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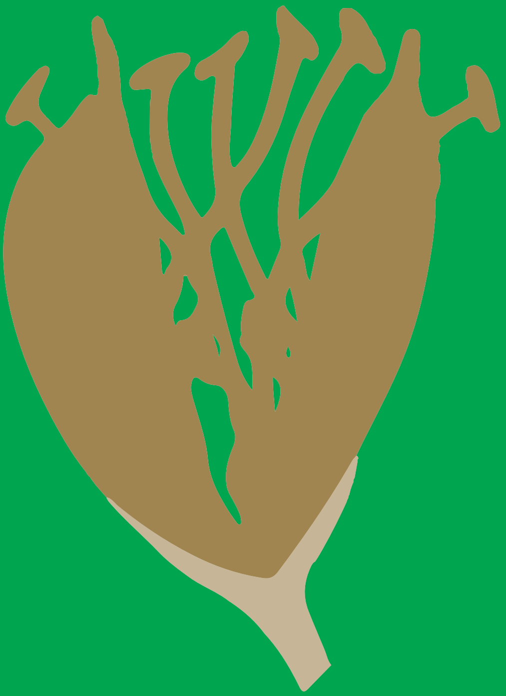

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Molecular and Phenotypic Characterization for Cold Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

Cold stress affects growth and development of rice plants from germination to reproductive stages. Thirty breeding lines/varieties including tolerant and susceptible check varieties were evaluated for cold tolerance at seedling and reproductive stage using artificially induced low temperature situations to understand the genetic relationship among them and to identify promising donors for cold tolerance. Three breeding lines, BR8253-9-3-4-3, IR77497-31-2-3-1and IR2266-42-6-2 demonstrated higher cold tolerance for the seedling stage (LD score <3.0). Molecular analysis revealed 68% genetic similarity among the genotypes. On average 64% of the breeding lines shared common alleles at any given locus ranging from 27% (RM22870) to 97% (RM186). UPGMA dendrogram showed that majority of the breeding lines showing 4-5 degrees of LD were grouped together. Two breeding lines, BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3 having 86% common alleles showed low leaf discoloration (LD: 4) at seedling stage coupled with relatively low reduction in spikelet fertility (45.8% and 48.1%) and grain yield (43.3% and 39.1%). The gene diversity and PIC value revealed that seven SSR loci (RM1282, RM600, RM5349, RM6972, RM5847, RM5911, and RM511) might be the best markers to differentiate between tolerant and susceptible genotypes at reproductive stage. The breeding lines with tolerance to cold stress at the seedling and the reproductive stages identified from this study, and the SSR markers mentioned above could be useful for developing cold tolerance rice.

Key words: Cold tolerance, leaf discoloration score, spikelet fertility, SSR marker, genetic diversity

INTRODUCTION

Rice is the staple food for more than half of the world's population providing 21% of the global per capita energy and 15% of per capita protein intake (Maclean *et al.*, 2002). Although originating in swampy areas of the tropics, rice is now grown globally in diverse ecologies and thus suffers a wide range of abiotic stresses. Low-temperature stress (LTS) or cold is a worldwide problem limiting rice yield. Rice crops normally suffer from two types of cold stresses; at the early vegetative stage of rice crop, LTS restrains seedling establishment and plant growth and development; at the reproductive stage, cold induces low fertility and poor grain filling (Andaya and Tai, 2006). In Bangladesh, around 2 million hectare of rice area becomes affected by low temperature stress or cold during the winter season causing seedling mortality in some years up to 90% when ambient temperature goes below 10°C and thereby increases cultivation cost. Worse, a bad cold spell during the reproductive phase of the rice crop sometimes causes partial to total loss of the crop due to the failure of the seed set. Yoshida (1980) reported that upon exposure to the temperature below 10°C for 5-6 days at early vegetative stage rice plant gets yellowing, stunting, reduced growth and dies ultimately due to wilting. The reproductive stage, particularly at booting stage of rice plants is the most sensitive stage to LTS of 15° - 20°C (Yoshida 1980). Complete failure of seed setting may occur upon exposure to LTS below 17°C. In the winter season in Bangladesh, ambient temperature usually goes below 10°C at majority of the Northern districts, which

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causes yellowing and stunted growth of Boro rice seedlings that ultimately turns into seedling mortality. On the other hand, reproductive stage of early grown Boro rice varieties in the *haor* areas becomes affected with low temperature which causes spikelet sterility up to 100% in some years (Rashid and Yasmeen, 2017; Kabir *et al.*, 2015).

Rice genotypes differ considerably in cold tolerance (Mackill and Lei, 1997). *Indica* rices that are widely grown in South Asia, are very much susceptible to cold stress. The development of high-yielding, cold-tolerant cultivars is the most effective way to overcome the problem of LTS. The progress to the improvement of cold tolerance in rice that has so far been made is based on phenotypic selection and conventional breeding strategy (Unkovich, 2008). However, considerable efforts have been taken by different groups of researchers (Qian *et al.*, 2000; Misawa *et al.*, 2000; Andaya and Mackill, 2003; Han *et al.*, 2004; Han *et al.*, 2007; Zhang *et al.*, 2005; Lou *et al.*, 2007; Jiang *et al.*, 2008; Baruah *et al.*, 2009; Ji *et al.*, 2010; Iwata *et al.*, 2010) to genetically dissect rice cold tolerance using DNA markers, which has resulted into the discovery and mapping of many quantitative trait loci (QTLs) associated with cold tolerance. Two major QTLs for cold tolerance (CT), *qCTS4* and *qCTS12*, have been fine-mapped onto rice chromosome 4 and the short arm of chromosome 12, respectively (Andaya and Tai, 2006 and Andaya and Tai, 2007). Kim *et al.* (2014) identified one candidate gene encoding calmoduline-binding transcription activator (CAMTA) from a japonica cultivar Jinbubyeo responsible for cold tolerance at seedling stage. From a map based cloning study with a cold tolerant variety Norin-PL8, Saito *et al.* (2010) showed that a gene encoding F-Box protein on chromosome 4 confers cold tolerance at reproductive stage. Recently, Sun *et al.* (2018) reported that *LOC_Os06g39750* was strongly associated with seed setting under cold water irrigation condition of 17°C. Unfortunately, results from these genetic studies have not yet been directly fruitful for marker-assisted selection to develop improved rice varieties tolerant to cold stress owing to possible epistasis and gene × environment interactions associated with the identified QTLs (Hospital, 2009). Furthermore, most of the reported QTLs are of *japonica* origin. Improvement of *indica* rice using QTLs from *japonica* donors restrains by linkage drag of undesired traits like spikelet sterility, low tillering (Ribaut and Hoisington, 1998; Salina *et al.*, 2003), although these can be resolved by using high resolution marker system for background recovery, which may entail huge expense in line development. Contrary, haplotypes of these genes in *indica* background might be a better option in this regard. Therefore, molecular characterization and phenotypic screening of *indica* varieties, breeding lines or germplasm for cold tolerance could reveal their usefulness in utilization of genetic improvement of existing varieties. Thus in this study, we investigated genetic diversity among the breeding lines using SSR marker to identify potential elite *indica* cold tolerant donor to use in the breeding programme for the developing cold tolerant rice.

MATERIALS AND METHODS

A set of 594 advanced breeding lines derived from the crosses between different genetically diverged parents were evaluated as the initial materials for cold stress tolerance at seedling and reproductive stages. BR1 and BR18 were used as the susceptible and tolerant check varieties. A subset of 22 genotypes selected from the initial screening against LTS was used in further evaluation for cold tolerance at both seedling and reproductive stages (Table 1). This subset of genotypes was also used in genotyping with SSR markers to assess genetic diversity among them.

Table 1. The breeding lines used in phenotypic evaluation for cold tolerance and genetic diversity analysis.

Designation	Parentage	Origin
BR8264-1-1-3B2-HR4	BR7166-5B-4/YUNLUN63	Bangladesh
BR7840-54-3-2-1	IR75382-32-2-3-3/ BR7166-4-5-3-2-5-5B1-92	Bangladesh
BR7840-54-5-3-4	IR75382-32-2-3-3/ BR7166-4-5-3-2-5-5B1-92	Bangladesh
BR7683-30-3-3-4	BRRI dhan32/BR7166-4-5-3-2-5-8B2	Bangladesh
IR83294-9-1-3-2-3-Gaz1	Daesanbyeo/IR56664-44-5-1	IRRI
BR8427-2-3-2	BR7166-5B-5/IR73001-13-2-2-2	Bangladesh
BR8260-16-2-3-1	BR7166-5B-4/BR7166-5B-1-RAN1	Bangladesh
BR8261-19-1-5-1	BR7166-5B-4/BRRI dhan29	Bangladesh
BR8331-13-3-1-3	BR7305-21-6-1/BRRI dhan45//BRRI dhan45	Bangladesh
BR8260-7-3-1-1B1	BR7166-5B-4/BR7166-5B-1-RAN1	Bangladesh
BR8261-19-1-1-3	BR7166-5B-4/BRRI dhan29	Bangladesh
BR8258-7-1-5-2B3	IR71780-1-1-3-2/BR7166-4-5-3-2-5B1-6-3//BRRI dhan29	Bangladesh
BR8261-19-5-2-4	BR7166-5B-4/BRRI dhan29	Bangladesh
A69-1	BG94-1/POKKALI	Srilanka
IR83294-9-1-3-2-3-P1	Daesanbyeo/IR56664-44-5-1	IRRI
BR7840-2-1-1-P2	IR75382-32-2-3-3/ BR7166-4-5-3-2-5-5B1-92	Bangladesh
BR8253-9-3-4-3	BR7305-21-6-1/ BRRI dhan29// BR7305-21-6-1	Bangladesh
BR8253-46-7-2-1B1	BR7305-21-6-1/ BRRI dhan29// BR7305-21-6-1	Bangladesh
BR8257-10-1-1-2	IR71780-1-1-3-2/BR7166-4-5-3-2-5B1-6-3// TCN58	Bangladesh
BR8258-7-1-5-2B2	IR71780-1-1-3-2/BR7166-4-5-3-2-5B1-6-3//BRRI dhan29	Bangladesh
BR8260-7-3-4-1B2	BR7166-5B-4/BR7166-5B-1-RAN1	Bangladesh
BR7988-10-4-3-2	BR6840-3-4-1/Tep Hanh 170DB	Bangladesh
IR77496-31-2-1-3-1	IR64680-81-2-2-1-3/IR68077-82-2-2-2-3	IRRI
IR2266-42-6-2	IR1529-123-1/GIZA173	IRRI
BR1 (Sus. Ck)	IR532-1-176 (IR262-24-3/TKM6)	IRRI
BR17 (MT. Ck)	Kn-1B-361-1-8-6-10 (Zerak/IR8)	Indonesia
BR18 (T. Ck)	B541-B-Kn-22-7-2 (Pelita1-1/IR1108-2)	Indonesia
BRRI dhan28 (Sus. Ck)	BR6 (IR28)/Purbachi	Bangladesh
BRRI dhan29 (Sus. Ck)	BG90-2/BR51-46-5	Bangladesh
BRRI dhan55 (MT. Ck)	IR73678-6-9-B (IR64/*Oryza rufipogon*)	IRRI

Sus. ck = Susceptible check, MT ck = Moderately tolerant check, T. ck = Tolerant check.

Evaluation of cold tolerance at the seedling stage

The breeding lines were evaluated under artificial cold stress condition of 13°C using cold-water irrigation in a water tank as described in Khatun *et al.* (2016). Briefly, 10 seedlings of each breeding lines/varieties were raised in 1-row plots spaced at 3.0 cm in the flat bottom plastic trays (60 cm × 30 cm × 2.5 cm) filled with gravel and crop residue free fertilized soil. The experiment was established following randomized complete block design with three replications. Forty-five entries along with two check varieties were grown at each tray. Same set of entries were grown in three trays with full randomization to consider as three blocks. BR18 and BR1 were grown as the tolerant and susceptible check varieties (Khatun *et al.*, 2016), respectively at every after 15 breeding lines in the trays. After seeding, a thin layer of fine granular soil was used to cover the germinating seeds. The seedlings were allowed to grow at ambient temperature. At 3-leaf stage (approximately 12 - 15 days after seeding), the plastic trays were placed in the cold water tank pre-set at 13°C. Sensitivity to cold stress was recorded using an arbitrary leaf discoloration (LD) scores (1 to 9) as described in Biswas *et al.* (2017). LD scores were recorded at seven days after cold treatment or when the susceptible check variety (BR1) died. A subset of 24 genotypes selected from the initial cold screening was evaluated thrice with six check varieties for confirmation of seedling stage cold tolerance.

Evaluation of cold tolerance at the reproductive stage

Twenty-four genotypes along with six check varieties were evaluated for the reproductive stage cold tolerance using controlled temperature in a growth chamber (Dian Corp. Inc, Korea). In this experiment, 25-day-old six seedlings of each genotype were transplanted individually in earthen pot in a circular fashion. Each genotype was transplanted in six pots. The plants were grown in ambient temperature until the meiotic phase of the reproductive stage triggers. Growing tillers from each plant were clipped off at alternate day to allow only the main culm to grow. Three pots out of six of each genotype were placed in the growth chamber adjusted at 15°C with 11/13 hours day (3000 lux)/night period and 60% relative humidity. Other three pots were kept at ambient temperature as control. After 10 days of cold treatment, the pots were further placed at ambient temperature until the plants get maturity. Days to heading, culm length, spikelet fertility, grain yield and panicle exertion were recorded from both treated and control plants. Reduction in percentage of the phenotypic value of a trait from a treated plant was estimated over a control plant. This experiment was repeated twice for further confirmation of cold response.

SSR genotyping

Thirty breeding lines/varieties including six check varieties were evaluated with 50 SSRs markers. The SSRs were evenly distributed over all 12 chromosomes. DNA was extracted from young leaves tissues of 8-week-old plants using a modified CTAB miniprep method (Virk *et al.*, 1995). PCR analysis was performed in 10 µl reactions containing 50 ng of DNA template, 1 µl 10X TB buffer (containing 200 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1 µl of 1 mM dNTP, 0.50 µl each of 5 µM forward and reverse primers and 0.2 µl of *Taq* DNA polymerase (5 U/µl) using thermal cycler. After initial denaturation for 5 min at 94°C, each cycle comprises 30 sec denaturation at 94°C, 30 sec min annealing at 55°C, and 30 sec extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR products were separated by electrophoresis on 6% polyacrylamide. The gels were stained in 0.5 mg/ml Ethidium bromide and DNA bands were visualized under UV light using a gel documentation system and saved as jpeg format. Allele scoring was performed considering the relative position of the bands in the gel images compared to the position of parental bands.

Phenotypic and molecular data analysis

The mean, range and standard deviation of LD scores were calculated using Microsoft excel 2010. Percent reduction in different growth trait was calculated using the formula given as,

$$\% \text{ reduction} = \frac{\text{Traits of control} - \text{traits of treatment}}{\text{Traits of control}} \times 100$$

Molecular weight for allele specific bands of the SSRs was measured using Alpha Ease FC5.0. Summary statistics, including number of alleles per locus, major allele frequency and Polymorphic information content (PIC) values were determined using Power Marker version 3.25 (Liu and Muse, 2005) based on the formula developed by Anderson *et al.* (1993)

$$PICi = 1 - \sum_{j=1}^{n} P^2{}_{ij}$$

where, P_{ij} is the frequency of the jth allele for the ith marker and is summed over n alleles.

The genetic distance was calculated using Nei distance (Nei and Takezaki, 1983). The similarity matrix was calculated with the Simqual sub-programme using the Dice coefficient (Dice, 1945) and subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the programme NTSYS-pc (Rohlf, 2002).

RESULTS

Cold tolerance at the seedling stage

Leaf discoloration is an important trait for discriminating breeding population under LTS and to isolate tolerant lines/progenies from intolerant one. LD scores of 594 breeding lines ranged from 1 to 9 with an average value of 6.49 and standard deviation of 1.69, while the susceptible (BR1) and tolerant (BR18) check varieties had LD scores ranging from 7-9 with 0.69 SD (standard deviation) and 1-4 with 0.89 SD, respectively (Table 2). A subset of 22 genotypes showing strong tolerance (LD: 1-3) to cold stress in the initial cold screening experiment along with three susceptible check varieties (BR1, BRRI dhan28 and BRRI dhan29), three tolerant check varieties (tolerant: BR18, moderately tolerant: BR17 and BRRI dhan55) and two IR lines, which showed strong tolerance to cold stress at seedling stage in a study reported by BRRI (2013), were used in the confirmatory cold screening. The results showed wide range of variation among the genotypes in average LD score (3.2 to 8.4). The lowest average LD score was obtained with IR77496-31-2-1-3-1 (3.2) followed by IR2266-42-6-2 (3.4), BR8257-10-1-1-2 (3.4), BR18 (3.4) and the highest LD was with BR1 (8.4) followed by BRRI dhan28 (7.3), BR17 (5.7). Out of 30 genotypes, twelve showed LD values less than 4, while another 12 genotypes showed LD values ranging from 4.0 to 5.0 (Table 3).

Cold tolerance at the reproductive stage

Upon exposure to LTS, various genotypic responses in different cold related traits at the reproductive stage were observed (Table 4). Heading delays (delay in 50% flowering than the control) due to cold stress was observed in the treated plants ranging from 2 - 12 days (Mean: 9). Reductions in culm length, spikelet fertility and yield varied from 4.5 – 43.1% (average: 17.7%), 48.1 – 88.4% (average: 69.9%) and 39.1 – 91.8% (average: 75.4%), respectively (Table 4). Panicle exertion is another important trait determining cold tolerance. The genotypes under cold stress showed variable response to this trait ranging from 1 - 7 with an average value of 4.6. Heading delays in cold stress compared to control was five days or less than five days in 2 genotypes (BR18 and BR8331-13-3-1-3) and 10 – 12 days in 12 genotypes. Culm length was reduced by more than 30% in six genotypes and less than 10% in five genotypes. The maximum reduction in culm length was observed with BR7683-30-3-3-4 (43.1%) followed by IR83294-9-1-3-2-3-Gaz1 (42.9%), while the least reduction was observed with BR8260-16-2-3-1 (4.5%) followed by BR8261-19-5-2-4 (5%), BR7840-2-1-1-P1 (5.0%), BR7840-2-1-1-P1 (5.4%), BR8264-1-1-3B2-HR4 (6.5%), BR8253-46-7-2-1B1 (8.1%) and thereafter increasing (Table 5). Although nearly 50% of the genotypes had good panicle exertion, but spikelet fertility and yield/panicle were reduced severely in most of the genotypes due to cold stress compared to the plants grown under ambient temperature. Under cold stress BR8258-7-1-5-2B2 showed the highest panicle yield coupled with comparatively higher seed set percentage (Table 6).

Table 2. Leaf discoloration response of 594 breeding lines/varieties to 13°C cold water treatment at seedling stage.

Genotype	Leaf discoloration score		
	Range	Average	SD (n =x)
Breeding line	1-9	6.49	1.69 (n=594)
BR1 (Susceptible check)	7-9	8.6	0.69 (n=19)
BR18 (Tolerant check)	1-4	2.5	0.89 (n =19)

Table 3. Leaf discoloration responses of 30 breeding lines/varieties to 13°C cold water treatment at seedling stage.

Designation	Batch 1	Batch 2	Batch 3	Average
	Av± SD (n=2)	Av ± SD (n=4)	Av ± SD (n=3)	
BR8264-1-1-3B2-HR4	6.0±1.4	3.0 ± 1.2	2.3±1.15	3.8
BR7840-54-3-2-1	6.0±1.4	5.0 ± 1.9	2.3±1.15	4.4
BR7840-54-5-3-4	4.0±1.4	6.0 ± 1.1	2.3±1.15	4.1
BR7683-30-3-3-4	3.5±2.1	5.7 ± 0.8	2.3±1.15	3.8
IR83294-9-1-3-2-3-Gaz1	6.0±1.4	5.0 ± 0.0	2.3±1.15	4.4
BR8427-2-3-2	6.0±1.4	5.7 ± 1.2	3.7±1.15	5.1
BR8260-16-2-3-1	6.0±1.4	5.3 ± 1.6	3.7±1.15	5.0
BR8261-19-1-5-1	5.0±0.0	4.0 ± 1.1	4.0±1.73	4.3
BR8331-13-3-1-3	5.0±1.4	4.2 ± 0.9	4.0±1.73	4.4
BR8260-7-3-1-1B1	2.0±1.4	4.3 ± 2.2	4.3±1.15	3.6
BR8261-19-1-1-3	3.0±2.8	6.0 ± 1.1	2.3±1.15	3.8
BR8258-7-1-5-2B3	4.0±1.4	5.2 ± 0.9	2.3±1.15	3.8
BR8261-19-5-2-4	2.5±2.1	5.3 ± 1.6	4.3±1.15	4.1
A69-1	2.0±1.4	6.0 ± 0.6	6.3±1.15	4.8
IR 83294-9-1-3-2-3-p1	4.0±1.4	5.2 ± 0.7	2.3±1.15	3.8
BR7840-2-1-1-P2	3.5±0.7	6.2 ± 1.1	4.0±1.00	4.6
BR8253-9-3-4-3	2.0±1.4	6.0 ± 1.1	2.3±1.15	3.4
BR8253-46-7-2-1B1	3.0±0.0	6.5 ± 1.0	4.3±1.15	4.6
BR8257-10-1-1-2	4.0±0.0	4.2 ± 0.9	2.3±1.15	3.5
BR8258-7-1-5-2B2	4.5±0.7	3.8 ± 0.9	2.3±1.15	3.6
BR8260-7-3-4-1B2	6.0±1.4	4.8 ± 1.3	4.3±1.15	5.1
BR7988-10-4-3-2	4.0±0.0	5.7 ± 1.5	4.3±1.15	4.7
IR77496-31-2-1-3-1	-	-	3.2±1.15	3.2
IR2266-42-6-2	-	-	3.4±1.53	3.4
BR1 (Sus. ck)	8.6±2.8	9.0 ± 0.0	7.7±0.58	8.4
BR17 (MT. ck)	-	-	5.7±1.15	5.7
BR18 (T. ck)	3.0±0.0	3.0 ± 0.0	4.3±1.15	3.4
BRRI dhan28 (Sus. ck)	7.0±2.8	7.7 ± 0.7	7.3±1.15	7.3
BRRI dhan29 (Sus. ck)	6.0±2.8	-	4.0±1.73	5.0
BRRI dhan55 (MT. ck)	6.0±2.8	-	2.3±1.15	4.2

Sus. ck = Susceptible check, MT ck = Moderately tolerant check, T. ck = Tolerant check.

Table 4. Estimates of variation among 30 genotypes in different indexes for cold tolerance in rice.

Cold tolerance index	Range	Mean
Leaf discoloration score (1-9)	3.2 - 8.4	4.4
Heading delay (day)	2 - 12	9.0
Culm length reduction (%)	4.5 – 43.1	17.7
Spikelet fertility reduction (%)	48.1 – 88.4	70.6
Yield reduction (%)	39.1 – 91.8	75.4
Panicle exertion score (1-9)	1 - 7	4.6

Table 5. Genotypic response to cold stress in different cold related traits of 30 breeding lines/varieties at reproductive stage.

Designation	Heading delay (day)	Culm length reduction (%)	Panicle exertion score (1-9)	Spikelet fertility reduction (%)	Yield reduction (%)
BR8264-1-1-3B2-HR4	6	6.5	7	81.5	82.6
BR7840-54-3-2-1	12	24.1	2	78.6	88.2
BR7840-54-5-3-4	9	13.7	3	73.7	85.3
BR7683-30-3-3-4	10	43.1	7	61.9	91.1
BR8427-2-3-2	11	35.1	1	66.6	82.5
BR8260-16-2-3-1	12	4.5	7	70.5	76.9
BR8261-19-1-5-1	9	23.5	3	88.4	77.5
BR8331-13-3-1-3	5	17.6	7	69.9	81.7
BR8260-7-3-1-1B1	8	21.6	7	62.6	61.4
BR8261-19-1-1-3	7	24.6	7	84.6	90.3
BR8258-7-1-5-2B3	9	11.5	7	48.1	39.1
BR8261-19-5-2-4	7	5.0	4	85.8	91.8
A69-1	9	25.4	3	71.2	83.1
IR83294-9-1-3-2-3-P1	9	5.4	3	58.8	77.7
BR7840-2-1-1-P1	9	5.0	4	79.6	89.1
BR8253-9-3-4-3	11	10.4	7	69.9	77.3
BR8253-46-7-2-1B1	10	8.1	7	56.2	71.8
BR8257-10-1-1-2	10	23.5	3	72.1	71.6
BR8258-7-1-5-2B2	10	34.1	1	45.8	43.3
BR8260-7-3-4-1B2	11	25.0	7	80.1	82.0
BR7988-10-4-3-2	10	19.6	3	77.4	75.3
IR83294-9-1-3-2-3-Gaz1	7	42.9	1	66.2	79.1
IR77496-31-2-1-3-1	7	36.4	6	64.4	43.2
IR2266-42-6-2	8	27.0	7	71.1	87.1
BR1	12	38.9	3	62.1	50.8
BR17	10	15.4	2	72.8	84.2
BR18	2	12.7	7	69.1	68.4
BRRI dhan28	9	20.3	3	78.3	80.4
BRRI dhan29	12	11.9	7	76.9	86.1
BRRI dhan55	8	20.9	3	73.2	62.8

Table 6. Agronomic performance of 30 breeding lines/varieties under cold stress.

Designation	Days to heading	Culm length (cm)	Panicle length (cm	No. of spikelet per panicle	Spikelet fertility (%)	Yield per plant (g)
BR8264-1-1-3B2-HR4	122	65.1	18.2	141	18.1	0.61
BR7840-54-3-2-1	120	52.0	19.1	118	17.0	0.39
BR7840-54-5-3-4	126	61.8	22.3	108	21.0	0.49
BR7683-30-3-3-4	127	56.0	16.6	108	29.6	0.76
BR8427-2-3-2	123	47.3	23.4	113	30.4	0.75
BR8260-16-2-3-1	134	50.1	19.7	163	20.3	0.66
BR8261-19-1-5-1	134	51.1	17.3	237	3.8	0.20
BR8331-13-3-1-3	123	49.0	19.4	133	18.5	0.49
BR8260-7-3-1-1B1	130	54.6	17.3	199	15.3	0.65
BR8261-19-1-1-3	127	61.9	17.6	159	11.8	0.41
BR8258-7-1-5-2B3	120	38.4	19.3	140	28.5	0.81
BR8261-19-5-2-4	127	63.7	19.0	145	14.0	0.52
A69-1	127	49.5	20.5	119	23.0	0.62
IR83294-9-1-3-2-3-P1	129	64.3	19.8	93	26.1	0.58
BR7840-2-1-1-P1	125	63.6	17.9	159	18.6	0.59
BR8253-9-3-4-3	127	59.8	20.8	185	17.0	0.63
BR8253-46-7-2-1B1	123	61.4	19.7	145	27.6	0.81
BR8257-10-1-1-2	-	42.1	16.8	176	19.6	0.76
BR8258-7-1-5-2B2	107	39.0	19.7	178	30.5	1.41
BR8260-7-3-4-1B2	142	55.1	18.8	151	10.8	0.43
BR7988-10-4-3-2	110	48.5	19.8	134	19.2	0.74
IR83294-9-1-3-2-3-Gaz1	121	56.0	19.8	102	24.3	0.61
IR77496-31-2-1-3-1	-	39.9	17.4	121	26.3	0.70
IR2266-42-6-2	111	44.7	20.6	105	20.1	0.57
BR1	-	32.0	16.5	55	26.4	0.32
BR17	102	71.4	20.7	183	19.4	0.74
BR18	125	59.8	21.7	158	19.7	0.69
BRRI dhan28	112	56.1	19.2	110	20.0	0.55
BRRI dhan29	122	50.9	19.7	123	18.0	0.53
BRRI dhan55	109	48.7	18.4	97	22.8	0.56

Molecular characterization

Fifty SSR markers distributed over the 12 chromosomes of rice were used to characterize and discriminate 30 genotypes having differential response to cold stress at the seedling and the reproductive stage. Among 50 SSRs, thirty eight SSRs were found polymorphic. Monomorphic SSR markers were excluded from the analysis. Table 7 summarizes the results obtained from the analysis of 38 SSR markers loci across the genotypes. Out of 38 polymorphic SSRs, two markers were present on each of chromosome 4, 9 and 10, three markers on each of chromosome 2, 3, 5 and 12, four markers on each of chromosome on 7 and 11, five markers on chromosome 8, and seven markers on chromosome 1. The SSR markers under this study was highly informative and polymorphic as evident from their polymorphism information content (PIC) values, which measures magnitude

Table 7. Summary results of 38 SSR markers across 30 genotypes.

Marker	Chromosome	Genetic distance (cm)	No. of alleles	Major allele frequency	Gene diversity	PIC
RM1282	1	0.5	6	0.43	0.71	0.67
RM600	1	9.5	7	0.43	0.72	0.68
RM595	1	15.3	4	0.68	0.49	0.44
RM7266	1	24.4	3	0.87	0.24	0.22
RM128	1	31.1	3	0.93	0.13	0.12
RM5362	1	41.1	3	0.93	0.13	0.12
RM6840	1	43.2	4	0.73	0.43	0.4
RM5764	2	1.6	4	0.87	0.24	0.23
RM290	2	10.8	3	0.72	0.44	0.4
RM498	2	35.4	3	0.83	0.29	0.27
RM523	3	1.3	6	0.63	0.56	0.54
RM251	3	9.9	3	0.93	0.13	0.12
RM186	3	28.8	2	0.97	0.06	0.06
RM6314	4	18.4	5	0.37	0.75	0.71
RM3288	4	27.3	4	0.6	0.56	0.5
RM1089	5	5.3	7	0.42	0.71	0.67
RM6024	5	17.8	4	0.78	0.37	0.35
RM6972	5	25.3	4	0.52	0.63	0.57
RM7454	7	1.1	3	0.6	0.55	0.48
RM6574	7	4.7	2	0.83	0.28	0.24
RM21333	7	9.0	3	0.73	0.41	0.35
RM5847	7	23.6	9	0.27	0.84	0.83
RM5911	8	0.1	5	0.57	0.61	0.56
RM3215	8	8.6	6	0.4	0.69	0.64
RM22780	8	10.8	5	0.37	0.72	0.68
RM22870	8	13.9	9	0.27	0.84	0.82
RM556	8	22.2	3	0.9	0.18	0.18
RM5799	9	3.8	2	0.87	0.23	0.2
RM3769	9	11.7	3	0.43	0.64	0.56
RM311	10	9.7	5	0.4	0.7	0.65
RM258	10	17.8	5	0.4	0.66	0.6
RM286	11	0.4	3	0.7	0.46	0.41
RM3701	11	8.1	2	0.93	0.12	0.12
RM5349	11	19.2	5	0.3	0.76	0.72
RM6094	11	28.4	3	0.57	0.58	0.51
RM511	12	17.4	2	0.77	0.36	0.29
RM313	12	20.8	5	0.63	0.55	0.51
RM6410	12	29.3	5	0.53	0.64	0.59
Total			161			
Mean			4	0.64	0.47	0.44

of polymorphism and diversity among the genotypes for a marker locus used in linkage analysis. The PIC values varied greatly for all 38 SSR loci ranging from 0.06 to 0.83 with an average of 0.44. The highest PIC value (0.83) was obtained for RM5847 followed by RM22870 (0.82), RM5349 (0.72), RM6314 (0.71), RM600 (0.68), RM1282 (0.67) and RM1089 (0.67). A total of 161 alleles across 30 genotypes were detected at 38 SSR loci. The number of alleles generated by each marker locus varied from 2.0 to 9.0 with an average value of 4.0. The highest number of alleles (9.0) was detected with RM22870 and RM5847, and the lowest number of alleles (2.0) was detected with RM511, RM5799, and RM3701and RM186. On average 64% of the genotypes shared a common major allele at any given locus ranging from 27% (RM22870) to 97% (RM186) common allele per marker locus. An overall genetic diversity of 0.47 with a range of 012 to 0.84 was obtained among the breeding lines/varieties. The gene diversity of each SSR locus appeared to be associated with the number of alleles detected per locus.

The UPGMA-based dendrogram obtained from the binary data deduced from DNA profiles of the samples showed that the genotypes which were derivatives of genetically similar types clustered together (Fig. 1). All genotypes clearly grouped into two major clusters in the dendrogram at 68 % genetic similarity based on Nei similarity index with additional sub-clusters in each group. Cluster-I contained three genotypes which shared 76% common alleles and further divided into two sub-clusters at 0.84 similarity coefficient. Twenty-seven genotypes were grouped into Cluster-II, which was further divided into two sub-clusters. BR18, the cold tolerant variety at the seedling stage was grouped singly in one sub-cluster, while the remaining 26 genotypes including cold susceptible BR1, BRRI dhan28 and BRRI dhan29 were grouped in another sub-cluster, which was further divided into many sub-groups. In these subgroups 79.25% common alleles were shared by 19 genotypes. BR18 shared 73.5% common alleles with the genotypes in Cluster-II. However, the genotypes in Cluster - I belonging 76% common alleles had 28% different alleles from BR18 of Cluster - II. The coaxial tree based on neighbour joining algorithm also showed that the genotypes in Cluster - I were genetically closer to BR18 than others (Fig. 2).

DISCUSSION

Rice plants are affected by cold stress at various stages of crop growth depending on severity and extent of cold wave (Li *et al.*, 1981). The sensitivity to cold stress varies between the stages of crop growth in rice. Yoshida (1981) showed that rice plants have a lower threshold temperature (10–13°C) for cold damage during the early stages of development (germination and vegetative), while threshold temperature for damage is higher (18–20°C) during the reproductive stage. Thus, vegetative stages are less sensitive to cold than the reproductive stage. Despite these differences among stages, cool weather and cold irrigation water can damage the rice plants during any developmental stage such as germination, seedling, vegetative, reproductive and maturity (Majumder *et al.*, 1989; Andaya and Mackill, 2003; Xu *et al.*, 2008, Ye *et al.*, 2009). The most common symptoms of LTS damage at the germination stage are delayed and lower percentage of germination (da Cruz and Milach, 2000). At the vegetative stage, cold damage is expressed through yellowing of the leaves, shorter stature and decreased tillering of rice plants. Cold stress at the reproductive stage commonly causes spikelet sterility; incomplete panicle exertion and spikelet abortion also may occur (Satake and Hayase, 1970), which ultimately lower the grain yield of rice. Therefore, leaf discoloration, % survivability and % recovery are considered as the three major criteria for

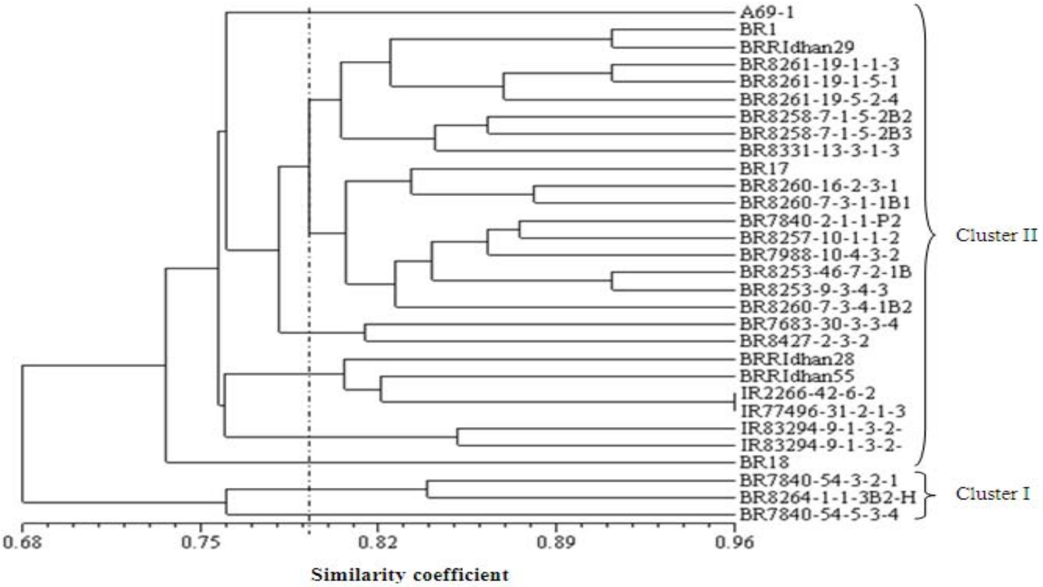

Fig. 1. Cluster of 30 rice genotypes based on Nei similarities index calculated from allele frequency data matrix of 38 SSR markers using UPGMA dendrogram.

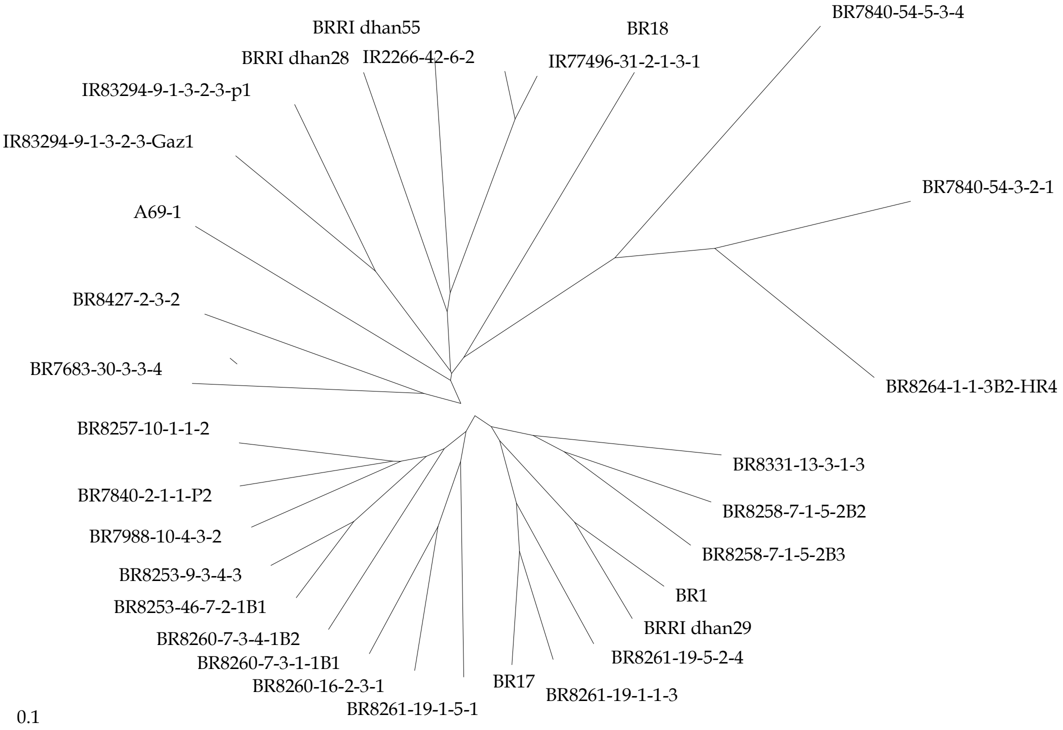

Fig. 2. Cluster of 30 rice genotypes based on allele sharing similarities index calculated from data matrix of 38 SSR markers using neighbour joining algorithm.

determining the level of cold tolerance at seedling stage in rice (NICS, 2004). While, spikelet fertility % and grain yield are considered to be selection criteria for reproductive stage cold tolerance.

In this study, we measure LD score for discriminating varieties or germplasm under cold stress. LTS of 13°C at 3-leaf stage to an initial population of 594 breeding lines showed well discrimination among themselves in leaf discoloration. The 22 breeding lines obtaining LD score (1 – 3) in the initial evaluation along with three susceptible check varieties, five tolerant check varieties showed wide range of variation in the LD score starting from 3.2 to 8.4. The variations were wider and more pronounced in the Batch 1 and Batch 2 ranging from 2 to 8.6 and 3.0 to 9.0, respectively (Table 3). In all three batches, the tolerant variety (BR18) and susceptible variety (BR1) obtained an average LD score of 3.4 and 8.4, respectively. The lowest average LD score was obtained with IR77496-31-2-1-3-1 (3.2) followed by IR2266-42-6-2 (3.4), BR8257-10-1-1-2 (3.5) indicating them to be strong cold tolerant at the seeding stage. Among the 30 genotypes evaluated in the final evaluation, twelve showed LD score less than 4 and other 12 genotypes showed LD values ranging from 4.0 to 5.0, indicating them to be tolerant and moderately tolerant, respectively. It is important to note that the majority of breeding lines which showed low LD values were derived from the crosses that involved at least one parent originated from a single cross BR7166, which was developed from a cross between BRRI dhan29 and a Chinese genotype, PL Youhong (Table 1). This finding indicated that the alleles from PL Youhong might have contribution for cold tolerance in the breeding lines.

Cold stress has noticeable consequence on reproductive stage as it induces spikelet sterility due to low seed setting, which ultimately turns into low yield of rice (Thakura *et al.*, 2010). Jena *et al.* (2010) also reported that cold stress not only affects spikelet fertility but also reduces culm length and delays heading. In this study most of all breeding lines showed higher reduction percentage in spikelet fertility and grain yield except BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3 (Table 5). Ye *et al.* (2009) and Shinada *et al.* (2013) also reported similar results of yield reduction under cold stress at the reproductive stages. Low temperature significantly affects yield attributing traits such as culm length, number of panicles, reduce panicle emergence and panicle length, and grain yield in rice genotypes (Ghadirnezhad *et al.*, 2004 and Farrell *et al.*, 2006). In this study, spikelet fertility and grain yield were relatively lower in all breeding lines including the check varieties (Table 6). BR1, the cold susceptible variety showed comparatively lower reduction in spikelet fertility and grain yield on the other hand, the breeding lines/varieties showing higher tolerance to cold stress at seedling stage had higher reduction in spikelet fertility and grain yield except few cases, like BR8258-7-1-5-2B2, BR8258-7-1-5-2B3, etc. which indicated the independence of genes underlying cold tolerance at seedling stage and reproductive stage. Biswas *et al.* (2019) also reported that genes or QTL conferring cold tolerance for different growth stages are independent but they might be co-localized in some cases. The seedling stage cold tolerant variety, BR18 (LD: 3.4) also showed relatively higher reduction in spikelet fertility and grain yield under cold stress at booting stage compared to the plants grown under ambient temperature. Relative comparison of cold sensitivity among the genotypes suggests that the genetic control on cold tolerance at both seedling and reproductive stage is absent in the test genotypes rather seedling stage cold tolerance is dominant except few cases (Tables 3, 5 and 6).

The UPGMA dendrogram based on Nei similarity index of shared SSR loci among the genotypes also showed that majority of the breeding lines showing 4-5 degrees of LD were grouped together. However, susceptible varieties, BR1, BRRI dhan28 and BRRI dhan29 shared 76% to 84% common alleles with these genotypes. Two breeding lines, BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3 originating from a three way cross that involved BRRI dhan29 as one of the three parents had 86% common alleles and showed cold tolerance at both seedling and reproductive stage. Another breeding line, BR8331-13-3-1-3 which was genetically very close to BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3 had only 2% different alleles from them and showed around 70% and 82% reduction in spikelet fertility and grain yield, respectively. These results indicated that the SSR loci, which contributed to this difference between the two cold tolerant breeding lines (BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3) and the cold susceptible BR8331-13-3-1-3, might have close association with the QTL responsible for the increased seed setting rate and grain yield under cold stress. The binary data of the shared alleles of the SSR (Table 8) showed difference in seven loci (RM1282, RM600, RM5349, RM6972, RM5847, RM5911, and RM511), which indicated these seven SSR makers might be useful in discriminating tolerant lines from susceptible ones for higher seed setting rate under cold stress. A distinction between the tolerant and susceptible breeding lines in both phenotyping and molecular analysis indicates the presence of specific alleles that could be useful for the improvement of cold tolerance in rice.

CONCLUSION

Three breeding lines, BR8253-9-3-4-3, IR77497-31-2-3-1and IR2266-42-6-2 demonstrated higher cold tolerance only for the seedling stage (LD score <3.0), while two breeding lines, BR8258-7-1-5-2B2 and BR8258-7-1-5-2B3 having 86% shared alleles showed cold tolerance both at seedling stage and reproductive stage in terms of low reduction in spikelet fertility (45.8% and 48.1%) and grain yield (43.3% and 39.1%). In addition, eight SSR loci showed differential allele call between the cold tolerant and susceptible genotypes at reproductive stage. The identified breeding lines and SSR markers in this study could be useful for developing cold tolerant rice.

Table 8. Binary values of allele distribution among 30 rice breeding lines/varieties deduced from DNA profiling of seven SSR loci showing allelic variation between BR8258-7-1-5-2B2 and/or BR8258-7-1-5-2B3 and BR8331-13-3-1-3.

SSR Loci	Chromosomal location	Genotype																													
		A69-1	BR1	BR17	BR18	BR7683-30-3-3-4	BR7840-2-1-1-P2	BR7840-54-3-2-1	BR7840-54-5-3-4	BR7988-10-4-3-2	BR8253-46-7-2-1B1	BR8253-9-3-4-3	BR8257-10-1-1-2	BR8258-7-1-5-2B2	BR8258-7-1-5-2B3	BR8260-16-2-3-1	BR8260-7-3-1-1B1	BR8260-7-3-4-1B2	BR8261-19-1-1-3	BR8261-19-1-5-1	BR8261-19-5-2-4	BR8264-1-1-3B2-HR4	BR8331-13-3-1-3	BR8427-2-3-2	BRRIdhan28	BRRIdhan29	BRRIdhan55	IR2266-42-6-2	IR77496-31-2-1-3-1	IR83294-9-1-3-2-3-Gaz1	IR83294-9-1-3-2-3-p1
RM1282	1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
RM600	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM5349	3	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0
RM6972	5	0	1	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1
RM5847	7	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1
RM5911	8	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
RM511	12	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1

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Response to Callus Induction and Regeneration of Newly Released BRRI Rice Varieties

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ABSTRACT

A study was carried out for developing an efficient callus induction and regeneration system for three newly developed BRRI varieties namely BRRI dhan86, BRRI dhan87 and BRRI dhan89. Dehusked seeds were plated onto MS and N6 media with two hormone combinations for callus induction. Calli obtained from each callus induction medium were transferred to four different regeneration media. Callus induction frequency and regeneration ability were significantly influenced by rice varieties, and interactions of variety and media. Among the media compositions, the highest callus (59.44%) were obtained from C_1 (MS+2mg/l 2,4-D) followed by C_2 (MS+2 mg/l 2,4-D+0.5 mg/l kinetin) , C_3 (N6+2 mg/l 2,4-D) and C_4 (N6+2 mg/l 2,4-D+0.5 mg/l kinetin) medium. The highest regeneration (45.74%) was obtained from R_2 (MS+4 mg/ml BAP+1.2 mg/ml kinetin+0.5 mg/ml NAA), followed by R_3 (1 mg/ml BAP+1 mg/ml Kinetin+1 mg/ml NAA), R_4 (2 mg/ml kinetin+1 mg/ml NAA+300 mg casein hydrolysate) and R_1 (2 mg/ml BAP+1 mg/ml kinetin+1 mg/ml NAA). BRRI dhan86 showed the highest regeneration ability (53.06%) than the other two varieties. It is observed that all varieties performed better in C_1 medium for callus induction and R_2 medium for regeneration. This study also revealed that BRRI dhan86 was more responsive to callus induction and regeneration of green plants than the other two varieties.

Key words: Rice, callus, regeneration, hormone

INTRODUCTION

Rice is consumed as a staple food by more than half of the world population. Asia produces 90% of rice to meet up the demand (Bandumula *et al.*, 2017). But rice production faces a threat of biotic (diseases and insects) and abiotic (salinity, drought, submergence, cold, heat) stresses due to rapid climatic changes (Das *et al.*, 2019). Various research approaches like conventional breeding, somaclonal variation and marker assisted selection are being carried out since the last decades to develop tolerant rice varieties to reduce above mentioned stresses. Though some remarkable progresses are achieved, those are not worthy of satisfaction. Rice has become the prime target for genetic manipulations due to much dependence upon it as a staple food. Genetic transformation is an important biotechnological tool for developing stress tolerant rice varieties. However, the success of genetic transformation depends on several factors like genotype, media, light, hormonal effect, etc. *Agrobacterium* mediated transformation of rice requires an efficient regeneration system from a transformed callus and ironically, shoot regeneration represents a major bottleneck in this endeavour (Lim *et al.*, 2017). Most of the *indica* rice genotypes, the world's most cultivated rice types, still remain less amenable to genetic transformation due to their poor regeneration potentiality (Sripriya *et al.*, 2017). Although reporting is abundant on callus induction, regeneration and also transformation in japonica rice but it is limited in *indica* rice. In this context, the evaluation of new factors and their manipulation for efficient callusing and green plant regeneration from the mature embryo in *indica*

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rice is still challenging (Pawar *et al.*, 2015). Researchers still struggle to optimize the callus induction procedures and efficiency of plant regeneration for rice mature embryos because it varies with variety to variety. In this context, the evaluation of varieties with different types of media and their manipulation for efficient callusing and green plant regeneration from the dehusked mature embryo in *indica* is still a challenging field (Pazuki and Sohani, 2013). Many studies have been conducted to optimize the techniques and composition of culture medium for callus induction from dehusked rice seed for various purposes (Benlioglu *et al.*, 2015). However, it's application is still limited by many factors influencing the culture efficiency such as medium composition explants source (Din *et al.*, 2016), genotype and environment (Islam *et al.*, 2014). Among them, the genotype and nutrient composition are considered to be the major sources of variation for *in vitro* culture (Kido *et al.*, 2015). For carrying out successful genetic transformation in rice, identification and screening of useful cultivars and the establishment of efficient regeneration protocols are very essential (Vennapusa *et al.*, 2015). With the concern of the above situations, the present study was carried out to compare the performance of three newly developed high yielding rice varieties to find out the best combination of callus induction media and regeneration media for each variety and to identify the best responsive variety among them for transformation study.

MATERIALS AND METHODS

This research work was conducted at the Biotechnology Division of BRRI following Complete randomized design with three replications. A total of 1200 dry seeds of each three rice cultivars viz BRRI dhan86, BRRI dhan87, BRRI dhan89 were used for this study. Sterilization was carried out in a laminar air flow cabinet. Dehusked seeds were first sterilized with 70% ethanol for one minute followed by washing with sterile water. Then it was sterilized with 50% sodium hypochlorite (v/v) containing one drop of 20 for 40 minutes with gentle agitation. Seeds were thoroughly washed five to six times with sterile distilled water to remove sodium hypochlorite. The sterilized seeds were finally placed on sterile filter papers also to remove excess water.

Both MS (Murashige and Skoog, 1962) and N6 (Nitsch and Nitsch, 1969) basal salts were used to supplement with two different hormone combinations for calli induction (Table 1). Sucrose 30 g/l and phytagel 4 g/l was used as a source of carbohydrate and solidifying agent, respectively. Four types of media (C_1, C_2, C_3 and C_4) were used. The pH of the media was adjusted to 5.8. The media was autoclaved at 15 psi at 121°C for 20 minutes. Four hundred sterilized seeds of each variety were placed on four callus induction media.

Then all culture plates were placed at 25±1°C under the dark condition for callusing. Data on % callus induction and size of callus were collected after 21 days and 30 days after plating the seeds. Three week's old calli were transferred onto magenta boxes containing regeneration media. Callus obtained from each combination of callus induction media (C_1, C_2, C_3 and C_4 were transferred onto four types of regeneration media (R_1, R_2, R_3 and R_4) (Table 2). Regeneration efficiency was observed on MS media supplemented with four different combinations of naphthalene acetic acid (NAA), Kinetin (Kn) and 6-benzylaminopurine (BAP). The pH of the regeneration media was adjusted to 5.8. Cultural conditions of the study was maintained at 25±1°C, light and dark cycle of 16:8 hours. After 30 days, % regenerated calli and number of regenerated green plants were recorded.

The data were subjected to ANOVA (Analysis of Variance) testing and the mean values were separated by the least significant difference (LSD) using MS Excel and R software (Table 3).

Table 1. Composition of callus induction media are as follows.

Callus induction media	Basal media	Media combination
C_1	MS	MS+2 mg/l 2,4-D
C_2	MS	MS+2 mg/l 2,4-D+0.5 mg/l kinetin
C_3	N6	N_6+2 mg/l 2,4-D
C_4	N6	N_6+2 mg/l 2,4-D+0.5 mg/l kinetin

Table 2. Four hormonal combinations in regeneration media are as follows.

Regeneration media	Basal media	Hormone combination
R_1	MS	2 mg/ml BAP+1mg/ml kinetin+1 mg/ml NAA
R_2	MS	4 mg/ml BAP+1.2 mg/ml kinetin+0.5 mg/ml NAA
R_3	MS	1 mg/ml BAP+1mg/ml Kinetin+1mg/ml NAA
R_4	MS	2 mg/ml kinetin+1mg/ml NAA+300 mg casein hydrolysate

Table 3. Analysis of variance (ANOVA) for callus induction and size of the calli.

Source of variation (SV)	DF	Mean sum of square (MS)	
		Calli induction	Size of calli
Variety (V)	2	1281.60***	0.05**
Calli induction media (C)	3	3041.60***	0.02
C:V	6	561.03***	0.01
Residuals	24	56.86	0.01

** and *** indicates significant at the 5% and the 1% level of significance respectively.

RESULTS AND DISCUSSION

Callus initiation in varieties

The highest callus induction (52.17%) was found in BRRI dhan86 followed by (43.50%) BRRI dhan87 and the lowest (31.58%) was in BRRI dhan89 (Table 4). Thus, BRRI dhan86 showed comparatively better potential in callus induction compared to other varieties. At the beginning, scutellum callus was compact almost in all varieties but turned into friable after two weeks of culture. Calli of BRRI dhan86 were bright yellowish, look most healthy compared to other varieties (Fig. 1a-f).

BRRI dhan87 performed better in media C_2 followed by C_4 and C_3. The results found of this study are in agreement with the findings of other researchers largely depending on the use of harmonious combinations of nutritional constituents and growth regulators. There are many factors influence callus induction in rice like genotype potentiality, P^H of the media, plant growth regulators (PGRs) supplement, solidification of culture medium, light intensity etc. Joyia *et al*., (2012). Yaqoob *et al*., (2016) reported that mature dehusked rice seeds

Table 4. Interaction between the genotypes and media for callus induction of three rice genotypes.

Calli induction media → Varieties↓	MS		N_6		Mean of varieties
	C_1	C_2	C_3	C_4	
BRRI dhan86	83.00d	70.67cd	26.00a	29.00ab	52.17A
BRRI dhan87	49.67bc	71.00cd	24.33a	29.00ab	43.50B
BRRI dhan89	45.67ab	29.67ab	24.67a	26.33a	31.58C
Calli induction media mean	59.44A	57.11A	28.11B	25.00B	-

*In interaction means in both row and column indicate a common small letter(s) are not different significantly. (P≤0.05)

*In a row, calli induction media mean indicates a common capital letter(s) are not different significantly. (P≤0.05)

*In a column mean of varieties indicate a common capital letter(s) are not different significantly. (P≤0.05)

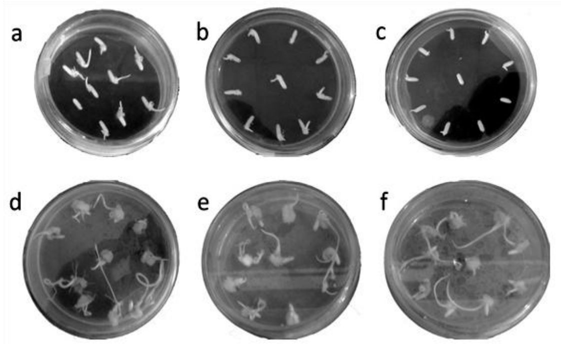

Fig. 1. Seed placement of BRRI dhan86 (a), BRRI dhan87 (b) and BRRI dhan89 (c) and callus initiation of BRRI dhan86 (d), BRRI dhan87 (e) and BRRI dhan89 (f) into media.

were good for callus induction because of callus initiated from scutellum of mature rice seeds have high embryogenic potentiality. Amer *et al*., (2016) and Ahmad *et al*., (2015) also reported that embryogenic calli have high regeneration capacity and excellent material for the transformation of rice by using *Agrobacterium*.

Effect of medium on callusing

From this study, it was found that the varieties, media compositions and their interactions significantly affected on callus induction at the 5% level of significance. Among the media composition, C_1 and C_2 were significantly higher than C_3 and C_4 media for effective callus induction. The rate of callus induction was similar in C_3 and C_4 media (Table 4). The rate of callus induction was also similar in C_1 and C_2 media, but calli obtained from C_1 medium were quite good in texture and friable in nature than that of C_2 medium. MS and N_6 are the most commonly used basal media for calli induction and regeneration (Azizi *et al*., 2015). BRRI dhan86 in MS medium gave better callus induction as compared to N_6 media, these findings were similar to the report by Islam *et al*., (2014) who indicated that variety was one of the major determinants in embryogenic callus induction. Kido *et al*. (2015), Narciso *et al*. (2010) and Roy *et al*. (2015) also reported that embryogenic callus formation and plantlet regeneration were influenced by culture medium and variety.

The mean frequency of calli size over media (Table 5) showed that the variety BRRI dhan87 produced larger calli (0.47%). Among the media composition we found, calli size on C_3 media were higher than C_1, C_2 and C_4 media. BRRI dhan87 produced larger size calli from C_3 (0.54%) and C_2 (0.51%) media and BRRI dhan89 produced lower size calli in C_2 (0.27%) than the other two varieties. Variations were observed among the three varieties but there was no significant differences.

The concentrations of the plant growth regulators also affect the callusing of the genotypes. In this study, 2, 4-D was used at the rate of 2 mg/l. Previous studies also showed that callus could be induced better in 2 mg/l (Shahsavari *et al*., 2010). These results are confirmatory to the findings of the other researchers (Htwe *et al*., 2011). Thus present investigation revealed that both genotypes and media compositions and their interaction largely affected the callus.

Table 5. Interaction between the varieties and media for calli size of three rice varieties.

Media→ Varieties↓	MS		N_6		Mean of varieties
	C_1	C_2	C_3	C_4	
BRRI dhan86	0.45a	0.39a	0.41a	0.33a	0.39AB
BRRI dhan87	0.47a	0.51a	0.54a	0.37a	0.47A
BRRI dhan89	0.37a	0.27a	0.41a	0.31a	0.34B
Calli induction media mean	0.43A	0.39A	0.45A	0.34A	-

*In interaction means in both row and column indicate a common small letter (s) are not different significantly. ($P \le 0.05$)

*In a row, calli induction media mean indicates a common capital letter (s) are not different significantly. ($P \le 0.05$)

*In a column mean of varieties indicate a common capital letter(s) are not different significantly. ($P \le 0.05$)

Regeneration of calli and green plant

In vitro plant regeneration was investigated on MS medium supplemented with different combinations of BAP, Kinetin, and NAA (Kaswan *et al*., 2012). Several factors, such as variety, developmental stage of cells in the explants, plant growth hormone composition in the medium, carbohydrates source, have been reported to improve the frequency of plantlet regeneration in rice (Muhammad *et al*., 2014). Plant regeneration was observed as early as three weeks and continued to occur up to four weeks after calluses were placed on regeneration media (Fig. 2a-e). Likewise callus proliferation, regenerative capacities varied considerably among rice genotypes. Table 4 shows the ANOVA results for the regeneration of calli and green plants. It was found that varieties were highly significant for regenerated calli induction and variety and regenerated media interaction showed highly significant differences for regenerated green plants.

The addition of day to the regeneration medium appeared to exert the least influence of all factors studied for plant regeneration frequency (Table 6). Nevertheless; day differentially affected subsequent plant regeneration of three varieties.

Mean frequency of regenerated calli induction over varieties (Table 7) showed that the variety BRRI dhan86 produced maximum callus (53.06%) which was not significantly higher than other varieties BRRI dhan87 (26.43%) and BRRI dhan89 (26.32%). Among the media composition, media R_2 (45.74%) was found to be most effective for regeneration. Although other varieties performed better in media R_3, R_4 and R_1, the quality of regenerated calli were not as good as those induced in R_2.

Mean frequency of green plants over varieties (Table 8) showed that the variety BRRI dhan86 produced maximum green plants (41.37%) which was not significantly different than the rest of the varieties BRRI dhan87 (32.71%) and BRRI dhan89 (31.01%). Among the media composition for producing green plant, R_2 was the found highest (47.34%) followed by R_3 (42.23%) which did not significantly differ from the other media R_1 and R_4. Although some varieties performed better in media R_3, R_4 and R_1, the quality of regenerated green plants was not as good as those induced in R_2. Given the highly significant genotype effect, the statistical analysis is shown separately for each of them (Table 8).

Table 6. Analysis of variance (ANOVA) for regeneration of calli induction and green plants.

Sources of variation	DF	Mean sum of square (MS)	
		Regenerated calli (%)	Green plants (%)
Variety (V)	2	15174.00***	1513.50
Calli induction media (C)	3	128.00	3962.67
Regeneration media (R)	3	1860.33	5258.33**
V:R	6	1480.17	9364.50***
V: D	2	2265.00	338.50
C:R	9	859.22	2184.33
V:C:R	12	1479.50**	1856.67
V: R: D	9	742.78	1592.44
Residuals	144	767.58	-

** and *** indicates significant at the 5% and the 1% level of significance respectively.

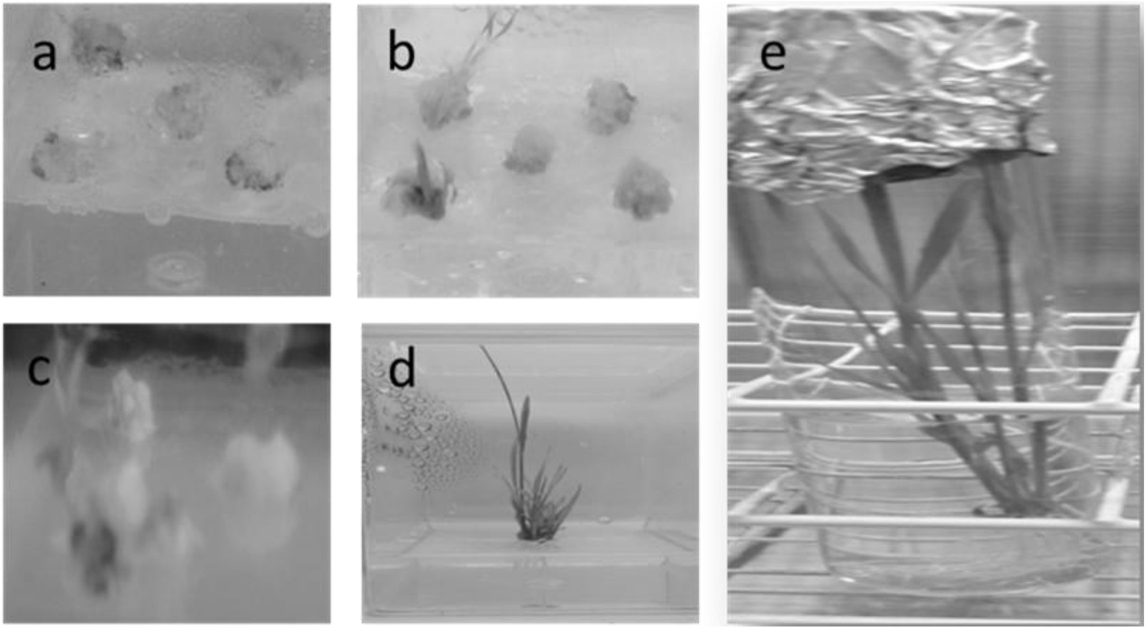

Fig. 2. Green bud initiation and induction of shoot (a-d), and established regenerated green plantlets of BRRI dhan86 (e).

Table 7. Interaction between the varieties and regeneration media for regenerated calli of three rice varieties.

Regeneration media (R) → Varieties (V)↓	R_1	R_2	R_3	R_4	Mean of varieties
BRRI dhan86	50.10a	76.10a	69.90a	67.00a	53.06A
BRRI dhan87	40.90a	38.10a	40.40a	37.30a	26.43B
BRRI dhan89	31.70a	72.80a	39.90a	41.40a	26.32B
Regeneration mean	29.26A	45.74A	38.64A	36.37A	-

*In interaction means in both row and column indicate a common small letter (s) are not different significantly. ($P \leq 0.05$)
*In a row, calli induction media mean indicates a common capital letter (s) are not different significantly. ($P \leq 0.05$)
*In a column mean of varieties indicate a common capital letter (s) are not different significantly. ($P \leq 0.05$)

Table 8. Interaction between the varieties and regenerated media for green plants of three rice varieties three rice varieties.

Plant regeneration media (R) → Varieties (V)↓	R_1	R_2	R_3	R_4	Mean of varieties
BRRI dhan86	31.20a	69.00a	82.70a	58.60a	41.37A
BRRI dhan87	69.40a	53.00a	40.20a	44.30a	32.71A
BRRI dhan89	38.80a	104.20a	71.00a	30.10a	31.01A
Regeneration media mean	27.55A	47.34A	42.23A	27.03A	-

*In interaction means in both row and column indicate a common small letter (s) are not different significantly. ($P \leq 0.05$)
*In a row, calli induction media mean indicates a common capital letter (s) are not different significantly. ($P \leq 0.05$)
*In a column mean of varieties indicate a common capital letter (s) are not different significantly. ($P \leq 0.05$)

In a subsequent study, in comparison to the results presented here, higher regeneration frequency was observed in scutellar calli of all the three genotypes tested when dehusked seeds used as explants. Dehusked seeds proved to be better explants than mature-embryos, possibly due to the involvement of endosperm factor(s) and some possible damage to the embryos during the process of embryo isolation from mature seeds. However, in the present study isolated embryos rather than dehusked seeds were used to eliminate any variation due to the involvement of endosperm. The results have demonstrated that the composition of basal media used for callus induction is not always optimum for plant regeneration as the nutritional requirements of the two phases of development may vary. Furthermore, this differential requirement was found to be variety dependent. Transfer of embryo-calli from callus induction medium to plant regeneration medium involved either a change of only the growth regulators or a change of both, growth regulators as well as the basal medium. Within a variety, significant differences affecting the regeneration parameters were observed between the two types of regeneration media (MS based regeneration medium and callus-induction basal medium based regeneration medium). In cultivar BRRI dhan86, variation was mainly due to regeneration media effect whereas in BRRI dhan87 and BRRI dhan89, callus induction medium affected the most. After transferring the calli into the regeneration medium, green spots became visible on the surface of the calli within 5-7 days were developed. MS medium supplemented with 4 mg/ml BAP+1.2 mg/ml kinetin+0.5 mg/ml NAA was found the best in respect to percent regenerated planlet (47.34%) as well as for the growth of plantlets *in vitro*. The addition of a small amount of Kinetin has been reported to improve embryogenic calli and shoot formation efficiency (Barbosa *et al*., 2014) in *indica* rice. Present finding agrees with the result of (Azizi *et al.,* 2016 and Barman *et al*., 2016) where it was reported that kinetin was found to be more effective for plantlets regeneration compared with BAP (6-benzylaminopurine). Combinations of Auxin and cytokinin along with the effect of basal salts played an important role in plant regeneration (Kumar *et al*., 2013).

The interaction effect of variety and medium on callus induction was not varied significantly. The highest callus induction was recorded in BRRI dhan86 (90.00%) in C_3 for callus induction and R_2 (Table 9). The lowest callus induction was observed in BRRI dhan87 (07.14%) in C_3 and R_1.

However, higher frequencies of regeneration were associated with certain calli induction media (Table 9). Interestingly, for BRRI dhan86 was appeared to be an equal relationship between callus induction and highest plant regeneration capacity.

CONCLUSIONS

In summary, rice varieties showed significant divergence for their *in vitro* response to callus induction. The quality and frequency of callus induction and subsequent plant regeneration, however, ultimately depend on the composition of initial callus induction treatment. Therefore, selection of better responsive rice variety like BRRI dhan86 and medium designated as C_1 for callus induction as like as R_2 for regeneration would offer great promise for the induction of higher level of desired somaclones and quality of callus for various means of genetic transformation as well as in the selection of stress tolerant cultivar development program and other relevant studies for improving this world's staple food crop.

Table 9. Interaction between the varieties and calli induction media on percent regenerated calli induction.

Variety	Callus induction media	Regeneration media			
		R_1	R_2	R_3	R_4
BRRI dhan86	C_1	47.08bc	61.07bc	50.00bc	75.00bc
	C_2	45.00bc	52.92bc	70.00bc	47.14bc
	C_3	39.58bc	90.00c	45.83bc	60.00bc
	C_4	8.33b	60.00bc	74.29bc	44.58bc
BRRI dhan87	C_1	22.5b	23.75bc	21.67bc	25.00bc
	C_2	25.00bc	23.75bc	46.67bc	20.00bc
	C_3	07.14b	23.33bc	20.00b	16.03bc
	C_4	63.75bc	35.00bc	30.00bc	30.83bc
BRRI dhan89	C_1	11.58b	0a	0a	0a
	C_2	0a	46.75bc	0a	0a
	C_3	0a	0a	19.72b	0a
	C_4	0a	0a	0a	20.30b

Zero (0) represented by non-regenerated plants.
*In interaction means in both row and column indicate a common small letter(s) are not different significantly. ($P \leq 0.05$)

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Genetic Variability, Heritability, Correlation and Path Coefficient Studies for Yield and Yield Components of Some Promising Rice Hybrids

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ABSTRACT

Eight promising hybrids along with three checks were evaluated for yield and yield contributing traits to observe their genetic variability, heritability, correlation and path coefficient analysis during T. Aman season 2014. The results indicated that the highest genotypic variance was recorded in spikelet panicle^{-1} followed by effective tiller m^{-2}. Similarly, the highest phenotypic variances were also found with these two characters. Phenotypic coefficient of variation (PCV) was slightly higher than genotypic coefficient of variation (GCV) for all the traits under this study. Hence, slight differences indicate less or minor environmental influence and greater role of genetic factors on the expression of the traits. High heritability was observed in all the characters studied except effective tiller m^{-2}. Highly significant and positive correlations of grain yield with effective tiller m^{-2}, spikelet panicle^{-1} at genotypic level were observed. Spikelet fertility was found significant at both genotypic and phenotypic level. Path analysis revealed that spikelet fertility had highly positive direct effect on grain yield followed by effective tiller m^{-2}. On the other hand, spikelet panicle^{-1} showed positive indirect effect on grain yield. Thus, the results suggested that effective tiller m^{-2}; number of spikelet panicle^{-1} and spikelet fertility (%) could be considered as effective selection criteria for the development of heterotic rice hybrids.

Key words: Genetic variability, heritability, correlation, path analysis, hybrid rice

INTRODUCTION

Rice occupies more than 75.01% of total cropped area of Bangladesh (BBS, 2015). It provides 75% of the calories and 55% of the proteins in the average daily diet of the people (Bhuiyan *et al*., 2002). Bangladesh is one of the most densely populated countries of the world. To feed the ever increasing population, there is no option other than breaking the yield ceiling of rice. Hybrids may offer to make a breakthrough in the yield ceiling of semi-dwarf rice that began in 1964. Currently, hybrid rice technology is considered as a viable option to increase rice yield globally. China is the first country to exploit of heterosis commercially in rice. During late 90s, about 55% of the rice area in China was under hybrid rice, producing 66% of the total rice production (Virmani *et al*., 1998). At present, hybrid rice covers 70% of the total rice area which is about 20 million hectares. The most promising hybrids yielded 20-30% and 15-20% (Yuan, 1998) higher than the best conventional and modern rice varieties, respectively. Initially, after the introduction of hybrid rice in Bangladesh in 1998, over the last one and half decades, about 174 rice hybrids were released for commercial cultivation in different rice growing regions of the country (SCA, 2018). The estimated area under hybrid rice in Bangladesh is about 0.7M ha has contributed 3-4 MT of additional rice to the total rice production in the country (AIS, 2018).

Grain yield is a complex trait, controlled by many genes, environmentally influenced and determined by the magnitude and nature of their genetic variability in which they grow

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(Singh *et al.*, 2000 and Selvaraj *et al.*, 2011). Hence, direct selection for yield is often misleading. Therefore, the characters, which are closely related to grain yield contribution, were identified (Rangare *et al.*, 2012). Genetic variation is the basis of plant breeding and provides a great array of genotypes that can be selected to develop new varieties or breeding materials (Pandey *et al.*, 2009). Variability in terms of genetic divergence for agronomic traits is the component of breeding programmes for broadening the gene pool of rice and requires reliable estimates of heritability to plan an efficient breeding strategy (Akinwale *et al.*, 2011). Path coefficient analysis partitions the genetic correlation between yield and its component traits into direct and indirect effects and hence has effectively been used in identifying useful traits as selection criteria to improve grain yield in rice (Sadeghi, 2011; Lakshmi *et al.*, 2017 and Singh *et al.*, 2018). Genetic improvement of plants for quantitative traits requires reliable estimates of heritability in order to plan an efficient breeding programme. The broad sense heritability is the relative magnitude of genotypic and phenotypic variance for the traits and it gives an idea of the total variation accounted to genotypic effect (Allard, 1960). Correlation coefficient determines the simple relations among the traits. It does not always determine decisive result about determination of plant selection criteria. But path coefficient analyses as well as correlation coefficient give more detailed information on the relations among the yield and yield contributing traits. Present research was conducted to evaluate the heritability and genetic advance for grain yield and some of its related components in order to select the more effective traits which may contribute for the improvement of hybrid rice. This experiment also aimed to study the variation of important agronomic traits and identify the characters of utmost importance which could be used as selection criteria in a hybrid rice breeding programme and to determine the direct and indirect contributions of important yield components on yield.

MATERIALS AND METHODS

Experimental site, seasons, materials and procedures

The experiment was conducted at the experimental farm of BRRI, Gazipur during July to November in T. Aman season 2014. Nine promising rice hybrids viz. IR79156A/BRRI20R, IR79156A/BasmatiR, IR58025A/BasmatiR, Jin23A/BasmatiR, BRRI7A/BRRI31R, BRRI28A/BRRI31R, BRRI33A/BRRI31R, BRRI28A/BRRI26R and three conventional checks viz BRRI dhan49, BR11, BRRI hybrid dhan4 were used as experimental materials in this study. All the promising hybrids along with three checks were sown separately into well prepared seed bed. Twenty-one–day old seedlings were transplanted with a spacing of 15 cm between plants and 20 cm between rows with single seedling per hill in the main field in randomized complete block (RCB) design with three replications. The plot size was 5 x 4 m^2. Fertilizers were applied @ 150:100:70:60:10 kg ha^{-1} Urea, TSP, MP, gypsum and $ZnSO_4$, respectively. Standard agronomic practices were followed and plant protection measures were taken as required following (Anonymous, 2014).

Data collection

An observation and data record for all traits studied was made based on the Standard Evaluation System for rice (IRRI, 1996). Five sample plants were selected randomly in the middle three rows from each plot and observations were recorded on days to maturity, plant height (cm), number of effective tiller m^{-2}, number of spikelet $panicle^{-1}$, panicle length (cm), spikelet fertility (%), panicle weight (g), thousand grain weight (g), grain yield (ton $hectare^{-1}$) and moisture content of grains were measured by moisture meter and adjusted at 14% moisture content. Two border rows were used to minimize the border effects.

Statistical analysis

Analysis of variance was performed using the plant breeding statistical programme MSTAT C software. The genotypic and phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2b), genetic advance in percentage of mean (GA), genotypic correlation coefficients (r_g) and phenotypic correlation coefficients (r_p) analysis were estimated following (Singh and Chaudhary, 1985). The estimates of GCV and PCV were classified as low, medium and high (Sivasubramanian and Madhavamenon, 1973). Heritability in broad sense and genetic advance were calculated according to methods given by (Allard, 1960). Path coefficient analysis was done using R-software (Ri386 3.1.2)

RESULTS AND DISCUSSION

Variation and performance of the genotypes

The analyses of variance of nine important quantitative characters for different genotypes showed significant variations among the genotypes for days to maturity, plant height (cm), effective tiller m^{-2}, no. of spikelet panicle^{-1}, panicle length (cm), spikelet fertility (%), panicle weight (g), thousand grain weights (g) and yield (t ha^{-1}) (Table 1). These results indicated that there were genotypic variations among the genotypes for these characters.

Genetic variability

Table 2 shows that the highest genotypic variance was obtained in spikelet panicle^{-1} followed by effective tiller m^{-2}. Similarly the highest phenotypic variances were also obtained for those two characters. On the other hand, the lowest magnitude of genotypic and phenotypic variance was recorded in panicle weight and panicle length respectively. This might be due to the presence of both positive and negative alleles in the hybrids studied. Similar results were reported by Iftekharuddaula *et al.* (2001). The extent of variability for any character is very important for the improvement of a crop through breeding. The variability of the characters was measured by range, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). The estimates of phenotypic coefficient of variation were slightly higher than those of genotypic coefficient of variation (GCV) for all the traits studied. The extent of the environmental influence on traits is explained by the magnitude of the difference between GCV and PCV. Large differences between GCV and PCV values reflect high environmental influence on the expression of traits. In this study, slight differences indicated environmental influence and consequently greater role of genetic factors on the expression of traits. Similar observations were also noted earlier by Mustafa and Elsheikh (2007) and Kole *et al.* (2008) in rice.

Table 1. Mean squares analysis for morphological traits in promising hybrid rice.

Character	df	Days to maturity	Plant height (cm)	Effective tiller m^{-2}	Spikelet panicle^{-1}	Panicle length (cm)	Spikelet fertility (%)	Panicle weight (g)	TGW (g)	Yield (t ha^{-1})
Genotypes	10	387.89**	87.38**	2157.44**	2344.38**	6.75**	55.59**	2.71**	31.64**	3.21**
Replication	2	0.21	5.68	425.08	58.30	0.79	5.83	0.01	0.00	0.26
Error	20	0.17	12.89	671.90	172.65	0.61	2.29	0.00	0.00	0.07
CV (%)		0.37	3.05	11.44	6.66	3.00	1.85	1.42	0.17	4.93

*Significant at the 5% level of probability; ** Significant at the 1% level of probability; TGW= Thousand grain weight.

Table 2. Estimate of genetic parameters for grain yield and its contributing traits of promising hybrid rice.

Character	Maximum	Minimum	Mean	Genotypic variance	Phenotypic variance	GCV (%)	PCV (%)	h^2b	GA
Days to maturity	140.00	102.00	114.303	129.239	129.418	9.95	9.95	99.86	23.40
Plant height (cm)	129.20	106.20	117.679	24.828	37.724	4.23	5.22	65.82	8.33
Effective tiller m^{-2}	283.80	158.00	226.539	495.180	1167.081	9.82	15.08	42.43	29.86
Spikelet $panicle^{-1}$	249.00	133.00	197.424	723.911	896.557	13.63	15.17	80.74	49.80
Panicle length (cm)	29.20	22.20	25.970	2.047	2.654	5.51	6.27	77.13	2.59
Spikelet fertility (%)	87.00	70.00	81.670	17.765	20.060	5.16	5.48	88.56	8.17
Panicle weight (g)	5.20	2.18	3.687	0.904	0.907	25.78	25.83	99.67	1.96
Thousand grain weight (g)	32.10	20.43	25.66	10.546	10.548	12.65	12.65	99.98	6.69
Yield (t ha^{-1})	6.84	2.59	5.374	1.046	1.116	19.03	19.66	93.73	2.04

GCV- Genotypic coefficient of variation; PCV- Phenotypic coefficient of variation; h^2b- Heritability in broad sense; GA- Genetic advance.

Heritability

The estimates of broad sense heritability varied from 42.43 to 99.98 % (Table 2). High heritability was observed in all the characters except effective tiller m^{-2}. Although high heritability suggests high component of heritable portion of variation that can be exploited by breeders in the selection of superior genotypes (Ali *et al.*, 2002). On the basis of phenotypic performance, heritability estimates along with genetic advance will be more useful in predicting the effect for selecting the best individual. Effective tiller m^{-2} exhibited moderate heritability which indicates the possibility of using for rice improvement programme but their expression can be influenced more by the environment. High to moderate heritability was reported for different quantative traits in rice (Zahid *et al.*, 2006; Kole *et al.*, 2008; Khan *et al.*, 2009 and Sadeghi, 2011). The estimate of genetic advance as percent of mean (Table 2) was high for spikelet $panicle^{-1}$, effective tiller m^{-2} and days to maturity; moderate for plant height, spikelet fertility and thousand grain weight; and very low for panicle length, panicle weight and yield. Therefore, the characters, viz effective tiller m^{-2}, spikelet $panicle^{-1}$, spikelet fertility and thousand grain weight (TGW) with moderate to high heritability and genetic advance could be considered effective traits in selection of F_1 genotypes. Similar result was reported by Iftekharuddaula *et al.* (2001). However, effective tiller m^{-2} and spikelet $panicle^{-1}$ could be the most effective selection criteria as these characters had high genotypic and phenotypic variance in addition to high genetic advance.

Correlation coefficient

Table 3 presents genotypic and phenotypic correlation coefficient among grain yield and yield components for promising rice hybrid. Genotypic correlation coefficients were found higher than the phenotypic correlation coefficients in most of the cases which suggested that character association had not been largely influenced by environmental factors. There were highly significant and positive correlations of grain yield with spikelet fertility both at genotypic and phenotypic levels. On the other hand, effective tiller m^{-2} and spikelet panicle^{-1} were significant and positive correlation of grain yield at genotypic levels, indicating the importance of these traits for yield improvement. The results are in agreement with Rokonuzzaman *et al.* (2008) and Khan *et al.* (2009) for spikelet panicle^{-1}, Kole *et al.* (2008) and Qamar *et al.* (2005) for effective tiller m^{-2} and Ramakrishman *et al.* (2006) for spikelet fertility. Grain yield had positive and non-significant correlation in days to maturity, plant height, panicle length and thousand grain weight both at genotypic and phenotypic levels. Similar observations were reported for panicle length by Kole *et al.* (2008). On the contrary, Khan *et al.* (2009) and Qamar *et al.* (2005) reported positive and non-significant association between grain yield and days to maturity. In our present study yield showed negative and non-significant association with panicle weight both at genotypic and phenotypic levels.

Interrelationships among yield contributing characters showed both significant positive and negative correlation. Days to maturity possesses significant and negative correlation with panicle length and TGW at both genotypic and phenotypic level. Plant height had significant and positive correlation with thousand grain weight at both genotypic and phenotypic level. Significant and positive correlation was observed for effective tiller m^{-2} with spikelet fertility and grain yield at genotypic level only. It was observed that there were significant and positive correlations of spikelet panicle^{-1} with spikelet fertility at both genotypic and phenotypic level but with grain yield at genotypic level only. Panicle length was highly significant and positive correlation with TGW was at both genotypic and phenotypic level. Finally, panicle weight showed significant and positive correlation with TGW at both the levels.

Table 3. Genotypic (G) and phenotypic (P) correlation coefficients among yield and yield components for promising hybrid rice.

Character		Plant height (cm)	Effective tiller m^{-2}	Spikelet panicle^{-1}	Panicle length (cm)	Spikelet fertility (%)	Panicle weight (g)	TGW (g)	Yield (t ha^{-1})
Days to maturity	G	-0.1087	0.2170	0.2859	-0.8804**	0.4890	0.0369	-0.7110*	0.0785
	P	-0.0843	o.1475	0.2576	-0.7728**	0.4602	0.0364	-0.7102*	0.0739
Plant height (cm)	G		-0.2872	0.1091	0.2123	0.2337	0.5690	0.8859**	0.1875
	P		-0.0557	0.2318	0.2456	0.1756	0.4549	0.7206*	0.1332
Effective tiller m^{-2}	G			0.5439	-0.1765	0.7178*	-0.2841	-0.3622	0.8275**
	P			0.3323	-0.0445	0.4888	-0.1630	-0.2322	0.4532
Spikelet panicle^{-1}	G				-0.0633	0.6681*	0.2028	0.2453	0.5669*
	P				-0.0235	0.5598*	0.1817	0.2219	0.4707
Panicle length (cm)	G					-0.2474	0.0439	0.9589**	0.2230
	P					-0.1698	0.0358	0.8415**	0.1356
Spikelet fertility (%)	G						0.0889	-0.1045	0.9103**
	P						0.0909	-0.0992	0.8169**
Panicle weight (g)	G							0.6943*	-0.0021
	P							0.6943*	-0.0056
Thousand grain weight (g)	G								0.1886
	P								0.1806

*Significant at the 5% level of probability; ** Significant at the 1% level of probability.

Path coefficient analysis

Genotypic correlation coefficients were partitioned by using path analysis method to find out the direct and indirect effects of yield contributing traits towards the grain yield. Path analysis (Table 4) revealed that the highest positive direct effect and genotypic correlation (r_g = 0.9103) with grain yield was obtained by spikelet fertility (1.117) followed by days to maturity (0.655), panicle length (0.404) and effective tiller m^{-2} (0.103). The highest positive direct effect has been reported for spikelet fertility (Agbo and Obi, 2005), days to maturity (Qamar *et al.*, 2005), productive tillers (Agahi *et al.*, 2005; Rokonuzzaman *et al.*, 2008) in rice. Spikelet panicle^{-1} had negative direct effect with significant genetic correlation with grain yield. On the other hand, effective tiller m^{-2} had positive indirect effect through all the traits except thousand grain weight but days to maturity, plant height, panicle weight and spikelet panicle^{-1} had negatibe indirect effect on grain yield through most of the other traits. The indirect effects of spikelet fertility for other traits indicated the importance of direct selection using spikelet fertility to select high yielding genotypes. The residual effect of the present study was 0.017, indicating that about 98 percent of variability in grain yield might be contributed by these eight yield contributing traits studied in the path analysis. This gives an impression that some other minor characters than those involved in the present study also contributed to the variability of grain yield.

CONCLUSION

The estimation of genetic parameters, correlation and path coefficient of some promising hybrid rice for grain yield and yield contributing characters indicated that spikelet panicle^{-1} had high both genotypic and phenotypic variance with high heritability and high genetic advance. Effective tiller m^{-2} had high phenotypic variance with high genetic advance. Spikelet fertility had the highest genotypic and phenotypic correlation coefficient on yield.

Table 4. Path coefficient showing direct and indirect effects of yield components on grain yield.

Character	Day to maturity	Plant height (cm)	Effective tiller m^{-2}	Spikelet panicle^{-1}	Panicle length (cm)	Spikelet fertility (%)	Panicle weight (g)	TGW (g)	Genotypic correlation with grain yield
Day to maturity	**0.655**	0.011	0.034	-0.044	-0.031	-0.635	0.008	-0.050	0.0785
Plant height (cm)	0.273	**0.027**	-0.010	-0.077	0.105	-0.939	0.040	-0.003	0.1875
Effective tiller m^{-2}	0.215	-0.003	**0.103**	0.074	0.060	0.252	0.018	-0.045	0.8275**
Spikelet panicle^{-1}	0.069	0.005	-0.018	**-0.418**	0.169	-0.128	0.051	0.020	0.5669*
Panicle length (cm)	-0.051	0.007	0.015	-0.176	**0.404**	-0.050	0.049	-0.016	0.2230
Spikelet fertility (%)	-0.372	-o.023	0.023	0.048	-0.018	**1.117**	-0.018	-0.004	0.9103**
Panicle weight (g)	0.063	0.013	0.021	-0.253	0.236	-0.241	**0.085**	-0.008	-0.0021
Thousand grain weight (g)	0.378	0.001	0.054	0.096	0.074	0.046	0.007	**-0.087**	0.1886

Bold figures indicate the direct effect, Residual effect=0.017.

On the other hand, effective tiller m^{-2} and spikelet panicle^{-1} had significant only genotypic correlation coefficient on grain yield. Spikelet fertility had high direct effect and highly significant positive correlation with grain yield followed by days to maturity, effective tiller m^{-2} and panicle length (cm). Hence these four important traits, could be considered as selection criteria for the development of high yielding hybrid rice varieties.

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Adaptation of Promising Rice Genotypes for Broadcast Aus Season

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ABSTRACT

Rice cultivation in Aus season is eco-friendly and essential for sustainable food security of Bangladesh utilizing rain water rather than extracting underground water. Four rice genotypes (BR6855-3B-12, BR6855-3B-13, BR6848-3B-12 and BR6976-2B-11-1) along with check variety BRRI dhan43 were evaluated at farmers' field during broadcast Aus season. The objectives were to investigate the adaptability of the rice genotypes and find out promising genotype(s) for Broadcast Aus rice. The experiment was conducted at farmers' field in eight agro-ecological zones of Bangladesh. Randomized complete block design (RCB) was used with three replications. Standard and uniform management practices were followed for all the locations. Data were taken on grain yield, days to 80% maturity, plant height, panicles m^{-2}, 1000-grain weight, grains $panicle^{-1}$, spikelet sterility (%), Phenotypic acceptance at vegetative and maturity stage, pest incidence; feedback of farmers and extension personnel. Genotypes, environments and interaction of genotypes by environments had significant effect on grain yield, yield components and agronomic parameters. Analysis of variance indicated that there were significant differences between the check (control) and improved genotypes except the genotype BR6976-2B-11-1 which was similar with the check BRRI dhan43 for all parameters. Correlation analysis was also performed to establish extent of association between yield and major yield components. Grain yield positively and significantly correlated with the agronomic parameters except spikelet sterility. Across the locations, BR6848-3B-12 produced significantly the highest grain yield (4.88 t ha^{-1}) at Feni while the lowest grain yield (1.58 t ha^{-1}) was obtained in BR6976-2B-11-1 followed by BRRI dhan43 (1.64 t ha^{-1}) at Kushtia. BR6848-3B-12 showed yield advantage of 1.0 t ha^{-1} over the check variety BRRI dhan43 with similar growth duration (104 day). AMMI stability value, yield stability index and GGE Bi-plot model indicated that BR6855-3B-12 is the most adaptable, suitable and stable genotype for broadcast Aus rice across the locations, and finally it is released in 2017 as BRRI dhan83 for broadcast Aus season.

Key words: AMMI, broadcast Aus, G×E interaction, GGE Bi-plot, grain yield, sterility, panicle, phenotypic acceptability

INTRODUCTION

Rice is the staple food crop in Bangladesh and occupies about 80% of the country's total cropped area (BRRI, 2019). The total area of the country is 14.86 million ha (147,570 sq. km) (Shelley *et al.,* 2016) and the cultivable area is 8.57 million ha (Kabir *et al.,* 2015). Rice is cultivating nearly in 11.42 m ha (Karmakar and Ali, 2019; BBS, 2018) of which Aus, Aman and Boro rice are covered 9.3, 48.9 and 41.8%, respectively (BRRI, 2019; BBS, 2019). There are three rice-growing seasons in Bangladesh namely Aus, Aman, and Boro. Aus rice is important for environments as it is less water requiring cultivated in pre-monsoon upland rice-growing season under rainfed environments in Bangladesh. Broadcast Aus rice is established through direct broadcasting the seeds in April after the pre-monsoon shower and harvested at July to August (Shelley *et al.,* 2016) but it is practically

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harmonized with the climatic season hot summer (Rahman *et al.*, 2018). There are two types of Aus rice such as broadcast/direct seeded Aus also known as upland Aus and partially irrigated Aus or transplanted Aus (TPR-Aus) in Bangladesh. Also transplanting cost is one of the major resource-consuming activities and it could be reduced through cultivating direct-seeded rice (DSR-Aus). Aus rice occupies about 12.53% of total cultivable area from where modern varieties cover 10.67% and local varieties cover 1.86%. The present status of total area and production of Aus rice is 1.08 million ha and 2.71 million MT (BBS, 2018).

Puddling soil in repeated manner for transplant rice adversely affects its fertility properties along with declining the subsurface absorptivity (Sharma *et al.,* 2003). This may cause destruction of soil mass and formation of hard aggregates underneath the superficial layer (Gathala *et al.,* 2011). Consequentially, it may affect deleteriously the production of other rotating crops rather than rice (Gopal *et al.,* 2010). Direct seeded broadcast rice would be an attractive alternative on the face of global water scarcity and escalating labour rates in future. In these points of view, direct seeded broadcast rice received much attention in many countries like Australia, America, Europe, Philippines, and Malaysia etc. because of its low input demand (Farooq *et al.*, 2011). Considering that lack of high yielding good quality broadcast rice variety is one of the major constraints. Rice cultivation in Aus season is somewhat decreasing while it is increasing in Boro season, but it is not good for sustainable agriculture. Drought is one of the major abiotic constraints for rice grown under rainfed conditions in Bangladesh and causes a substantial reduction in yield (Shelley *et al.,* 2016). Rice cultivation in Boro season (Dry season) will be a great challenge in future due to the falling down of underground water level in different areas of Bangladesh. Due to global warming, manipulation of evapotranspiration takes place in soil, green-house gas emission and other adverse effects pertinent to this are motivating broadcast seeding of rice (Monaco *et al.,* 2016). Aus rice could suffer from drought any time from the seedling to reproductive stages, as the crop is direct-seeded and grown under rainfed upland conditions (Biswas, 2014). However, the traditional Aus varieties have some tolerance to drought and can overcome drought if some rain occurs in June. The yield potential of these rice varieties is very low. The improvement in the yield potential of upland rice may increase production and reduce its cost (Hossain *et al.,* 2013). We have to give more emphasis on the Aus (Pre-wet season rice) and Aman (Wet season rice) based cropping pattern because of uncertainty of water availability for Boro cultivation. Although we have limited scope of horizontal expansion for rice cultivation but still we have some areas in Aus season to explore for rice cultivation. Many fields in our country remain fallow in Aus season which could be used for rice cultivation and it is now very much essential for our sustainable food security. Moreover, rice cultivation in Aus season is also environment-friendly because of its use of rain water without extracting undergroud water. But it is comparatively complex than the other seasons due to unfavourable climatic condition which favours the insect attack and disease incidence. Dry direct seeding of rice is profitable if sufficient weed control approaches are maintained (Rao *et al.,* 2007). Akhgari and Kaviani (2011) reported that DSR can help rice farming communities bring down the labour cost but the only challenge is the weed pressure. With the short duration of growth and high yielding properties of Aus varieties and new herbicides controlling weeds to a large extent has made the transformation of seeding methods from transplanting to broadcast technically viable (Mortimer *et al.,* 2008).

DSR could be a potential substitute for transplanted rice, if weeds are well managed

(Johnkutty, 2002). Mann (2007) in Pakistan also obtained rice yield of 3.70 ton/ha in a weed free direct seeded rice trial. Similarly, there are many literatures and studies that mention potential of direct seeded rice (Manjunatha *et al.,* 2009 and Sanusan *et al.,* 2010). On the other hand, we do not have potential good broadcast Aus variety with higher yield and shorter growth duration. Sometimes farmers do not show interest to cultivate rice in Aus season, especially broadcast Aus as they feel that it may not be cost effective for them. Despite controversies, if properly managed and developed high yielding varieties, comparable yield may be obtained from direct seeded broadcast rice compared with transplanting rice (Farooq *et al.,* 2011). That is why, we need potential broadcast Aus variety characterised by higher yield, shorter growth duration, good grain quality and also drought tolerent, which are the main criteria for our farmers' attraction to cultivate rice. Therefore, some promising rice genotypes were evaluated along with existing broadcast Aus variety BRRI dhan43 as check in eight different locations of Bangladesh. Instability is the result of cultivars response in different environments which usually indicates a high interaction between genetic and environmental factors (Lone *et al.,* 2009). Grain yield depends on genotype, environment and management practices and their interaction with each other (Messina *et al.,* 2009). Under the same management conditions, variation in grain yield is principally explained by the effects of genotype and environment (Dingkuhn *et al.,* 2006). The development of rice and other crops is affected by effects of the environment (E), genotype (G) and their interaction (GE) (Mattos *et al.,* 2013). Several statistical methods were used separately to evaluate genotypes and environments of different crops of multi-location trials and GEI data (Malosetti *et al.,* 2013). There are two multivariate analysis such as Additive main effect and multiplicative interaction (AMMI) model and genotype plus genotype by environment interaction (GGE) biplot analysis has been used in this study. These two statistical methods (AMMI and GGEBiplot) have wider importance for agricultural researchers because they affect to any two-way data matrices (Farhad *et al.,* 2017; Crossa, 1990). AMMI analysis can also be used to find out the stability of the genotypes across locations using the PCA (principal component axis) scores and AMMI stability value (Sandhu *et al.,* 2019; Purchase, 1997). Research on adaptability of the Aus rice varieties would help farmers to cultivate right varieties in right environments as well as the policy makers to formulate decision in this regard. The major objectives of the present study were to assess the stability and yield performance of promising broadcast Aus rice genotypes evaluated in multi-environmental conditions and to discover stable high yielding variety(s) for possible release using various statistical tools.

MATERIALS AND METHODS

Four advanced lines BR6855-3B-12 (G1), BR6855-3B-13 (G2), BR6848-3B-12 (G3) and BR6976-2B-11-1 (G4) along with BRRI dhan43 (G5) as check were tested in Gazipur (E1), Kapasia (E2), Noakhali sadar (E3), Feni (E4), Sylhet (E5), Faridpur (E6), Magura sadar (E7) and Kushtia (E8) during B. Aus 2014. The experiment was conducted in RCB design with three replications. The unit plot size for each entry was 15 m^2 (3 m × 5 m). The time of seed sowing in main field at different locations was not same and the dates were within 15-30 April 2014. Direct seeding in line was done with 25 cm row spacing. Fertilizers urea, triple super phosphate, Muriate of potash, gypsum and zinc sulfate were applied @ 60, 10, 40, 10 and 4 kg N, P, K, S and Zn ha^{-1}, respectively. All the fertilizers except urea were applied as basal and urea was applied in three equal splits at 20, 35 and 45 days after seeding

(DAS). Standard and uniform crop management practices were followed in all the locations. Appropriate measures were taken to control insect but diseases were not controlled to identify susceptibility and tolerance level of the genotypes.

Date of seeding, transplanting, flowering and maturity, plant population m^{-2}, phenotypic acceptance at vegetative and ripening stage, plant height, lodging tolerance, yield and yield components, disease and insect incidence were recorded. Plant population (stand) m^{-2} was counted at 30 days after seeding (DAS). Feedback from farmers and extension personnel were also recorded. For yield estimation, 10 m^2 sample area from each plot was harvested at maturity and grain yields were adjusted to 14% moisture content. Data were analyzed using the statistical software CropStat7.2.

A combined analysis of variance was performed treating genotype as fixed effect and environment as random effect. The most recent statistical method is the AMMI (Additive Main Effect and Multiplicative Interaction) described by (Zobel *et al.*, 1988) was used to investigate the main effects (G and E) and G×E interactions for grain yield of multi-environment data which includes analysis of variance and principal component analysis. A formula of AMMI stability value (ASV) developed by Purchase *et al.,* (2000) based on the AMMI model's IPC1 and IPC2 scores for each genotype was used to find the stable genotype:

$$ASV = \sqrt{\frac{SSPC1}{SSPC2}(PC1)^2 + (PC2)^2}$$

To integrate both yield performance and stability in a single index namely yield stability index (YSI) statistic (Farshadfar *et al.,* 2011) was applied for selecting high-yielding and stable genotypes. YSI was calculated based on the rank of mean yield of genotype (RY) and rank of ASV (RASV) in a single criterion (YSI) as:

$$YSI = RASV + RY$$

Ranks were assigned for mean yield and stability parameter, so that the genotype with the highest yield and the lowest estimated value for each statistic given a rank of 1 (Farshadfar *et al.,* 2011; Roostaei *et al.*, 2014). The genotypes with the lowest ASV value would be more stable. Also GGEbiplot is used to interpret the GE interaction of grain yield of multi-environment trial data (Yan, 2002; Yan *et al.*, 2000). GGEbiplot analysis is a visualization method which graphically displays a GE interaction in two-way table for mega-environment analysis (e.g., which-won where pattern) that means specific genotypes can be recommended to specific mega-environments; the mean performance and stability (genotype evaluation) and environmental evaluation.

RESULTS AND DISCUSSION

The interaction effect of location and genotype for grain yield, growth duration, plant height, panicles per unit area, 1000-grain weight, sterility, grains $panicle^{-1}$ and plant population m^{-2} at 30 DAS (days after seeding) were highly significant at the 0.01 level. The individual effects of genotypes and locations on different parameters were also highly significant at 0.01 levels. As interaction effect for all parameters are significant, the individual effect of genotypes or location is not important for discussion. However, tables 1 and 2 help to have an overall idea about the performance of lines. Grain yield of the tested genotypes varied significantly in different locations due to environmental effect and the tested entries were also varied within the locations (Table 3).

Yield stability index (Ysi)

The rank of ASV and mean grain yield are incorporated in a single stability index namely yield stability index (YSI). YSI allows the simultaneous selection for yield and stability performance; on this basis genotypes with the least YSI represent stable genotypes with the high performance (Farshadfar *et al.*, 2011). YSI values indicated that BR6855-3B-12 and BR6855-3B-13 genotype with YSI=3 were high-yielding and stable, in contrast BRRI dhan43 (ck), BR6976-2B-11-1 and BR6848-3B-12 with the highest values (9, 8 and 7 respectively) were identified as unstable genotypes based on YSI (Table 1). Table 2 shows that yield had positive correlation with yield components like plant height, panicle m^{-2}, 1000-grain weight, grains panicle but it had negative correlation with the spikelet sterility of rice genotypes. This finding is in close agreement with the findings of Ndebeh *et al.*, 2018.

Table 1. AMMI stability value (ASV) and mean performance for grain yield ($t\ ha^{-1}$) of the rice genotypes grown at eight environments in Bangladesh.

Genotype code	Genotype	ASV	YSI	rASV	rYSI	Grain yield ($t\ ha^{-1}$)
G3	BR6855-3B-12	0.282	3	2	1	3.64
G2	BR6855-3B-13	0.085	3	1	2	3.40
G1	BR6848-3B-12	0.720	7	4	3	3.21
G4	BR6976-2B-11-1	0.715	8	3	5	2.63
G5	BRRI dhan43 (ck)	0.970	9	5	4	2.68

Table 2. Correlation coefficient analysis of yield and yield components of the rice genotypes.

	GY	GD	PHT	PPM	TGW	STER	GPP
GY	1						
GD	0.07	1					
PHT	0.70***	0.04	1				
PPM	0.98***	0.06	0.82***	1			
TGW	0.54**	0.87***	0.40*	0.55**	1		
STER	-0.95***	0.19	-0.81***	-0.97***	-0.31	1	
GPP	0.99***	0.04	0.81***	0.99***	0.52**	-0.97***	1

***= 1% level of significance, **= 5 % level of significance, GY= Grain yield ($t\ ha^{-1}$), GD= Growth duration, PHT= Plant height, PPM= Panicle per m^2, TGW= 1000 grain weight, STER= Sterility (%), GPP= Grain per panicle.

Table 3. Performance of yield components of the rice genotypes, Broadcast Aus 2014.

Gen	Genotype	GY	rGY	GD	rGD	PHT	rPHT	PPM	rPPM	GPP	rGPP	TGW	rTGW	STER	rSTER
G1	BR6855-3B-12	3.21	3	109	1	109	2	252	3	70	3	28.2	2	28.9	3
G2	BR6855-3B-13	3.40	2	108	2	108	3	255	2	71	2	28.5	1	27.3	4
G3	BR6848-3B-12	3.64	1	104	5	112	1	258	1	75	1	24.3	3	24.1	5
G4	BR6976-2B-11-1	2.63	5	106	3	91	5	241	5	60	5	23.3	4	32.6	1
G5	BRRI dhan43 (ck)	2.68	4	105	4	108	4	245	4	64	4	23.0	5	30.2	2
$LSD_{0.05}$		0.53		2.0		2.5		17.1		7.0		0.6		5.7	
CV (%)		10.4		1.1		1.5		4.2		6.3		1.3		12.0	

GY= Grain yield ($t\ ha^{-1}$), GD= Growth duration, PHT= Plant height, PPM= Panicle per m^2, TGW= 1000 grain weight, STER= Sterility (%), GPP= Grain per panicle, r= rank of (GY, GD, PHT, PPM, GPP, TGW, STER).

Grain yield

Grain yield was significantly influenced by genotypes, environment and their interaction (Table 4 and Fig. 1). This result was in conformity with the earlier findings of Babu *et al.* (2012), Karmakar *et al.* (2015) and Vanisree *et al.* (2013). Irrespective of locations and genotypes, the highest mean grain yield (3.64 t ha^{-1}) was obtained in BR6848-3B-12 and it was almost 1.0 t ha^{-1} higher than the standard check variety BRRI dhan43 (2.68 t ha^{-1}). The genotype BR6848-3B-12, BR6855-3B-12 and BR6855-3B-13 performed better in almost all the locations possibly due to their higher adaptation to different environments. These findings corroborate with the report of Ndebeh *et al.*, 2018. Across the locations, the highest grain yield (4.88 t ha^{-1}) obtained in BR6848-3B-12 at Sonagazi, Feni that followed by Magura (4.81 t ha^{-1}). It is might be due to the environments was suitable for obtaining higher yield performances. Grain yield of the genotypes varied from location to location might be due to environmental effect (Karmakar *et al.*, 2015; Sakai *et al.*, 2010). The lowest yield (1.58 t ha^{-1}) was found in BR6976-2B-11-1 followed by BR6855-3B-13 (2.10 t ha^{-1}) at Veramara, Kushtia (Table 4). Overall yield performance of the genotypes was good and consistent without some exceptions in Veramara, Kushtia and Madhukhali, Faridpur where the trials were suffered by drought and scarcity of rain water. In respect to yield and growth duration, most of the tested genotypes except BR6976-2B-11-1 performed better than the check variety BRRI dhan43.

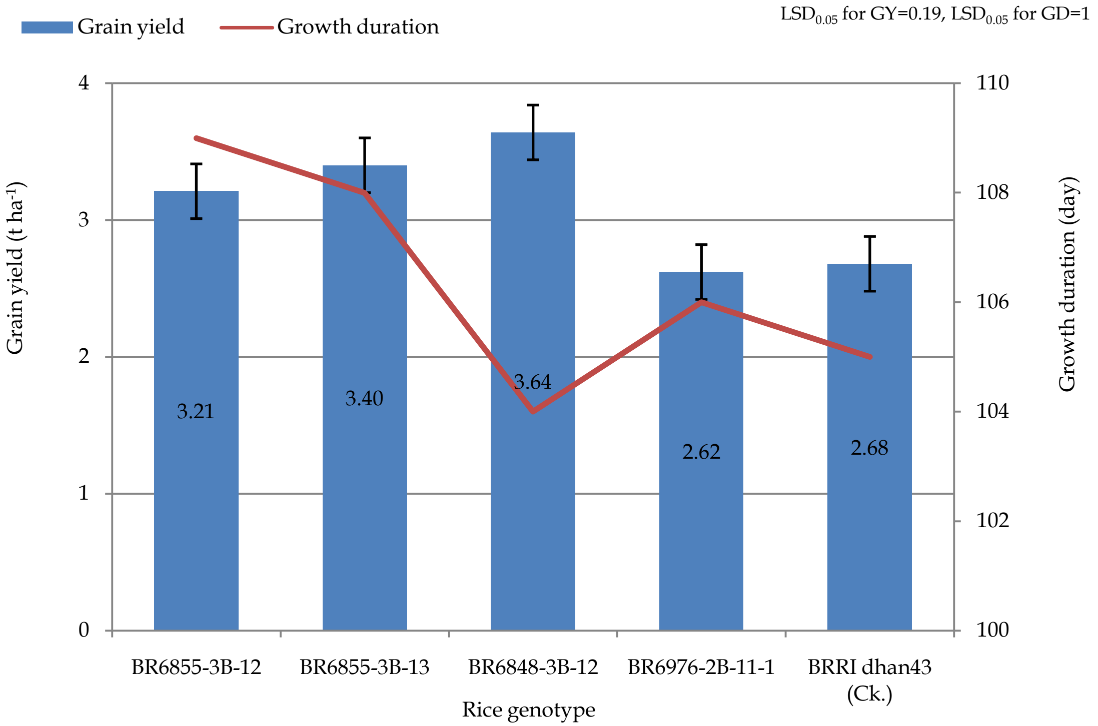
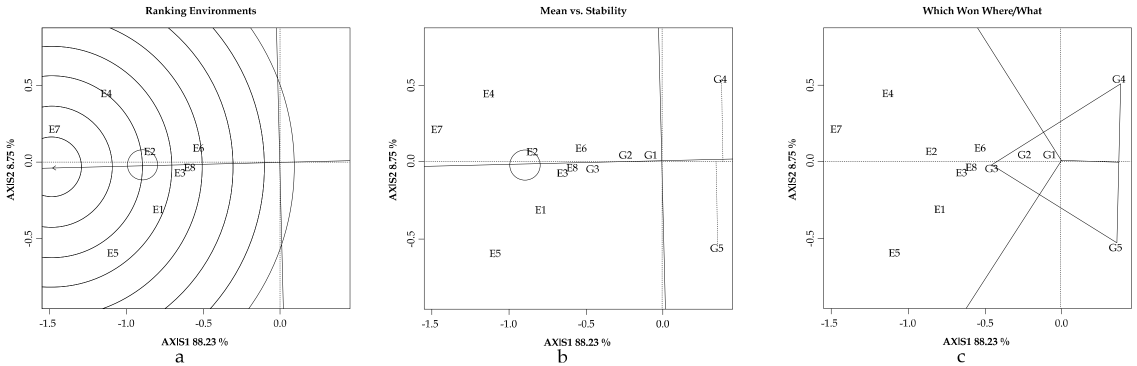


Fig. 1. Mean grain yield and growth duration of genotypes, broadcast Aus 2014.

Table 4. Interaction effect of genotypes and environments (G x E) on grain yield and growth duration of the rice genotypes, Broadcast Aus 2014.

Location Genotype	Gazipur (BRRI)	Gazipur (Kapasia)	Noakhali (Sadar)	Feni (Sonagazi)	Sylhet (Golapganj)	Faridpur (Madhukhali)	Magura (Sadar)	Kushtia (Veramara)
				Grain yield (t ha^{-1})				
BR6855-3B-12	2.84	2.90	3.32	4.29	3.31	2.74	4.08	2.18
BR6855-3B-13	3.05	3.32	3.46	4.31	3.47	2.90	4.56	2.10
BR6848-3B-12	3.37	3.60	3.34	4.88	3.94	2.96	4.81	2.26
BR6976-2B-11-1	2.23	2.65	2.68	3.83	2.25	2.45	3.34	1.58
BRRI dhan43 (ck)	2.68	2.57	2.78	3.22	3.11	2.34	3.10	1.64
F-test	**	**	**	**	**	**	**	**
LSD$_{0.05}$					0.53			
				Growth duration (day)				
BR6855-3B-12	108	109	112	112	106	107	108	107
BR6855-3B-13	107	109	108	111	106	107	109	107
BR6848-3B-12	104	106	105	107	101	103	106	102
BR6976-2B-11-1	105	107	108	107	103	106	107	106
BRRI dhan43 (ck)	103	105	106	108	102	105	107	106
F-test	**	**	**	**	**	**	**	**
LSD$_{0.05}$					2			

The GGE biplot explained 88.23% and 8.75% accounted 96.98% of the total variation of the environments for grain yield. GGE biplot showed three distinct clusters in one containing four locations Kapasia Gazipur (E2); Noakhali (E3); Kushtia (E8) and Faridpur (E6), and the second containing Gazipur sadar (E1) and Sylhet (E5), third one containing Feni (E4); and Magura (E7) (Fig. 2.a). Among experimental location first cluster of environments are more stable environments and Kapasia Gazipur (E2) is the ideal location among them for testing genotypes based on GGEBiplot analysis. The closest association was observed between the environments Noakhali (E3), and Kushtia (E8); and Kapasia Gazipur (E2) and Faridpur (E6). To evaluate the genotypes according to ranking with reference to the mean value of grain yield stability across environments, the AEC method can be used in the GGE biplot graph (Fig. 2.b). BR6848-3B-12 (G3) recorded the highest average grain yield. BR6848-3B-12 (G3), BR6855-3B-13 (G2), and BR6855-3B-12 (G1) were the most stable genotypes with above-average yields. Thus, the BR6848-3B-12 (G3) was the most ideal genotype with the highest mean yield and stability among the tested genotypes. Also BR6976-2B-11-1 (G4) and BRRI dhan43 (ck) (G5) were unstable and low yielder genotypes. The biplot showed one sector containing all the test environments and BR6848-3B-12 (G3), BR6855-3B-13 (G2) and BR6855-3B-12 (G1) were the winning genotypes and Kapasia Gazipur (E2), Noakhali (E3), Noakhali (E6) and Kushtia (E8) were the best for tested locations of these three genotypes (Fig. 2.c).



a b c

Fig. 2. GGE Biplot for (a) ranking environments (b) mean vs stability (c) which won where for broadcast Aus 2014.

Breeding View software (Breeding Management System v3.9) (https://integratedbreeding.net/) a version of GenStat software was used for analyzing grain yield data for characterizing environments and interactions to the genotypes performance through Finlay-Wilkinson regression (Malosetti, *et al.,* 2013). Based on grain yield, two environments (Feni and Magura) showed high performing environment having median yield >4.0 t ha^{-1} with characteristics with higher variances. Kushtia performed poor showing median yield about 2 t ha^{-1} but the rest five environments perform moderately median yield ranged from 2.75 to 3.25 t ha^{-1}) (Fig. 3). Correlation between environments showed all eight environments have strong positive correlations (Fig. 4) indicating any better genotypes selected from this study could be recommended for similar environments. Finlay-Wilkinson regression represents the yield performances of the genotypes across environments in visual mode (Fig. 5). Among the tested four genotypes, three (BR6848-3B-12, BR6848-3B-13 and BR6848-3B-12) performed superiorly compared to BR6976-2B-11-1 and check BRRI dhan43. Among the three best performing genotype, BR6848-3B-12 was found superior for all three categories of environments (poor, moderate and high performing environment). Moreover, the superior genotype (BR6848-3B-12) out yielded in the high performing environments indicating it has potentiality to perform superiorly in all high performing environments as well as performing better in moderate and poor environments (Fig. 5).

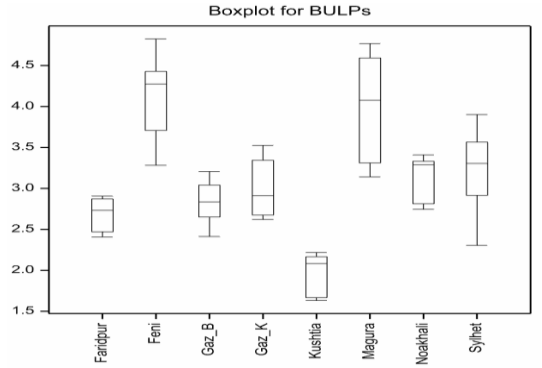


Fig. 3. Boxplot for grain yield of the rice genotypes representing performance of the environments based on yield of B. Aus rice (Gaz_B = BRRI, Gazipur and Gaz_K=Kapasia, Gazipur).

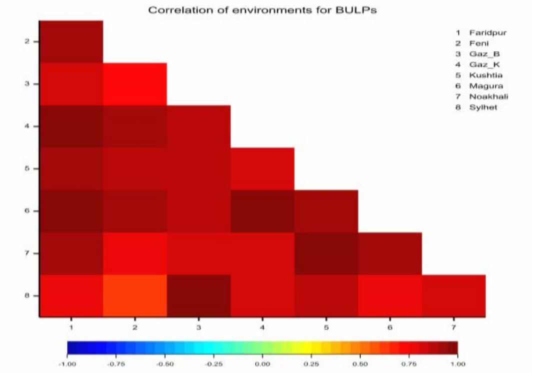


Fig. 4. Correlation between environments based on yield performances of the rice genotypes (Gaz_B = BRRI, Gazipur and Gaz_K=Kapasia, Gazipur).

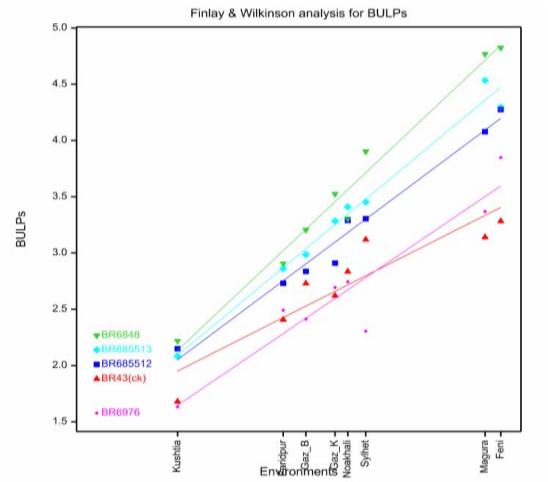


Fig. 5. Finlay-Wilkinson Regression for grain yield of the rice genotypes representing performances and interactions between genotype (BR6848 = BR6848-3B-12, BR685513 = BR6858-3B-13, BR685512 = BR6858-3B-12, BR43 (ck) = BRRI dhan43 and BR6979 = BR6979-2B-11-1) and environments (Gaz_B = BRRI, Gazipur and Gaz_K = Kapasia, Gazipur).

Growth Duration

Growth duration of the genotypes widely and significantly varied across the genotypes and locations (Table 4). These findings are in alignment with Karmakar *et al.* (2015) and Sakai *et al.* (2010). Mean growth duration of the tested genotypes ranged from 104 to 109 days whereas it was 106 days for the check variety BRRI dhan43 (Table 4). The highest yielder, BR6848-3B-12 matured within the shortest period of time (104 days) which was two days earlier than that of check the variety BRRI dhan43 (106 days). The growth duration of 2nd (BR6855-3B-13) and 3rd (BR6855-3B-12) highest yielder were 108 and 109 days, respectively which were little bit longer (1-3 days) than BRRI dhan43.

Plant population

Plant population (stand) counted at 30 DAS was significantly affected by genotypes, locations and their interactions (Table 5). Plant population per unit area (per square meter) varied from 169 to 215 for the tested advanced lines and it was 165 for the check variety BRRI dhan43 (Table 5). Across the locations, the highest plant population m^{-2} (250) was found in BR6848-3B-12 at Magura and the lowest (130) was in BRRI dhan43 at Veramara, Kushtia. Depending on rain water, rice cultivation in Aus season by broadcasting is comparatively difficult. Especially seed germination is hampered due to unavailability of soil moisture which might be affected the homogenesity of this experiment for all locations.

Plant height

Genotypes by environments interaction had significant effect on plant height of the genotypes (Table 5). Across the locations, the longest plant (113 cm) was found in the genotype BR6848-3B-12 at Noakhali and Feni that was followed by BRRI dhan43 (112 cm) at Feni. Plant height of BR6976-2B-11-1 was horter at alomost all the locations. It might be due to its genetical characteristcs. Plent height of the genotypes ranged from 85 to 113 cm across the locations. Plant height of the highest yielder was significantly taller than all other the geontypes including check variety BRRI dhan43. However, plant height of 2nd and 3rd yielder were was similar to that of the check variety. The plant height of the tested genotypes were also very much expective and similar to that of check variety which might be a promising one for Broadcast Aus season in Bangladesh.

Table 5. Interaction effects of genotypes and environments on plant stand m^{-2} at seedling stage (30 DAS) and plant height at maturity stage.

Genotype	Gazipur (BRRI)	Gazipur (Kapasia)	Noakhali (Sadar)	Feni (Sonagazi)	Sylhet (Golapganj)	Faridpur (Madhukhali)	Magura (Sadar)	Kushtia (Veramara)	Mean
				Plant stand m^{-2}					
BR6855-3B-12	206	210	216	231	223	189	228	133	205
BR6855-3B-13	202	208	211	236	225	232	231	151	212
BR6848-3B-12	204	197	227	244	238	232	250	146	217
BR6976-2B-11-1	146	162	168	224	192	215	231	136	184
BRRI dhan43 (ck)	176	180	183	212	207	152	201	130	180
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			33						12
				Plant height (cm)					
BR6855-3B-12	109	111	110	111	108	107	110	105	109
BR6855-3B-13	108	111	109	111	106	107	107	106	108
BR6848-3B-12	112	112	113	113	111	111	112	111	112
BR6976-2B-11-1	92	89	93	96	86	90	95	85	91
BRRI dhan43 (ck)	109	107	110	112	106	106	109	104	108
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			3						1

Yield components

Yield components like panicle m^{-2}, 1000-grain weight (TGW), grains panicle^{-1} and spikelet sterility were significantly affected by the genotypes, environments and their interactions (Table 6). Panicles production per unit area (m^2) varied from 206 to 281 for different genotypes including check variety across the locations. This finding was in conformity with the findings of Ndebeh *et al.* (2018). The highest number of panicles m^{-2} (281) obtained in BR6855-3B-13 at Sonagazi, Feni that was followed by BR6855-3B-12 (280) and BR6848-3B-12 (279). The lowest number (206) of panicles m^{-2} was recorded in BR6976-2B-11-1 at Veramara, Kushtia followed by BRRI dhan43 (212). In general, panicles production per unit area in Veramara, Kushtia was lower for all the genotypes compared to the other locations as the crop of Kushtia severely affected by drought stress that reflected also in grain yield.

Grain weight of the tested genotypes significantly affected by the interaction of genotypes and environments (Table 6). Lack *et al.* (2012) also stated that there was a significant difference between cultivars regarding grain weight. Across the locations, the highest TGW (28.8 g) was found in BR6855-3B-13 at Magura followed by Feni (28.7 g). The lowest 1000-grain weight (22.7 g) was obtained in BRRI dhan43 at Veramara, Kushtia followed by Madhukhali, Faridpur and Gazipur (22.8 g). Grain weight of the tested genotypes including the check variety was lower compared to the other locations. It was happened might be due to drought effect at Veramara, Kushtia as the crop suffered severe drought stress at that location. In general, grain weight of all the genotypes was higher than the check variety BRRI dhan43. Mean TGW of the highest yielder was 24.3 g which was much lower than that of 2nd and 3rd highest yielder (28.7g) and it indicates the fineness and slenderness of the advanced line BR6848-3B-12. Although grain weight is one of the important genetic characteristic, however it was remarkedly influenced by the locations might be due to environmental effect.

Grains production per panicle of the genotypes was substantially variable across the genotypes which were affected by genotypes, environments and their interaction (Rai and Kushwaha, 2008). Grains panicle^{-1} ranged from 52 to 85 across the locations. The highest yielder BR6848-3B-12 produced the highest number of grains (85) at Feni while the lowest yielder BR6976-2B-11-1 produced the lowest number of grains panicle (52) at Kushtia and Gazipur (Table 6). Grains panicle^{-1} was in generally, lower in trend as the crop was grown under rainfed condition, and the crop suffered different stresses especially water stress in some locations.

Spikelet sterility varied significantly among the genotypes, environments and their interaction (Table 6). Sterility was ranged from 21 to 42% across the locations (Table 6). Genotypes had strong significant effect spikelet sterility (Karmakar *et al.,* 2015) The highest sterility (42%) observed in the lowest yielder BR6976-2B-11-1 at Veramara, Kushtia that was followed by BRRI dhan43 (40%). Spiklets sterility negtively correlated with grain yield and it affected higher yield of BR6976-2B-11-1 and BRRI dhan43. Sterilty increased with increasing drought stress across the locations.

Table 6. Interaction effect of genotypes and environments on yield components of the rice genotypes.

Genotype	Gazipur (BRRI)	Gazipur (Kapasia)	Noakhali (Sadar)	Feni (Sonagazi)	Sylhet (Golapganj)	Faridpur (Madhukhali)	Magura (Sadar)	Kushtia (Veramara)	Mean
				Panicles m^{-2} (no.)					
BR6855-3B-12	243	247	259	280	264	244	261	215	252
BR6855-3B-13	245	249	260	281	263	253	272	218	255
BR6848-3B-12	254	256	253	279	265	261	268	230	258
BR6976-2B-11-1	237	241	245	262	247	228	260	206	241
BRRI dhan43 (ck)	245	242	255	260	251	231	267	212	245
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			17						6
				1000-grain weight (g)					
BR6855-3B-12	28.2	28.4	28.1	28.6	28.4	28.1	28.4	27.5	28.2
BR6855-3B-13	28.3	28.5	28.6	28.7	28.5	28.6	28.8	27.6	28.5
BR6848-3B-12	24.3	24.6	24.4	24.5	24.0	23.9	24.5	23.8	24.3
BR6976-2B-11-1	23.1	23.2	23.0	23.5	23.3	23.1	23.6	23.0	23.2
BRRI dhan43 (ck)	22.8	23.1	23.0	23.3	23.1	22.8	23.4	22.7	23.0
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			0.6						0.2
				Grains panicle^{-1} (no.)					
BR6855-3B-12	65	68	71	80	71	68	77	56	70
BR6855-3B-13	70	72	68	81	73	69	80	57	71
BR6848-3B-12	69	71	72	85	75	81	82	66	75
BR6976-2B-11-1	52	55	65	68	66	60	61	52	60
BRRI dhan43 (ck)	63	61	64	69	66	62	67	58	64
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			7						3
				Sterility (%)					
BR6855-3B-12	31	28	27	24	27	33	26	35	29
BR6855-3B-13	26	23	27	26	24	32	27	34	27
BR6848-3B-12	25	25	24	21	23	24	22	30	24
BR6976-2B-11-1	36	32	30	27	33	32	29	42	33
BRRI dhan43 (ck)	29	31	28	25	28	35	26	40	30
F-test	**	**	**	**	**	**	**	**	**
LSD$_{0.05}$			5						2

Insect infestation

Proper control measures were taken as and when necessary so that the trials were almost free from insect's damage. However, stem borer (3-10%) and rice bug infestations (5-15%) were common in most of the locations.

Disease infection

Most of the genotypes including check variety BRRI dhan43 were found to be disease free in most of the locations except Gazipur and Sylhet where almost all the entries were affected by some diseases. BRRI dhan43 was less infected by sheath blight (10-15%) and BR6855-3B-13 was affected by sheath rot (20%). In Sylhet, all the entries were affected by Sheath blight ranged from 5 to 90% and Brown spot (3-10%). In Kushtia, brown spot (5-10%) was found in all entries.

Lodging incidence

The tested genotypes were lodging tolerant in all locations except at Gazipur and Feni sites. Due to heavy rain and stagnant water, almost all the genotypes including BRRI dhan43 were lodged by 25-60%, however the highest yielder BR6848-3B-12 was found to be less lodged (25%) at Gazipur site. Moreover, BRRI dhan43 was lodged by 50% but the tested genotypes showed lodging tolerant at Sonagazi of Feni district.

Feedback of farmers and extension personnel

Most of the farmers and extension personnel preferred BR6848-3B-12 for its higher yield, shorter growth duration and medium bold reddish grain. Some farmers also preferred BR6855-3B-12 and BR6855-3B-13 for their good yield and shorter growth duration.

CONCLUSION

Among the tested genotypes, BR6848-3B-12 was the most productive and it had about 1.0 t ha^{-1} yield advantage over the check variety BRRI dhan43. All the tested genotypes were found almost disease free in all the locations except Gazipur and Sylhet while BRRI dhan43 was infected by sheath blight disease at BRRI Gazipur and Sylhet. Based on overall performances, yield advantage, grain size, growth duration, farmers preference, pest tolerance and phenotypic acceptance; BR6848-3B-12 recommended for proposed variety trial and finally the genotype is released as BRRI dhan83 in 2017 for broadcast Aus season. It is concluded that BRRI dhan83 would have good potential to increase yield and area of broadcast Aus throughout the country.

Table 7. Phenotypic acceptance of the rice genotypes.

Genotype	Comments on phenotypic acceptance	*PAcp Score	
		Veg.	Mat.
BR6855-3B-12	Rice plant remains green up to maturity. Flag leafs are erect. Grains are bold with uniform flowering and maturity.	5	7
BR6855-3B-13	Uniform flowering and maturity, bold grain. Long panicle with green and erect flag leaf	5	7
BR6848-3B-12	Plants remain green up to maturity. Uniform flowering and maturity with medium bold grain. Panicle long and it remains above flag leaf. Rice grain turns in golden radish color at maturity stage.	3	5
BR6976-2B-11-1	Irregular flowering and maturity, Plant too short.	5	9
BRRI dhan43 (ck)	Uniform flowering and maturity, Grain medium bold and poor yield.	5	5

*Phenotypic acceptability (PAcp): 1=Excellent, 3=Good, 5=Fair, 7=Poor, 9=Unacceptable (IRRI, 2014).

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Effect of Nitrogen Rates and Leaf Clipping on Forage and Grain Yield, and Seed Quality of Transplant Aman Rice

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ABSTRACT

Sustainable fodder production is difficult from limited cultivable land occupied for food grain production. This paper presents the results of the experiment designed to test the hypothesis that forage could be produced along rice grain from the same rice field. The aim of the present study was to investigate the influence of N-rates and leaf clipping on forage and grain yield; and seed quality of transplant Aman (wet season) rice. Four nitrogen (N) rates (N_1=46, N_2=69, N_3=92 and N_4=115 kg N ha^{-1}) and four times of leaf clipping viz, C_0=No leaf clipping, C_1=leaf clipping at 25 DAT (Days after transplanting), C_2=40 and C_3=55 DAT were evaluated following split-plot design with three replications. BRRI dhan41 was used in the experiment. N rates, leaf clipping times and its interaction had significant effects on forage and grain yield, yield components and seed quality except thousand grain weight. The results revealed that forage yield increased with increasing N rates and leaf clipping times. Grain yield was higher in higher N rates and early leaf clippings, however, it was decreased in the late clipping (55 DAT). The highest mean grain yield (5.25 t ha^{-1}) was obtained from the treatment combination of 115 kg N ha^{-1} (N_4) and no leaf clipping (N_4C_0) which was statistically similar (5.18 t ha^{-1}) to N_3C_0 (5.18 t ha^{-1}), N_3C_1 (5.10 t ha^{-1}) and N_4C_1 (5.06 t ha^{-1}). The lowest mean grain yield (3.17 t ha^{-1}) was obtained from N_1C_3. Grain yield was reduced by 3, 6 and 24% in C_1, C_2 and C_3, respectively compared to C_0 (No leaf clipping). Qualitative characters (germination and vigour index) of seed increased with increased N rates, however it decreased in the delayed leaf cuttings. Application of 92 to 115 kg N ha^{-1} with early leaf clipping (25 to 40 DAT) might be allowed to attain moderate forage yield, higher grain yield, and quality seed of transplant Aman rice.

Key words: Leaf clipping, forage yield, grain yield, harvest index, vigour index.

INTRODUCTION

The population of Bangladesh is increasing at an alarming rate and the cultivable land is reducing @ 0.4% as it is converting to non-cultivable land due to urbanization and industrialization (Hasan *et al.,* 2013; Karmakar and Ali, 2019). Thus, it is an urgent need to increase rice production through maximization of yield for sustainable food security. For maximizing yield and quality of rice seed, proper agronomic management is required. N-fertilization is an important factor which considerably affects yield, yield components and seed quality of transplant Aman rice and its defficiency impairs the growth and yield of rice (Ahmed *et al.,* 2001a). Enormous efforts are, therefore, needed to formulate nitrogen recommendation for high yielding rice varieties that will technically be feasible, economically viable, socially acceptable and environmentally sound. Moreover, vegetative growth and developmant of rice is generally highly affected by N-fertilization (Ahmed *et al.*, 2001b).

Livestock is an integral part of agriculture in Bangaldesh and plays an important role in agriculture (Ahmed *et al.*, 2001a). Conversely, severe shortage of fodder and feeds, is the biggest constraint of livestock

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production in the country. Farmers use their land for crop production to fullfill the requirement of human consumption. Therefore, they could not able to leave enough land for fodder production due to high pressure on land for crop production for human consumption. So, farmers have limited opportunity to grow fodder crop in single field. Non-availablity of fallow land and pasture for green grass due to high pressure on land for crop production for human consumption create problem for the improvement of livestock sector in Bangladseh. The farmers are not interested to grow fodder crop in these lands because most of the farmers of our country are small holders and they do not show interest to use their land for fodder crop instead of food crop. Severe crisis of green leaf for livestock consumption occurs during August to October, when entire fields are occupied by transplant Aman rice. Moreover, there is a deficit of straw of that time for livestock. To overcome the scarcity of green forage, it is necessary to explore the feasibility of using green leaves of rice as forage without affecting grain yield. It is probable that rice leaves could be used as green fodder to meet fodder deficit because most of the area is covered by rice plants in year round. In rainy season when most of the fields are under water, rice plants remain above water. To overcome the scarcity of food-cum forage, crop like rice seems to be one of the most feasible and economically viable practice to serve the needs of human, cash income and animal feed, particularly for those who have limited resources (Topark-Ngram *et al.*, 1988).

Flag leaf plays an imperative role on grain yield through synthesis and translocation of photo-assimilates to the rice grain (Das *et al.,* 2017; Fatima *et al.,* 2019). The rice crop at vegetative stage may be used as forage, then further allowed for regrowth; and finally it could be harvested for grain at maturity. Use of rice as forage at the vegetative phase of the plant may encourage small farmers to raise more animals on farm. Usually, excess leaf growth of long duration rice plants is grazed at the early vegetative stage. In some deep-water areas of Bangladesh, a traditional deep-water rice variety is grown as fodder (Magor, 1986). If leaf cutting really has no injurious effect on grain production, it may become one of the most economical ways of increasing the total productivity of land by providing additional green feeding materials for the animals. The success of rice cultivation as dual purpose crop, is mostly dependent on different improved agronomic techniques like leaf clipping time, application of N-fertilizer etc. Therefore, the present study was undertaken to investigate the effect of N-rates, leaf clipping timings and their interaction on forage and grain yield of transplant Aman rice.

MATERIALS AND METHODS

The experiment was conducted at the Agronomy Field and Laboratory (24°75/N latitude, 90°50/E longitude and 18 meter above from sea level) of the Bangladesh Agricultural University, Mymensingh, which belongs to the Sonatola soil series under Old Brahmaputra Floodplain (AEZ 9), characterized by medium high silty loam soil with pH 6.5 (BARC, 2012). BRRI dhan41, a high yielding modern rice variety of Aman season (wet season) developed by Bangladesh Rice Research Institute (BRRI) was used in the experiment. Four nitrogen (N) rates, $N_1 = 46$, $N_2 = 69$, $N_3 = 92$, $N_4 = 115$ kg N ha^{-1} and four times of leaf clipping (cutting) viz $C_0 = 0$ (No leaf clipping), C_1 = leaf clipping at 25 DAT (Days after transplanting), C_2 = leaf clipping at 40 DAT, and C_3 = leaf clipping at 55 DAT. In the clipping treatments, all the leaves (100%) were removed from rice plant and the leaves were cut from 12 hills selected diagonally of each plot. Experiment was laid out in split-plot design with three replications, assigning N rates in the main plots and leaf clipping times in the sub plots. Unit plot size was 3 m × 4 m. Seedbed management was done according to the traditional farm practice (BRRI, 2011). Land preparation was conducted according to the common practice of wetland soil preparation with puddling. Thirty-day-old seedlings were transplanted with 25 × 15 cm spacing using three seedlings hill^{-1}. Fertilizers containing P, K, S and Zn were applied @ 15, 38, 10.6 and 2.7 kg ha^{-1}, respectively using TSP, MOP, gypsum and zinc sulfate. The full amount of TSP, MOP, gypsum and zinc sulfate were applied basally during the final land preparation and incorporated into the soil. Urea fertilizer was top-dressed according to the treatments at three equal splits at 25, 40 and 55 DAT, respectively syncronyzing to immediate after every leaf clipping. Due to frequent rains occurred during cropping period, no irrigation was needed. The crop was protected from pest infestation according to standard management practices. Furadan 10G was applied at panicle initiation stage of the crop to protect from stem borer infestation. Green and dry fodder yield data were collected from 6 m^2 area of each plot and converted into t ha^{-1}. From the center of each plot, 6 m^2 area was harvested for determination of grain yield when 85% of the grains appeared yellowish (IRRI, 2014). Grain yield was adjusted to 14% moisture content, and expressed in t ha^{-1}. Yield component data were collected following recommended procedures (IRRI, 1994). Harvest index was calculated by using the following formula (Fageria *et al.*, 2011; Karmakar and Sarkar, 2015):

$$\text{Harvest index} = \frac{\text{Grain yield}}{\text{Grain yield} + \text{Straw yield}}$$

Germination test was conducted following the rules and procedures of ISTA (ISTA, 1976). Germinated seeds were counted on 7, 10 and 14th days after placement of seed vigour index of seedling was calculated using the following formula of Maguire (1962):

$$\text{Vigour Index} = \frac{\text{No. of seeds germinated at first count}}{\text{Duration of first count}} + ..$$
$$...... + \frac{\text{No. of seeds germinated at last count}}{\text{Duration of last count}}$$

Analysis of variance was conducted using the statistical software MStatC. Mean differences among the treatments were tested using Duncan's Multiple Range Test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant height

Plant height was significantly affected by N rates, leaf clipping times and their interaction (Table 1). Plant height was significantly increased with increasing nitrogen rates which is corroborating with Fageria *et al.* (2011). The highest plant height (130.10 cm) at harvest was recorded from 115 kg N ha^{-1} (N_4) followed by 92 kg N ha^{-1} (126.90 cm). The lowest plant height (121.22 cm) was found from 46 kg N ha^{-1} (N_1). This result indicates that plant height increased significantly with increased N application. These results are in confirmation of the findings of Fageria *et al.* (2011); Awan *et al.* (1984) and Mannan *et al.* (2010). The highest plant height (128.95 cm) was recorded at C_0 (No leaf clipping) and the lowest plant height (116.83 cm) was found C_3 (leaf clipping time at 55 DAT). The results also showed that plant height was significantly decreased in later leaf cut treatments compared to those with no and early cuts. Similar results were found by Bandyopadhyay *et al.* (1998). The plant height at harvest ranged from 113.20 to 137.60 cm in the interaction of nitrogen rates and leaf clipping timings. The longest plant (137.60 cm) was observed in the interaction of 92 kg N ha^{-1} with leaf clipping at 40 DAT (N_3C_2) at harvest and the shotrtest plant (113.20 cm) was recorded in 92 kg N ha^{-1} with leaf clipping at 55 DAT (N_3C_3).

Table 1. Effect of nitrogen rates, leaf clipping times and their interactions on plant height of transplant Aman rice cv. BRRI dhan41 at different dates after transplanting (DAT).

Treatment	Plant height (cm) at different DAT			
	25 DAT	40 DAT	55 DAT	At Harvest
N rates (kg ha^{-1})				
N_1= 46	42.79c	60.66b	78.54c	121.22d
N_2= 69	45.85b	62.90a	80.84bc	124.45c
N_3= 92	47.03a	62.05a	81.18b	126.90b
N_4= 115	47.76a	62.97a	82.94a	130.10a
$LSD_{0.05}$	0.94	1.66	1.09	2.24
F-test	**	**	**	**
Leaf clipping times (DAT)				
C_0= 0	47.15	64.43a	81.72a	128.32a
C_1= 25	48.35	63.78b	79.37b	125.57b
C_2= 40	47.21	59.58d	79.32b	123.95bc
C_3= 55	48.22	60.75c	78.09c	116.83c
$LSD_{0.05}$	0.81	1.53	0.99	1.65
F-test	ns	**	*	**
Interaction effect of N rates and leaf clipping times				
$N_1 \times C_0$	45.87c	63.97b	84.07a	124.73bcdef
$N_1 \times C_1$	47.13bc	61.32	78.47de	126.33bcde
$N_1 \times C_2$	47.40b	56.73f	75.53efg	119.20efg
$N_1 \times C_3$	46.75bc	60.60def	76.10ef	114.60fg
$N_2 \times C_0$	46.73bc	63.60bc	80.73c	128.93abcde
$N_2 \times C_1$	49.20a	67.47a	82.40b	131.67abcd
$N_2 \times C_2$	47.17bc	61.53de	60.88g	125.40bcdef
$N_2 \times C_3$	42.10d	59.00e	79.40cd	121.60defg
$N_3 \times C_0$	48.47a	64.13c	81.17bc	124.27bcdef
$N_3 \times C_1$	47.41b	61.07de	76.67ef	122.73cdefg
$N_3 \times C_2$	46.27bc	61.85	79.73cd	137.60a
$N_3 \times C_3$	45.93c	61.13de	79.11cd	113.20g
$N_4 \times C_0$	47.53bc	66.00ab	80.93c	135.33ab
$N_4 \times C_1$	49.40a	65.27ab	79.93c	133.53abc
$N_4 \times C_2$	48.00a	58.20e	81.17bc	133.60abc
$N_4 \times C_3$	46.10bc	62.40cd	77.73ef	117.93efg
$LSD_{0.05}$	1.63	3.06	1.98	3.30
F-test	*	**	**	**

Ns=Not significant, * = Significant at the 5% level of probability and ** = Significant at the 1% level of probability.

Green forage yield

Green forage yield of BRRI dhan41 was significantly affected by the nitrogen rates, leaf clipping timings and their interaction (Table 2). Green forage yield increased with increasing N rates. The highest green forage yield (14.5 t ha^{-1}) was recorded from 115 kg N ha^{-1} at 55 DAT and the lowest green forage yield (1.1 t ha^{-1}) was obtained from 46 kg N ha^{-1} (N_1) at 25 DAT (Table 2). In case of leaf clipping timings, the highest green forage yield (53.6 t ha^{-1}) was observed from leaf clipping time at 55 DAT (C_3) and the lowest green forage yield (6.5 t ha^{-1}) was observed from leaf clipping time at 25 DAT (C_1). The difference in green forage yield was mainly due to difference in duration of cuts from the transplanting times. Forage yield increased with delayed leaf cutting which was also reported previously by Ahmed *et al.* (2001b). This indicated that leaf clipping time at 55 DAT could produce a large amount of green leaves for animals. The crops were allowed to grow further for seed production. The highest green forage yield (57.8 t ha ^{1}) was found from 115 kg N ha^{-1} and clipping time at 55 DAT (N_4C_3) and the lowest (6.0 t ha^{-1}) was found from 46 kg N ha^{-1} and clipping time at 25 DAT (N_1C_1).

Dry forage yield

Dry forage yield of rice was significantly affected by the nitrogen fertilizer and the clipping time (Table 2). Dry forage yield increased with increasing N rates. The highest dry forage yield (0.990 t ha^{-1}) was recorded from 115 kg N ha^{-1} collected at 55 DAT while the lowest dry forage yield (0.421 t ha^{-1}) was noticed from 46 kg N ha^{-1} (N_1) collected at 25 DAT. In case of leaf cutting timings, the highest dry forage yield (3.764 t ha^{-1}) was observed from leaf clipping time at 55 DAT (C_3) while the lowest dry forage yield (2.127

Table 2. Forage yield of BRRI dhan41 as affected by N rates, leaf clipping times and their interactions.

Treatment	Green and dry forage yield (t ha^{-1}) at different DAT					
	25 DAT		40 DAT		55 DAT	
	Green FY[1] (t ha^{-1})	Dry FY (t ha^{-1})	Green FY (t ha^{-1})	Dry FY (t ha^{-1})	Green FY (t ha^{-1})	Dry FY (t ha^{-1})
N rates (kg ha^{-1})						
N_1= 46	1.1c	0.421c	4.8d	0.532c	11.0d	0.720d
N_2= 69	1.4b	0.500b	5.4c	0.561c	12.5c	0.839c
N_3= 92	1.6a	0.535ab	5.9b	0.650b	13.7ab	0.905b
N_4= 115	1.7a	0.542a	6.3a	0.767a	14.5a	0.990a
$LSD_{0.05}$	0.02	0.19	0.04	0.61	0.18	0.39
F-test	*	*	*	*	**	**
Leaf clipping times (DAT)						
C_0= 0	0.0b	0.000b	0.00b	0.000b	0.0b	0.000b
C_1= 25	6.5a	2.127a	0.00b	0.000b	0.0b	0.000b
C_2= 40	0.0b	0.000b	24.0a	2.835a	0.0b	0.000b
C_3= 55	0.0b	0.000b	0.00b	0.000b	53.6a	3.764a
$LSD_{0.05}$	0.01	0.23	0.04	0.39	0.07	0.62
F-test	**	**	**	*	**	**
Interaction effect of N rates and leaf clipping times						
$N_1 \times C_0$	-	-	-	-	-	-
$N_1 \times C_1$	6.0b	2.014d	-	-	-	-
$N_1 \times C_2$	-	-	22.7d	2.600c	-	
$N_1 \times C_3$	-	-	-	-	52.0d	3.680d
$N_2 \times C_0$	-	-	-	-	-	-
$N_2 \times C_1$	6.7ab	2.149b	-	-	-	-
$N_2 \times C_2$	-	-	24.5b	2.744b	-	
$N_2 \times C_3$	-	-	-	-	54.0b	3.722c
$N_3 \times C_0$	-	-	-	-	-	-
$N_3 \times C_1$	6.5b	2.140c	-	-	-	-
$N_3 \times C_2$	-	-	23.7c	2.930ab	-	-
$N_3 \times C_3$	-	-	-	-	50.7c	3.756b
$N_4 \times C_0$	-	-	-	-	-	-
$N_4 \times C_1$	7.0a	2.267a	-	-	-	-
$N_4 \times C_2$	-	-	25.0a	3.067a	-	-
$N_4 \times C_3$	-	-	-	-	57.8a	3.996a
$LSD_{0.05}$	0.03	0.06	0.08	0.09	0.14	0.11
F-test	*	*	**	**	*	**

[1] FY=Forage yield, * Significant at the 5% and ** Significant at the 1% level of probability.

t ha^{-1}) was observed from leaf cutting at 25 DAT (C_1). These results are in good harmony with Ahmed *et al.* (2001a). Interaction of 115 kg N ha^{-1} (N_4) and leaf cutting at 55 DAT (C3) produced the highest dry forage yield (3.996 t ha ^{1}) followed by N_3C_3 (2.930 t ha^{-1}) and the lowest dry forage yield (2.014 t ha^{-1}) was found from 46 kg N ha^{-1} and clipping time at 25 DAT (N_1C_1). These results are corroborating with findings of Abou-khalifa *et al.*, 2008.

Grain yield

Grain yield was influenced significantly by nitrogen rates, leaf clipping times and their interaction (Table 3). Results revealed that, the highest grain yield (4.88 t ha^{-1}) was recorded from 115 kg N ha^{-1} (N_4) that was statistically similar (4.75 t ha^{-1}) with 92 kg N ha^{-1} (N_3). The lowest grain yield (3.35 t ha^{-1}) was recorded when the crop was fertilized with 46 kg N ha^{-1} (N_1). Nevertheless, grain yield increased with increased nitrogen rates. The results confirmed the findings of Fageria *et al.* (2011), Prudente *et al.* (2008), Karmakar *et al.* (2002) and Oo *et al.* (2007) who reported that grain yield increased significantly with increasing N levels. In respect of leaf clipping, the highest grain yield (4.36 t ha^{-1}) was observed in control plot (C_0=leaf was not clipped) and the lowest grain yield (3.33 t ha^{-1}) was obtained from the delayed leaf cutting C_3 (at 55 DAT). Grain yield of C_1 (Leaf cliping at 25 DAT) and C_2 (at 40 DAT) was statistically similar, however it was statistically higher than C_3. These findings indicated that leaf clipping in early stage of crop did not reduce yield significantly. These results are in alignment with Das *et al.*, (2017) and Das and Mukherjee, (1992). Moreover, grain yield was reduced by 3, 6 and 24% in C_1, C_2 and C_3, respectively compared to C_0 (No leaf cutting). The results were consistent with reports by Ros *et al.* (2003) who found that pruning 30% of leaves depressed grain yield by 20%. Grain yield was highly influenced by the interaction of N rates and leaf clipping times at the 1% level of probability. The highest mean grain yield (5.25 t ha^{-1}) was obtained from the treatment combination of 115 kg N ha^{-1} and no leaf clipping (N_4C_0) which was statistically similar to N_3C_0 (5.18 t ha^{-1}), N_3C_1 (5.10 t ha^{-1}) and N_4C_1 (5.06 t ha^{-1}). The lowest mean grain yield (3.17 t ha^{-1}) was obtained from N_1C_3. The results indicated that moderate forage yield and higher grain yield could be obtained from higher N rates (92 to 115 kg N ha^{-1}) with early leaf clippings (25 DAT).

Harvest index

Harvest index was significantly influenced by nitrogen rates, leaf clipping timings, and their interaction (Table 3). In case of N application rates, harvest index varied from 0.42 to 0.46. Harvest index increased with increased N rates which was similar to the findings of Fageria *et al.* (2011). The highest harvest index (0.46) was obtained from the interaction effect of N_4C_0 and N_3C_0 that was statistically similar with N_3C_1 and N_1C_0 (0.45); and the lowest (0.38) was found in N_1C_3.

Panicle production

N rates, leaf clipping times and their interaction had significant effect on panicle production (Table 3). The highest mean number of panicles $hill^{-1}$ was produced (8.7) when 115 kg N ha^{-1} (N_4) was applied and that was statistically similar (8.5) with N_3 (92 kg N ha^{-1}) and the lowest (7.8) was in 46 kg N ha^{-1} (N_1). Panicle production increased with increased nitrogen rates. Leaf clipping timings had also remarkable effect on panicle production. Panicles $hill^{-1}$ ranged from 7.3 to 8.3 across the leaf cutting treatments. The maximum number of effective tillers $hill^{-1}$ (9.2) was performed from the interaction of 115 kg N ha^{-1} with no leaf clipping (N_4C_0) and the lowest number of panicles $hill^{-1}$(6.3) was obtained from N_1C_3.

Grains per panicle

Number of grains $panicle^{-1}$ was significantly influenced by the nitrogen rates, leaf clipping times and their interaction (Table 3). The highest mean number of grains panicle^{-1} (122) was recorded from 115 kg N ha^{-1} (N_4). The lowest number of grains panicle^{-1} (112) was observed form 46 kg N ha^{-1} (N_1). In case of leaf clipping timings, the highest mean number of grains panicle^{-1} (118) was found in C_0 (no leaf clipping) and the lowest (106) was obtained from C_3 when leaf clipped at 55 DAT (Table 3). This indicated that leaf clipping negatively affected in producing grains panicle^{-1} which was similar to the findings of Das *et al.* (2017). Without leaf clipping and early clipping produced more grains panicle^{-1} compared to the delayed leaf clippings. The present results explicitly confirm previous results obtained by Ghosh and Sharma (1998) who reported higher number of grains panicle^{-1} from early leaf clipping time than late leaf clipping. This might be attributed due to the fact that forage removal at later stages of crop growth did not produce sufficient photosynthetic leaf area. The lesser amount of dry matter production in the late leaf cut plants failed to supply sufficient amount of carbohydrates. The highest number of grains panicle^{-1} (124) was recorded from the interaction of 115 kg N ha^{-1} and no leaf clipping (N_4C_0) followed by N_3C_0 (123), and the lowest (90) was in the combination of N_1 x C_3.

Thousand grain weight (TGW)

Nitrogen rates, leaf clipping times and the interaction had no significant effect on 1000-grain weight (Table 3). It might be that the grain weight is genetical and stable varietal character, and the management practices has less effect on its variation (Yoshida, 1981). The results are in agreement with the findings of Mannan *et al*. (2010); Karmakar *et el.* (2002) who reported that nitrogen has no significant effect on TGW. Moreover, DAS *et al.* (2017) reported that leaf clipping had non-significant effect on TGW of modern variety while it was significant in local variety. On the contrary, Fagerial *et al.* (2011) found that TGW was significantly influenced by N fertilization. The highest TGW (23.16 g) was recorded in the interaction of 92 kg N ha^{-1} fertilizer (N_3) with no leaf clipping (C_0). Although treatments had no significant effect, however it varied in some extent across the treatments.

Seed germination

Germination of seed was significantly affected by N rates, leaf clipping times and their interaction (Table 3). The highest germination percentage (91.0%) was observed in N3 (92 kg N ha^{-1}) followed by N_4 (89.8%). However, the lowest germination percentage (85.6%) was recorded in 46 kg N ha^{-1}. Ahmed *et al.* (2001b) also reported that leaf cutting timings had significant effect on seed germination. The highest seed germination (89.0%) was found in C_0 followed by C_1 when leaves were cut at 25 DAT. Seed germination ranged from 80 to 93% in the interaction of N rates and leaf cutting timings.

Vigour index

Nitrogen rates, leaf clipping timings and their interaction had significant effect on vigour index of seedlings (Table 3). Among the N rates, seedling of N_3 (92 kg N ha^{-1}) performed the highest vigour (10.77) and the lowest (9.14) was in N_1 (46 kg N ha^{-1}). Seeds without leaf cutting treatment (C_0) showed the highest vigour index (10.33) while the lowest (9.60) was in C_3 (Leaf clipping at 55 DAT). The highest vigour index (11.78) was found in the interaction of 92 kg N ha^{-1} (N_3) with C_0 (no leaf cutting) that was followed by N_3C_1, N_2C_0 and the lowest vigour index was (6.17) for N_4C_3, respectively.

Table 3. Effect of nitrogen (N) rates, leaf clipping times and their interaction on yield and yield components of transplant Aman rice cv. BRRI dhan41 .

Treatment	Panicle hill^{-1} (no.)	Grain panicle^{-1}	1000-grain wt (g)	Grain yield (t ha^{-1})	Harvest index	Germination (%)	Vigour index
N rates (kg ha^{-1})							
N_1= 46	7.8c	112d	22.41	3.35c	0.42d	85.6d	9.14d
N_2= 69	8.2b	117c	22.81	4.52b	0.44c	86.5c	9.91c
N_3= 92	8.5ab	120b	22.74	4.75a	0.45b	91.0a	10.77a
N_4= 115	8.7a	122a	22.50	4.88a	0.46a	89.8b	10.33b
LSD$_{0.05}$	0.3	1	0.42	0.15	0.01	0.1	0.02
F-test	*	**	ns	**	**	**	**
Leaf clipping times (DAT)							
C_0= 0	8.3a	118a	22.72	4.36a	0.44b	89.0a	10.33a
C_1= 25	7.8b	115b	22.70	4.21ab	0.44a	88.0b	10.22ab
C_2= 40	7.5c	110c	22.54	4.10b	0.43b	87.3ab	10.10b
C_3= 55	7.3d	106d	22.64	3.33c	0.41c	86.0b	9.60c
LSD$_{0.05}$	0.2	2	0.27	0.13	0.01	0.80	0.15
F-test	**	**	ns	*	*	*	*
Interaction effect of N rates and leaf clipping times							
$N_1 \times C_0$	8.3ab	115c	22.05	4.25c	0.45a	81.7g	10.55cd
$N_1 \times C_1$	6.5de	108cde	22.59	4.10cd	0.44b	92.0ab	9.92de
$N_1 \times C_2$	6.8cde	97def	22.37	3.82de	0.40e	84.0f	10.75bc
$N_1 \times C_3$	6.3e	90f	22.61	3.17e	0. 38f	89.0d	10.40cd
$N_2 \times C_0$	8.0abc	122ab	22.86	4.65bc	0.43c	87.0ef	11.70a
$N_2 \times C_1$	8.2abc	108ef	23.15	4.17dc	0.42d	92.0ab	10.85bc
$N_2 \times C_2$	7.7bcd	98ef	22.83	3.42e	0.41cd	87.0e	10.09d
$N_2 \times C_3$	6.5de	94ef	22.97	3.30ef	0.41cd	80.0h	7.46g
$N_3 \times C_0$	7.7bcd	123a	23.16	5.18a	0.46a	92.4ab	11.78a
$N_3 \times C_1$	7.7bcd	121ab	22.43	5.10a	0.45a	93.0a	11.71a
$N_3 \times C_2$	7.1bcde	110de	22.60	4.84b	0.44b	92.0ab	10.39cd
$N_3 \times C_3$	6.9cde	97edf	22.36	3.58d	0.42c	89.0bcd	10.57cd
$N_4 \times C_0$	9.2a	124a	22.42	5.25a	0.46a	90.2bc	10.63cd
$N_4 \times C_1$	7.8bcd	122b	22.61	5.06a	0.44b	91.0bc	10.58cd
$N_4 \times C_2$	7.7bcd	111d	22.35	4.75bc	0.43bc	89.5d	9.18e
$N_4 \times C_3$	7.1bcde	93e	22.61	3.92d	0.42c	89.0d	6.17h
LSD$_{0.05}$	0.40	3	1.15	0.21	.02	0.6	0.10
F-test	**	**	ns	**	**	**	**

ns = Not significant; * = Significant at the 5% and ** = Significant at the 1% level of probability.

CONCLUSION

Application of 92 to 115 kg N ha^{-1} with leaf clipping at early stage (25 to 40) days after transplanting could be recommended to obtain moderate forage yield, higher grain yield and quality seed of transplant Aman rice BRRI dhan41. Moreover, further investigation on leaf cutting and nitrogen management is very much important especially for the rainfed lowland rice environment to meet up enormous demand of forage as well as better grain yield with quality seed from same land in Aman season.

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Genetic Diversity of Bacterial Blight Resistant Rice (*Oryza sativa* L.) Genotypes from INGER

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ABSTRACT

Genetic diversity of 65 rice genotypes was studied from IRBBN (International Rice Bacterial Blight Nursery) of INGER (International Network for Genetic Evaluation of Rice) materials through Mahalanobis D^2 statistic for grain yield and yield contributing characters. The genotypes were grouped into five clusters. The inter-cluster distances were higher than intra-cluster distances indicating wider genetic diversity among the genotypes of different clusters. The intra-cluster distances were lower in all the cases reflecting homogeneity of the genotypes within the clusters. The cluster III contained the highest number of genotypes (23) and the clusterv contained the lowest (8). The highest intra-cluster distance was noticed for the cluster I and lowest for cluster III. The highest inter-cluster distance was observed between cluster I and V, followed by cluster IV and V, cluster II andV and the lowest between cluster I and IV. Regarding inter-cluster distance, the genotypes of cluster V showed high genetic distance from all other clusters. The genotypes from cluster V could be hybridized with the genotypes of other clusters for producing transgressive segregants. Based on canonical vector analysis, panicle number per plant had maximum contribution towards genetic divergence. The highest cluster means for yield, grain/panicle and spikelet fertility were obtained from cluster V. The highest means for 1000 grain weight, second higher yield and the lowest growth duration were found in cluster II, while the lowest mean value for yield and 1000 grain weight and higher mean value for growth duration were found in cluster IV. The crosses between the genotypes/parents of cluster V and cluster II, cluster V and cluster I would exhibit high heterosis as well as higher level of yield potential. Therefore, more emphasis should be given for selection of the genotypes from clusters II and V for future breeding programme.

Key words: Genetic diversity, D^2 analysis, cluster analysis, rice (*Oryza sativa* L.), INGER

INTRODUCTION

Rice (*Oryza sativa* L.) is considered as the major crop in Bangladesh as it constitutes 91.8% of the total food grain (rice, wheat and maize) production of 37.50 million metric tons (BBS 2012-2013). Although Bangladesh is now on the verge of attaining self sufficiency in cereal production, there is still a large gap between the production and demand. Among other ecosystems, rainfed condition (T. Aman) is the most suitable for growing rice in this country. Area under T. Aman is the highest which is about 49.63% of total rice land and contributes 40.57% of the total rice production (BBS 2011-2012). Agronomic value of rice variety depends on many characteristics. The most important features include high yielding ability, resistance to diseases and pests, resistance to undesirable environmental factors, and high quality of the products (Aljumaili*et al*., 2018).

Genetic diversity studies occupy an important position in breeding and improvement programme as they ensure efficient utilization of germplasm resources and effective breeding system for the improvement of closely related crop species. Genetic variation analysis helps breeders in observing germplasm as well as predicting possible genetic potentials. Geneticallydistant parents usually able to produce higher

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heterosis(Chakravarthi *et al.*, 2006). It is a powerful tool to determine the genetic discrimination among the genotypes which can be used to select appropriate parental genotypes for hybridization to develop high yielding potential variety (Bhatt 1970). A clear understanding of genetic diversity is essential for the effective conservation and utilization of rice genetic resources (Tu *et al*. 2007, Siddique *et al*. 2011 and Islam *et al*. 2017). Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization (Samsuddin 1985) as cited by Biswash *et al.* 2016. This genetic diversity is essential to decrease crop vulnerability to abiotic and biotic stresses, ensure long-term selection grain in genetic improvement and promote rational use of genetic resource (Cortese *et al*., 2010).

The assessment of genetic diversity among parents used in plant breeding programmes is a necessary prerequisite to assure continuous improvement (Fuentes *et al.* 2005 and Islam *et al.* 2017). Considering this point, about 65 rice genotypes from INGER materials were cultivated. Classify of these genotypes into clusters to select entries as prospective parents to develop transgressive segregants. The present study was undertaken to assess the extent of genetic variability in 65 rice genotypes from INGER.

MATERIALS AND METHODS

Sixty-five rice genotypes from IRBBN (International Rice Bacterial Blight Nursery) of the INGER (International Network for Genetic Evaluation of Rice) materials were grown at BRRI, HQ, Gazipur farm during T. Aman 2016 season. The trial was conducted in a randomized complete block design with three replications. Thirty-two-day-old seedlings were transplanted using single seedling per hill at a spacing of 25 cm × 15 cm. The unit plot size was 5.4 m × 4 rows. Fertilizers urea, TSP, MOP, gypsum and zinc sulphate were applied @ 160:52:85:60:12 kg ha^{-1}. All the fertilizers except N were applied at final land preparation. Nitrogen was applied in three equal splits, at 15 days after transplanting (DAT), at 30 DAT and just before flowering. Intercultural operations and pest control measures were done as and when necessary. Days to 50% flowering, 80% maturity, plant height (cm), panicle number per plant and grain yield (kg/plot) data were taken and adjusted at 14% moisture level. Ten plants from each entry were randomly selected for recording 1000 grain weight (g), filled grains/panicle and unfilled grains/panicle data.The data were analyzed following principal component analysis (PCA) and Mahalanobis's (1936) generalized distance (D_2) extended by Rao (1952). Intra and inter-luster distances were calculated by the methods of Singh and Chaudhary (1985). All statistical analyses were carried out using Genstat 5.5.

RESULTS AND DISCUSSION

Analysis of variance showed significant differences among the 65 advanced lines of IRBBN (International Rice Bacterial Blight Nursery) for all of the seven characters under study, indicating the presence of notable genetic variability among the genotypes. The principal component analysis showed that the first component accounted for 80% of the total variation. Based on the degree of divergence, 65 genotypes were grouped into five clusters on the basis of cluster analysis (Table 1). Maximum 23 entries were grouped into the cluster III followed by 13 in cluster II and 12 in cluster I. The cluster I and II contained the lowest (8-9) number of genotypes.

Table 1. Distribution of some advanced lines of IRBBN (International Rice Bacterial Blight Nursery) in different clusters on the basis of seven characters.

Cluster no.	No. of genotypes	Entry no.	Designation
I	12	1,3,7,9,10,19,21,22,23,25, 26,42	IRBB1, IRBB3, IRBB8, IRBB11, IRBB13, IRBB57, IRBB59, IRBB60, IRBB61, IRBB63, IRBB64, IR09N516
II	13	2,4,5,6,8,11,12,14,17,27, 28,30,64	IRBB2, IRBB4, IRBB5, IRBB7, IRBB10, IRBB14, IRBB21, IRBB51, IRBB54, IRBB65, IRBB66, HHZ5-DT20-DT3-Y2, IR10L139
III	23	13,18,20,32,33,34,35,36, 40,41,45,48,49,51,52,53, 54,57,60,61,62,63,65,	IRBB50, IRBB55, IRBB58, IR05N412, IR81373-119-2-2-1, IR81852-120-2-1-3, IR82492-7-2-3-3, IR08N194, IR09A133, IR09N127, IR10A199, IR10A314, IR10A318, IR10F221, IR11A314, IR10F365, IR10L185, IR10N375, IR78101-68-1-1-2-2, IR82786-43-3-2-1, IR07A166, IR09L324, TN1
IV	9	16,31,38,39,43,44,47,50,55	IRBB53, IRO5A272, IR04A216, IR09L337, IR10A237, IR10A136, IR10A287, IR10A325, IR10N278
V	8	15,24,29,37,46,56,58,59	IRBB52, IRBB62, 12DS-GMET-22, IR08N210, IR10A231, IR10N305, IR10N396, IR11A108

Table 2 presents intra and inter-cluster distances. Average intra- and inter-cluster distance for the entire genotypes based on phenotypic traits was determined. Considerable variation occurred in intra-cluster distances (ranging from 0.2456 in cluster III to 0.5134 in cluster I), indicating significant diversity prevailing among genotypes within clusters. Here the highestintra cluster value was 0.5134 and the highest inter-cluster value was 9.86, which clearly indicated variability in the genotypes of different clusters. However, lower intra cluster values in clusters III (0.2456) and V (0.3064) was observed due to lower variation in all morpho-agronomic data within these groups. The intra-cluster distances were low for all the five clusters with the range of 0.2456 in cluster III to 0.5134 in cluster I which indicated homogeneous nature of the genotypes within the clusters.

The results are supported by those of Islam *et al.* (2014) in rice. The inter-cluster distances ranged from 3.061 to 9.860 and PCA scores also indicated a high degree of genetic diversity among the genotypes. Regarding inter-cluster distance, cluster V showed the maximum genetic distance (9.860) from cluster I followed by 7.834 from cluster IV and 6.837 from cluster II and so on. It is obvious that in most of the cases cluster V produced the highest inter-cluster distances than the other clusters suggesting wide diversity of the genotypes within cluster V with the genotypes of other clusters. The genotypes in these clusters could be used as parents in hybridization programme for getting transgressive segregants (Saini and Kaiker, 1987). Moderate inter-cluster distance was observed between cluster III and I (5.882), followed by cluster V and III (4.271). The minimum inter-cluster diversity was observed between cluster IV and I (3.061), cluster IV and II (3.233), cluster II and I (3.301), cluster III and II (3.504) and cluster IV and III (3.565) indicating that the genotypes of these clusters were genetically close. The inter-cluster distances in all the clusters were higher than the intra-cluster distances suggesting wider genetic diversity among the genotype of different groups. The results were in agreement with Islam *et al*. (2019) and Akter *et al.* (2016).

Table 2. Intra (bold) and inter-cluster distances (D_2) of 65 advanced lines from IRBBN (International Rice Bacterial Blight Nursery).

Cluster	I	II	III	IV	V
I	**0.5134**	3.301	5.882	3.061	9.860
II		**0.3786**	3.504	3.233	6.837
III			**0.2456**	3.565	4.271
IV				**0.3817**	7.834
V					**0.3064**

The highest cluster means for yield, grain/panicle and spikelet fertility were obtained from cluster V (Table 3). The highest (TGW), second highest yield and lowest growth duration and plant height were found in cluster II. While the lowest mean value for yield, TGW and second lower grain/panicle and longer growth duration were found in cluster IV and the lowest panicle number/plant and highest mean value for plant height and growth duration were found in cluster III.

Mean performance of different clusters for the characters studied revealed that lower TGW, grain/panicle, longer growth duration and low yielding (t ha^{-1}) genotypes were clubbed into cluster IV whereas higher yield, grains/panicle and spikelet fertility and other second highest yield contributing characters (taller plant, TGW) were obtained in cluster V. Maximum good characters were accumulated in cluster V and as a result higher yield (4.36 t ha^{-1}) was obtained in this cluster. It was interesting that considering cluster distances, the cluster V showed comparative higher estimates of inter cluster-values than all other clusters. Therefore, the genotypes of cluster V can be used in hybridization programme to produce higher yielding genotypes with all other clusters.

Joshi and Dhawan (1966) reported that inclusion of more diverse parents (within a limit) is believed to increase the chances for obtaining stronger heterosis and give broad spectrum of variability in segregating generations. Therefore, more emphasis should be given on cluster V and II for selecting genotypes as parents for crossing with the genotypes of cluster IV which may produce new recombinants with desired traits. Cluster IV had the lowest cluster mean value for grain/panicle, TGW and also lower grain yield. It was also noticed that yield performance in six clusters was highly variable. On the other hand, the highest panicle number/panicle was observed in cluster I. However, yield was comparatively lower due to lower grain/panicle and spikelet fertility. So yield improvement of cluster I is possible by crossing with cluster V. Such results reflected that all these advanced lines are genetically distant from each other.

Table 3. Cluster means for seven characters of 65 advanced lines from IRBBN.

Character	Cluster number					
	I	II	III	IV	V	CV
Plant height	88.08	87.54	108.26	106.67	108.12	12.86
Panicle number/plant	9.58	9.38	9.00	9.56	9.25	16.44
Growth duration (day)	123.92	122.38	129.83	128.33	128.25	3.81
Yield (t ha^{-1})	3.51	3.73	3.62	3.22	4.36	17.43
Grains/panicle	83.50	111.23	131.00	98.00	178.87	25.64
Spikelet fertility (%)	58.33	76.85	80.96	70.56	87.87	16.98
1000 grain weight (g)	21.08	21.85	21.70	19.56	21.50	11.28

Table 4 presents contributions of the characters towards divergence. The canonicalvector analysis revealed that the vectors (vector I and II) were positive only for panicle number per plant. Such result indicated that this character contributed maximum towards divergence. It is interesting that the greater divergence in the present materials due to this character will offer a good scope for improvement of yield through rational selection of parents for producing heterotic rice hybrids. Similar findings also reported by Islam *et al.* (2014) and Akter *et al*. (2018).

It is assumed that, maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. However, for a practical plant breeder, the objective is not only high heterosis but also to achieve high level of production and reducing the growth duration. In the present study, the maximum distances existed between cluster V and I. However, considering the yield, grain/panicle and spikelet fertility, crosses involving the genotypes of cluster V and cluster I may exhibit high heterosis for yield.Again, considering growth duration and plant height, crosses between the genotypes of cluster III and II mayexhibit higher heterosisfor earliness and dwarf stature.

Table 4. Relative contributions of the seven characters towards total divergence in 65 advanced lines from IRBBN.

Trait	Vector 1	Vector 2
Plant height	-0.0327	-0.0774
Panicle number/plant	0.1208	0.0058
Growth duration	-0.0388	-0.1067
Yield (t ha^{-1})	-0.0485	0.6809
Grains /panicle	-0.0792	0.0121
Spikelet fertility (%)	-0.0462	0.0228
1000 grain weight (g)	-0.0616	0.1744

Similarly, the genotypes of cluster V might be crossed with the genotypes of cluster II to get higher grain yield along with earliness. Mian and Bahe (1989) reported that the parents separated by medium magnitude of inter-cluster distance generally showed higher heterosis in chickpea. Similar findings were also reported by Khalequzzaman *et al.* (2008) for rice.

CONCLUSION

Based on the results of the current study, it was observed that there was a high level of genetic diversity among the genotypes included in cluster V. In addition highest yield and yield contributing characters were produced by the genotypes of cluster V. The crosses involving parents belonging to maximum divergent clusters were expected to manifest maximum heterosis and also wide genetic variability. A higher heterosis could be produced from the crosses between genetically distant parents (Ghaderi *et al*., 1984). Keeping this in view, it appeared that crosses between the genotypes belonging to cluster V and cluster I, cluster V and cluster II would exhibit high heterosis as well as higher level of yield potential. So, based on this result, the genotypes under cluster II (IRBB2, IRBB4, IRBB5, IRBB7, IRBB10, IRBB14, IRBB21, IRBB51, IRBB54, IRBB65, IRBB66, HHZ5-DT20-DT3-Y2, IR10L139) and cluster V (IRBB52, IRBB62, 12DS-GMET-22, IR08N210, IR10A231, IR10N305, IR10N396, IR11A108) have been identifed as diverse genotypes and suitable as a parent in the future breeding programme.

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Custom Hire Service Business of Rice Combine Harvester in *Haor* Basin of Bangladesh

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ABSTRACT

Mechanization is a vital issue to modernize agriculture. The entrepreneurship development is imperative to provide service to the farmers at an affordable price, especially in *haor* areas. The study was conceived to estimate the combine harvester's rental charge and payback period. Data were collected from 86 rice fields harvested by a whole-feed combine harvester (Model: Zoomlion) in Mithamain upazila under the Kishoreganj district representing *haor* area of Bangladesh. Land size, operational time, loss time, repair time, idle time, daily area coverage and constraints of harvester machine were also recorded to predict the business viability of combine harvester. The rental charge and payback period was calculated following standard protocol to make business venture profitable. The perception of farmers regarding the prospect of using combine harvester in harvesting has been recorded through personal interviews. The field capacity of combine harvester was observed as 0.20 ha hr^{-1}. Field efficiency of combine harvester depended on the land size. The daily area coverage can be increased after careful selection of the plots. Fuel consumption was found 4.18 l ha^{-1}. Operational consolidation is the best approach to reduce the time of movement resulting in increased daily area coverage. Seasonal use of combine harvester in one locality of the study area was observed for 22 days. The rental charge of the machine was estimated as 10,000 Tk ha^{-1} and payback period was 3.5 years for the area coverage of 40 ha rice fields. Farmers would be able to save 40% harvesting cost for the rental charge of Tk 10,000 ha^{-1}. Combine harvester rental service has emerged as a viable business model in the *haor* basin. Whole-feed (Zoomlion model) combine harvester appeared as an effective, economical, and labour-saving harvesting machine in the *haor* region land tenure system.

Key words: Field capacity, annual use, cost and time savings, rental charge, payback period

INTRODUCTION

The *haor* basin in the north-eastern zone of Bangladesh is an important wetland ecosystem. *Haors* are large back swamp or bowl-shaped depressions between the natural levees of rivers subject to monsoon flooding every year, mostly found in north eastern part of Bangladesh, collectively known as *haor* basin (Miah, 2013; Irfanullah *et al*., 2011). Bangladesh has as many as 423 small or big *haors*. In the district of Sunamganj the highest number of *haors* (133) falls followed by Kishoregonj (122) (Alam *et al*., 2010). As far as the ecosystem is concerned, crop production practices, economic activities and the livelihood of *haor* farmers are quite different from those of other parts of the country. It is a single rice cropped area. The livelihood of this region is mostly depended on Boro rice cultivation.

In the current Boro season (2019-20), paddy has been planted in 50.34 lakh ha of land which is 2% more than the target, and produced about 20 million metric tons of paddy. About 10 percent of paddy has been planted in the districts of the *haor* region alone, which produces about 1.7 million metric tons of rice (Khatun, 2020). However, a major portion of Boro rice is prone to damage due to early flash flood during the harvesting period. Climate in *haor* areas is most vulnerable during harvesting season. Timely harvesting of the crop is very much important to get the desired yield. Harvesting operation is known as critical issues in this region due to natural calamities. It is necessary to find ways to

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harvest the crop quickly so that the damage can be reduced to a minimum level.

Rice harvesting in *haor* areas is a significant issue for farmers because of labourer scarcity and high labour costs leading to increased production cost during rice harvesting period. Farmers are compelled to practice delayed harvesting due to shortage of labour, which results in yield loss; sometimes incurred total loss of field crops due to natural disasters. Harvesting operation is done by manually in *haor* region. Plots are very far from the locality. Moreover, farm roads are absent in this areas. Head and shoulder is the only option to carry harvested crops from field to threshing yard. Threshing is done by close drum thresher. Iqbal *et al*. (1980) found that harvesting losses increased linearly with time, ranging from 3% in the first week to 7% in the third week after maturity of the crop. Appropriate harvesting machine is a major constrain in the *haor* areas. In this situation, rice combine harvester services have played an important role in the harvesting of Boro rice especially in this eco-system.

Harvesting is the process of obtaining the mature crop from the farm which involves different exercises like cutting, collecting, threshing, cleaning, and bagging. These all exercises can be performed manually and in addition to the aid of harvesting machines (Mali *et al*., 2015). Mechanizing those activities can serve to achieve greater efficiencies, resource conservation, and reduction in the cost of crop production (Islam *et al*., 2017a). Harvesting of paddy using combine harvester required 34% labour than manual operation (Hasan, 2019). The Government of Bangladesh took serious efforts to introduce combine harvester of different makes and models having varying harvesting capacity under the farm mechanization project during 2009. Government provided funds to Bangladesh Rice Research Institute (BRRI) to procure combine harvester. BRRI and Department of Agriculture Extension (DAE) arranged large scale demonstrations in the farmers' field to create awareness among the farmers on mechanized harvesting. Entrepreneurs purchased the combine harvester at a subsidized price and operated in the farmers' field in custom hire basis. In the meantime, DAE distributed more than 2000 combine harvesters at a subsidized price (Islam, 2018). The government recently declared 70% subsidy for farmers of the *haor* region, up from the previous 50%, for the purchase of agriculture machinery (MoA, 2020). In Boro 2019-20 season, government has allotted Tk 200 crore as assistance to procure combine harvester and reaper (MoA, 2020). However, the combine harvester is unaffordable to most of the farmers in Bangladesh due to socio-economic conditions. In most of the cases, it is impossible to buy at a subsidized price. On the other hand, combine harvester is season based machine and due to limited annual use, most of the time of the year it sits idle. In that case, the farmer should get the service on a rental basis. There is a need to assess the business viability of custom hire service of combine harvester in *haor* areas. Information on technical and economic performances offers the opportunity to accept the combine harvester in harvesting the crops. This will consequently ensure the timeliness of harvesting operation and reduce the drudgery of the farmers. However, there is no study that has been done in a while to focus on the custom hire service of rice combine harvester for farmers in *haor* areas.

Therefore, the study was undertaken to estimate the rental charge and payback period of the combine harvester in *haor* areas that it is a challenging issue in the economic aspect and factor affecting farmers' use of rice combine harvester. These results will be important for policy makers, extension officers and related agents to improve this business for farmers' benefits and use.

METHODOLOGY

Study area

This study was conducted in Maisherkandi, Mithamain upazila under Kishoreganj district during Boro 2019-20. Crops were harvested by Zoomlion combine harvester from 16 March to 10 May 2020. Data were collected from 86 rice fields. Soft land with a water height of 25 mm was typically prevailed in the study region during harvest time. Most of the crops were not in standing position. Ninety percent crops were observed at an inclination of 70 degrees. Plate 1 shows field operation of Zoomlion combine harvester in study area.

Technical specifications of combine harvester

Table 1 presents technical specification of Zoomlion model combine harvesters. Zoomlion model combine harvester is originally developed in China.

Field performances

Effective field capacity was calculated on the harvester's productivity under actual working conditions (ASAE Standards, 2005; Amponsah *et al.*, 2017).

$$\text{Effective field capacity (ha h}^{-1}) = \frac{\text{Total area harvested (ha)}}{\text{Total operation time (h)}} \quad (1)$$

Fuel consumption

Fuel tank was refilled when fuel level goes to the minimum position. Fuel consumption was recorded after completion of each day operation and measured by weighing refilled volume.

Time record

Digital stopwatch was used to record time parameters. Time parameters included operational time, movement time from machinery shed to plot and return from plot to shed, and other time (harvester movement time from one plot to another, idle time-repairing time, time spend in unloading grain

Plate 1. Field operation of Zoomlion combine harvester in *haor* areas.

Table 1. Technical specifications of Zoomlion combine harvester.

Item	Specification
Name	Zoomlion
Model	4LZT-4.0ZD
Country of origin	China
Price, Tk	20,00,000
Max power, kW	67
Rated speed, rpm	2400
Total weight, kg	3260
Tracking tire	Full track
Minimum ground clearance, mm	325
Fuel type	Diesel
Fuel tank capacity, l	40
Cutting width, m	2.0
Feed quantity, kg s^{-1}	4.0
Grain tank capacity, kg	650
Unloading discharge, kg s^{-1}	3.6
Header auger	Spiral blade+ Eccentric telescopic rod rack
Bagging facility	No
Grain cleaning (depending on moisture level)	Good
Worker	1-2 person

tank, unable to work due to rain, operator resting time, machinery maintenance time etc). The average of time was determined as the summation of the time in all the plots divided by the total number of the plots.

Harvesting time in *haor* areas

Harvesting time started from 22 April 2020 and ended on 10 May 2020. Initially, farmers harvested medium duration rice variety such as BRRI dhan28 and BRRI dhan58. Then, the entrepreneur went for harvesting popular long duration rice variety BRRI dhan29.

Economic feasibility assessment

Costs in various segments of harvesting (both mechanized and manual methods) were classified into two groups, fixed cost and variable cost.

Economic analysis

The cost of operating the combine harvester was computed using the following equation involving the fixed and variable cost items.

$$AC = FC + V \qquad (2)$$

where,

AC = annual operating cost, Tk yr^{-1}

FC = annual fixed cost, Tk yr^{-1}

VC= variable or operation cost, Tk yr^{-1}

Fixed cost (FC)

It is independent of machine use and calculated on the basis of capital consumption method (CC). A capital recovery factor (CRF) was used to combine the total depreciation and interest changes into a series of equal annual payments at compound interest. The capital recovery factor can be interpreted as the amount of equal (or uniform) payments to be received for n years such that the total present value of all these equal payments is equivalent to a payment of one taka at present, if interest rate is i. This payment is used to estimate the capital consumption for farm machinery (Hunt, 2001).

$$CC = (P - S)\,CRF + S \times i \qquad (3)$$

$$CRF = \frac{i(i+1)^n}{(1+i)^n - 1} \qquad (4)$$

where,

CC = capital consumption

P = purchase price of the combine harvester, Tk

S = salvage value, Tk

CRF = capital recovery factor

i = interest on rate of investment, %

n = life of machine, yr

Variable cost (VC)

These costs are associated with use of the harvester and calculated on the basis of the following equation (Hunt, 1995).

$$VC = \frac{A}{C}[(R \,\&\, M) \times P + L \qquad (5)$$

where,

A = annual area coverage, ha

C = effective field capacity of the harvester, ha hr^{-1}

R and M = repair and maintenance cost, %

P= purchase price of the combine harvester, Tk

L = labour cost, Tk hr^{-1}

Rental charge calculation and assumptions

The rental charge for unit (1 ha) area depends on machine price, fuel, labour, transport cost, annual usage and profit. Following assumptions are taken for calculating rental charge of combine harvester;

- Purchase price of the combine harvester is Tk 20,00,000
- Government assistance is 70% of the purchase price in *haor* area (MoA, 2020)
- The machine is in good condition to perform its task in same rate (ha hr^{-1}) without varying over its lifetime
- The bank rate for interest on investment is 12%
- The repair and maintenance cost of rice harvester is taken as 5% of its purchase price

- The life of the harvester is considered 8 years
- Fuel price is taken as Tk 70 l^{-1}
- Operator charge is considered Tk 900 day^{-1}
- Cost of manual harvesting and threshing was Tk 16,500 ha^{-1}

Break-even analysis

The break-even point (BEP) is that point at which neither profit is made nor loss incurred. The total costs of the farm enterprise would be the same as the gross income. It is important to continuously investigate the cost of operation of the farm machine. The farm's fixed costs have to be covered by the income: the higher the fixed costs, the longer it will take for the business to reach break-even and make a profit. Therefore, it is important to keep fixed costs down to a minimum level. The following formula was used to estimate the BEP of the machine.

$$\mathrm{BEP} = \frac{\mathrm{FC}}{\mathrm{CR} - \mathrm{VC}} \qquad (6)$$

where,

BEP = break-even point, ha yr^{-1}

FC = fixed cost, Tk yr^{-1}

VC = variable cost, Tk ha^{-1}

CR =custom hire rate, Tk ha^{-1}

Payback period

The payback period is the length of time required to recover the cost of an investment. The payback period of a given investment or project is an important determinant of whether to undertake the position or project, as longer payback periods are typically not desirable for investment positions. Payback period measures the time required for total cash outflows to equal total cash inflows, that is, the time required to break even.

$$\text{Payback period, yr} = \frac{\text{Investment ,Tk}}{\text{Profit ,Tk yr}^{-1}} \qquad (7)$$

Data collection and analysis

Plot length and width (m) was measured using plastic tape and recorded carefully. Daily area coverage of harvesting, travelling distance (km) from machine shed to field, machinery trouble was collected by direct monitoring. Data were analyzed using MS-Excel 2013 and presented in tabular and graphical form.

RESULTS AND DISCUSSION

Land size

Figure 1 shows the land size of the plots in the study areas. It has been observed that the majority of the plot size exceeds 700 m^2 while the plot size of just a few lands is below 700 m^2. Fifty percent of the plot size is greater than 2000 m^2. Larger land sizes have a major impact on the daily coverage of combine harvester areas. Islam (2020) mentioned that combine harvester having 2.0 m cutting width should be operated in land size having 800 m^2 to get the highest performance of the machine. Daily area coverage of harvesting operation depended on land size and shape (Islam *et al.*, 2015).

Length-width (L/W) ratio

Figure 2 shows the length width ratio of rice fields under the study area. In *haor* regions, the L/W ratio for 70 percent of the plots was 1.5-2.0. Taniyama (1975) and Islam (2018) mentioned that machinery performance dependent on the plot length-width ratio. The higher the L/W ratio lesser the turning events of the farm machine. Islam *et al.* (2017a) studied the plot L/W ratio in eight northern districts of Bangladesh and observed that 25% of plots belong to L/W ratio 1.6-2.0. The plot L/W ratio in *haor* areas is comparatively higher than those areas. Islam *et al.* (2017b) observed that the least number of turns were obtained for L/W ratio of 2-2.5 and length-wise transplanting. With the increasing of L/W ratio, the number of turning events reduced in length-wise operation and increased in width-wise transplanting layout. Lengthwise operation of farm machinery is preferable to minimize the turning events. The author suggested that the plot length should be increased by keeping the same plot size (Islam *et al*., 2017b). It was preferable to select the plot having L/W ratio greater than 1.5 to operate the machine at full capacity.

Variety grown in *haor* area

Figure 3 shows the proportion of rice variety harvested using combine harvester in the study area. BRRI dhan29 is the largest proportion (54%) followed by BRRI dhan58 and hybrid dhan (14%). BRRI dahan28 is the smallest proportion. BRRI dhan58 and BRRI dhan28 having growth duration of 150 days and 140 days respectively are grown early in the study areas. However, BRRI dhan29 having growth duration of 160 days is widely grown in *haor* areas. The result revealed that BRRI dhan29 is a dominant variety in this region. BRRI dhan89 as newly released variety having similar characteristics of BRRI dhan29 may introduce in those areas. Information on type of variety and growth duration is very much important for proper planning of the harvester operation.

Daily area coverage

Figure 4 illustrates the daily area coverage of combine harvester. Harvesting period was recorded as 22 days and harvester machine effectively operated in 17 days in *haor* areas due to unavoidable reason like rain, machine maintenance and unavailability of fuel. In the peak time, machine can run in day and night. Area coverage increases with careful selection of the plot. Operational consolidation i.e harvesting in adjacent plots increases the daily area coverage. Sazzad (2014) studied the field performance of DAEDONG combine harvester in Dhamrai upazila under Dhaka district during 2015 and observed that the seasonal use of combine harvester was 16 days in one locality. After completion of harvesting in *haor* areas, machine can be shifted to other non-*haor* areas to increase annual areas coverage.

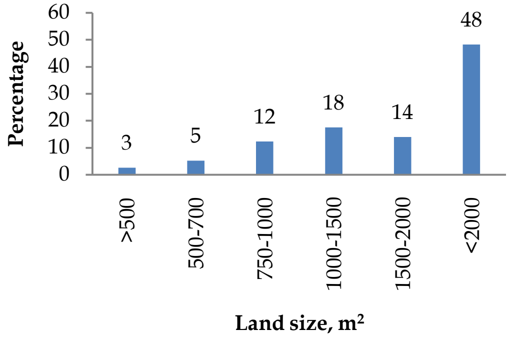

Fig. 1. Land sizes in study location.

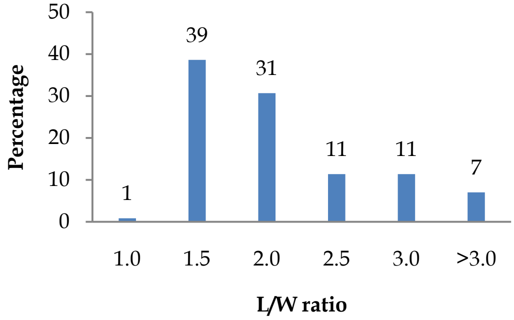

Fig. 2. Length-width ratio of rice land in *haor* areas.

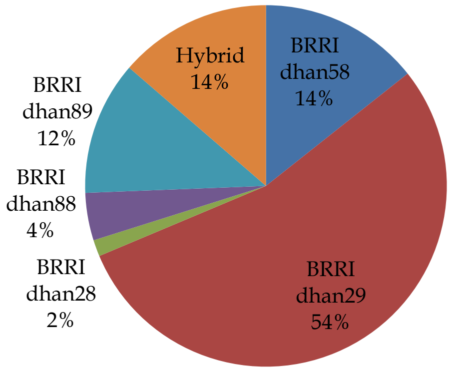

Fig. 3. Variety-wise harvesting area.

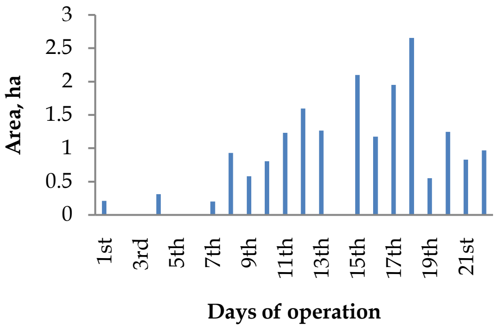

Fig. 4. Daily area coverage of combine harvester.

Field capacity

Figure 5 shows the field capacity in different plots in the study areas. It was found that the maximum occurrence of events at 0.17 followed by 0.19, 0.21 and 0.23 ha hr^{-1} (Fig. 5). The highest field capacity (more than 0.30 ha hr^{-1}) was observed in the fewer plots due to operated day and night. This might be an exceptional situation in *haor* areas. Field capacity having less than 0.15 ha hr^{-1} was observed in 6% percent plot which might be due to lodged crop, soft land, smaller size of lands etc. On average, the actual field capacity of combine harvester was observed as 0.20 ha hr^{-1}. According to Islam (2018) field capacity increased with the increase in plot size due to the reduction of turning loss.

Time distribution pattern

Figure 6 shows the time distribution of harvesting operation. Harvesting activity was categorized as harvesting time, machinery movement time from shed to plot and return from plot to shed, plot to plot movement, and idle time. In the study area, paddy fields were very far from the machinery shed. Most of the time (41%) spent in machinery movement including shed to plot, plot to plot. After harvesting, the machine should return from the plot to avoid stolen of spare-parts. The layout of farm roads should be re-adjusted according to Taniyama (1975) to facilitate the movement of farm machinery to each field plot without causing harm to machinery, levees and irrigation channels. Some plots were very far and damaged the irrigation channel and levees due to the machine movement. Unavoidable idle time included heavy rainfall and machine breakdown (Islam *et al.*, 2015).

Fuel consumption

Fuel consumption depended on area coverage, distance from shed to rice field, plot to plot movement. It was estimated that total 700 l (4.18 l ha^{-1}) fuel was consumed to harvest 22.39 ha of land. Fuel was fully utilized in easily accessible regular shape and large size plots because of high field capacity. Additional fuel was required due to long distance of field from machinery shed. The average distance travelled in each day by the harvester from machinery shed to plot was 1.4 km in the study area.

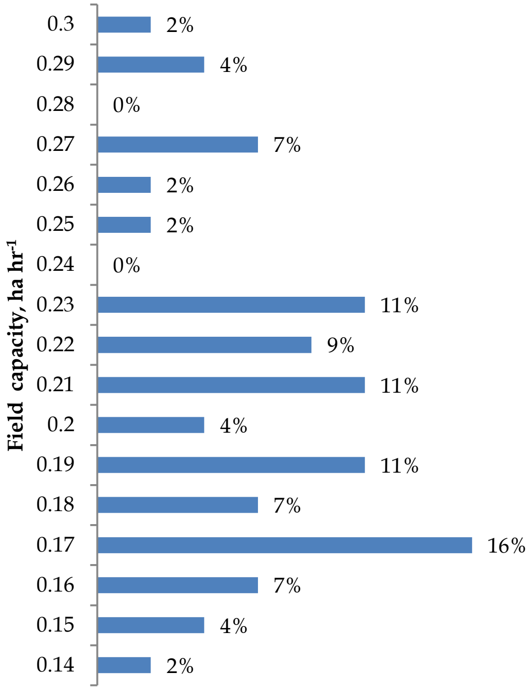

Fig. 5. Percentage of field capacity in different plots in *haor* areas.

Operational cost

Figure 7 presents cost distribution of harvesting operation. Operation cost was categorized as operator's charge, fuel and oil cost, and repairing charge of the combine harvester. The fuel cost (50 percent) was the highest in items with operating costs including harvesting, movement in plot to plot, machinery shed to plot and return from plot to machinery shed. Transport cost may be minimized to operate harvester in adjacent plots. Fuel consumption may be decreased by avoiding small sizes of plots. The operating cost may be reduced through careful selection of plots having larger in size and regular in shape.

Payback period

Figure 8 was drawn from the entrepreneurs' point of view and shows the return and payback period of combine harvester. Profitability of rental service of combine harvester varied with the area coverage and rental charge. Graphs were drawn with five level of profit ranging from 10,000-12,000 Tk ha^{-1}. Profitability started after yearly area coverage of harvesting is 22 ha with rental charge of 10,000 Tk ha^{-1}. The payback period would be 3.5 years for the same rental charge if yearly areas coverage is expected to 40 ha. Profit margin increases with the increase in area coverage. Business will not be viable below the annual area coverage of 40 ha for the same rental charge. The entrepreneurs can get early return on investment if offered rental charge 10,000 Tk ha^{-1} for the annual areas coverage of 50 ha. It is possible to harvest 50 ha of land in three rice seasons by Zoomlion combine harvester. Farmers would be able to save 40% harvesting cost for the rental charge of Tk 10,000 ha^{-1}.

Break-even area

Figure 9 displays the mechanized harvesting costs using the Zoomlion combine harvester and manual harvesting based on relevant assumptions. Mechanized harvesting offered a total annual fixed cost of Tk 1,51,132 at an investment cost of Tk 20.00,000. However, the same conventional system procedure will involve 16,500 Tk ha^{-1}. Break-even area would be 36 ha for Tk 10,000 rental fee. The traditional method is intended for sickle picking, carrying paddy by head or shoulder, threshing with close drum threshers and winnowing with conventional winnower.

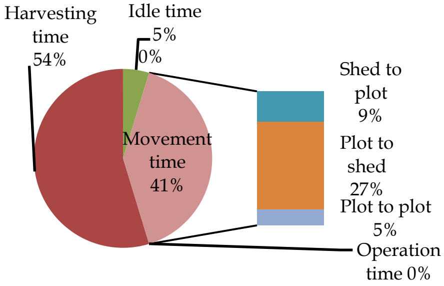

Fig. 6. Time distribution pattern in harvesting operation.

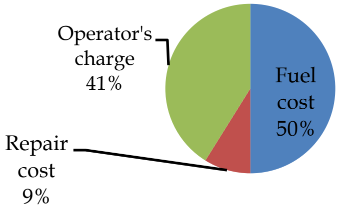

Fig. 7. Cost distribution in operation of combine harvester.

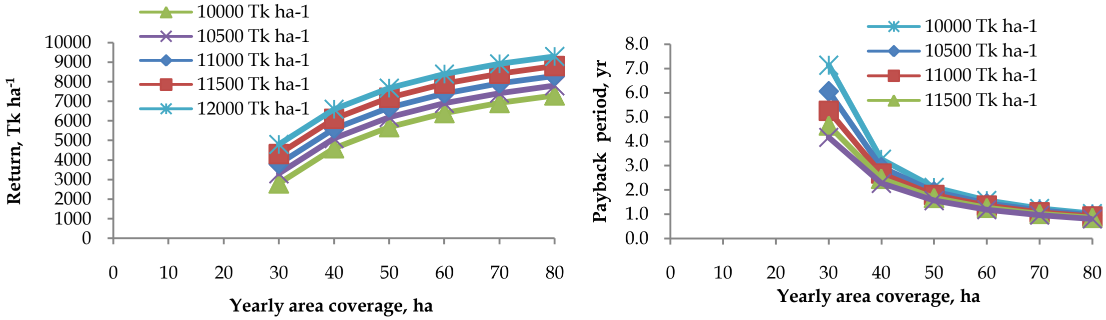

Fig. 8. Return and payback period of combine harvester.

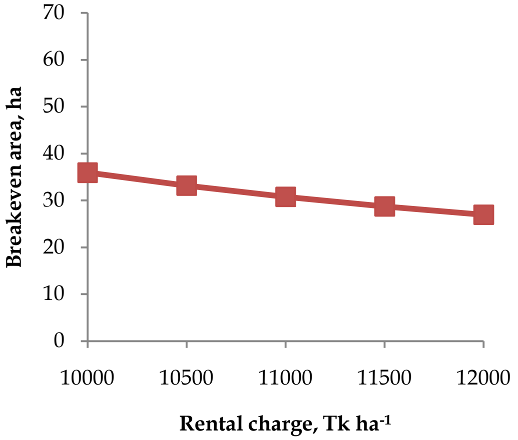

Fig. 9. Break even area with respect to rental charge.

Proposed business model of harvester

Local service providers/entrepreneurs will get the facility to purchase the combine harvester through government assistance (70%) and provide custom hire services to the farmers at an affordable price. Before the harvesting season, the entrepreneur will contract the expected farmers to harvest the crop by combine harvester and prepare harvesting schedule.

Precaution

In *haor* eco-system, the market place is very far from the rice field. During the peak harvesting period, every day is a very crucial time to complete the harvesting works smoothly. Entrepreneurs as well as farmers' desire to run the combine harvester every day and night without causing any trouble. Care should be taken to investigate the combine harvester thoroughly before the harvesting season. Carefully check all moving parts, engine power, power transmission system, crawler movement, reel rotation, crop gathering, cutter bar movement and sharpness, conveyer belt movement, threshing drum rotation, blower, cleaner, auger and delivery chute. Most vulnerable parts should be identified and maintain the stock before going to the field.

Harvester model selection criteria in *haor* area

- Built-in grain tank facility. After harvesting, grain can be unloaded in the side of the plot.
- Bigger size having high harvesting capacity
- High capacity engine is required to develop sufficient traction force in wet land
- Low repair and maintenance time
- Parts failure frequency is minimum
- Trouble free operation
- Low noise and vibration (Operators' comfortability)

Challenges/Limitation in harvesting in *haor* areas

The big problem of running the harvester in *haor* areas is that the plots are far away from locality. There is no road for machine movement. Variety having various growth durations were grown in *haor* areas and matured in different time. Paddy ripens at different times as the growth duration is different for different varieties. If the grains of the inner plot ripen earlier, the machine cannot be taken to that plot over another field. Farmers can follow the synchronize farming to overcome this barrier. Harvesting schedule often broken down or delayed due to rain and hail storm.

Government policy in up-scaling combine harvester

The government has given utmost importance to mechanized agriculture to rising harvesting costs and operational timeliness. The *haor* region currently operates 362 combine harvesters and 1056 reapers. One hundred and sixty (160) new combine harvesters and 137 reapers were allocated for seven districts of the *haor* region on an emergency basis in the Boro 2019-20 season. Additionally, 220 old repairable combine harvesters and 487 reapers were repaired. The government recently adopted an action plan to promote mechanized harvesting in the region. In 2020, they provided 70% assistance to the *haor* areas and 50% aid to the other areas for harvester procurement (MoA, 2020).

CONCLUSION AND RECOMMENDATION

Combine harvester reduced the time required in harvesting, cost of harvesting and drudgery of the farmers. There is an ample opportunity to invest money on combine harvester with the government assistance and make the business profitable. The custom hire service business, however, will not be viable if the annual area covers less than 40 hectares for rental charges of 10,000 Tk ha^{-1}. With the increase in area coverage, the profit margin increases. Larger sizes of Zoomlion model combine harvester appeared as business viable machine in *haor* areas of Bangladesh. Additionally, a network of *haor* combine harvester service operators should be formed to create approaches to promote the business in the region, to develop organizational strength, and to increase the sustainability of the business in the *haor* region. Larger size of land is preferable to operate the farm machinery at full capacity. Farmers can introduce synchronize farming techniques i.e. transplant the variety having the same growth duration at the same time in certain areas to facilitate the harvesting crops by machine. It may also increase daily harvest area coverage that effectively make harvesting business profitable and reduce the farmer's cost of production. The findings of study give contributions in development of entrepreneurship of combine harvester custom hire service to the farmers, particularly in *haor* ecosystem. However, government assistance should be continued to procure combine harvester in *haor* areas for timely and faster harvesting of rice crop.

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Identification of Ideal Trial Sites and Wide Adaptable T. Aus Rice Genotypes Suitable for Bangladesh

M Khatun[1*], M M E Ahmed[2], M A Syed[3], F Akter[4], S Das[5], M E Haq[5] and S S Dipti[6]

ABSTRACT

GGE biplot study is an effective tool for many crops including rice to identify mega-environments, ideal trial site and suitable genotypes for general and specific adaptation. An attempt was made to find promising T. Aus rice genotypes having suitable grain quality, better agronomic parameters and ideal test location for T. Aus rice growing areas. In this study, a total of 11 promising genotypes along with two popular cultivars BR26 and BRRI dhan48 were tested across six locations; Cumilla, Gazipur, Rajshahi, Rangpur in T. Aus 2016 and 2017 whereas Habiganj and Kushtia only in T. Aus 2017 season. From GGE biplot study, BRRI RS, Rajshahi (E8) was the most discriminating and ideal location for evaluating T. Aus rice genotypes in Bangladesh condition while BRRI RS, Cumilla 2016 (E1) showed the least discriminating ability and the least representative location. From two years combination data, BR9011-19-1-2 (G6) recorded the highest average grain yield (5.11±0.68 t ha^{-1}) but BR9011-46-2-2 (G2) was the most stable genotype having grain yield (4.97±0.62 t ha^{-1}) and other stable genotypes with above average yield were BR9011-67-4-1 (G5), BR9011-34-3-2 (G1), BRRI dhan48 (G13), BR9039-28-3-2 (G9) and BR9039-9-1-3 (G8) indicated that these genotypes adapted to favourable environments. BR26 (G12) was found highly variable and less stable across the test environments. In addition, agronomic trait (plant height, growth duration) and grain quality traits were also considered for suitability and wider adaptation in T. Aus growing areas in Bangladesh. BR9011-46-2-2 (G2) and BR9011-19-1-2 (G6) were the better genotypes in most of the locations but, considering all of the parameters, the newly developed rice breeding line BR9011-67-4-1 (G5) has been identified as suitable genotype to release as a new variety for sustainable T. Aus rice production in Bangladesh.

Key words: GGE biplot analysis, ideal trial sites, stability, T. Aus rice, wider adaptation

INTRODUCTION

Bangladesh has a tropical monsoon climate characterized by wide seasonal variations in rainfall, high temperatures and high humidity. The dominant food crop of Bangladesh is rice; around 67 percent of the country's cultivated land area is used for rice production (Shelly *et al.*, 2016). Rice grows our three seasons in Bangladesh namely; Aus, Aman and Boro. Aus rice is sown in April, benefits from summer rains (April and May), matures and harvest during July to August, Aman cultivated in wet (monsoon) season and Boro cultivated in dry season applying irrigation. Therefore, T. Aus rice is also called partially irrigated rice that means irrigation requires only at the time of land preparation and initial crop establishment in April while limitation of rainfall and growing period has sufficient rainfall in the rainy season (mid-June to mid-August). There are two Aus dominating cropping pattern in Bangladesh. Boro-Aus-T. Aman is the major cropping pattern occupied 0.21 million hectares land of 47 districts including Cumilla, Bogura, Habiganj, Naogaon, Kishoreganj,

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[1]PSO, Plant Breeding Division, BRRI, Gazipur; [2]SO, Plant Breeding Division, BRRI; [3]SSO, BRRI RS, Barishal; [4]SO, BRRI RS, Rajshahi; [5]SO, TRB-BRRI Project; [6]PSO, Grain Quality and Nutrition Division, BRRI. *corresponding author's E-mail: mahmuda.breeding@gmail.com

Jashore, Bhola, Kushtia etc. The Aus based cropping pattern Fallow-Aus-T. Aman ranking 2nd and possessed 0.19 million hectares land of 30 districts namely Sylhet, Moulovibajar, Habiganj, Jhalkathi, Noakhali, Barishal etc. (Nasim *et al.*, 2017). It is short duration crop having field duration only 80-85 days. It is grown only in 7% of farm land compared to mega season Boro (55%) and Aman (38%) in Bangladesh (The Daily Star, 2017). This area is increasing day by day as farmers are being motivated to cultivate Aus rice due to less irrigation, less fertilizer and least management requirement. In 2017, rice production in Boro experienced a loss of 20 lakh tons due to flashflood in *haor* areas and fungal attacks (mainly blast) in some northern parts of the Bangladesh. In addition, 6.11 lakh hectares area of Aman rice was completely damaged by heavy flood but, this loss was compensated by harvesting 23 lakh tons of Aus rice from 10 lakh hectares area (The Daily Star, 2017). Recently, Aus season is becoming popular due to favourable environment throughout the growing period especially less rainfall in harvesting period to ensure post-harvest activities (drying and storage).

Genotype-environment interactions are major importance to the plant breeders for developing improved genotypes. Even for similar ecological conditions, general adaptability is an important quality of a variety. According to Sharma (1994), adaptability refers to the capacity of a genotype to macro-environmental factors in its favour in order to a consistent performance over time and locations. Development of location specific variety has been considered for future challenge and sustainable food production. Performance of a genotype in diverse environments is a true evaluation of its inherent potential adaptiveness (Panday *et al.*, 1981).

Plant Breeding Division of BRRI has been working extensively to develop short duration T. Aus rice variety with high yielding performance and good acceptance to farmers. In accordance with this, a number of field trials are being conducted across promising T. Aus growing areas of Bangladesh. Thus, ideal trial site and genotype identification is needed through effective statistical tools. In biplot study, both G (genotype main effects) and G × E (genotype × environment interaction) are considered simultaneously for genotype and environment evaluation (Yan *et al.,* 2003). Through data visualization, a 'which-won-where' view of biplot helps to mark distinct mega-environments to identify the best performing genotypes in the respective environments (Gauch *et al.,* 1997). Additionally, ranking of environments and genotypes means stability and comparing the genotype with the popular cultivar and the biplot analysis paved the way to more reliable and proper data interpretation in multi-location trials.

Thus, the present study was undertaken to identify best location as a representative of T. Aus growing areas as well as select stable and superior genotypes for commercial cultivation.

MATERIALS AND METHODS

Plant materials and testing locations

A total of 11 advanced breeding lines were evaluated across six locations of Bangladesh in two consecutive years. In the study, BRRI developed two popular T. Aus rice varieties namely, BR26 and BRRI dhan48 were used as standard checks. Plant height (95-112cm) and growth duration (111-114 days) of the promising T. Aus genotypes were in the acceptable range (Table 1**)** except for BR8776-17-4-2 and BR8781-5-2-3 (117 days). Yield range of the advanced lines was 4.64-5.11 t h^{-1} that was higher than the check BR26 but similar to the check BRRI dhan48 (Table 1).

Table 1. List of the partially irrigated (T. Aus) rice genotypes with some important features.

Genotype	Parentage	Plant height (cm)	Growth duration (day)	Yield (t h^{-1})
BR9011-34-3-2	MLT-145-2/HR17512-11-2-3-1-4-2-3	106±6.14	111±5.63	5.05±0.67
BR9011-46-2-2	"	105±9.42	113±6.58	4.97±0.62
BR9011-48-4-3	"	109±5.83	114±7.19	4.76±0.65
BR9011-64-1-2	"	108±5.35	111±6.01	4.70±0.82
BR9011-67-4-1	"	107±3.49	112±6.88	4.83±0.81
BR9011-19-1-2	"	109±4.67	114±6.59	5.11±0.68
BR9011-9-4-2	"	104±6.50	114±7.55	4.71±0.59
BR9039-9-1-3	BR26/BRRI dhan48	95±5.04	114±5.82	4.84±0.67
BR9039-28-3-2	"	95±4.55	111±5.23	4.93±0.92
BR8776-17-4-2	MOROBEREKAN/BR5563-3-3-4-1	112±7.36	117±6.76	4.64±1.09
BR8781-5-2-3	MOROBEREKAN/IR50	107±7.76	117±6.70	4.68±0.83
BR26 (ck.)	IR18348-36-3-3/IR25863-61-3-2//IR58	103±9.65	112±5.27	4.02±0.94
BRRI dhan48(ck.)	BR1543-9-2-1/IR13249-49-3-2-2	106±6.34	109±4.46	4.90±0.56

In the first year (2016), yield trials were conducted in four locations (Cumilla, Gazipur, Rajshahi and Rangpur) as an initial multi-location trial. In the second year (2017), same lines were tested in the same locations with an addition to Habiganj and Kushtia as a trial site. The ten locations/environments belong to two T. Aus growing conditions such as BRRI regional station (RS) Cumilla (E1 and E2) under stagnant water condition and rest of the environments such as BRRI headquarter (HQ), Gazipur (E3 and E4), BRRI RS Habiganj (E5), BRRI RS, Kushtia (E6) and BRRI RS, Rajshahi (E7 and E8) and BRRI RS, Rangpur (E9 and E10) under favourable condition (Table 2).

Field experiment and experimental design

The experiment was conducted in T. Aus season in the year 2016 and 2017. Seeding was done at different sites from mid-April to last week of April and transplanting was done from first to third week of May. Three-week-old seedlings were transplanted at a spacing of 20 × 15 cm with 2-3 seedlings per hill. The unit plot size was 5.4 m × 2.4 m. The field layout was in RCB design with three replications. Fertilizers were applied @ 195:50:75:40:5 kg urea, TSP, MP, gypsum, zinc sulphate/ha respectively. The fertilizers, other than urea, were applied as basal during final land preparation. Urea was applied in three splits at final land preparation, 4-5 tillering stage (10 DAT) and 5-7 days before PI stages (30 DAT). Crop management such as weeding, irrigation etc was done in time. Insects, diseases and other pests were controlled properly.

Table 2. Locations/Environments and year of the trials of high yielding T. Aus rice genotypes in Bangladesh.

Code	Location/Environment	Season	Remark
E1	BRRI RS, Cumilla	Transplanted Aus	Stagnant water
E2	BRRI RS, Cumilla	"	Stagnant water
E3	BRRI HQ, Gazipur	"	Favourable
E4	BRRI HQ, Gazipur	"	Favourable
E5	BRRI RS, Habiganj	"	Favourable
E6	BRRI RS, Kushtia	"	Favourable
E7	BRRI RS, Rajshahi	"	Favourable
E8	BRRI RS, Rajshahi	"	Favourable
E9	BRRI RS, Rangpur	"	Favourable
E10	BRRI RS, Rangpur	"	Favourable

STATISTICAL ANALYSIS

The grain yield data for 13 genotypes in ten environments were used to perform combined analysis of variance (ANOVA) to determine the effects of environment (E), genotype (G) and their interactions. Combined analysis of variance, standard error of mean, co-efficient of variation was measured by STAR software (version 2.0.1) and broad sense heritability for each trial was generated by PB Tools software (version 1.3). Pooled analysis was performed with two years combination data. GGE biplot was constructed through principal component analysis by R Programming (R development core team, 2013).

RESULTS AND DISCUSSION

Combined analysis of variance

Combined analysis of variance was performed using two years yield data. In combined analysis, the results indicated that the effects of genotype, environments and genotype × environment were significant at 1% level (Table 3). The highly significant G × E effects suggest that genotypes may be selected for adaption to specific environments, which is in harmony with the findings of Aina *et al*. (2009) and Xu-Fei *et al.* (2014). The significant genotype × environment interaction effects clearly exhibited that genotypes responded differently to the variation in environmental conditions of the locations. It indicates the necessity of testing rice varieties at multiple locations. In the present study, highly significant mean squares were found which indicated presence of sufficient variability among the genotypes and environments for grain yield of rice. Similar results were reported by Iftekharuddaula *et al.* (2002) and Hossain *et al.* (2007) in rice.

The quality of the data could be considered reliable due to considerable CV (8.79%) and moderate broad sense heritability (0.69) in pooled analysis (Table 4).

Table 3. Combinedanalysis of variance of grain yield for 11 T. Aus rice genotypes in different sites of Bangladesh.

Source of variation	Degree of freedom (df)	Sum of square (SS)	Mean square (MS)	F value
Environment (E)	9	91.5514	10.1724**	55.15
Replication within	26	6.5161	0.2506	1.36
Genotype (G)	12	25.7723	2.1477**	8.57
Genotype × Environment	108	83.7586	0.7755**	4.20
Pooled error	221	40.7661	0.1845	
CV %		8.79		

**Indicate significant at the confidence level of 1%.

Table 4. Standard error of mean, co-efficient of variation (CV) and heritability in broad sense (h^2) of 13 T. Aus rice genotypes in ten different environments.

Code	Locations and year	Std. error of mean	CV (%)	Trial heritability in broad sense (h^2)
E1	Cumilla 2016	0.330894	10.89	0.67
E2	Cumilla 2017	0.380046	15.33	0.57
E3	Gazipur 2016	0.306069	9.07	0.90
E4	Gazipur 2017	0.199357	7.05	0.58
E5	Habiganj 2017	0.092910	2.85	0.90
E6	Kushtia 2017	0.186051	6.85	0.90
E7	Rajshahi 2016	0.289159	8.16	0.91
E8	Rajshahi 2017	0.161457	5.77	0.68
E9	Rangpur 2016	0.227355	8.73	0.68
E10	Rangpur 2017	0.213196	7.16	0.83
Pooled			8.79	0.69

Evaluation of test environments

Multi-location trials are being conducted by plant breeders around the respective trial sites to assess superior genotypes with wider adaptability. A numerous studies have been reported on GGE analysis in rice, cotton, sorghum and sugarcane (Krishnamurthy *et al.*, 2017; Mohammadi *et al.*, 2013; Samonte *et al.*, 2004; Luo *et al.*, 2015). In the present study, the portioning of GE interaction through GGE biplot analysis showed that PC1 and PC2 accounted for 31.78% and 18.36% of GGE sum of squares, respectively, explained 50.14% of the total variation of the tested environments in two years (Fig. 1).

Three distinct clusters were observed in the GGE biplot study. Among them, Cumilla 2017 (E2), Gazipur 2016 (E3), Habiganj 2017(E5), Rajshahi 2017 (E8) and Rangpur 2017 (E10) comprised in the first cluster, the second cluster consisted of Cumilla 2016 (E1), Rajshahi 2016 (E7) and Rangpur 2016 (E9) and the remaining environments Gazipur 2017 (E4) and Kushtia 2017 (E6) were included in the third cluster (Fig. 1). Among ten environments, the closest association was observed between the environments Habiganj 2017 (E5) and Rangpur 2017 (E10) which belongs to first cluster. Considering all clusters only Cumilla 2016 (E1) showed negative correlation therefore, it can be considered as distinct compared with above locations (Fig. 1). Yan *et al.* (2003) described that the degree and causes of G × E interaction is useful for identifying ideal test locations and best performing genotypes from multi environment trials.

Identification of ideal location

In the present study, Rajshahi 2017 (E8) had the narrowest angle with average environment axis (Fig. 2). Thus, it was highly representative location followed by Rangpur 2017 (E10) and Habiganj (E5). On the other hand, Cumilla 2016 (E1) showed the least discriminating ability and was the least representative location. Yan and Tinker (2006) explained that the narrower angle of two arrows means the closer relation between two environments. The wider angle of two arrows means the more different of results, because of the bigger effect of G × E of the observed traits.

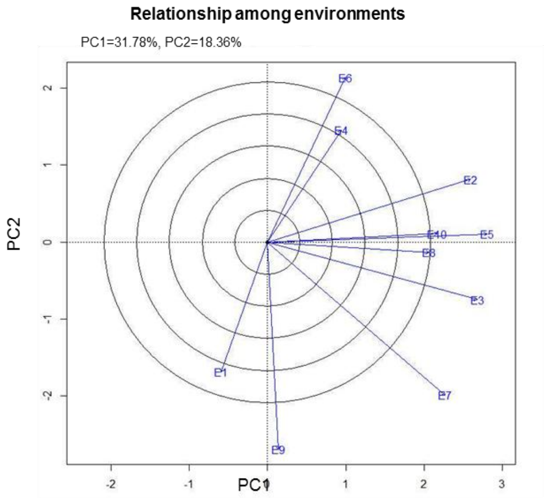

Fig. 1. Association among the test environments of 2016 and 2017 growing season combinations.

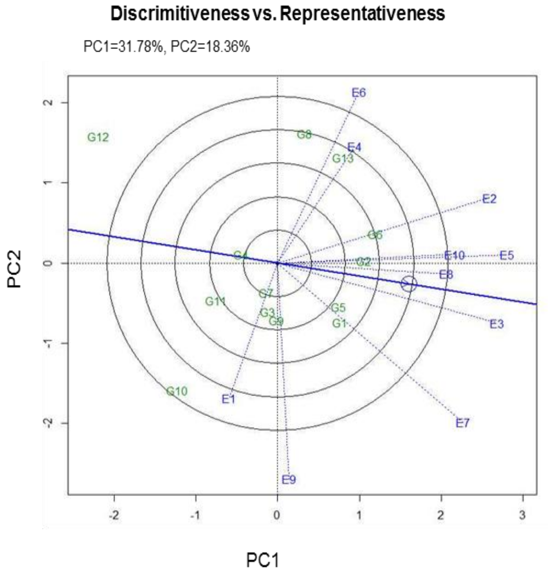

Fig. 2. GGE Biplot power to discriminate (*discrimitiveness*) and power of representation of an environment (*representativeness*).

Ideal test environment is a virtual environment that has the longest vector of all test environments (most discriminating) and it is located on the AEA (Yan *et al.,* 2000). In the present study, BRRI RS, Rajshahi (E8) was identified as the most ideal location with significant discriminating ability. Eventhough Gazipur 2016 (E3), Rajshahi 2016 (E7) and Rangpur 2016 (E9) had the bigger angles to AEA, the locations laid in the same quadrant and tended to have the same results with Rajshahi (E8). Jambormias (2008) reported that locations laid in the same quadrant which is the same with AEA vector line had the same tendency of results. It agreed with the variance analysis of yield in E8, E10, E5, E3, E7 and E9 which had lower average standard error compared to the environment from different quadrant (E1, E2, E4 and E6) (Table 4). Coefficient of variation (CV%) of Cumilla 2016 (E1) and Cumilla 2017 (E2) 10.89 and 15.33 respectively, was higher than other sites. It might indicated that more sever stagnant condition tend to give higher variation on the observed trait (Table 4).

Identification of 'which-won-where' and mega environment (Mega-E)

Mega-E is environmental group which has similarity to support performance of some genotypes simultaneously (Crossa *et al.* 2002). Mega-E is determined by the vertex genotype, i.e. the highest yield genotypes in each quadrant developed by GGE analysis visualization (Yan and Hunt, 2001). Position of the vertex were connected by connecting lines, i.e. a linear line started from the base of biplot that cross perpendicularly each connecting line and separated the biplots into some sectors. Sectors containing environments, i.e. sectors containing dots representing environments, called as mega-E (Jambormias, 2011). In the present study, biplot showed four sectors including all the test environment combinations which indicated four mega environments (Fig.3). Firstly, Mega-E1 had five locations namely Cumilla 2017 (E2), Gazipur 2016 (E3), Habiganj 2017 (E5), Rajshahi 2017 (E8) and Rangpur 2017 (E10).

The second (mega-E2) consisting of Cumilla 2016 (E1) and Rangpur 2016 (E9) and the third (mega-E3) had Gazipur 2017 (E4) and Kushtia 2017 (E6). Finally, fourth (mega-E4) had only one location Rajshahi 2016 (E7). Thus, mega-E4 could not be termed as a mega environment. In GGE study, genotypes located nearer to the ideal genotype are preferable in most cases. In the Mega-E1, BR9011-46-2-2 (G2) and BR9011-19-1-2 (G6) were the winning genotypes indicating the advanced lines were widely adaptable in most of the environments. On the other hand, BR8776-17-4-2 (G10) was the winner in the mega-E2. The genotype BR8776-17-4-2 (G10) having taller plant height (112±7.36) might be benefitted to adapt in the stagnant water condition of the environment Cumilla 2017 (E2) (Table 1). Lastly, BR9039-9-1-3 (G8) and the popular variety BRRI dhan48 (G13) were the winning genotypes in the mega-E3 that was consisting of two environments Gazipur 2017 (E4) and Kushtia 2017 (E6) indicating the genotypes showed their specific adaptability in these locations (Fig. 3).

Performance and stability of genotypes across the test environments

Visualization of GGE biplot is very useful to evaluate and find the most stable genotypes (Farshadfar *et al.*, 2013). Genotypes laid in the concentric area were more stable in giving the yield compared to the genotypes laid outside, even though the environmental effect was very strong. Figure 4 shows the average environment axis (AEA) view of GGE biplot of grain yield for two years combinations (2016 and 2017). In this figure, the genotype BR9011-19-1-2 (G6) produced the highest yield average of 5.11±0.68 t ha^{-1} (Table 1) based on the average from all the environments, and it was visualized by its position in the most right end of AEA line, indicating that the genotype was stable (Fig. 4).

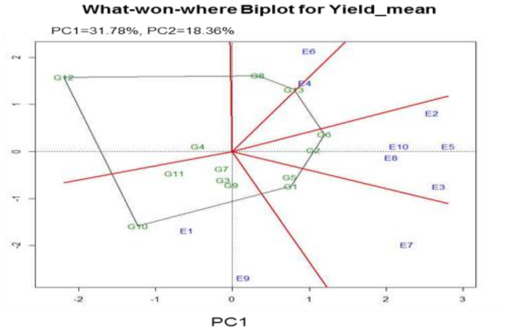

Fig. 3. Visualization of the difference of Mega-E on GGE biplot on yield trait of 13 T. Aus rice genotypes.

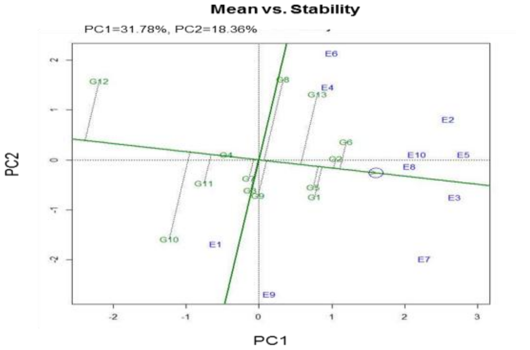

Fig. 4. Visualization of GGE biplot showing the stability of genotypes in which the linear line showing the axis of environment mean and interrupted circle is confidential range.

There were other five genotypes laid in the right end of AEA line, i.e. BR9011-34-3-2 (G1), BR9011-67-4-1 (G5), BRRI dhan48 (G13), BR9039-28-3-2 (G9) and BR9039-9-1-3 (G8) had yield above the average and were considered as relatively stable. These results are in agreement with those obtained by Susan to *et al.* (2015) in rice. The other genotype, BR9011-46-2-2 (G2) had the shortest vector which produced the yield average of 4.97±0.62 t ha^{-1} (Table 1). Therefore, it was identified as the most stable genotype based on GGE biplot study. It meant that BR9011-46-2-2 (G2) had the widest adaptability and stable yield across the environment compared to other 11 genotypes. These results are in agreement with those obtained by Akter *et al.* (2015) in hybrid rice. Akmal *et al.* (2014) reported that genotype with the highest yield average was not necessarily be the most stable and vice versa. The T. Aus variety BR26 (G12) was located far from the AEA and was found highly variable and less stable across the test environments.

Table 5 presents the physicochemical properties such as total milled rice (%), head rice (%), L/B ratio, size and shape, chalkiness and cooking properties viz amylose (%), elongation ratio (ER) and imbibition ratio (IR). Ranges of total milled rice (%) was 62.7-68.7, head rice (%) 21.4-59.9, L/B ratio 2.5-3.4, amylose % 20.5-27.9, elongation ratio (ER) 1.1-1.5, Imbibition ratio (IR) 2.6-3.3 and most of the genotypes having long slender grain (Table 5).

Table 5. Physico-chemical and cooking properties of the advanced T. Aus genotypes.

Designation	Total milled rice (%)	Head rice (%)	L/B ratio	Size and shape	Chalkiness	Amy-lose (%)	Elongation ratio (ER)	Imbibition ratio (IR)
BR9011-34-3-2	59.6	49.0	3.4	LS	Wb9	23.0	1.2	3.3
BR9011-46-2-2	68.7	50.7	3.2	LS	Wc5	26.7	1.3	2.9
BR9011-48-4-3	68.0	58.1	3.2	LS	Wb1	26.8	1.1	2.7
BR9011-64-1-2	67.7	57.7	3.1	LS	Wb1	27.3	1.2	2.7
BR9011-67-4-1	66.0	51.6	3.2	LS	Wb1	27.9	1.3	2.9
BR9011-19-1-2	68.0	50.7	3.1	LS	Wb5	22.4	1.3	2.6
BR9011-9-4-2	68.7	55.3	3.2	LS	Wb5	20.5	1.2	2.9
BR9039-9-1-3	65.0	56.0	3.2	LS	Wb5	26.8	1.5	3.0
BR9039-28-3-2	62.7	21.4	3.4	LS	Wb5	20.7	1.3	3.0
BR8776-17-4-2	67.2	59.9	2.5	MB	Wb5	25.6	1.2	2.9
BR8781-5-2-3	67.5	59.0	2.8	LB	Wb1	26.4	1.3	2.9
BR26 (ck)	65.1	52.4	3.9	LS	Wb1	22.7	1.1	2.9
BRRI dhan48 (ck)	66.3	58.4	2.7	MB	Wb5	26.0	1.3	2.9

Wb1- White belly; Wc- White center; Tr- Translucence; L/B ratio- Length/Breadth ratio

It is well known that the preference of Bangladeshi people is high amylose content (%) as well as non-sticky rice. Therefore, developing an adoptable variety should have high yield with acceptable grain qualities. The acceptable grain qualities such as amylose content must have more than 25%, total milled rice more than 66%, head rice more than 50% with some additional traits viz long slender grain (LS), high elongation ratio (ER) and no-chalk or translucence (Tr) are to ensure high market price. In the study, the genotype BR9011-19-1-2 produced the highest yield on average but amylose content was low (22.4%) (Table 5). Another genotype, BR9011-46-2-2 was observed an adoptable genotype but considering grain quality having chalkiness (Wc_5), it should not be identified as promising one. Therefore, the next adoptable genotype BR9011-67-4-1 was the most acceptable promising line considering higher grain yield (4.83±0.81 t ha^{-1}), intermediate plant height (108±5.35 cm), shorter growth duration (112±6.88 days) and high amylose (27.9%), milling outturn (66%), long slender grain with moderate elongation (1.3) and imbibition ratio (2.7) (Tables 1 and 5).

However, plant breeders need to develop not only high yielding stable genotypes but also consider higher acceptability (high amylose percentage, export quality grain and short duration) to farmers for faster adaptation. In this regards, agronomic traits and physico-chemical properties could help us to draw better inferences for selecting most outstanding genotype with better grain quality. Considering yield, some agronomic characters (plant height, growth duration) and grain quality, the promising line BR9011-67-4-1 was the superior genotype. Thus, the newly developed rice breeding line BR9011-67-4-1(G5) could be further advanced to release as a new T. Aus rice variety for commercial cultivation in Bangladesh.

CONCLUSIONS

From GGE biplot study, the environment of BRRI RS, Rajshahi (E8) was the most discriminating one and could be used as an ideal test site for evaluating newly developed T. Aus genotypes. The genotype, BR9011-67-4-1 (G5) had the above average yield with stability compared to popular cultivar BRRI dhan48 (G13). Thus, the line could be promoted for commercial cultivation across T. Aus growing areas in Bangladesh. The present study supported the fact that which-won-where can be used to identify winners from multi-location trials but agronomic and grain quality traits should also be considered to identify adaptable genotype for making them acceptable to farmers.

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Agroclimatic Indices of Rice (*Oryza sativa* L.) Influenced by Different Planting Times

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ABSTRACT

Meteorological variables have an important role in rice production. Considering this study was carried out in Aman season of 2016 at the research field of Bangladesh Rice Research Institute, Gazipur. The research was conducted in order to investigate the effects of planting time on various agrometeorological indices viz., accumulated growing degree days (GDD), heliothermal units (HTU), photo thermal units (PTU), and heat use efficiency (HUE) the yield of advanced/variety grown under different time of transplanting in the Gazipur climatic zone (AEZ, 28). The research was conducted as a split plot using randomized complete block design (RCBD) with three replications. In this research, three planting times (1st Aug, 16th Aug, and 31th Aug) and the Advanced lines/varieties BR (Bio) 9786-BC2-119-1-1, BR (Bio) 9786-BC2-132-1-3 and BRRI dhan49 (check variety) were used in the experiment. The results showed that planting time significantly affected on grain yield. Among the genotypes, BR (Bio) 9786-BC2-119-1-1 and BR (Bio) 9786-BC2-132-1-3 produced significantly higher grain yield than check variety BRRI dhan49 in all the planting times. The variety (BRRI dhan49), transplanted at the first August, took maximum calendar days (136 days) to attain different phenological stages till physiological maturity coupled with highest growing degree days (2420 degree day) for maturity, lowest heat use efficiency (2.25 kg ha^{-1} c^{-1} day^{-1}), highest heliothermal units (15141.01 degree day hours) and lowest photothermal units (29678.81 degree day hours) resulting lower yield. Among the advanced lines the line BR (Bio) 9786-BC2-119-1-1 took higher calendar day (134 days) to attain different phenological stages till physiological maturity coupled with higher growing degree days (2393 degree day) for maturity, heat use efficiency (2.76 kg ha^{-1} c^{-1} day^{-1}), heliothermal units (13759.75 degree day hours) and photothermal units (30112.51 degree day hours) resulting higher yield. Taken together, the results indicate that the advanced line, BR (Bio) 9786-BC2-119-1-1, could be select as a best advanced line to release as a variety due to its higher grain yield.

Kew words: Rice, planting time, advanced lines/variety and agroclimatic indices

INTRODUCTION

In Bangladesh, traditionally, T. Aman occupied the largest rice harvested area. The broadcasting Aman is mostly planted in deeply flooded lowland areas. The transplanted Aman crops are grown mostly under rainfed conditions (FAO, 2002). Aman rice, is the monsoon-season rainfed rice which is the most widespread, including along the coastal areas. Two ways: direct seeding with Aus in March and April and transplantation between July and August is planted in Aman season. Both types are harvested from November through December (Shelley, 2016). Aman rice previously contributed a major portion of total rice, but Boro is now the major contributor to total rice production in the country, despite Aman coverage area being greater. Aus, Aman, and Boro rice were recently reported to account for 7%, 38%, and 55%, respectively, of the total rice production in Bangladesh (Risingbd, 2014). Total Aman rice covers 55, 90, 340 hectares land with a production of 1, 34, 83, 437 metric tons (BBS, 2015).

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An increase in total rice production is required to feed the ever-increasing population of Bangladesh as the population growth rate of Bangladesh is 2 million per year, and if the population increases at this rate, the total population will reach 238 million by 2050. On the other hand, the total cultivable land is decreasing at rate an alarming per year owing to the construction of industries, factories, houses, roads, and highways (Shelley, 2016).

Rice production is affected by various meteorological variables like rainfall, temperature etc. (Ji *et al.,* 2007). Temperature is an important environmental factor that influences the growth, phenology and yield of crop (Bishnoi *et al.,* 1995). The extreme agroclimatic conditions, particularly high temperature, reduce the plant growth and yield significantly (Satake and Yoshida, 1978). The crop growth response is mainly influenced by the microclimate of crop canopy. Temperature and light radiation are key factors affecting crop production. During crop growth period, the occurrence of various phenological events can be estimated by computing accumulated growing degree days (GDD) (Gouri *et al.*, 2005). GDD provides an estimate of harvest date as well as development stages of crop (Ketring and Wheless, 1989). Thermal time can be used as a tool for characterizing thermal responses in different crops as it is an independent variable to describe plant development (Dwyer and Stewart, 1986). Heat use efficiency (HUE), i.e., efficiency of heat utilization in terms of dry matter accumulation, depends on genetic factors, crop type and sowing time and has great practical application (Rao *et al.,* 1999). The duration of each phenophase determines the dry matter accumulation and its partitioning into different organs. Dalton, 1967; Wang, 1960 reported that the duration of growth stage of any particular species was directly related to temperature and it could be predicted using the sum of daily air temperature, it becomes imperative to have the knowledge of exact duration of various phenological stages of crop in a particular growing environment and their impact on its yield.

Before releasing a variety it is very important to determine optimum time of transplanting date to get higher yield. The promising lines have high yield potentials to be selected by planting rice seeding under varied planting dates in a particular season. Therefore, considering the above facts, the present study was conducted to determine the optimum transplanting time and yield with agroclimatic indices viz GDD, HUE, helio thermal units (HTU) and photothermal units (PTU) of T. Aman advanced rice lines to select best advanced line for release as variety.

MATERIALS AND METHODS:

The experiment was conducted at BRRI farm, Gazipur in T. Aman 2016 to find out optimum planting time and to select the best advanced line. Advanced lines were BR (Bio) 9786-BC2-119-1-1 and BR (Bio) 9786-BC2-132-1-3 along with the check variety BRRI dhan49 (Table 1). Transplanting was started from 1 August to 31 August with 15 days intervals. Twenty-five-day-old seedling was transplanted with 20 cm × 20 cm spacing and one seedling per hill. The experiment was laid down in spilt-plot design, where planting date was in the main plot and the advanced lines were in the sub-plot. Fertilizers were applied @76-12-52-12 kg ha^{-1} N-P-K-S respectively. All fertilizers were applied during final land preparation but urea was applied as top dress in three equal splits at 10, 30 and 45 DAT (day after transplanting). The soil at the study site is of fine silty clay loam type. The location previously had three season rice. The field was cleared and manually ploughed to provide a fine tilth for cultivation. Each plot was demarcated with a 40 cm walk way and a plot size of 3.2m x 2.2m.

Table 1. Transplanting time with growth duration of three genotypes in T. Aman rice.

Advanced line/varietiy	Growth duration		
	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	134	131	130
BR (Bio) 9786-BC2-132-1-3	133	134	132
BRRI dhan49 (ck)	136	135	130

Weeds were manually controlled (2 times) 20 days after sowing and at maximum tillering stage. Also, Dursband was applied at 50 ml/16 litres at panicle initiation stage to control stem borer infestation within the field. At harvest, the middle portion of each plot (5 m^2) was harvested and the grain weight (grain yield was reported at 14% moisture) represent the plot after drying (Saito *et al.*, 2006). The agroclimatic indices, namely, growing degree-days, heliothermal units and heliothermal use efficiency, photo-thermal units, and heat use efficiency were worked out. Weather data were collected from Physiology Division. BRRI, Gazipur.

Growing degree days (GDD):

Cumulative growing degree days were determined by summing the daily mean temperature above base temperature, expressed in degree day. This was determined by using the following formula as per (Nuttonson, 1995).

$$GDD = \frac{(Tmax + Tmin)}{2} - Tbase$$

Where,

Tmax = Daily maximum temperature (°C), Tmin = Daily minimum temperature (°C).

T base = Minimum threshold/base temperature (°C), A base temperature of 10°C was used for rice basis as per (Gao, 1992).

Heat use efficiency (HUE):

Heat use efficiency (HUE) = Grain yield (kg/ha)/GDD

Helio-thermal units (HTU):

The product of GDD and corresponding actual sunshine hours for that day were computed on daily basis as per (Satake, 1978).

HTU = GDD × Actual sunshine hours

Photothermal units (PTU) :

The product of GDD and corresponding day length for that day were computed on daily basis as follows as per (Gouri, 2005).

PTU = GDD × Day length

Day length refers to maximum possible sunshine hours.

Growing degree-days, heat use efficiency, heliothermal units and photothermal units were accumulated from the date of planting to each phenological stage and accumulated from transplanting to physiological maturity.

RESULTS AND DISCUSSION

Grain yield

Rice grain yield is depends on total number of panicles, number of grains per panicle and thousand grain weight, which was significantly influenced by the time of planting (Table 2). First planting time (1 Aug) produced the maximum grain yield in all the genotypes compare to the second (16 Aug) and third (31 Aug) planting time (Table 2). The reason can be attributed to the suitable growth duration, coincidence of the phenological stages, especially the heading and grain filling stages with day length and more favorable temperature. Positive influence of temperature on dynamic formation of the grain yield components resulting higher dry matter accumulation capacity. The minimum grain yield was found in all the genotypes at 3rd planting time. Grain yield decreased in the 3rd planting time due to the reduction in yield attributing characteristics with delay transplanting and excessive heat during the pollination period and grain filling stage caused disorder in grain formation and grain weight which reduced the grain yield. The results were in accordance with the findings of (Sharma *et al.* 2011), Noorbakhshian (2003), Pirdashti *et al*. (2003) and Gines *et al.* (1987). There was a significant difference among the genotype and variety in terms of grain yield (Table 2). The highest grain yield (6630 kg ha^{-1}) was obtained in the BR (Bio) 9786-BC2-119-1-1 genotype and followed by 6020 kg ha^{-1} in the BR (Bio) 9786-BC2-132-1-3 genotype. The lowest grain yield (5450 kg ha^{-1}) was observed in the check variety, BRRI dhan49. This disparity was caused by the difference between genotypes in terms of the growth period duration, panicle emergence time, fertility rate, the number of grains per panicle, the 1000 grains weight, and heat-sensitivity level (Table 2). The results were similar with the findings of Kawakata and Yajima (1995) and Yoshida (1978) who reported that a determining role for temperature and day duration on panicle emergence and their impacts on physiological, growth and maturity processes resulting higher grain yield. The interaction effect of transplanting time and genotypes significantly differ on grain yield (Table 3). Reduction of grain yield due to delayed planting was to an extent of 1.5% and 6.33% in crop planting during second (16 August) and third (31 August) planting time respectively, compared to the first (1 August) planting time (Table 3).

Table 2. Interactive effect of planting time on yield (Kg/ha) of advanced lines/varieties in T. Aman rice.

Advanced lines/varieties	Yield (kg ha)		
	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	6630	6400	6000
BR (Bio) 9786-BC2-132-1-3	6020	6000	5570
BRRI dhan49 (ck)	5450	5440	5080
CV%		2.86	
Lsd (0.05) for transplant time		0.12	
Lsd (0.05) for variety		0.14	
Lsd (0.05) for transplant time* variety		0.26	

Table 3. Extent of reduction in yield of genotypes due to delay in planting time.

Advanced lines/varieties	Reduction in grain yield (kg/ha)	
	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	4%	6%
BR (Bio) 9786-BC2-132-1-3	0.33%	7%
BRRI dhan49 (ck)	0.18%	6%

Table 4. Growing degree days requirement of genotypes under different planting times.

	GDD (Growing degree days)		
Advanced line/variety	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	2399	2234	2088
BR (Bio) 9786-BC2-132-1-3	2388	2272	2109
BRRI dhan49 (ck)	2420	2283	2088

Table 5. Heat use efficiency (HUE) requirement of genotypes under different planting times.

	Heat use efficiency (HUE)		
Genotype	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	2.76	2.86	2.87
BR (Bio) 9786-BC2-132-1-3	2.52	2.64	2.64
BRRI dhan49 (ck)	2.25	2.38	2.43

Table 6. Heliothermal units (HTU) requirement of genotypes under different planting times.

	Helio-thermal units (HTU)		
Advanced line/variety	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	13759.75	11467.93	1052.026
BR (Bio) 9786-BC2-132-1-3	13712.14	11636.24	10663.14
BRRI dhan49 (ck)	15141.01	11467.93	10743.62

Table 7. Yield response of genotypes to heliothermal units and heliothermal use efficiency across planting time and genotypes.

	Time of planting		
	1 Aug	16 Aug	31 Aug
Genotypes BR (Bio) 9786-BC2-119-1-1			
Yield (Kg/ha)	6630	6400	6000
HTU	13759.75	11467.93	10520.26
HTUE (kg/HTU)	0.48	0.55	0.57
Genotype BR (Bio) 9786-BC2-132-1-3			
	1 Aug	16 Aug	31 Aug
Yield (kg/ha)	6020	6000	5570
HTU	13712.14	11636.24	10663.1
HTUE (kg/ HTU)	0.43	0.51	0.50
Genotype BRRI dhan49 (ck)			
	1 Aug	16 Aug	31 Aug
Yield (kg/ha)	5450	5440	5080
HTU	15141.01	11467.93	10743.62
HTUE (kg/ HTU)	0.35	0.47	0.47

Table 8. Photothermal units (PTU) requirement of genotypes under different planting times.

	Photo-thermal unit (PTU)		
Advanced lines/varieties	1 Aug	16 Aug	31 Aug
BR (Bio) 9786-BC2-119-1-1	30112.51	25653.48	23359.90
BR (Bio) 9786-BC2-132-1-3	30060.07	26044.92	23579.26
BRRI dhan49 (ck)	29678.81	26156.42	23359.90

Growing degree days

Table 4 presents accumulation of growing degree days (GDD) were calculated at different planting times of the advanced lines/varieties during crop seasons. The higher number of GDD was accumulated by the first (1 August) planting time followed by second (16 August) and third (31 August) transplanting time in all of the advanced lines/varieties. Among the advanced lines/varieties, the higher GDD (2420 degree C/day) was recorded in the variety BRRI dhan49 followed by the BR (Bio) 9786-BC2-119-1-1 (2399 degree C/day) at first transplanting time. Growing degree days (GDD) were decreased with late transplanting for all the advanced lines/varieties during the crop season. In general, earlier transplanting time crop availed higher degree days and with each delay in transplanting the degree day consumption decreased during the crop seasons. It is reported that GDD accumulation is higher under early transplanting. Reduction in heat units with delay in transplanting has been reported by Singh and Pal (2003), Rajput *et al.* (1987) and Abhilash *et al.* (2017). Khan *et al.* (2006) found that the GDD requirement for the maturity was the highest in Lal Swarna (2385 degree C/day), followed by IR-36 (1975 degree C/day) and Kshitish (1913 degree C/day).

Heat use efficiency

Total heat energy available to any crop is never completely converted to dry matter even under most favorable agro climatic conditions (Table 5). Heat use efficiency (HUT) was calculated at various time of transplanting of the used advanced lines/varieties and is presented in Table 5. HUE was increased with delay the transplanting time. Among the transplanting time, 31st August transplanted crop exhibited maximum HUE followed by 16th August and 1st August transplanting during the crop season for all the advanced lines/variety. Among the three advanced line/varity, BR (Bio) 9786-BC2-119-1-1 was recorded maximum HUE (2.87-2.76 kg ha^{-1} $°C^{-1}$ day^{-1}). The second highest HUE (2.64-2.52 kg ha^{-1} $°C^{-1}$ day^{-1}) was found in the advanced line, BR (Bio) 9786-BC2-132-1-3. The lowest HUE (2.43-2.25 kg ha^{-1} $°C^{-1}$ day^{-1}) was recorded in BRRI dhan49.

Heliothermal units

Table 6 presents heliothermal units (HTU) that are required for attaining different transplanting times of the advanced lines/varieties during the crop seasons. It was observed that HTU accumulation increased in all the advanced lines/variety transplanted at first (1st) August and HTU accumulation decreased with delay the transplanting time in all the advanced lines/variety. Maximum heliothermal units were calculated in the advanced lines/variety transplanted on 1st August followed by the lines/variety transplanted on 16th August and 31st August, respectively. The maximum helio-thermal units (15141.01°C day hour) were recorded in the BRRI dhan49 followed by (13759.75°C day hour) in the advanced line BR (Bio) 9786-BC2-119-1-1 and the lowest (10520.26°C day hour) in the advanced line BR (Bio) 9786-BC2-119-1-3 at the first transplanting time (1st August). These findings are similar with Kaur and Dhaliwal (2014) who reported that heliothermal units was maximum in crop that are transplanted on 15th June as compared to the transplanting time 30th June and 15th July.

Heliothermal use efficiency

Table 6 presents heliothermal use efficiency was recorded by the different planting times and advanced lines/variety of rice. At the first planting time, the advanced line BR (Bio) 9786-BC2-119-1-1 has exhibited high HTUE than the advanced line BR (Bio) 9786-BC2-119-1-3 and BRRI dhan49. Similar trend of HTUE were also found in the second (16th August) and third (31th August) planting time. This could be due to exposure of long duration genotype to bright sunny days combined with long dry spells at their later growth period (grain filling stage). In all, early planting rice has taken the advantage of optimum temperature and sunlight during the early stage of plant development and thereby avoided adverse situations during its life cycle.

Photothermal units

The data on variation of photothermal units (PTU) with respect to the transplanting time and advanced lines/varieties were observed. The PTU was higher in the first transplanting time (1st August) followed by second transplanting time (16th August) and then third planting time (31 August) in all the advanced lines/varieties (Table 7). Among the advanced lines/varieties, higher PTU was recorded in advanced line for BR (Bio) 9786-BC2-119-1-1 (30112.51°C day hour) followed by BRRI dhan49 (29678.81°C day hour) at the first transplanting time (1st August). PTU decreased in late transplanting for all advanced lines/varieties (Table 7). Earlier transplanted rice accumulated higher photothermal units for all the advanced lines/varieties and with delay in transplanting time, PTU requirements decreased (Table 8).

CONCLUTION

Taken together, it may be concluded that maximum grain yield can be obtained from the advanced line BR (Bio) 9786-BC2-119-1-1 and BR (Bio) 9786-BC2-132-1-3 by using the 1st planting time (1st August). Our result indicates that the different agroclimate indices *viz*. GDD, HTU, and PTU were higher in the advanced line/verity of rice transplanted at 1st August that would be the positive yield regulator of rice. It provides a scientific basis for determining higher yield that could be the effect of temperature, radiation or photoperiod on phenological behavior of a standing crop.

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