	0.48	49.45	30.82	80
120	0.85	0.51	57.46	34.83	93
160	0.99	0.53	71.58	38.48	110
200	1.05	0.55	74.76	40.48	115
LSD (0.05)	0.08		0.04		
T X V	4.16		4.33		
CV (%)	3.21		3.67		

Nitrogen use efficiency

Agronomic N use efficiency (ANUE) decreased with the increasing doses of N application steeply (Table 3). Varietal difference was distinct at the 40 kg N ha^{-1} level, but at 160 kg N level, varietal difference diminishes for ANUE. The BBRAL had 22.6 kg/kg compared 22.4 kg/kg. It means that the BBRAL would produce 22.6 kg grain per kg of the applied N and that would be 22.4 for BRRI dhan28. BBRAL showed similar apparent recovery (41%) of applied N compared to BRRI dhan28 which had 43% apparent recovery of N. In the N-control plot, BBRAL showed slightly lower internal use efficiency of N (82 kg of grain per kg of N uptake) compared to 89 in case of BRRI dhan28. At 160 kg N ha^{-1} level, internal use efficiency of N of the tested genotypes were similar, 55 kg

kg^{-1} for BBRAL and 52 kg kg^{-1} for BRRI dhan28. The fact indicates that the BBRAL was less efficient to utilize native N from soil, slightly more efficient to utilize applied N.

The reciprocal of internal use efficiency of N indicate the amount of N required to plants absorb to produce one ton of rice grain. The lower the N absorb, the higher the utilization efficiency, means less amount of N require per ton of rice grain. N uptake in N-control plots was the lowest (Table 2), Table 3 shows that the least amount of N was required to produce 1 ton of grain. BBRAL and BRRI dhan28 required only 12 and 11 kg N, respectively, to produce 1 ton grain, while at 160 kg N ha^{-1} level, 1 ton of rice grain production required 18 and 19 kg N for BBRAL and BRRI dhan28, respectively.

Partial factor productivity (PFP) is another important parameter of N use efficiency, which tells how much of rice grain is produced per kg of N application through fertilizer. Both the check variety BRRI dhan28 and the new genotype had similar PFP. At 160 kg N ha^{-1} level, BBRAL had PFP of 43 compared to 45 with BRRI dhan28. At 80 – 200 kg N ha^{-1} level, the PFP of BBRAL was about 2 kg consistently lower than that of BRRI dhan28.

Nitrogen harvest index (NHI) translates the proportion of absorbed N translocated to grain. According to this index, BBRAL showed slightly superiority to BRRI dhan28. From 0 – 160 kg N ha^{-1} level, NHI in BBRAL was about 0.02 greater than that of BRRI dhan28.

Table 3. Nitrogen use efficiencies of BR8938-19-4-3-1-1-P2-HR3 and BRRI dhan28.

N kg ha^{-1}	Agron. N use (kg/kg)		Apparent Recovery (%)		Internal use efficiency (kg/kg)		kg N req. for ton grain		Partial factor productivity		Nitrogen harvest index	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
0	-	-	-	-	82	89	12	11	-	-	-	-
40	52.8	42.5	70	60	75	71	13	14	134	134	0.64	0.61
80	32.5	33.8	51	49	63	69	16	14	73	79	0.64	0.62
120	26.4	26.0	46	43	58	60	17	17	54	56	0.66	0.62
160	22.6	22.4	41	43	55	52	18	19	43	45	0.66	0.62
200	16.8	17.4	34	37	49	47	20	21	33	36	0.65	0.65

V1 = BR8938-19-4-3-1-1-P2-HR3, V2 = BRRI dhan28

CONCLUSIONS

Application of N doses greatly increase the yield and yield contributing characters of tested two rice genotypes. Comparatively higher grain yield obtained in the check variety BRRI dhan28 than the advanced line BR8938-19-4-3-1-1-P2-HR3 in the same dose of applied nitrogen. The calculated maximum doses of N for advanced line BR8938-19-4-3-1-1-P2-HR3 and the check variety BRRI dhan28 in Boro season were 158 and 167 kgha^{-1}, respectively, while the economic optimum doses were 153 and 162 kgNha^{-1}, respectively. The bacterial blight disease resistant line BR8938-19-4-3-1-1-P2-HR3 can give a good yield with medium doses of N which may be a good rice variety in bacterial blight disease proon area of Bangladesh.

REFERENCES

Akram, H. M., Ali, A., Nadeem, M. A., & Iqbal, M. S. (2007). Yield and yield components of rice varieties as affected by transplanting dates. *Journal of Agricultural Research* 45(2), 105-111.

Attanandana, T. Kongton, S., Boonsowphan, B.,

Plowatana, A., Verapatananirund, P., & Yost, R. (2010). Site Specific Nutrient Management of Irrigated Rice in the Central plain of Thailand. *Journal of Sustainable Agriculture*, 34(3), 258-269. DOI: 10.1080/10440041003613297

Baral, B. R., Pande, K. R., Gaihr, Y. K., Baral, K. R., Sah, S. K., & Thapa, Y. B. (2019). Farmers' fertilizer application gap in rice based cropping system: A case study of Nepal. *SAARC Journal of Agriculture*, 17(2), 267-277.

Bhuiyan, M. K. A., Bhuiya, S. U., Saleque, M. A., & Khatun, A. (2017). Nitrogen application in direct wet-seeded rice under alternate wetting and drying irrigation condition: Effects on grain yield, dry matter production, nitrogen uptake and nitrogen use efficiencies. *Journal plant nutr.*, 40 (17), 2477-2493.

Conry, M. J., (1995). Comparisons of early, normal and late sowing at three rates of nitrogen on the yield, grain nitrogen and screenings content of Blenheim spring malting barley in Ireland. *Journal of Agricultural Science, Cambridge*. 125, 183–189.

Djaman, K., Mel, V. C., Ametonou, F. Y., El-Namaky, R., Diallo, M. D., & Koudahe, K. (2018). Effect of nitrogen fertilizer dose and application timing on yield and nitrogen use efficiency of irrigated hybrid rice under semi-arid conditions. *Journal of Agricultural Science and Food Research*, 9(2), 223. https://hdl.handle.net/10568/102040

FAO. (2024). Food and Agriculture Organization (FAO). Rome, Italy. Current world fertilizer trends and outlook to 2024. https://www.fao.org.

Gairhe, S. (2018). Dynamics of Major Cereals Productivity in Nepal. *Journal of Nepal Agricultural Research Council*. 4(1), 60–71. doi https://doi.org/10.3126/jnarc.v4i1.19691

Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research. Second Edition. John Willy & Sons, 324 – 325.

Hasanuzzaman, M., Ahamed, K. U., Rahmatullah, N. M., Akhter, N., Nahar, K., & Rahman, M. L. (2010). Plant growth characters and productivity of wetland rice (*Oryza sativa* L.) as affected by application of different manures. *Emirates Journal of Food and Agriculture*, 22(1), 46-58.

Heluf, G., & Mulugeta, S. (2006). Effects of mineral N and P fertilizers on yield and yield components of flooded lowland rice on Vertisols of Fogera Plain, Ethiopia. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 107(2), 161-176.

Hirzel, J., Jose, P., & Karla, C. (2011). Effect of Nitrogen Rates and Split Nitrogen Fertilization on Grain Yield and its Components in Flooded Rice. *Chilean Journal of Agricultural Research*, 71(3), 437. doi: 10.4067/S0718-58392011000300015

Hutchinson, C., Simonne, E., Solano, P., Meldrum, J., & Livingston-Way, P. (2003). Testing of controlled release fertilizer programs for seep irrigated Irish potato production. *J. Plant Nutr.*, 26, 1709–1723.

Islam, S. M. M., Gaihre, Y. K., Islam, M. R., Ahmed, M. N., Akter, M., Singh, U., & Sander B. O. (2022). Mitigating greenhouse gas emissions from irrigated rice cultivation through improved fertilizer and water management. *Journal of Environmental Management*, 307, doi.org/10.1016/j.jenvman.2022.114520

Islam, S. M. M., Khatun, A., Hossain, A. T. M. S., Naher, U. A., & Saleque, M. A. (2015). Rice response to nitrogen in tidal non-saline soil. *Bangladesh Rice J.*, 19 (2), 65 – 70.

Jing, H. C., Darren, L., Richard, G., Daniel, J., Dmitry, K., Olga, P. M., Gert, H. J. K., & Kim, E. H. K. (2008). Phenotypic and genetic analysis of the Triticum monococcum–Mycosphaerella graminicola interaction. *New Phytologist*, 179(4), 1121-1132. https://doi.org/10.1111/j.1469-8137.2008.02526

Kausar, K, Akbar, M., Rasul, E., & Ahmad, A. N. (1993). Physiological responses of nitrogen, phosphorus and potassium on growth and yield of wheat. *Pakistan J. Agric. Res.*, 14, 2–3.

Khatun, A., Bhuiya, M. S. U., & Saleque, M. A. (2014). (2014). Response of nitrogen on yield and seed quality of Boro Rice. *Bangladesh Rice J.*, 18(1&2), 24-32.

Ladha, J. K., Tirol-Padre, A., Reddy, C. K., Cassman, K. G., Verma, S., Powlson, D. S., Van Kessel, C., de B Richter, D., Chakraborty, D., & Pathak, H. (2016). Global nitrogen budgets in cereals: A 50-year assessment for maize, rice and wheat production systems. *Scientific Reports*, 6(1), 1-9.

Meena, R. K., Singh, Y. V., Parsana, L. R., Kaur, C., Kumar, A., & Bana, R. S. (2014). Influence of plant growth promoting rhizobacteria inoculation on nutrient availability soil microbial property and defence enzyme in rice crop. *Indian J. Agril. Sci*. 84, 761-764.

Meng, X., Hongyao, L., Shanshan, Z., Runqi, Z., Guoyu, L., Weiya, X., Jiazheng, Y., Yufeng, Z., Zhongfu, N., Qixin, S., Jiewen, X., & Baoyun, L. (2024). TaNAM-6A is essential for nitrogen remobilisation and regulates grain protein content in wheat (*Triticum aestivum* L.). *Plant cell & environment*. DOI: 10.1111/pce.14878

Mohan, S., Singh, M., & Kumar, R. (2015). Effect of nitrogen, phosphorus and zinc fertilization on yield and quality of Kharif fodder -A review. *Agril. Reviews*. 36, 218-226.

Moro, B. M., Nuhu, I. R., Ato, E., & Naathanial, B. (2015). Effect of nitrogen rates on the growth and yield of three rice (*Oryza sativa* L.) varieties in rain-fed lowland in the forest agro-ecological zone of Ghana. *International Journal of Agricultural Science*, 5(7), 878–885.

Pamela, A., Claudia, B., & Francisco, J. M. (2009). Nitrogen Application in Irrigated Rice Grown in Mediterranean Conditions: Effects on Grain Yield, Dry Matter Production, Nitrogen Uptake, and Nitrogen Use Efficiency. *Journal of Plant Nutrition*, 32(9), 1574-1593. doi. 10.1080/01904160903094339.

Rajput, M. K. K., Ansari, A. H., Mehdi, S., & Hussain, A. M. (1988). Effect of N and P fertilizers alone and in combination with organic matter on the growth and yield of Toria. *Sarhad J. Agri. Res.*, 4, 3–6.

Saleque M.A., Naher, U.A., Choudhury, N.N., Hossain, A.T.M.S. (2005). Variety-specific nitrogen fertilizer recommendation for lowland rice. *Commun. Soil. Sci. Plant Anal*, 35, 1891-1903.

Sarkar, M. I. U., Islam, M. N., Jahan, A., Islam, A, and Biswas, J. C. (2016). Rice straw as a source of potassium for wetland rice cultivation. *Geology, Ecology, and Landscapes*, 1(3), 184-189. https://doi.org/https://doi.org/10.1080/24749508.2017.1361145

Schorder, W., & Roland, P. (2011). Mapping Carbon sequestration in forest at the regional scale- a climate bio monitoring approach by example of Germany. *Environmental Science Europe*, 23, Article 31.

Tayefe, M., Gerayzade, A., Amiri, E., & Zade, A. N. (2014). Effect of nitrogen on rice yield, yield components and quality parameters. *African Journal of Biotechnology*, 13(1), 91–105. https://doi.org/https://doi.org/10.5897/AJB

Timsina, J., Dutta, S., Devkota, K. P., Chakraborty, S., Neupane, R. K., Bishta, S., Amgain, L. P., Singh, V. K., Islam, S., & Majumdar, K. (2021). Improved nutrient management in cereals using Nutrient Expert and machine learning tools: Productivity, profitability and nutrient use efficiency. *Agricultural Systems*. 192, 103181. https://doi.org/10.1016/J.agsy.2021.103181

Wood, R. M., Dunn, B. W., Jeanette, L. B., Daniel, L. E. W., Christopher, L. B., Andrew, J. M., & Prakash, O. (2021). Effect of agronomic management on rice grain quality Part II: Nitrogen rate and

timing. *Journal of Food Science*, 98 (2), 234-248. https://doi.org/10.1002/cche.10372

Yesuf, E., & Balcha, A. (2014). Effect of nitrogen application on grain yield and nitrogen efficiency of rice (*Oryza sativa* L.). *Asian Journal of Crop Science*, 6(3), 273–280. https://doi.org/https://doi.org/10.3923/ajcs.2014.273.280

Yoshida, S., Cock, J. H., & Parao, F.T. (1972). Physiological aspects of high yield. Int. Rice Res. Inst. *Rice breeding*, pp. 455-469.

Yu, W. J., Li, X. S., Chen, Z. J., & Zhou, J. B. (2018). Effects of nitrogen fertilizer application on carbon dioxide emissions from soils with different inorganic carbon contents. Ying Yong Sheng tai xue bao. *The Journal of Applied Ecology*, 29(8), 2493-2500.

Bangladesh Rice J.28(1): 91-99, 2024, doi.org/10.3329/brj.v28i1.86965

Potassium Fertilizer Effect on the Growth and Yield of Boro Rice in Old Himalayan Piedmont Plain Soil of Bangladesh

ATM S Hossain[1*], F Rahman[1], R R Debnath[1] and J Khatun[2]

ABSTRACT

Increasing cropping intensity increases nutrient mining from the soil, especially potassium (K), if nutrient management is not properly maintained. A field experiment was conducted at Hajee Mohammad Danesh Science and Technology University (HSTU) farm soil, Dinajpur district under the Agro Ecological Zone (AEZ 1) the Old Himalayan Piedmont Plain soil in Boro 2020-21 to evaluate the soil test-based K fertilizer application on Boro rice to increase rice yield as well as maintain soil fertility. Six potassium doses namely, K control, 50%, 75%, 100%, 125% and 150% of soil test based (STB) K, were tested with balanced doses of other chemical fertilizers. Rice growth, yield, and yield-contributing characters were recorded during the panicle initiation and maturity stages of rice, which were greatly influenced by potassium fertilizer application. The grain yield increased significantly with increasing K rates up to 100% STB dose, and excess K application did not show any yield advantage. The N, P and K uptake and the K use efficiencies were influenced largely by the right amount of K application. The optimum K fertilizer dose was around 106 kg K ha-1 for the deficient piedmont rice soil. The 100% STB K fertilization might be suitable for obtaining higher rice yield and maintaining soil fertility in the HSTU farm soil under the Old Himalayan Piedmont Plain of AEZ 1.

Keywords: Piedmont soil, potassium, rice, grain yield, K efficiencies.

INTRODUCTION

Rice (*Oryza sativa*) is the most extensively cultivated cereal crop in Bangladesh, which covers about 76% of the total cropped area (BBS, 2018), and occupies first position as staple food. To satisfy the demands for food due to both population growth and diet diversity, production improvement is an urgent issue. Rice yield is predicted to double by 2030 (Zhang *et al.*, 2018). Fertilizers are usually applied to soil to increase or maintain crop yields to meet the increasing demand for food. The need for K fertilizer in the last decade has increased sharply along with the increase in food demand (Hartati *et al.*, 2018). Potassium (K) is an essential plant nutrient that contributes to the quality and plant resistance to water stress because of lower transpiration, so that water consumption is low. Potassium's role in the process of transpiration or water transport within the plant body is as a regulator of cell osmotic potential (Taize *et al.*, 2010; Zhang *et al.*, 2019). Rice crop requires large quantities and a continuous supply of K for the heading growth stage after completion of the reproduction stage (Atapattu *et al.*, 2018). A requirement of 200-300 kg K2O ha^{-1} is necessary to obtain 5-10 t ha^{-1} of cereal crop yield (Atapattu *et al.*, 2018). Modern high-yielding rice varieties remove much higher amounts of K than phosphorus (P) or even nitrogen (N) (Choudhury *et al.*, 1997; Liu *et al.*,

[1]Soil Science Division, Bangladesh Rice Research Institute, Gazipur
[2]Hazee Mohammed Danesh Science and Technology University, Dinajpur
[*]Corresponding author's E-mail: sakhawatbrri@gmail.com (ATM S Hossain)

2009; Sharma *et al.*, 2013). It is essentially required to stabilize the yield at a higher level. Compared to N and P fertilizers, K fertilizer is often ignored by farmers, particularly in Asia (Li *et al.*, 2014). On the other hand, rice crops remove about 103 kg K for a yield level of 7.0 t ha^{-1} (FRG, 2018). Islam and Muttaleb (2016) reported that K fertilization significantly increased rice yield and also mentioned the optimum dose of K for rice cultivation ranged from 78 to 93 kg ha^{-1}. Rice crop removes about 100 kg K for a yield level of 5 t ha^{-1} (Saha *et al.*, 2009). The balanced use of organic and inorganic fertilizers is the best practice to maintain soil health and crop productivity (Sarkar *et al.*, 2017). The general recommendation of K fertilizer for rice is often less than 100 kg-ha^{-1}, which causes mining of soil K. The K reserve of any soil is certainly limited, and no soil can supply K to crops adequately for an indefinite period of time. Intensive cropping and the use of modern rice varieties for high yield caused heavy depletion of K in soils, particularly in the absence of K application. Most of the area of AEZ 1 (Panchagarh, Thakurgoan, and part of Dinajpur districts) is deficient in potassium, where the dominant land types are high land to medium high land. In both land types, the soil potassium status is low to medium. Light-textured soils of this area have low exchangeable K, and farmers use a low amount of K fertilizer. Increasing cropping intensity increased the K deficiency in those areas. Understanding of K supplying power of soil is necessary for judicious recommendation of K fertilizer, compromising economic return and maintaining soil K reserve. In AEZ 1, the dominant clay minerals are Mica and Chloride, which are inherently deficient in K. At present, a soil test-based fertilizer application method was suggested by FRG, BARC, (2024). The application of fertilizer following STB would benefit the respective areas of farmers. The present investigation aimed to evaluate the soil test-based K fertilizer application on Boro rice to increase rice yield as well as maintain soil fertility.

MATERIALS AND METHODS

The field experiment was conducted at Hajee Mohammed Danesh Science and Technology University (HSTU) farm, Dinajpur (AEZ 1: Old Himalayan Piedmont Soil) during the Boro 2020-21 season. Before setting the field experiment, the initial soil sample was collected from 0-15 cm depth in the respective field. The soil sample was mixed properly and dried in a shaded conditions. Then the soil sample was ground, sieved by a 10 mesh and analyzed for different physical and chemical properties in the laboratory. The soil of the experimental field was loamy in texture, having 45% sand, 40% silt, and 15% clay. The pH of the experimental field was 5.8 (moderately acidic), organic carbon 1.2% (low), total N 0.09% (very low), available phosphorus 12 mg kg^{-1} (low), exchangeable potassium 0.10 meq/100g soil (low), available sulfur 10 mg kg^{-1} (low), and available zinc 2 mg kg^{-1} (medium). The soil test-based fertilizer for each nutrient was calculated.

Six potassium rates, including control, were assigned in an RCB design with three replications. The individual plot size was 4m × 4m. The treatments were K control, 50% STB K (40 kg ha^{-1}), 75% STB K (69 kg ha^{-1}), 100% STB K (92 kg ha^{-1}), 125% STB K (115 kg ha^{-1}), and 150% STB K (138 kg ha^{-1}). The N-P-S and Zn were used @ 150-20-10 and 2 kg ha^{-1}, respectively. The tested variety was BRRI dhan88. Forty-day-old seedlings of BRRI dhan88 were transplanted, maintaining 20 cm × 20 cm spacing. Urea was applied in three equal splits; one-third as basal, one-third at 25 days after transplanting (DAT) and the rest one-third at 5 days before panicle initiation (PI) stage. Standard cultural practices were followed for raising the rice crop. All plots were surrounded by a 30 cm soil levee to avoid fertilizer contamination between plots. Plant samples were collected at the panicle initiation stage for plant height, tiller number per square meter, shoot weight, and for measuring nitrogen, phosphorus, and potassium content and uptake. At maturity, the crop was harvested manually

from 5 m^2 from the middle of each plot at 15 cm above ground level for grain yield, 16 hills from each plot at ground level for tiller, panicle and total straw yield data. Grain yield was recorded at 14% moisture content, and straw yield on an oven-dry basis. Tiller and panicle number per square meter were recorded. Percent filled grain and unfilled grain were also documented.

Grain and straw samples were chemically analyzed in the Soil Science Division's laboratory, BRRI, to determine the nitrogen, phosphorus, and potassium content and uptake. The agronomic use efficiency, physiological use efficiency, partial factor productivity, and recovery use efficiency were calculated. All experimental data were statistically analyzed using STAR software.

RESULTS AND DISCUSSION

Plant growth and biomass yield at the PI stage of Boro rice

Potassium fertilization showed a significant effect on rice plant height, tiller m^{-2}, and shoot yield at the panicle initiation (PI) stage of Boro rice (Table 1). The tallest plant (105.29 cm) was observed in T_6 treatment, where 150% STB potassium fertilizer was applied, followed by T_5 treatment (103.88 cm), where 125% STB K was applied. Medium-tall plants were observed in the T_4 treatment (96.37 cm), where 100% STB K was applied, which was significantly shorter than T_6 and T_5. The reduced doses of K application shortened plant height significantly, and the shortest plant (73.19 cm) was observed in the K control plot; i.e., in T_1. Nitrogen and K have a synergistic effect to increase plant height. The tiller m^{-2} was also influenced significantly by the application of K at different rates (Table 1). The number of effective tillers increased with the increasing potassium doses. The maximum tiller m^{-2} (405) was observed where the highest dose of K was applied. On the other hand, with decreasing K doses, tiller number decreased significantly. The minimum tiller number was found in the K control treatment. Similar findings were also reported by Kalita *et al.* (2002). It was evident that shoot biomass was significantly influenced by potassium application. Shoot weight increased gradually with increasing the K doses. Significantly higher shoot yield was obtained in T_4 (100% STB-K), which was statistically similar to T5 (125% STB-K) and T_6 (150% STB-K) treatment (Table 1). Significantly lower shoot yield was obtained with T_2 treatment (5.12 t ha^{-1}), where 75% STB-K was applied, and the K control (T_1) treatment performed the lowest (4.75 t ha^{-1}) shoot yield. This finding is at per with the results reported by Islam *et al.*, (2015).

Table 1. Effect of potassium fertilizer on plant height, tiller number and shoot yield in panicle initiation (PI) stage of BRRI dhan88 in Boro 2020-21 season at HSTU farm, Dinajpur.

Treatment	Plant height (cm)	Tiller No. (m^{-2})	Shoot yield (t ha^{-1})
T_1 = K control	73.19	342	4.75
T_2 = 50% STB K	85.73	374	5.12
T_3 = 75% STB K	92.56	384	5.34
T_4 =100% STB K	96.37	389	5.44
T_5 = 125% STB K	103.88	396	5.56
T_6 = 150% STB K	105.29	405	5.59
LSD (0.05)	7.32	28	0.28
CV (%)	2.78	2.61	1.89

N.B. T_1 = 0, T_2 = 40, T_3 = 69, T_4 = 92, T_5 = 115, T_6= 138 kg K ha^{-1}, respectively

Shoot N, P, and K uptake at the PI stage of Boro rice

The nitrogen, phosphorus, and potassium uptake by the shoot at the panicle initiation stage of Boro rice are illustrated in Table 2. The N uptake increased significantly with increased K doses. Although the highest N uptake (65.49 kg N ha^{-1}) was found in T_6 treatment, where 150% STB-K was applied but the result was insignificant with other K doses. The N uptake was lowest (50.87 kg N ha^{-1}) in the K control treatment. Actually, a synergistic effect was observed between N uptake and K doses. The highest shoot P uptake (12.97 kg P ha^{-1}) was obtained in T_5 treatment, where 125% STB-K was applied, but the result was not significantly higher with the application of other K treatments. The significantly lowest shoot P uptake (9.50 kg P ha^{-1}) was found in the K control treatment. Actually, K fertilizer had a good impact on increase K uptake of rice at the panicle initiation stage. Potassium fertilizer application greatly influenced the K uptake of rice shoots at the panicle initiation stage (Table 2). The highest K uptake (135.93 kg K-ha^{-1}) in the shoot was observed in the T_6 treatment (150% STB-K), which was statistically similar to the T_5 (130.78 kg K ha^{-1}) treatment (125% STB-K). The 100% STB-K (T_4) produced significantly lower K uptake than the T_5 and T_6 treatments. The other lower K doses, i.e., T_3 (75% STB-K) and T_2 (50% STB-K) treatment, gave significantly lower K uptake in the shoot at the PI stage of rice than T_4 (100% STB-K). The significantly lowest K uptake (75.72 kg K ha^{-1}) was achieved in T_1 treatment, where no K fertilizer was applied for rice production in the Boro season. The N, P, and K uptake was maximum at the panicle initiation stage of rice as reported by Payman *et al.,* (2017).

Table 2. Effect of potassium on shoot N, P, and K uptake at PI stage of BRRI dhan88 in Boro 2020-21 season at HSTU farm, Dinajpur.

Treatment	Shoot N uptake (kg ha^{-1})	Shoot P uptake (kg ha^{-1})	Shoot K uptake (kg ha^{-1})
T_1 = K control	50.87	9.50	75.72
T_2 = 50% STB K	57.20	11.28	95.95
T_3 = 75% STB K	61.26	12.29	110.25
T_4 =100% STB K	63.11	12.88	119.56
T_5 = 125% STB K	65.23	12.97	130.78
T_6 = 150% STB K	65.49	12.86	135.93
LSD (0.05)	4.83	1.47	11.39
CV (%)	2.81	4.34	3.61

N.B. T_1 = 0, T_2 = 40, T_3 = 69, T_4 = 92, T_5 = 115, T_6= 138 kg K ha^{-1}, respectively.

Growth, yield, and percent sterility at the maturity stage of rice

At maturity or harvest stage, tiller number per square meter and panicle number per square meter, grain yield and straw yield, and percent sterility were influenced significantly with the application of different K doses (Table 3). Regarding tiller production, the highest tiller number per square meter was found in the T_6 (150% STB-K) treatment, but it was statistically similar to T_5 (125% STB-K), T_4 (100% STB-K), and T_3 (75% STB-K) treatments. Significantly lower tiller was obtained with T_2 (50% STB-K) treatment, and the lowest tiller per square meter was in T_1 (without K fertilizer). The number of panicles per square meter also increased significantly with increasing doses of K at the maturity stage of rice (Table 3). The highest

panicle number was observed in T_6 (401) treatment, where 150% STB-K was applied, but the result was statistically similar to that obtained in T_5 (398) and T_4 (386) treatment, where 125% STB-K and 100% STB-K were applied, respectively. The T_3 (75% STB-K) and T_2 (50% STB-K) treatments gave significantly lower panicle numbers than T_4, and the lowest panicle number was observed in the K control (346) treatment (T_1). Increasing potassium rates resulted in the longest panicle of rice, which could bear a higher number of spikelets per panicle. It was supported by Zayed *et al.* (2007). Grain yield, straw yield, and percent filled grain were influenced significantly by the application of K doses at different rates on a soil test basis in the Boro rice of BRRI dhan88 (Table 3). The K control plot yielded only 5.52 t ha^{-1} grain, and with the application of K fertilizer, the grain yield increased significantly. Grain yield increased with the increasing in potassium level up to T_6 and thereafter decreased. The grain yield significantly increases with increasing the K rates up to 100% STB, and further increasing the K rates, grain yield remains more or less similar and static. The highest grain yield was obtained with T_4 (7.91 t ha^{-1}) treatment, where 100% STB-K was applied, but the result was statistically identical with T_5 (7.83 t ha^{-1}) and T_6 (7.73 t ha^{-1}) treatment, where 125% and 150% STB-K were applied, respectively. The T_3 treatment (75% STB-K) gave significantly lower grain yield (7.39 t ha^{-1}) than T_4 (7.91 t ha^{-1}). Potassium fertilization influenced grain yield due to the assimilation of carbohydrates increased from source to sink. Increase in grain yield for the application of K was mainly due to improvement in yield components, i.e., number of effective tillers, panicle length, and grains per panicle. The highest grain yield was recorded in T_4 because of the highest number of effective tillers and the maximum number of grains per panicle also produced by T_4, which was significant with other higher K doses. Several workers reported a significant response of the grain yield of rice to the application of potassium (Bahmanyar and Mashaee, 2010). Saha *et al.* (2009) reported that application of chemical K fertilizer or rice straw increased the grain and straw yield of rice in Boro-Fallow-T. Aman cropping pattern in Modhupur Tract soil of AEZ-28. Saha *et al.* (2010) also reported that application of K @ 66 $kgha^{-1}$ for rice wheat pattern was not adequate and needed to increase the K dose for better K reserve in soil. It was evident that straw yield was significantly influenced by potassium rates, and it was the highest (8.03 t ha^{-1}) in T_6, and the lowest straw yield was obtained in T_1 (5.56 t ha^{-1}) treatment (Table 3). The higher K doses gave comparatively higher straw yield, and the result was statistically similar among T_3 to T_6 treatments. Significantly lower straw yield was observed in the T_2 treatment, where 50% STB-K was applied. All the treatments produced significantly higher straw yield than the K control treatment. Grain filling also increased due to proper K fertilization. The percent filled grain increased sharply with the application of K fertilizers at different rates. Actually, all the K-fertilized plots gave statistically similar filled grain percent over the K control plot (Table 3). Here, it was clear that K fertilization increased the number of filled grains in the panicle and thus yield increased. A similar result was found by Krishnappa *et al.* (2006) and who reported that applying K increased the number of filled grains per panicle. Basal application of K fertilizer showed a positive effect on the percentage of filled grains. Potassium helped in proper filling of seeds, which resulted higher number of plump seeds and thus increased the number of grains per panicle. Esfehani *et al.* (2005) showed that potassium fertilizer has positive effects on filled grains in rice, while its deficiency causes pollen sterility and decreases the number of filled grains per panicle.

Table 3. Response of BRRI dhan88 to potassium fertilizer on tiller and panicle production, grain and straw yield, and % filled grain in Boro 2020-21 season at HSTU farm, Dinajpur.

Treatment	Tiller m^{-2}	Panicle m^{-2}	GY (t ha^{-1})	SY (t ha^{-1})	Filled grain (%)
T_1 = K control	363	346	5.52	5.56	82.07
T_2 = 50% STB K	390	372	7.07	7.15	85.61
T_3 = 75% STB K	404	379	7.39	7.46	86.66
T_4 =100% STB K	414	386	7.91	7.97	87.23
T_5 = 125% STB K	423	398	7.83	8.00	87.63
T_6 = 150% STB K	425	401	7.73	8.03	87.78
LSD (0.05)	24	20	0.49	0.59	2.85
CV (%)	2.07	1.89	2.39	2.83	1.17

T_1 = 0, T_2 = 40, T_3 = 69, T_4 = 92, T_5 = 115, T_6= 138 kg K ha^{-1}, respectively.

Total N, P, and K uptake at the maturity stage of Boro rice

The total N uptake by rice (grain and straw) at the maturity stage was significantly influenced by the application of different doses of STB-K fertilizer in rice in the Boro season. Although higher N uptake was found in T6 (150% STB-K) but the result was statistically similar to that obtained in T_5 (125% STB-K) and T_4 (100% STB-K) treatment (Table 4). Nitrogen uptake at maturity was significantly lower in those treatments where K fertilizer was used below the 100% STB level. The total P uptake by rice (grain and straw) at the maturity stage was also significantly influenced by the application of different doses of STB-K fertilizer. Although higher P uptake was found in T_6 (150% STB-K) but the result was statistically similar to that obtained in T_5 (125% STB-K), T_4 (100% STB-K), and T_3 (75% STB-K) treatment (Table 4). The total P uptake was significantly lower in those treatments where K fertilizer was used below the 75% STB level. Significantly less P uptake was obtained in the K control treatment. The total K uptake by rice (grain and straw) at the maturity stage was also significantly influenced by the application of different doses of STB-K fertilizer in Boro rice (Table 4). The highest K uptake (193 kg ha^{-1}) in grain and straw was obtained in T_6 (150% STB-K), and the lowest K uptake (90 kg ha^{-1}) was in T_1 (K control). Although higher K uptake was found in T_6 (150% STB-K) but the result was statistically similar to that obtained in T_5 (125% STB-K) and T_4 (100% STB-K) treatment (Table 4). Potassium uptake was significantly lower in those treatments where K fertilizer was used below the 100% STB level. Application of K significantly increased the straw K content and total K uptake of both wheat and rice crops as reported by Saha *et al.* (2008). The potassium uptake was much higher than the nitrogen and phosphorus uptake by rice. Hossain *et al.* (2013) also reported that application of soil test based inorganic K (STB-K) fertilizer increased the grain yield of wheat as well as total N, P, and K uptake in Old Himalayan Piedmont Plain Soil of AEZ^{-1}.

Potassium use efficiency of rice

Application of K fertilizer in rice field in Old Himalayan Piedmont Plain Soil, i.e., in AEZ^{-1} in Boro season, greatly influenced the K use efficiencies. The agronomic efficiency (AE) (kg grain yield increased per kg of applied K) decreased with increasing K levels (Table 5). The higher AE were observed in lower doses of K application. A similar trend was also found in

the case of partial factor productivity for applied K (PFP-K) and recovery efficiency of K (RE-K). Nevertheless, the physiological efficiency of K (PE-K) was almost the same level regardless of K application (Table 5). Agronomic use efficiency of K, partial factor productivity and recovery efficiency of K decreased with the increase of K levels. A similar result was obtained by Saha *et al.* in 2008, who also reported that the AE, PFP, RE, etc., were decreased with increased K fertilizer doses.

Table 4. Response of BRRI dhan88 to potassium fertilizer application on N, P and K uptake in Boro 2020-21 season at HSTU farm, Dinajpur.

Treatment	Total N uptake (kg ha^{-1})	Total P uptake (kg ha^{-1})	Total K uptake (kg ha^{-1})
T_1 = K control	74.14	12.84	90.06
T_2 = 50% STB K	99.82	18.84	139.05
T_3 = 75% STB K	108.96	20.89	159.87
T_4 =100% STB K	119.24	23.24	179.68
T_5 = 125% STB K	121.53	23.13	187.10
T_6 = 150% STB K	121.99	23.71	192.99
LSD (0.05)	5.42	3.84	19.13
CV (%)	1.78	6.62	4.27

N.B. T_1 = 0, T_2 = 40, T_3 = 69, T_4 = 92, T_5 = 115, T_6= 138 kg K ha^{-1}, respectively

Table 5. Influence of K fertilization on different potassium use efficiencies of Boro rice of BRRI dhan88 at HSTU, Dinajpur.

Treatment	PFP-K	AE-K	PE-K	RE-K
T_1= K control	-	-	-	-
T_2= 50% STB-K	177	39	31.64	122
T_3= 75% STB-K	107	27	26.79	101
T_4= 100% STB-K	86	26	26.67	97
T_5= 125% STB-K	68	20	23.80	84
T_6= 150% STB-K	56	16	21.47	75

PEP-K = Partial factor productivity of K, AE-K = Agronomic efficiency of K, PE = Physiological efficiency of K, RE-K (%) = Recovery efficiency of K.

CONCLUSION

Potassium is becoming the second most limiting nutrient for crop production in many parts of Bangladesh, including AEZ 1. Potassium fertilizer increased grain yield with increasing rate of K up to 100% STB dose. The 100% STB-K (92 kg ha^{-1}) performed the best in terms of rice yield. From the quadratic equation of the response curve, the economic optimum dose of K was found to be 106 kg ha^{-1}, which is almost closer to the STB dose; therefore, soil test-based fertilizer should be applied for optimum rice production in those areas. As the Old Himalayan Piedmont Plain Soil of AEZ 1 is inherently

K-deficient, potassium fertilizer must be used judiciously to obtain higher rice yield and maintain soil K supplying capacity. More research work should be conducted on K fertilizer management in different rice-based cropping patterns and locations of the piedmont soils of AEZ 1.

REFERENCES

Atapattu, A. J., Prasantha, B. D. R., Amaratunga, K. S. P., & Marambe. (2018). Increased rate of potassium fertilizer at the time of heading enhances the quality of direct seeded rice. *Chemical and Biological Technologies in Agriculture*, 5(22). Doi.10.1186/s40538-018-0136-x

Bahmanyar, M. A., & Mashaee, S. S. (2010). Influences of nitrogen and potassium top dressing on yield and yield components as well as their accumulation in rice (*Oryza sativa*). *African Journal of Biotechnology*, 9(18), 2648-2653.

BBS (Bangladesh Bureau of Statistics). (2018). Statistical yearbook of Bangladesh 2018. Bangladesh Bureau of Statistics, Statistics and Information Division, Ministry of Planning, Government of the People's Republic of Bangladesh.

Choudhury, A. T. M. A., Zaman, S. K., & Bhuiyan, N. I. (1997). Prospect of mixed fertilizer application in wetland rice culture. *Thai Journal of Agricultural Science*, 30, 477-482.

Esfehani, M., Sadrzade, S. M., Kavoosi, M., & Dabagh-Mohammad-Nasab, A. (2005). Study on the different levels of nitrogen and potassium fertilizers on growth, grain yield, yield components of rice (*Oryza sativa*) cv. Khazar. *Iran Agronomy J*. 7(3), 226-241.

FRG. (2024). Fertilizer Recommendation Guide. Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka-1215, 274 pp.

Grzebisz, W., Gransee, A., Szczepaniak, W., & Diatta, J. (2012). The Effects of Potassium Fertilization on Water-Use Efficiency in Crop Plants. *J Plant Nutr Soil Sci*, 176(3), 355-374.

Hartati, S., Suryono, & Purnomo, D. (2018). Effectiveness and efficiency of potassium fertilizer application to increase the production and quality of rice in Entisols. *IOP Conference Series: Earth and Environmental Science*.142, 012031. doi:10.1088/1755-1315/142/1/012031

Hossain, A. T. M. S., Rahman, F., Panaullah, G. M. & Saleque, M. A. (2013). Fertilizer management for wheat crop in the Himalayan Piedmont soil. *Bangladesh J. Agril. Res*. 38(2), 241-246.

Islam, A., & Muttaleb, A. (2016). Effect of potassium fertilization on yield and potassium nutrition of Boro rice in a wetland ecosystem of Bangladesh, *Archives of Agronomy and Soil Science* http://dx.doi.org/10.1080/03650340.2016.1157259

Islam, A., Biswas, J. C., Karim, A.J.M.S., Pervin, M. S., & Saleque, M. A. (2015). Effects of Potassium Fertilization on Growth and Yield of Wetland Rice in Grey Terrace Soils of Bangladesh. *Research on Crop Ecophysiology Journal*, 10 (2), 64-82.

Kalita, U., Das, J. R., & Suhrawardy, J. (2002). Effect of seed priming with potassium salt and potassium levels on growth and yield of direct seeded summer rice under rainfed upland condition. *Indian J. Hill Farming*, 15(1), 50-53.

Krishnappa, M., Gowda, K. N., Shankarayan, V., Maharudrappa, & Khan, M. M. (2006). Effect of graded levels and split application of potassium on its availability and yield of rice. *J. Potassium Research*, 6(4), 156-161.

Li, J., Lu, J., Li, X., Ren, T., Cong, R., & Zhou, L. (2014). Dynamics of potassium release and adsorption on rice straw residue. PLOS ONE 9: e90440. Doi, 10.1371/journal.

Liu, G., Li, Y., & Porterfield, D.M. (2009). Genotypic differences in potassium nutrition in lowland rice hybrids. *Commun Soil Sci Plant Anal*. 40, 1803–1821.

Payman, G., Reddy, P. R. R., Sreenivas, A., Reddy, S. N., & Madhavi, A. (2017). Accumulation of nutrients (NPK) at different growth stages of machine

transplanted rice (*Oryza sativa* L.) under different levels of nitrogen and Split Schedules. *Int. J. Curr. Microbial. App. Sci*. Special Issue-4, 172-181.

Saha, P. K., Hossain, A. T. M. S., & Miah, M. A. M. (2010). Effect of potassium application on wheat in Old Himalayan Piedmont plain. *Bangladesh J. Agril. Res.* 35(2), 207-216.

Saha, P. K., Hossain, A. T. M. S., Miah, M. A. M., Rahman, M. M., & Saleque, M. A. (2008). Contribution of crop residue to supply K in rice-wheat system. *Bangladesh Rice J*. 14 (1 & 2), 43-48.

Saha, P. K., Miah, M. A. M., Hossain, A. T. M. S., Rahman, F., & Saleque, M. A., (2009). Contribution of rice straw to potassium supply in rice fallow rice cropping pattern. *Bangladesh J. Agril. Res.* 34(4), 633-643.

Sarkar, M. I. U., Islam, M. N., Jahan, A., Islam, A., & Biswas, J. C. (2017). Rice straw as a source of potassium for wetland rice cultivation. *Geology, Ecology, and Landscapes*, 1(3), 184-189. doi:10.1080/24749508.2017.1361145

Sharma, S., Chander, G., Verma, T. S., & Verma, S. (2013). Soil potassium fractions in rice-wheat cropping system after twelve years of Lantana residue incorporation in a northeast Himalayan Alfisol. *Journal of Plant Nutrient*. 36, 1809-1820.

Zayed, B. A., Elkhoby, W. M., Shehata, S. M., & Ammar, M. H. (2007). Role of potassium application on the productivity of some inbred and hybrid rice varieties under newly reclaimed saline soils. African Crop Science Conference Proceedings, *African Crop Science Society*, 8, 53-60.

Zhang, H., Liu, H., Hou, D., Zhou, Y., Liu, M., Wang, Z., Liu, L., Gu, J., & Yang, J. (2019). The effect of integrative crop management on root growth and methane emission of paddy rice. *Crop J*., 7, 444–457.

The *Bangladesh Rice Journal* is published in June and December by the Bangladesh Rice Research Institute (BRRI). The journal is a peer reviewed one based on original research related to rice science. An article submitted to the *Bangladesh Rice Journal* must not have been published in or accepted for publication by any other journal.

Changes of address should be informed immediately. Claims for copies, which failed to reach the paid subscribers must be informed to the Chief Editor within three months of the publication date.

Authors will be asked to modify the manuscripts according to the comments of the reviewers and send back corrected copies and the original copy together to the Chief Editor within the specified time, failing of which the paper may not be printed in the current issue of the journal.

BRJ: Publication no. : 456; 500 copies, Published in December 2025
Published by the Director General, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh
Printed by Akkhor Printing Press, West Joydebpur, Gazipur-1700