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CONTENTS

S. Yeasmin, M. M. Islam and S. N. Begum

M. R. Islam, R. Ashrafi and M. A. Salam

S. Roknuzzaman, S. N. Begum, M. M. Islam and A. K. Patwary

H. A. Begum and M. B. Meah

M. M. Islam, M. M. Hassan Sohel, M. N. Hoque, S. N. Begum and M. S. R. Khanom

M. S. Islam, M. A. R. Sarkar and M. A. Salam

H. A. Begum and M. B. Meah

- M. A. Haque, M. A. Sattar, M. R. Islam, M. A. Hashem and M. K. Khan
- M. M. Islam, M. Ahmed, S. A. Fakir and K. Begum

M. E. Haque, H. K. Sumon, M. A. Sattar, M. K. Khan, M. E. Hossain, M. M. Rahman and M. M. Hossain

M. Ibrahim Khalil and M. H. Rashid

M. A. Rahman, A. A. Sarkar, B. Siddiqui, P. Biswas and N. N. Karim

M. A. Mannan, M. S. U. Bhuiya, H. M. A. Hossain, M. I. M. Akhand and M. E. Haque

- 1 MOLECULAR CHARACTERIZATION OF SOME RICE GENOTYPES USING RAPD MARKERS
- 13 SCREENING FOR DROUGHT TOLERANCE OF WHEAT GENOTYPES
- **39** MANAGEMENT OF FUSAURIM WILT OF TOMATO WITH SOIL AMENDMENTS
- **49** EVALUATION OF SUBMERGENCE TOLERANT RICE GENOTYPES FOR FLOOD PRONE AREAS OF BANGLADESH
- 59 SCREENING OF DIFFERENT AROMATIC AND NON-AROMATIC FINE RICE VARIETIES IN AMAN SEASON IN TERMS OF YIELD
- 67 PERFORMANCE OF TOMATO MUTANTS AND VARIETIES AGAINST BACTERIAL WILT
- 73 EVALUATION OF PHOSPHATE SOLUBILIZING BACTERIA IN RELATION TO PHOSPHORUS SOLUBILIZATION AND PHOSPHATASE ACTIVITY
- 85 EFFECT OF CULTIVAR AND ROW SPACING ON THE YIELD AND YIELD CONTRIBUTING ATTRIBUTES OF CHICKPEA
- **91** ASSESSMENT OF DIFFERENT CHEMICAL PROPERTIES IN BALU RIVER WATER DURING WET AND DRY SEASON
- 105 EVALUATION OF FUNGICIDES ON THE GROWTH OF RHIZOCTONIA SOLANI, RHIZOCTONIA ORYZAE AND RHIZOCTONIA ORYZAE- SATIVAE
- 113 STUDIES ON AGRO-CLIMATIC PARAMETERS OF GANGES RIVER FLOODPLAIN AND ITS LONG-TERM TREND ANALYSIS USING "MAKESENS" MODEL
- 127 EFFECT OF TRANSPLANTING DATE ON THE GROWTH AND YIELD OF MODERN AROMATIC FINE RICE VARIETIES IN AMAN SEASON

MOLECULAR CHARACTERIZATION OF SOME RICE GENOTYPES USING RAPD MARKERS

S. Yeasmin¹, M. M. Islam² and S. N. Begum³

Abstract

Molecular markers are useful tools for evaluating genetic diversity and DNA fingerprinting. This investigation was aimed at exploring the genetic diversity and relationship among eight rice accessions namely, Binadhan-4, Binadhan-5 and RcSTL-20, BRRI dhan42, BRRI dhan43, BRRI dhan44, BRRI dhan45 and BRRI dhan47 using Random Amplified Polymorphic DNA (RAPD) markers. In total, 44 reproducible DNA bands were generated by three arbitrary selected primers (OPA01, OPA02 and OPB01), of which 34 (77.30%) bands were proved to be polymorphic. The highest proportion of polymorphic loci and gene diversity values were 13.064% and 0.067, respectively for Binadhan-4 and the lowest proportion of polymorphic loci and gene diversity values were 0.00% and 0.00, for both BRRI dhan43 and BRRI dhan47 respectively. Binadhan-4 showed highest gene diversity could be used in new breeding programs to widen the genetic base of rice varieties. The average coefficient of gene differentiation (Gst), gene flow (Nm) and Shannon's Information Index values were 0.902, 0.054 and 0.274, respectively. Based on pair-wise comparison of RAPD amplification products, genetic similarity was estimated using Nie's (1972) genetic distance and a dendrogram was constructed using unweighted pair group method of arithmetic mean (UPGMA). The result of cluster analysis indicated that the eight accessions were capable of being classified into two major groups; Binadhan-4, BRRI dhan42, BRRI dhan43 and BRRI dhan44 grouped in cluster 1 and BRRI dhan47, BRRI dhan45, Binadhan-5 and RcSTL-20 were grouped in cluster 2. The BRRI dhan42 variety was closer to the variety BRRI dhan43 with the lowest genetic distance (0.088) and the highest genetic distance was found between Binadhan-4 and BRRI dhan47 (0.504). Each cluster further divided into two sub-clusters. The varieties with higher genetic distance could be utilized in future breeding programme for improving trait of interest.

Key Word: Molecular characterization, RAPD and Marker

Introduction

Rice, *Oryza sativa* (2n = 24) belongs to the Gramineae family and the genus *Oryza*. Bangladesh is mainly a rice growing country. The contribution of the crop sector to GDP is 12.10% while rice alone contributes to about 10% i.e. about 83% of the total contribution of the crop sector (The Bangladesh Observer, 04 March, 2007). Per hectare

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yield of rice in Bangladesh is low only 2.42 t ha⁻¹ (BBS, December 2004) in contrast to about 7-8 t ha⁻¹ in China. It clearly indicates that without adoption of modern rice varieties along with associated increased rice yield, it would not have been possible to achieve food security for the 140 million people of Bangladesh. Assessment of genetic diversity and identification of superior genotypes are important prerequisites for any crop improvement programme. With the development of a wide range of molecular techniques, marker assisted breeding is now used to enhance traditional breeding programs to improve crops (Frey et al., 2004; Lu et al., 2004). Due to their abundance, DNA or molecular markers have been used extensively to assess the level of genetic diversity in most crop species (Islam et al. 2007). Random Amplified Polymorphic DNA (RAPD) based on polymerase chain reaction are also promising for this purpose, has been readily adopted in gene mapping and finger printing studies (Williams et al., 1993), requires minute quantities of DNA for detection of polymorphism based on the presence (dominant) or absence (recessive) of particular bands in electrophoresis. The RAPD technique has several advantages over other DNA marker methodologies, such as speed, low cost, small amounts of DNA and non-requirement of sequence information (Karp et al., 1997).

In the present study, we have used RAPD markers to determine the pattern and extent of genetic variation within accessions of rice, as it is important particularly for variety selection for breeding purpose, hybridization evaluation and conservation of their diverse gene pool. Moreover, it is of great interest to determine the degree of genetic differentiation between populations of *Oryza sativa* because the taxonomic status of taken accessions is unclear. Thus the present study was conducted with the following objectives in view:

- 1. To reveal nuclear DNA level variation within each of the rice genotype.
- 2. To establish the genetic relationship among eight rice genotypes at molecular level.

Materials and Methods

The experiment was carried out at the Biotechnology Laboratory, of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh from January to May, 2007. One advanced rice line RcSTL-20, two BINA released mutant varieties (Binadhan-4, Binadhan-5) and five BRRI varieties (BRRI dhan42, BRRI dhan43, BRRI dhan44, BRRI dhan45, BRRI dhan47) were used in the study. Seeds were collected from the Bangladesh Rice Research Institute (BRRI), Gazipur and Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Genomic DNA samples of three individuals per variety (a total of 24 individuals) were extracted from young actively growing leaf (10-15 day old) tissues following Phenol: Chloroform: Isoamyl alcohol purification and ethanol precipitation method (Sambrook, 1989). Short identities of the germplasm used in the experiment are listed in Table 1.

Name of genetype	Variety	Maturiy period	Plant hight	Yield	Varietal
Name of genotype	collected from	(days)	(cm)	$(t ha^{-1})$	type
Binadhan-4	BINA	140	80	4.5	Aman
Binadhan-5	BINA	145	94	5.4	Boro
RcSTL-20	BINA	148	88	4.4	Boro
BRRI dhan42	BRRI	98	95	3.5	Aus
BRRI dhan43	BRRI	101	95	3.5	Aus
BRRI dhan44	BRRI	150	125	4.5	Aman
BRRI dhan45	BRRI	145	98	6.5	Boro
BRRI dhan47	BRRI	152	101	6.1	Boro

Table 1. Details of the eight rice germplasms used for RAPD analysis

Source: Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA).

DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The reaction mix was preheated at 94°C for 3 minutes followed by 40 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 34°C and elongation or extension at 72°C for 2 minutes. After the last cycle, a final step of 7 minutes at 72°C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°. Upon completion of the amplification, reaction composition was loaded onto a 1.5% agarose gel and electrophoresed at 220V for 1 hr in TBE buffer. Bands were visualized by ethidium-bromide staining and sizes of the identified bands were derived relative to 20 kb DNA ladder. DNA bands were observed under UV light using a Gel DOC and photographed by Image Documentation System.

Eleven primers of random sequences were screened on randomly chosen individuals from different germplasm to evaluate their suitability for amplifying DNA sequences. A final subset of three primers exhibiting good quality banding patterns and sufficient variability were selected for further analysis.

Since RAPD markers are dominant, we assumed that each band represented the phenotype at a single allelic locus (Williams *et al.*, 1990). A molecular weight marker, 20bp DNA ladder was used to estimate the size of the amplification products by comparing the distance traveled by each fragment with that of the known sized fragments of molecular weight markers. All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on gel and scored visually on the basis of their presence (1) or absence (0), separately for each individual and each primer.

The scores obtained using all primers in the RAPD analysis were then pooled to create a single data matrix. This was used to estimate polymorphic loci, Nei's (1973), gene

diversity, population differentiation, (G_{ST}), gene flow (N_m), genetic distance (GD) and to construct a UPGMA (Unweighted Pair Group Method With Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) (Yeh *et al.*, 1999). The same program was also used to perform test of homogeneity in different locus between population pairs. Gene frequency estimation for RAPD loci was based on the assumption of a two-allele system.

Under the assumption of Hardy-Weinberg equilibrium, the null allele frequency (q) may be (N/n) ¹/₂ where N and n are the number of band negative individuals observed and the sample size, respectively. The frequency of the other allele (P) is 1-q. The assumption of the two allele system enables us to calculate the Nei's, genetic distance (Nei, 1972) from the RAPD pattern.

Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually RAPD markers of the same molecular weight on the data matrix according to the following formula,

Similarity index (SI) = $2 N_{xy}/N_x + N_{y}$.

Where, N_{xy} is the number of RAPD bands shared by individuals x and y respectively, and Nx and N_y are the number of bands in individual x and y, respectively (Chapco *et al.*, 1992; Wilde *et al.*, 1992; Lynch, 1990).

Results and Discussion

Among eleven primers three primers (OPA01, OPA02 and OPB01) were selected, as they showed comparatively maximum number of high resolution bands. Selected three primers generated 44 bands and 34 bands were considered as polymorphic (Table 2). Maximum numbers of polymorphic bands (12) were generated from OPA01 suggested high level of polymorphism. Average polymorphism across primers was found 77.30% with the highest 80% in OPA01 followed by OPB01 (78.57%) and OPA02 (73.33%).

The present experiment produced 14.66 scorable bands per primer and 11.33 polymorphic RAPD markers per primer (Table 2). This is relatively high level of polymorphism detected by the arbitrary primers compared to the previous reports in other RAPD studies on rice varieties, such as 5.5 scored per primer in rice (Ferdowsi, 2004), 6.2 polymorphic bands per primer in 20 rice germplasms of Bangladesh (Khan, 2006). The reasons of the increased number of average scorable and polymorphic bands could be that the primers used in this study, consist of 60-70% GC content. Fukuoka *et al.* (1992) observed an increase in the number of bands with increasing GC content of the primer.

Drimor anda	Total number of	Polymorphic	Polymorphism	Mean
Finner Code	band scored	bands	(%)	polymorphism
OPA01	15	12	80	
OPA02	15	11	73.33	
OPB01	14	11	78.57	77.30%
Total	44	34	-	
Average	14.66	11.33	-	

Table 2. Number and Percentage of polymorphic loci across primers in studied rice germplasm

The banding patterns of different rice genotypes using primers OPA01, OPA02 and OPB01 are shown in Figs. 1, 2 and 3.



Fig. 1. RAPD profiles of five rice genotypes using primer OPA01. Lane 1-3: Binadhan-4, lane 4-6: Binadhan-5, lane 7-9: RcSTL-20, lane 10-12: BRRI dhan42, lane 13-15: BRRI dhan43. M: Molecular weight marker (20 bp DNA ladder).



Fig. 2. RAPD profiles of eight rice genotypes using primer OPA02. Lane 1-3: Binadhan-4, lane 4-6: Binadhan-5, lane 7-9: RcSTL-20, lane 10-12: BRRI dhan42, lane 13-15: BRRI dhan43. M: Molecular weight marker (20 bp DNA ladder).



Fig. 3. RAPD profiles of six rice genotypes using primer OPB01. Lane 1-3: Binadhan-4, lane 4-6: Binadhan-5, lane 7-9: RcSTL-20, lane 10-12: BRRI dhan42, lane 13-15: BRRI dhan43. M: Molecular weight marker (20 bp DNA ladder).

The highest polymorphic loci (13.06%) was found in Binadhan-4 which gave 6 polymorphic bands, whereas the lowest values (00.00) of these traits was recorded in both the varieties of BRRI dhan43 and BRRI dhan47 and performed no polymorphic band (Table 3). Ferdowsi (2004) found 13 polymorphic loci with four selected primers in some high yielding rice varieties released by BINA and BRRI among them Binadhan-4 showed the highest polymorphic loci (59.09%).

The variety, Binadhan-4 showed highest level of gene diversity (0.067) than other germplasm. Gene diversity across all germplasm for all loci was 0.196. BRRI dhan43 and BRRI dhan47 showed the lowest (0.000) gene diversity. Shannon information index (I) of all rice germplasms was 0.274. The variety Binadhan-4 showed the highest I value (0.093) and BRRI dhan43 and BRRI dhan47 showed the lowest (0.000).

Since the variety Binadhan-4 exhibited higher percentage of polymorphic loci, gene diversity and Shannon's information index suggested higher polymorphism. The variety Binadhan-4 produced also higher yield with novel characters. Considering this higher genetic distance Binadhan-4 and BRRI dhan47 could be utilized in future breeding programme.

8F				
	Number of	Percentage of	Gene	Shannon's
Accession ID	polymorphic	polymorphic	diversity	Information index
	loci	loci	(h)	(I)
Binadhan-4	6	13.06	0.067	0.093
Binadhan-5	2	4.55	0.022	0.031
RcSTL-20	3	6.82	0.033	0.046
BRRI dhan42	2	4.55	0.022	0.031
BRRI dhan43	0	0.00	0.000	0.000
BRRI dhan44	3	6.82	0.033	0.046
BRRI dhan45	2	4.55	0.018	0.026
BRRI dhan47	0	0.00	0.000	0.000
Total	-	-	0.196	0.274

Table 3. Estimated genetic variation in percentage of polymorphic loci; Nei's (1973) gene diversity (h); and Shannon's information index (I) obtained from studied rice germplasms

Intra-cultivar similarity index (Si) for the BRRI dhan43 and BRRI dhan47 were the highest (100%) followed by Binadhan-5 (98.05%), BRRI dhan42 (97.94%) and BRRI dhan45 (97.77%). This highest Si value reflects the lowest genetic variability within the variety. From this study, it is likely that individuals of BRRI dhan43 and BRRI dhan47 varieties were the most homogenous. The lowest Si value (93.06%) was found in Binadhan-4 (Table 4). Thus, Binadhan-4 indicated highest genetic variability among the populations.

Conotypa		Band sharin	g values (%)	
Genotype	OPA01	OPA02	OPB01	Average
Binadhan-4	94.53	93.49	91.168	93.06
Binadhan-5	97.33	96.82	100	98.05
RcSTL-20	91.031	97.33	97.33	95.23
BRRI dhan42	96.49	97.33	100	97.94
BRRI dhan43	100	100	100	100
BRRI dhan44	96.825	97.33	96.825	96.99
BRRI dhan45	96.825	100	96.49	97.77
BRRI dhan47	100	100	100	100
Average	96.628	97.78	97.726	97.75

Table 4. Similarity between (Si) individuals of the 8 rice varieties (intra)

Similarities (Sij) between different varietals pair (inter) are shown in the Table 5. The S_{ij} values were ranged from (62.74-93.39%). The highest similarity indices of 93.39% were found between BRRI dhan42-BRRI dhan43. Thus, lower levels of genetic distance between these cultivar pairs exist. On the other hand lowest inter-variety similarity indices

7

found between Binadhan-4 and BRRI dhan47 and it was 62.74% indicates greater genetic distance between these cultivar pairs. Among the three primers, OPA02 showed the highest intra- and inter-variety similarity indices (Tables 4 and 5).

Band sharing based intra-varietal similarity indices were higher average 97.75% than inter-varietal similarity indices average 93.39% indicating low level of genetic variation between two individuals of same variety and significant genetic variation between two individuals of different varieties using RAPD technique. In a previous study, Ferdowsi (2004) found inter-variety similarity indices ranged from 63.93-86.43% and intra-variety similarity indices ranged from 72.34-86.11% among 6 cultivars of rice. This difference can be ascribed to the genotypes assessed.

Variatals pair		Band sharing	values (%)	
vanetais pair	OPA01	OPA02	OPB01	Average
Binadhan-4 – Binadhan-5	76.72	90.90	78.26	81.96
Binadhan-4 – RcSTL-20	84.395	81.81	88.00	84.735
Binadhan-4 – BRRI dhan42	76.767	91.66	88.00	85.475
Binadhan-4 – BRRI dhan43	74.56	90.66	86.95	84.39
Binadhan-4 – BRRI dhan44	83.88	94.66	86.65	87.39
Binadhan-4 – BRRI dhan45	77.56	81.81	86.95	82.10
Binadhan-4 – BRRI dhan47	79.34	53.33	55.55	62.74
Binadhan-5 – RcSTL-20	83.28	72.72	75.00	77.00
Binadhan-5 – BRRI dhan42	85.63	83.33	83.33	84.09
Binadhan-5 – BRRI dhan43	84.183	83.33	72.72	81.05
Binadhan-5 – BRRI dhan44	83.272	91.66	72.72	82.55
Binadhan-5 – BRRI dhan45	83.717	81.81	82.81	82.44
Binadhan-5 – BRRI dhan47	86.09	84.33	70.588	80.00
RcSTL-20 – BRRI dhan42	86.855	83.33	92.30	87.49
RcSTL-20 – BRRI dhan43	83.073	80.33	86.33	83.244
RcSTL-20 – BRRI dhan44	87.656	83.33	83.33	84.77
RcSTL-20 – BRRI dhan45	87.274	72.72	91.66	83.88
RcSTL-20 – BRRI dhan47	93.18	91.66	63.15	82.66
BRRI dhan42 – BRRI dhan43	88.524	100	92.66	93.39
BRRI dhan42 – BRRI dhan44	80.257	92.30	90.66	88.072
BRRI dhan42 – BRRI dhan45	84.31	83.33	93.66	86.44
BRRI dhan42 – BRRI dhan47	83.525	84.61	63.15	77.095
BRRI dhan43 - BRRI dhan44	79.626	92.30	90.90	87.60
BRRI dhan43 – BRRI dhan45	80.55	83.33	90.90	84.92
BRRI dhan43 – BRRI dhan47	80	84.61	70.58	78.39
BRRI dhan44 – BRRI dhan45	74.26	98.66	90.90	87.60
BRRI dhan44 – BRRI dhan47	82.836	92.30	58.82	77.98
BRRI dhan45 – BRRI dhan47	86.956	83.33	70.58	80.28
Average	82.795	86.11	80.39	83.09

Table 5. Pair-wise similarity indices (Sij) among eight rice germplasms (Inter)

Out of 28 varietal pairs, 26 varietal pairs were heterogeneous at maximum number of loci. Therefore, this study clearly indicates that there was a significant level of genetic variation among different varieties.

The values of pair-wise comparisons of Nei's genetic distance (1972) were calculated from combined data sets for three primers ranging from 0.088 to 0.504 (Table 6). The highest genetic distance (0.504) was observed between Binadhan-4 vs. BRRI dhan47. BRRI dhan47 is a salt tolerant high yielding modern variety developed by BRRI for south east coastal region while Binadhan-4 is an advanced mutant line of BINA. As the Binadhan-4 and BRRI dhan47 are genetically apart so, can be used in breeding programme.

The lowest (0.088) genetic distance was revealed between BRRI dhan42 and BRRI dhan43. The variety BRRI dhan42 and BRRI dhan43 showed significantly alike agronomic performances viz. yield, maturity period and growing season (Table1) and also they are sister mutants were derived from same parent.

Genotypes	Binadhan-4	Binadhan-5	RcSTL-20	BRRI dhan42	BRRI dhan43	BRRI dhan44	BRRI dhan45	BRRI dhan47
Binadhan-4	***							
Binadhan-5	0.429	***						
RcSTT-20	0.274	0.297	****					
BRRI dhan42	0.325	0.301	0.252	****				
BRRI dhan43	0.331	0.281	0.293	0.088	****			
BRRI dhan44	0.208	0.385	0.294	0.207	0.233	****		
BRRI dhan45	0.376	0.284	0.287	0.316	0.322	0.267	****	
BRRI dhan47	0.504	0.344	0.258	0.446	0.452	0.357	0.286	****

Table 6. Nei's genetic distance (below diagonal) values among studied rice germplasms

The unweighted pair group method of arithmetic means (UPGMA) dendrogram was constructed based on Nei's (1972) original measures of genetic distance (GD) (Fig. 4). The UPGMA dendrogram segregates the rice genotypes into two major clusters: Binadhan-4, BRRI dhan42, BRRI dhan43 and BRRI dhan44 in a cluster and two mutant advanced lines of BINA along with BRRI dhan45 and BRRI dhan47 in another cluster. Each cluster again divided into two sub-clusters. Binadhan-4and BRRI dhan47 was distinctly located in the dendrogram and the highest genetic distance (0.504) was found between them. BRRI dhan 47 is a salt tolerant variety of boro season for the coastal area. Different result was found in another study by Ferdowsi (2004) with six rice cultivars: all the four mutants developed by BINA were grouped in one cluster and two BRRI released varieties were grouped in another cluster.

The results of this study can be used as a baseline of relationships for future diversity assessment and genetic analysis of rice varieties in Bangladesh. The genotypes showed highest genetic distance could be used in breeding programme to produce novel varieties that are intended towards the improvement of crop productivity and able to endure from biotic and abiotic stress. In this case Binadhan-4 and BRRI dhan47 can be used as a parental source for breeding programme. Overall, RAPD markers provided a fast, efficient technique for variability assessment that complements methods currently being used in genetic resources management and in plant biosystematics to confirm morphological differences among populations and/or to differentiate apparently similar populations.



Fig. 4. UPGMA dendrogram based on Nei's (1972) original measures of genetic distance, summarizing the data on differentiation between rice germplasm according to RAPD analysis

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SCREENING FOR DROUGHT TOLERANCE OF WHEAT GENOTYPES

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Abstract

The pot and field experiments were conducted to evaluate drought tolerance of 25 wheat genotypes with respect to three different moisture stress conditions. The moisture stress treatments for field condition were (i) Irrigated (control), (ii) Rainfed and (iii) Water stress treatment. The 30%, 55% and 80% field capacity (FC) were used in pot condition. The genotypes Shatabdi, Kanchan, BAW-944, BAW-969, GPB-48 and BAW-56 were identified to be the most tolerant to drought and they showed their highest growth and yield in both pot and field conditions. In pot condition Shatabdi and Kanchan demonstrated the highest dry matter stress index (DMSI) (Shatabdi = 66.8%, Kanchan = 66.1%) and yield stability (YS) (Shatabdi = 69.6%, Kanchan = 68.6%) while Agrani and BAW-944 possessed comparatively lower in DMSI (36.6) and YS (10.2) in 30% FC treatment. The lowest drought susceptibility index (S) was observed in Shatabdi under 55% and 30% FC (55% \overrightarrow{FC} = 0.26, 30% FC = 0.50) which demonstrated the most tolerant to drought stress. In field condition, Shatabdi also showed the highest DMSI (71.0) and YS (70.8) under stress treatment suffered minimum yield reduction under stress condition. The genotype, Agrani resulted in the lowest DMSI (33.2%) and YS (19.43%) in stress treatment. The least drought susceptibility index was observed in Shatabdi (0.42) and Kanchan (0.49) under stress treatments which clear indicated most tolerant to water stress. The genotype, Agrani had shown S value >1.00 under stress treatment indicated susceptible to water stress. From the results of pot as well as field experiments, it may be concluded that the two genotypes Shatabdi and Kanchan appeared to be the most tolerant in terms of growth and drought susceptibility indicators in stress level.

Key words: Wheat, Drought tolerance, Genotype

Introduction

Drought stress is one of the most limiting factors to crop production world wide. Drought stress factor negatively affects plant growth and development and causes a sharp decrease of plants productivity (Pan *et al.*, 2002). Among all the factors limiting wheat productivity, drought is the single most important factor affecting the wheat productivity, food security in terms of wheat and sustainability in agricultural production. For improving yield under dry land conditions, development of new wheat cultivars with high grain yield potential through identifying drought tolerance mechanism is of great significance (Noorifarjam, 2013). Wheat cultivars respond differently to water stress in the

¹Horticulture Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh ²Director (Research), Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh form of physiological and biochemical changes (Sanjari-Pireavatlou *et al.*, 2011). Improving drought tolerance of wheat has long been a major objective of most breeding programs because water deficit during some part of the growing period are common to most regions of the world where wheat is produced. Effective selection of genotypes to increase the productivity of rainfed wheat may be a convenient and efficient approach to meet this challenge (Talebi *et al.*, 2009). The best option for crop production, yield improvement and yield stability under moisture deficient conditions is to develop drought tolerant crop varieties (Abdullah *et al.*, 2011). Drought resistance is not a simple phenomenon. A single trait cannot make a plant resistant to water stress (Razzaq *et al.*, 2013).

Techniques suggested for drought screening includes also plant height stress index, dry matter stress index (Bauslama and Schapaugh, 1984), yield stability ratio (Lewis, 1954) and drought susceptibility index (Fischer and Maurer, 1987). Several physiological characteristics have been reported as being reliable indicators for selection of germplasm possessing drought tolerance. Most widely used criteria for selection are based on yield performance under stress and non-stress conditions (Rashid, 2003). Drought resistance index to assess drought tolerance of a genotype reflects drought resistance and high yield (Hu *et al.*, 2007) indicating the performance of wheat cultivars under water deficit conditions (Dong and Lui, 2005).

At present a good number of modern high yielding and local cultivars of wheat are being grown by the farmers of Bangladesh. Potentiality may exist among these cultivars for further genetic improvement so that new wheat varieties may come out with higher degree of drought tolerance. Physiological potentials of these wheat cultivars for their adaptability to drought have not yet been thoroughly studied. Success in such effort would be able to make tremendous increase in a wheat production in the rainfed area of the country. Development of stress tolerant varieties is always major objective of many breeding programs but success has been limited by adequate screening techniques and the lack of genotypes that show clear differences in response to various environmental stresses (Khakwani et al., 2011). The measurement of morphological characters or enumeration of organs gives a quantitative description of the morphological changes that take place during the growth of the crop, but this little light on the physiological explanations of yield problems as caused by drought. So it is essential to know the changes occurred during growth period from sowing to harvest and the peak period of growth which required maximum input. Therefore, the present research work was undertaken to categorize wheat genotypes according to their drought tolerance ability in terms of growth, yield and drought indexing parameters and to identify wheat genotypes which can be used for drought affected areas of Bangladesh.

Materials and methods

Screening experiments under pot and field conditions were conducted at the Bangladesh Institute of Nuclear Agriculture (BINA) experimental farm and at the field laboratory of Genetics and Plant Breeding Department, Bangladesh Agricultural University, Mymensingh respectively, during November 10 to March 5, 2007.

Pot condition: The soils used for filling the pots were taken from the area belong to Brahamaputra dark grey floodplain where the topsoil is usually silt-loam or sandy loam. It is slightly acidic to mildly alkaline in reaction. The soil was silty loam in texture having pH 6.7, organic C 0.65%, total N 0.09% and available P 9.3 μ gg⁻¹. Twenty five wheat genotypes were used for screening of drought tolerance under three moisture stress treatments *viz.*, 30, 55 and 80% field capacity (FC).

Field condition: The experiment was conducted at field laboratory of Genetics and Plant Breeding Department in Bangladesh Agriculture University. Twenty five wheat genotypes were grown in the same field under three moisture regimes viz. (i) Irrigated treatment (control) (ii) Rainfed treatment and (iii) Water stress (transparent polyethylene house). The experiment was laid out in a split plot design with three replications. Stress treatments were assigned in the main plot and genotypes were in subplot. The three moisture regimes were maintained from 25 days after sowing till the end of the experiment. The plots of stress treatment were covered with transparent polyethylene (PVC film 15 mm) with the help of bamboo to protect the rain and dews. The transparent polyethylene shade was about 10 ft. from the ground level for good aeration. The soil was collected (15 cm depth) at 15 days interval from each treatment for measuring soil moisture content by weight basis method in the entire cropping season. The average air temperature fluctuated about $0.5-1.0^{\circ}$ C between open field and transparent polyethylene covered treatment plots. The fluctuation of light intensity was high till 10 a.m. due to presence of dews but after 10 a.m., light intensity were almost equally sprayed between open field and poly house field. The plot size was 2.5 m \times 2 m and spacing was 20 cm \times 5 cm. The plots were fertilized with NPKS@ 101, 15, 33 and 14 kg ha⁻¹, respectively from the source of urea, TSP, MP and Zypsum. Moisture stress developed in the plots of stress treatment at tillering were 39 to 52% field capacity (FC) and upto maturity the soil moisture content was 23-30% FC. Data on growth, yield and indexing parameters were recorded from five randomly selected plants. The data were analyzed using the Computer Software Program MSTAT-C and the means were compared by Least Significant Difference (LSD) (Gomez and Gomez, 1984).

Results

Pot condition: The genotypes showed no significant variation in days to anthesis and maturity under different moisture regimes. However, all genotypes got anthesis 8-10

days earlier in 30% FC treatment. The highest grain weight plant⁻¹ was found in Shatabdi as 12.06 g plant⁻¹, 11.02 g plant⁻¹ and 8.40 g plant⁻¹ (Table 1) under 80%, 55 % and 30% FC treatments, respectively. The lowest grain yield $(1.00 \text{ g plant}^{-1})$ was exhibited by Agrani under 30% FC treatment. The genotypes Kanchan, BAW-969 and BAW-56 produced statistically alike grain yields (5.29-6.80 g plant⁻¹) in 30% FC treatment. The rest genotypes showed poor grain yield ranging from 1.60 to 3.5 g plant⁻¹ in 30% FC treatment (Table 1). Absolute growth of different plant characters (plant height, total tiller, leaf area index and total dry matter) were determined to measure genotypic variation in drought tolerance (Table 2). For growth characters, all the genotypes showed decreased score with the increase in soil moisture depletion. But the genotypes Shatabdi, Kanchan, BAW-969, BAW-56 could sustain better score under 55% FC and 30% FC moisture regimes. Genotypes BAW-923, Paban-76, GPB-118 and Agrani had lower plant height growth rate 0.461, 0.463, 0.401 and 0.260, respectively under 30% FC treatment, though they could maintain higher score in 80% FC treatment. Kanchan showed the highest total tiller growth rate (0.141) in 80% FC treatment. The rest of them had shown almost statistically similar total tiller growth rate ranging from 0.127 to 0.133 under the same moisture regime. The genotype BAW-56 showed the highest total tiller growth rate (0.047) under 30% FC treatment and held equal statistical rank with the second highest genotype Shatabdi (0.043), BAW-969 (0.040), Kanchan (0.040) and GPB-121 (0.040) in 30% FC moisture regime. In contrast, Agrani showed the lowest growth rate of total tiller under 30% FC treatment. The 55% moisture regime exhibited almost similar and intermediate growth rate of total tiller ranged (0.085-0.097). The genotype Shatabdi showed the highest growth rate of LAI (80% FC = 0.109, 55% FC = 0.093 and 30% FC = 0.069) in all moisture stress conditions and held equal rank with the second highest genotypes Kanchan (0.063), BAW-56 (0.058) and BAW-969 (0.057) in 30% FC severe stress condition. Agrani showed higher growth rate of LAI (0.095) in 80% FC treatment but the lowest LAI was found in 55% (0.051) and 30% FC (0.021) treatment. The growth rate of TDM was also highest in the genotype Shatabdi under the moisture stress treatments. Kanchan, BAW-969 and BAW-56 possessed statistically alike which was the second highest. Agrani also produced higher TDM growth rate in 80% FC and the least (0.065g) was found in 30% FC treatment. All the genotypes had shown intermediate TDM growth rate under 55% FC treatment (Table 2).

	Da	ve to anthe	acie	Da	ve to matu	ritz	Grain weight $plant^{-1}(g)$		
Genotypes	80% EC	55% EC	30% FC	80% EC	55% EC	30% EC	80% EC	55% FC	30% FC
DAW 044	68.1	66.2	50.1	106	106	102	7.64	6 5 5	2 56
DAW-944	72.0	69.1	62.2	110	100	105	7.04	0.55	2.15
Prouva	75.2	08.1	02.2	110	108	100	7.91	0.08	5.15
BAW-969	70.2	69.2	62.3	107	107	104	9.51	8.45	6.30
Sourav	72.3	68.0	63.3	107	106	104	7.97	6.65	2.84
BAW-970	69.4	65.2	60.4	107	104	102	8.56	5.40	2.01
Kanchan	70.2	69.1	63.5	106	105	104	9.91	8.95	6.80
BAW-1005	74.1	71.2	64.4	111	109	106	8.20	5.50	1.88
Gourav	70.2	67.0	60.1	106	104	102	7.96	5.60	3.30
BAW-1004	73.4	71.0	66.2	111	109	107	8.65	656	3.05
GPB-119	75.5	73.3	68.0	116	113	110	8.54	5.58	1.72
Ananda	68.2	65.4	61.0	110	109	107	8.06	6.29	2.81
GPB-118	75.1	71.1	66.3	115	113	111	6.69	4.50	1.70
Paban-76	75.3	73.2	67.3	110	107	106	7.06	4.10	1.06
GPB-48	76.2	74.3	69.2	115	113	112	7.37	5.79	2.90
Shatabdi	73.3	70.2	65.1	106	106	104	12.06	11.02	8.40
GPB-110	74.2	73.0	66.0	115	113	111	7.90	5.56	2.13
Agrani	70.1	72.0	61.2	110	107	105	9.04	3.10	1.00
GPB-121	75.0	69.1	66.2	115	112	110	6.73	5.00	1.90
BAW-1003	73.0	73.2	65.5	110	108	106	7.45	5.55	2.33
GPB-148	75.2	71.3	65.4	116	114	111	7.26	5.20	1.80
BAW-1002	71.2	72.3	64.2	110	108	106	7.94	5.90	2.25
BAW-56	72.3	70.4	63.2	107	106	105	8.51	7.70	5.29
BAW-966	69.1	69.2	62.1	107	105	104	7.27	5.30	2.30
BAW-917	75.2	72.1	65.2	112	110	108	7.54	5.29	1.95
BAW-923	75.2	72.1	65.2	112	110	108	7.39	4.90	1.60
LSD 0.05	7.44	7.44	7.44	9.57	9.57	9.57	0.93	0.93	0.93
SE(±)	3.78	3.78	1.78	4.86	4.86	4.86	0.47	0.47	0.47

 Table 1. Interaction effect of moisture regime and genotype on days to anthesis, days to maturity and grain yield of wheat (Pot Expt.)

The genotype had significant differences on drought indexing parameters in respect to moisture regimes. Shatabdi showed the highest DMSI (87.4 %) and YS value (91.3%) which was statistically alike with Kanchan at 55% FC. The genotypes BAW-969, BAW-56 and BAW-944 showed both DMSI and YS values in the second highest ranging 79 to 82.4% and 87.0-90.0%, respectively under the same moisture regime. The genotype Agrani showed the lowest DMSI (69.6%) and YS (56.4%) values in 55% FC. Under 30% FC treatment, Shatabdi and Kanchan had shown the highest DMSI as 66.8% and 66.1% and YS value as 69.6%, and 68.6%, respectively. The lowest DMSI (36.6%) and YS (10.2%) values were found from Agrani under 30% FC treatment (Table 3). The genotypes showed significant differences drought susceptibility index (S) values under different soil moisture regimes.

]	Plant height	:	Тс	otal tiller hil	1-1		Leaf area		Total	dry matter j	plant ⁻¹
Genotypes		(cm)			(no.)			index			(g)	
	80%FC	55%FC	30%FC	80%FC	55%FC	30%FC	80%FC	55%FC	30%FC	80%FC	55%FC	30%FC
BAW-944	0.930	0.661	0.532	0.127	0.087	0.037	0.096	0.079	0.055	0.137	0.126	0.079
Protiva	0.951	0.932	0.534	0.128	0.086	0.037	0.090	0.072	0.049	0.174	0.136	0.071
BAW-969	0.800	0.730	0.735	0.132	0.087	0.040	0.098	0.081	0.057	0.184	0.179	0.156
Sourav	0.502	0.533	0.130	0.133	0.088	0.037	0.094	0.074	0.050	0.180	0.160	0.109
BAW-970	0.861	0.634	0.331	0.132	0.089	0.020	0.093	0.060	0.034	0.178	0.173	0.085
Kanchan	0.961	0.931	0.802	0.141	0.097	0.039	0.105	0.088	0.063	0.192	0.191	0.186
BAW-1005	0.860	0.530	0.203	0.126	0.088	0.040	0.097	0.065	0.034	0.160	0.160	0.103
Gourav	0.600	0.500	0.465	0.127	0.091	0.023	0.093	0.070	0.050	0.157	0.151	0.133
BAW-1004	0.831	0.562	0.260	0.127	0.089	0.039	0.092	0.071	0.046	0.191	0.133	0.106
GPB-119	0.832	0.863	0.460	0.130	0.089	0.017	0.093	0.062	0.030	0.169	0.191	0.096
Ananda	0.861	0.762	0.404	0.132	0.095	0.038	0.085	0.065	0.043	0.166	0.101	0.058
GPB-118	0.933	0.765	0.401	0.132	0.088	0.037	0.087	0.061	0.033	0.166	0.134	0.057
Paban-76	0.980	0.733	0.463	0.125	0.089	0.026	0.085	0.057	0.026	0.148	0.126	0.059
GPB-48	0.975	0.934	0.602	0.130	0.088	0.033	0.087	0.065	0.038	0.158	0.133	0.085
Shatabdi	0.963	0.930	0.861	0.133	0.091	0.043	0.109	0.093	0.069	0.223	0.213	0.205
GPB-110	0.901	0.730	0.460	0.133	0.090	0.027	0.087	0.063	0.028	0.139	0.115	0.096
Agrani	0.974	0.801	0.260	0.135	0.093	0.030	0.095	0.051	0.021	0.181	0.174	0.065
GPB-121	0.900	0.763	0.463	0.131	0.087	0.040	0.090	0.065	0.035	0.169	0.127	0.091
BAW-1003	0.834	0.702	0.401	0.131	0.089	0.039	0.086	0.058	0.030	0.137	0.114	0.079
GPB-148	0.905	0.761	0.462	0.116	0.085	0.026	0.095	0.061	0.034	0.154	0.148	0.100
BAW-1002	0.831	0.730	0.404	0.127	0.087	0.029	0.092	0.060	0.031	0.152	0.127	0.108
BAW-56	0.731	0.630	0.603	0.133	0.091	0.047	0.095	0.079	0.058	0.182	0.159	0.123
BAW-966	0.761	0.662	0.400	0.127	0.087	0.037	0.093	0.073	0.032	0.143	0.134	0.090
BAW-917	0.930	0.860	0.462	0.126	0.091	0.039	0.088	0.059	0.033	0.166	0.140	0.092
BAW-923	0.99.0	0.801	0.461	0.127	0.088	0.039	0.089	0.062	0.029	0.147	0.114	0.075
LSD 0.05	0.135	0.135	0.135	0.005	0.005	0.005	0.006	0.006	0.006	0.016	0.016	0.016
SE(±)	0.006	0.006	0.006	0.003	0.003	0.003	0.002	0.002	0.002	0.008	0.008	0.008

 Table 2. Absolute growth rates on different plant characters as affected by genotype and imposed moisture stress (Pot Expt.)

The lowest S value was found in Shatabdi under 55% treatments (55% FC = 0.26) which demonstrated most tolerant to moisture stress. Kanchan showed the second highest S value in 55% (0.29) and 30% FC (0.051) treatment and held equal and statistically similar to BAW-56 (0.29) under same treatments. The genotype BAW-969 had third position in S value (0.62) in 30% FC treatment. The genotype BAW-56, BAW-944 showed S value intermediate under 30% FC treatment. The remainders had shown S value >1.00 under 30% FC treatment and thus indicated highly susceptible to 30% FC moisture stress treatment (Table 3).

	Dry matter	stress index	Yield s	tability	Drought susceptibility		
Genotypes	(%	6)	(%	6)	index	x (%)	
-	55% FC	30% FC	55% FC	30% FC	55% FC	30% FC	
BAW-944	79.6	51.0	87.0	46.6	0.39	0.88	
Protiva	78.6	46.9	84.4	39.8	0.47	0.99	
BAW-969	82.4	61.1	88.8	66.2	0.34	0.54	
Sourav	79.2	50.2	83.4	35.6	0.50	1.06	
BAW-970	72.4	40.0	63.0	23.4	1.11	1.25	
Kanchan	84.7	66.1	90.3	68.6	0.29	0.51	
BAW-1005	75.0	42.1	67.0	22.9	0.99	1.26	
Gourav	79.6	50.6	82.9	41.4	0.51	0.96	
BAW-1004	70.3	44.5	75.8	35.2	0.73	1.06	
GPB-119	72.6	40.6	65.3	20.1	1.04	1.31	
Ananda	73.4	43.2	78.0	34.8	0.66	1.07	
GPB-118	74.3	40.1	67.2	25.4	0.99	1.22	
Paban-76	71.7	36.4	67.6	17.4	0.97	1.35	
GPB-48	75.8	45.7	78.5	39.3	0.65	1.99	
Shatabdi	87.4	66.8	91.3	69.6	0.26	0.50	
GPB-110	71.3	41.9	70.3	29.9	0.89	1.20	
Agrani	69.6	36.6	56.4	10.2	1.31	1.47	
GPB-121	75.4	45.9	74.2	28.2	0.77	1.18	
BAW-1003	74.7	44.1	74.5	31.2	0.77	1.13	
GPB-148	73.1	44.5	71.6	24.7	0.85	1.23	
BAW-1002	74.1	45.9	74.3	28.3	0.77	1.17	
BAW-56	81.7	57.9	90.4	62.1	0.29	0.62	
BAW-966	75.4	46.0	72.9	31.6	0.82	1.12	
BAW-917	73.5	42.9	70.1	25.8	0.90	1.22	
BAW-923	70.7	39.8	66.3	21.6	1.01	1.28	
$LSD_{0.05}$	5.63	5.63	3.230	3.230	0.051	0.051	
SE(±)	2.64	2.64	1.151	1.151	0.018	0.018	

 Table 3. Interaction effect of moisture regime and genotype on dry matter stress index, yield stability and drought susceptibility index (Pot Expt.)

Field condition: In the field condition, irrigated, rainfed and stress (Polyethylene shade) treatments were used instead of 80%, 55% and 30% FC soil moisture regimes used in the pot experiment. The genotypes did not show significant differences on days to anthesis and maturity influenced by moisture regimes. The genotype Shatabdi performed the best of all in grain yield under all moisture regimes (irrigated = 3.43 t ha⁻¹, rainfed = 3.15 t ha⁻¹ and stress = 2.43 t ha⁻¹). The genotype Agrani produced the least yield in both rainfed (1.95 t ha^{-1}) and stress treatments (0.61 t ha^{-1}) . Hence the lowest yield reduction in Shatabdi was found as 8.16% and 29.15% under rainfed and stress treatments, respectively where the highest yield reduction was found in Agrani 37.89% and 80.57% under rainfed and stress treatments, respectively. The genotype Kanchan, BAW-969 and BAW-56 produced grain yield ranging from 2.00 to 2.35 t ha⁻¹ in stress treatment (Table 4). The genotype Shatabdi sustained the highest absolute growth rate of plant height, total tiller, LAI and TDM under rainfed and stress treatments followed by Kanchan, BAW-969, BAW-56. Agrani possessed comparatively lower of all regarding the growth characters under stress condition, yet had maintained higher score in irrigated condition. The remainders showed the absolute growth intermediate under rainfed and stress condition (Table 5).

Drought indexing parameters: From the Table 6, it is clear that the genotypic differences in drought indexing parameters were found significant with respect to moisture regimes. The genotypes Shatabdi, Kanchan, BAW-969 and BAW-56 showed S values as 0.37, 0.39, 0.46 and 0.48, respectively which were <0.50 demonstrated tolerant under moisture stress condition. The genotype BAW-944 showed S value as 0.52 which was >0.50 and exhibited medium tolerant to water stress in field condition. Agrani exhibited the highest S value (1.40) of all genotypes resulted in most susceptible type and possessed lowest grain yield production under stress condition. The remainders showed S values ranging from 0.67 to 1.23 and thus resulted in susceptible to drought stress. In field condition, the drought susceptibility index values (S) were lower than the pot experiment. In field experiment there was no such high soil moisture stress conditions as developed in pot condition. In case of indexing parameters, the maximum genotypes showed higher DMSI and YS values and lower S value under rainfed condition. Shatabdi showed the highest DMSI (71.0) and YS value (70.8) under stress treatment, similar to that of Kanchan. The DMSI and YS values of genotypes BAW-969, BAW-56 and BAW-944 showed second highest ranging from 51.6 to 57.1% and 62.7 to 65.5%, respectively under the same treatment. The genotype Agrani exhibited the lowest DMSI (33.2%) and YS (19.43%) in stress treatment (Polyethylene shade). The lowest S (0.42) value was found in Shatabdi under stress treatment and Kanchan also showed lesser S (0.49) value in the same treatment demonstrated and both of them were tolerant to soil moisture stress condition. The genotypes BAW-56 and BAW-944 showed S value intermediate under stress

treatment. The remainders had shown S value >1.00 under stress treatment indicated susceptible to water stress (Table 6).

Construnce	Days to anthesis Days to maturity				rity	Grai	n yield (t l	1a ⁻¹)	
Genotypes	Irrigated	Rainfed	Stress	Irrigated	Rainfed	Stress	Irrigated	Rainfed	Stress
BAW-944	67	66	65	107	105	105	2.29	2.32	1.65
Protiva	71	69	68	110	108	106	2.97	2.30	1.33
BAW-969	69	69	69	107	107	107	3.30	2.93	2.21
Sourav	70	67	67	108	105	105	2.92	2.50	1.26
BAW-970	68	65	63	107	104	105	3.26	2.23	0.92
Kanchan	70	69	69	107	105	105	3.40	3.08	2.35
BAW-1005	73	71	69	111	109	107	3.35	2.36	0.95
Gourav	67	66	65	105	102	102	3.18	2.75	1.45
BAW-1004	73	71	69	107	109	107	3.36	2.55	1.33
GPB-119	75	73	71	115	113	111	3.38	2.29	0.88
Ananda	65	63	62	110	108	107	2.87	2.20	1.05
GPB-118	75	71	70	115	113	111	2.70	2.00	0.90
Paban-76	75	73	71	109	107	106	2.71	1.85	0.70
GPB-48	76	74	72	117	115	113	2.95	2.20	1.15
Shatabdi	72	72	72	108	108	108	3.43	3.15	2.43
GPB-110	74	72	70	115	113	111	2.80	1.94	0.83
Agrani	67	66	64	109	107	105	3.14	1.95	0.61
GPB-121	75	73	71	115	113	111	3.33	2.45	1.10
BAW-1003	73	71	69	110	108	106	3.21	2.30	1.00
GPB-148	75	72	71	115	113	111	2.51	1.78	0.75
BAW-1002	71	69	68	110	108	106	3.11	2.25	0.99
BAW-56	70	69	68	108	106	106	3.05	2.70	2.00
BAW-966	69	67	66	107	105	104	3.12	2.30	1.04
BAW-917	75	72	70	111	109	107	3.30	2.35	1.02
BAW-923	72	72	69	110	109	107	2.90	2.00	0.80
LSD0.05	2.68	2.68	2.68	5.05	5.05	5.05	0.216	0.216	0.216
SE(±)	1.36	1.36	1.36	2.56	2.56	2.56	0.109	0.109	0.109

 Table 4. Effects of genotypes and different moisture stress on yield and yield contributing characters of wheat genotypes (Field Expt.)

]	Plant height		То	tal tiller hil	1 ⁻¹		Leaf area		Total	dry matter p	olant ⁻¹
Genotypes		(cm)			(no.)			index			(g)	
	Irrigated	Rainfed	Stress	Irrigated	Rainfed	Stress	Irrigated	Rainfed	Stress	Irrigated	Rainfed	Stress
BAW-944	0.680	0.573	0.519	0.127	0.086	0.038	0.085	0.069	0.63	0.247	0.213	0.200
Protiva	0.613	0.533	0.480	0.115	0.084	0.037	0.071	0.057	0.051	0.213	0.167	0.155
BAW-969	0.713	0.613	0.546	0.132	0.087	0.04	0.093	0.078	0.071	0.297	0.267	0.234
Sourav	0.587	0.520	0.475	0.121	0.082	0.037	0.082	0.062	0.055	0.260	0.203	0.163
BAW-970	0.659	0.478	0.381	0.121	0.077	0.020	0.085	0.051	0.043	0.277	0.156	0.132
Kanchan	0.717	0.600	0.552	0.133	0.091	0.039	0.091	0.077	0.070	0.308	0.280	0.243
BAW-1005	0.628	0.451	0.367	0.120	0.078	0.027	0.086	0.053	0.048	0.261	0.165	0.118
Gourav	0.659	0.519	0.482	0.124	0.082	0.036	0.084	0.063	0.053	0.253	0.207	0.163
BAW-1004	0.628	0.501	0.443	0.127	0.080	0.032	0.087	0.059	0.052	0.277	0.188	0.125
GPB-119	0.658	0.468	0.376	0.130	0.076	0.021	0.091	0.053	0.048	0.283	0.170	0.133
Ananda	0.589	0.520	0.467	0.120	0.082	0.034	0.063	0.047	0.041	0.202	0.160	0.110
GPB-118	0.587	0.491	0.396	0.095	0.078	0.028	0.073	0.050	0.041	0.200	0.129	0.099
Paban-76	0.586	0.458	0.389	0.099	0.080	0.022	0.066	0.041	0.038	0.190	0.177	0.095
GPB-48	0.591	0.519	0.458	0.101	0.081	0.033	0.070	0.050	0.044	0.225	0.173	0.133
Shatabdi	0.740	0.667	0.625	0.141	0.097	0.043	0.110	0.09	0.088	0.324	0.306	0.273
GPB-110	0.660	0.499	0.421	0.110	0.080	0.027	0.062	0.042	0.034	0.183	0.123	0.097
Agrani	0.695	0.442	0.333	0.119	0.063	0.018	0.079	0.032	0.020	0.225	0.102	0.087
GPB-121	0.590	0.508	0.400	0.105	0.080	0.032	0.083	0.053	0.048	0.236	0.167	0.123
BAW-1003	0.593	0.493	0.405	0.105	0.080	0.030	0.069	0.045	0.040	0.215	0.153	0.119
GPB-148	0.607	0.498	0.412	0.116	0.079	0.029	0.083	0.051	0.043	0.255	0.169	0.130
BAW-1002	0.652	0.504	0.396	0.102	0.081	0.029	0.08	0.053	0.047	0.242	0.155	0.134
BAW-56	0.695	0.580	0.526	0.112	0.087	0.038	0.087	0.071	0.063	0.267	0.231	0.190
BAW-966	0.654	0.513	0.413	0.110	0.082	0.031	0.081	0.052	0.047	0.243	0.173	0.158
BAW-917	0.652	0.497	0.420	0.108	0.091	0.025	0.080	0.051	0.041	0.232	0.159	0.115
BAW-923	0.641	0.480	0.391	0.101	0.078	0.025	0.061	0.038	0.029	0.233	0.153	0.121
LSD0.05	0.072	0.072	0.072	0.0161	0.0161	0.0161	0.0169	0.0169	0.0169	0.0085	0.0085	0.0085
SE(±)	0.036	0.036	0.036	0.0081	0.0081	0.0081	0.0096	0.0096	0.0096	0.011	0.011	0.011

Table 5. Absolute growths of different plant characters as affected by interaction of genotype and imposed moisture stress (Field Expt.)

Genotypes	Dry matter s	stress index	Yield s	tability	Drought susceptibility index (%)		
constypes .	Rainfed	Stress	Rainfed	Stress	Rainfed	Stress	
BAW-944	69.7	51.6	88.2	62.7	0.430	0.600	
Protiva	69.0	44.7	87.1	50.3	0.470	0.880	
BAW-969	70.6	53.8	88.7	66.9	0.410	0.510	
Sourav	65.6	48.5	85.6	43.1	0.420	1.030	
BAW-970	56.7	39.0	68.4	28.2	1.15	1.22	
Kanchan	71.8	65.0	90.5	69.1	0.340	0.490	
BAW-1005	59.1	39.2	70.4	28.3	1.07	1.24	
Gourav	66.3	50.1	86.4	45.6	0.490	0.980	
BAW-1004	65.8	44.4	75.8	39.5	0.880	1.00	
GPB-119	58.1	38.9	67.7	26.0	1.17	1.28	
Ananda	60.9	43.4	76.6	36.5	0.850	1.09	
GPB-118	55.8	36.7	74.0	33.3	0.940	1.15	
Paban-76	50.0	35.6	68.2	25.8	1.15	1.30	
GPB-48	65.2	44.7	74.5	38.9	0.920	0.990	
Shatabdi	76.7	71.0	91.8	70.8	0.300	0.427	
GPB-110	56.3	37.5	69.2	29.6	1.120	1.19	
Agrani	51.7	33.2	62.1	19.4	1.380	1.43	
GPB-121	61.6	40.1	73.5	33.0	0.950	1.15	
BAW-1003	55.2	39.1	71.6	31.1	1.030	1.18	
GPB-148	60.5	39.5	70.9	29.8	1.06	1.21	
BAW-1002	62.4	40.9	72.3	31.8	1.01	1.17	
BAW-56	69.6	57.1	88.5	65.5	0.420	0.540	
BAW-966	64.8	39.9	73.7	33.3	0.960	1.14	
BAW-917	60.6	39.0	71.2	30.9	1.05	1.18	
BAW-923	56.8	36.6	68.9	27.5	1.13	1.23	
LSD _{0.05}	5.53	5.53	6.95	6.95	0.240	0.240	
SE(±)	2.78	2.78	7.499	7.499	0.157	0.157	

 Table 6. Drought indexing parameters of wheat genotypes under rain fed and stress conditions as a proportion of irrigated condition

Discussion

All growth characters under the study were significantly affected by the imposed moisture stresses, gradually and significantly decreased with the imposed gradual increase in stress levels. Many workers observed reduced growth rate of plant height, total tiller, LAI and TDM (Bayoumi *et al.*, 2008; Blum and Pnuel, 1990; Chandler and Singh, 2008; Razzaq *et al.*, 2013). Wahid and Rasul (2005) also found decreased fresh biomass and dry matter production due to reduce photosynthates production under water deficit conditions. Babu *et al.* (1984) observed lower growth of plant height when their crops experienced moisture stresses. Total dry matter results are in conformity with Turk and Hall (1980); Pandey *et al.* (1984) and Ball *et al.* (1994) who observed lesser growth of TDM with

increasing level of drought stress. This means genotypes possessing higher growth rate of dry matter under moisture stress could be drought tolerant (Arjunan et al., 1992). Moreover, Collinson et al. (1996) had the opinion from their study that reduced absolute growth of dry matter production actually resulted from decline in intercepting radiation and thereby photo-assimilation. Drought indexing parameters such as dry matter stress index, yield stability and drought susceptibility index were also affected by the moisture stress treatments. Genotypic responses under moisture stress were found different for most of the characters. This result agrees with Islam et al. (1998) who found that moisture stress resulted in reduced total dry mater and yield. Water deficit decreased photosynthesis and comparatively produced less total dry matter. Stone et al. (1986) observed that TDM reduced with increasing drought duration. Cruz and O'Toole (1985) observed yield loss from water stress (17-80%) among different rice genotypes. Dong and Liu (2005) calculated drought resistance index values for seven wheat cultivars grown under irrigated and non irrigated conditions and found that cultivars having more drought resistance index values were more resistance to drought. Observations indicated that for different soil moisture regimes genotypic performances in terms of their susceptibility to drought was different. However, average values might be used to classify them for their relative resistance to stress keeping yield in view. Such classification is given where genotypes with S<0.5, between 0.5 to 0.7 and >0.7 were classified as tolerant, medium susceptible, susceptible, respectively. Shatabdi showed the lowest S value (0.38) while it was statistically similar to Kanchan (0.40). Shatabdi and Kanchan had shown S value <0.50demonstrated tolerant to severe stress condition. The genotypes BAW-969 and BAW-56 exhibited similar S values ranging from 0.44 to 0.45 which was <0.50 exhibited also tolerant to water stress. The genotypes BAW-944 had shown S value as 0.63 which was >0.50 and demonstrated medium tolerant to water stress. Agrani had shown the highest S value (1.39) among the genotypes while it produced lower grain yield. This genotype demonstrated most susceptible to water stress. The remainders showed S values ranging from 0.73 to1.32 and these genotypes had shown susceptible to drought stress. Fischer and Maurer (1987) reported similar findings.

Genotype discrimination for drought tolerance on the basis of absolute growth rates, grain yield and indexing parameters such as dry matter stress index, yield stability and drought susceptibly index demonstrated consistent results for stress tolerant. On the basis of above given approach, the genotypes Shatabdi, Kanchan fell into tolerant class, BAW-969, BAW-56 and BAW-944 moderately tolerant and reminders demonstrated susceptible to 30% FC moisture stress condition. The experimental results mentioned above, reveals that out of all the genotypes Shatabdi, Kanchan, BAW-944, BAW-56, and BAW-969 demonstrated better tolerance against water stress and the rest genotypes were susceptible to drought stress. This suggests that further in detail investigation and mechanism of drought tolerance from the biochemical point of view is required.

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INTROGRESSION AND MARKER ASSISTED SELECTION OF SALT TOLERANT GENE IN BC_1F_1 POPULATION OF BINADHAN-7 × FL - 478

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Abstract

Marker Assisted Selection (MAS) technique is very effective and reliable which can help selection of several traits associated with abiotic stresses such as salinity and also accelerates the breeding process and increases selection efficiency. The experiment was performed in two steps. At the first step, phenotypic performance of BC₁F₁ populations of Binadhan-7 x FL-478 along with their parents was determined from a pot experiment using biotechnology laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. In BC₁F₁ population, average plant height, total number of tillers plant ¹, productive tillers plant⁻¹, days to flowering and maturity were 85.5 cm, 39, 34, 93 days and 125 days, respectively. One salt tolerant rice genotype (FL-478) was selected as parent for transferring salt tolerant genes to high yielding short duration rice genotype (Binadahan-7). Backcrossing programme was conducted during Aman season in 2011, where Binadhan-7 was the recurrent parent and FL-478 was the nonrecurrent donor line. Sixty seven BC1F1 populations were backcrossed with the recurrent parent Binadhan-7 and produced 285 BC2F1 seeds. At the second step, foreground selection was performed and 32 BC1F1 populations were selected with tightly linked salt tolerant markers RM585, RM10720 and RM310. Out of 32 BC1F1 populations, the marker RM585 identified 20 lines, RM10720 identified 16 lines as tolerant, and the marker RM310 identified 17 lines as salt tolerant. This finding could be used for improvement of salt tolerant rice lines through SSR markers.

Key words: Rice salinity tolerance, Marker assisted selection, BGF, Population, Genetic distance

Introduction

Rice (*Oryza sativa* L.) belongs to the family Gramineae and subfamily *Oryzoidea* and it is one of the most important food crop grown worldwide and is the staple food of more than 50% of the world's population (Sasaki and Burr, 2000). About 85% of the rice production is used for human consumption (IRRI, 1997). This crop is being cultivated in at least 95 countries throughout the world and is the second agricultural crop plant in the world (FAO, 2004). Over 800 million hectares of land throughout the world are salt-

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affected, either by salinity (397 million ha) or the associated condition of sodicity (434 million ha) and this is over 6% of the world's total land area (FAO, 2005). It is the main source of carbohydrate for 40% of the world population. In Bangladesh, it is not only the main source of carbohydrate but also provides 75% of the calories and 55% of the average daily diet of the population (Bhuiyan *et al.*, 2002). Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. It occupies almost one-fifth of the total land area under cereals next to wheat which accounts to approximately 11% of the world's arable land (Chakravarthi and Naravaneni, 2006).

In a Marker Assisted Selection (MAS) scheme, molecular markers can be used to tag Quantitative Trait Loci (QTLs) and evaluate their contributions to the phenotype by selecting for favorable alleles at these loci. Identifying molecular markers that are linked to genes controlling salinity tolerance could facilitate selection for rice variety for salinity tolerance having low heritability expressivity. Salt stress is a major constraint to cereal production worldwide. Salt tolerant cultivars have generally been considered as the most economical and effective way of increasing crop production in saline soils (Flowers, 2004). The total saline area covers one third of the 9 million hectares of total national cultivated area in Bangladesh (ABSPII, 2006). Increase in salinity intrusion and increase in soil salinity have serious negative impacts on agriculture. The food production does not seem to have a better future in the event of climate change. In Bangladesh, rice production may fall by 10% and wheat by 30% by 2050 (IPCC, 2007). The objective of this study is to identify salt tolerant rice genotypes using molecular markers.

Materials and Methods

Plant materials

One salt tolerant rice genotype (FL-478) was selected as parent for transferring salt tolerant genes to Binadahan-7 a high yielding short duration variety. Backcrossing programme was conducted during Aman season in 2011, where Binadhan-7 was the recurrent parent and FL-478 was the non-recurrent donor line. BC_1F_1 populations were backcrossed with the recurrent parent Binadhan-7. In this study for phenotypic performance, a total of 67 BC_1F_1 populations of Binadhan-7 × FL-478 were used. Data were taken for plant height, number of effective tillers per plant, total number of tillers per plant, days to flowering and days to maturity.

Among these 67 plants 41 BC_1F_1 plants were successfully crossed with Binadhan-7 (recurrent parent) and 285 BC_2F_1 seeds were developed. Of these 41 BC_1F_1 plants, 32 BC_1F_1 plants were randomly selected to make foreground selection genotypically at the molecular level by using SSR markers.

Collection of leaf sample

The seedlings of two parents (Binadhan-7, FL-478) and BC_1F_1 populations of rice were raised in pots of BINA. Young vigorously growing fresh leaf samples from these seedlings were collected from 25 day old seedlings to extract genomic DNA. Initially, healthy portion of the youngest leaves of the tiller were cut apart with sterilized scissors and washed in distilled water and ethanol (70%). The collected leaf samples were then kept in polythene bags and for avoiding any damage of the leaf tissues the bags were placed in an ice box to carry it in Lab. and finally, the samples were stored in -80° C freezer with marking.

Genomic DNA extraction

DNA was extracted from the leaves of each genotype using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method (Zheng *et al.*, 1995). The simplified mini scale procedure for DNA isolation in PCR analysis developed at IRRI was followed. Out of 67 BC_1F_1 rice leaf samples, randomly 32 leaf samples were used for genotyping performance. For genotyping, healthy leaf samples were collected from 25 days old seedlings. DNA sample preparation, electrophoresis, PCR, documentation, microsatellite scoring were done as follows.

Polymorphism survey for primer selection

Polymorphism survey of 32 BC_1F_1 populations were carried out using 8 microsatellite markers: RM585, RM18, RM127, RM152, RM10720, RM155, RM169, and RM310. Out of 8 SSR primers, 3 primers (RM585, RM10720, and RM310) showed clear polymorphisms which were used in genotyping the Foreground selection of the 32 BC_1F_1 rice lines for salt tolerant genotypes.

Primer Name	Expected PCR product size (bp)		Sequence	Annealing temperature (°C)
RM585	233	Forward Reverse	CAGTCTTGCTCCGTTTGTTG CTGTGACTGACTTGGTCATAGG	55
RM10720	204	Forward Reverse	GCAAACGTCTACGTGAGAAACAAGC GCATGTGGTGCCTTAACATTTGG	55
RM310	105	Forward Reverse	CCAAAACATTTAAAATATCATG GCTTGTTGGTCATTACCATTC	55

Table 1	The sequence an	d size of the micross	tellite markers	(SSRs) used f	or BC.F. analysis
Table 1.	The sequence an	u size of the incrosa	tennte markers	(SSKS) used I	or $\mathbf{D}\mathbf{C}_1\mathbf{F}_1$ analysis

Components of PCR cocktail

The following components were used to prepare PCR cocktail (Table 2). The total volume of PCR cocktail for this study was 12.75 μ L per sample.

SL	Component		Quantity (for single reaction)
1	10 _× Buffer		1.5 μL
2	dNTPs		0.75 μL
3	Primer forward		1.0 µL
4	Primer reverse		1.0 µL
5	Taq polymerase		0.25 μL
6	ddH2O		8.25 μL
		Total =	12.75 μL

Table 2.	Comp	onents	of P	CR	cocktail
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1 μ L genomic DNA was added with 12.75 μ L PCR cocktail and finally, total volume was 13.75 μ L.

Thermal profile

The PCR tubes were set on the wells of the thermocycler. Then the machine was run according to the following setup

- Initial denaturation at 94°C for 3 min
- Denaturation at 94°C for 1 min
- Annealing at 34°C for 1 min
- Elongation or extension at 72°C for 2 min
- Step 2 to step 4 reaped 40 more cycles
- Final extension at 72°C for 7 min
- Completion of cycling program, reactions were held at 4°C.

Agarose gel electrophoresis of the amplified product

Agarose gel (1.5%) was prepared and poured into platform carefully when the gel solution cooled at 55°C. Let the gel polymerize for at least 30 minutes before removing the combs. After removing the casters, gel with platform was placed at the tank and poured 0.5X TBE buffer into the tank to submerge the gel. Then the combs were removed cautiously that gel slots were not injured.

Then the PCR products from each sample were confirmed by running 1.5% agarose gel. The PCR products were mixed with 3 µl of 2X gel loading dye. 13 µl of the mixture was loaded slowly per well on the gel in the gel tank and allowed them to sink in the bottom of the wells. The molecular weight marker (20 bp DNA ladder) was loaded at the first well on the gel. The tank was covered and all connections were checked. Electrophoresis machine run for 1.5-2.0 hr. The separation process was monitored by the migration of the dyes in the loading buffer. When the bromophenol blue dye had reached about three-fourth of the gel length, the electrophoresis was switched off.

Ethidium bromide staining

After completion of electrophoresis the gel was soaked in ethidium bromide (10 mg ml^{-1}) solution for 20-25 minutes.

Documentation of the DNA samples

After staining, the gel was taken out carefully from the staining tray and placed on high performance ultraviolet light box (UV transilluminator) of gel doc for checking the DNA bands. The DNA was observed as band and saved the records.

Scoring of Bands

The pattern of bands obtained after amplification with the primers was scored with reference to those of FL-478 and Binadhan-7, since FL-478 is salt tolerant designated as Binadhan-7 is salt susceptible designated as S and H for heterozygous.

Analysis of SSR Data

The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWER MARKER version 3.23 (Liu and Muse 2005), a genetic analysis software. Molecular weights for microsatellite products, in base-pairs, were estimated with Alpha Ease 4C software. The individual fragments were assigned as alleles of the appropriate microsatellite loci.

Polymorphism Information Content (PIC) value described by Botstein *et al.* (1980) and modified by Anderson E. (1993) for self-pollinated species were calculated as follows:

$$PIC_i = 1 - \sum_{j=1}^{n} p^2 i j$$

Where, Pij is the frequency of the jth allele for ith marker, and summed over n alleles.

The 34 accessions were clustered based on the matrix of genetic similarities using the unweighted pair group method with the arithmetic averages (UPGMA). The cluster analysis and dendogram construction were performed with NTSYS-PC (version 2.1).

Nei's (1973) genetic distance value was computed using the formula as described in the NTSYS-PC (version 2.1) software user manual.

Results and Discussion

Agronomic study of BC₁F₁ populations of Binadhan-7 x FL 478

In BC₁F₁ population, the range of plant height and total number of tillers $plant^{-1}$ were 78.7-93.9 cm and 32-45, respectively. The average plant height was 85.5 cm and average number of productive tillers $plant^{-1}$ was 34. This sort of result was not unexpected as because it was a pot experiment where there was no competition for nutrition, air, water, light etc. Average days to flowering and were 93 days and 125 days, respectively, and the range was between 90-97 days and 121-128 days, respectively (Table 3).

	Plant	Total tillers	Effective	Days	Days
Parents/progenies	height	plant ⁻¹	tillers plant ⁻¹	to 50%	to
	(cm)	(no.)	(no.)	flowering	maturity
Binadhan-7 (P)	93.4	29	26	95	127
FL-478 (P)	91.2	31	27	101	133
Mean	85.5	39	34	93	125
SD	3.4	2.8	2.7	1.9	1.3
CV (%)	4.0	7.2	8.0	2.0	1.0
Range	78.7-93.9	32-45	26-39	90-97	121-128

Table 3. Average performance of 67 BC_1F_1 rice populations of Binadhan-7 \times FL-478 along with their parents grown in Aman season in 2011

Genotypic performance of backcross population BC₁F₁ along with their two parents

The banding patterns were scored with reference to those of FL-478 and Binadhan-7, since FL-478 is salt tolerant and Binadhan-7 is salt susceptible genotype. For each of the marker, allelic bands were scored based on the parental bands and designated as T for tolerant, S for susceptible and H for heterozygous (Table 4).

 Table 4. Thirty two BC1F1 populations with respect to the alleles amplified by the RM585, RM10720 and RM310 microsatellite primer

Nama of	Total number	Patterns of BC_1F_1 population				
primers of BC_1F_1 lines		Susceptible type (Binadhan-7)	Tolerant type (FL478)	Heterozygous		
RM585		9	20	3		
RM10720	32	14	16	2		
RM310		14	17	1		

Banding pattern of salt tolerance of 32 BC₁F₁ lines using RM585 markers

In respect of the marker RM585, nine lines showed similar band as Binadhan-7, 20 lines showed similar band as FL 478 i.e. tolerant band and three lines showed heterozygous band (Fig. 1).



Fig. 1. Banding pattern of BC₁F₁ population of Binadhan-7/FL-478 using

Banding pattern of salt tolerance of 32 BC₁F₁ lines using RM 10720 marker

In respect of the marker RM10720, 14 lines showed similar band as Binadhan-7, 16 lines showed similar band as FL478 and two lines showed heterozygous band (Fig. 2).



Fig. 2 Banding pattern of BC₁F₁ population of Binadhan-7/FL-478 using RM10720 marker

Banding pattern of salt tolerance of 32 BC1F1 lines using RM 310 marker

In respect of the marker RM 310, 14 lines showed similar band as Binadhan-7, 17 lines showed similar band as FL-478 and one line showed heterozygous band (Fig. 3).



Fig. 3. Banding pattern of BC₁F₁ population of Binadhan-7/FL-478 using RM310 marker

Overall performance of BC₁F₁ rice lines against the RM585, RM10720 and RM310

Foreground selection was performed and 32 BC_1F_1 populations were selected with tightly linked salt tolerant markers RM585, RM10720 and RM310. Out of 32 BC_1F_1 populations, the marker RM585 identified 20 lines, RM10720 identified 16 lines and the marker RM310 identified 17 lines as salt tolerantolerant.

Genetic distance based analysis

The values of pair-wise comparisons of Nei's (1983), genetic distance (D) between varieties were computed from combined data for the 3 primers, ranged from 0.0000 to 1.0000 (Table 5).

Genetic similarity analysis using UPGMA (Ungeighted Pair Group Method of Arithmetic Means)

A dendrogram was constructed based on the Nei's genetic distance calculated from the 32 BC_1F_1 population of Binadhan-7 × FL 478 along with their parents. The dendrogram grouped all the 32 BC_1F_1 population of Binadhan-7 × FL 478 along with their parents in two major clusters (Fig. 4).
BC ₁ F ₁ populations	BCIF1-1	BCIF1-10	BCIF1-12	BCIF1-14	BCIF1-17	BCIF1-18	BCIF1-19	BCIF1-21	BCIF1-22	BCIF1-24	BCIF1-25	BC1F1-28	BCIF1-3	BCIF1-30	BCIF1-33	BCIF1-34	BCIF1-37	BCIF1-39	BCIF1-4	BCIF1-41	BCIF1-43
BC1F1-1																					
BC1F1-10	0.3333																				
BC1F1-12	0.3333	0.6667																			
BC1F1-14	0.3333	0.6667	0.0000																		
BC1F1-17	0.0000	0.3333	0.3333	0.3333																	
BC1F1-18	0.6667	0.3333	0.3333	0.3333	0.6667																
BC1F1-19	0.3333	0.0000	0.6667	0.6667	0.3333	0.3333															
BC1F1-21	0.6667	0.3333	0.3333	0.3333	0.6667	0.0000	0.3333														
BC1F1-22	1.0000	0.6667	0.6667	0.6667	1.0000	0.3333	0.6667	0.3333													
BC1F1-24	0.3333	0.0000	0.6667	0.6667	0.3333	0.3333	0.0000	0.3333	0.6667												
BC1F1-25	0.3333	0.6667	0.0000	0.0000	0.3333	0.3333	0.6667	0.3333	0.6667	0.6667											
BC1F1-28	0.3333	0.6667	0.0000	0.0000	0.3333	0.3333	0.6667	0.3333	0.6667	0.6667	0.0000										
BC1F1-3	0.3333	0.0000	0.6667	0.6667	0.3333	0.3333	0.0000	0.3333	0.6667	0.0000	0.6667	0.6667									
BC1F1-30	0.6667	0.3333	0.3333	0.3333	0.6667	0.0000	0.3333	0.0000	0.3333	0.3333	0.3333	0.3333	0.3333								
BC1F1-33	0.6667	0.3333	0.3333	0.3333	0.6667	0.0000	0.3333	0.0000	0.3333	0.3333	0.3333	0.3333	0.3333	0.0000							
BC1F1-34	0.0000	0.3333	0.3333	0.3333	0.0000	0.6667	0.3333	0.6667	1.0000	0.3333	0.3333	0.3333	0.3333	0.6667	0.6667						
BC1F1-37	0.3333	0.6667	0.0000	0.0000	0.3333	0.3333	0.6667	0.3333	0.6667	0.6667	0.0000	0.0000	0.6667	0.3333	0.3333	0.3333					
BC1F1-39	0.6667	0.3333	0.3333	0.3333	0.6667	0.0000	0.3333	0.0000	0.3333	0.3333	0.3333	0.3333	0.3333	0.0000	0.0000	0.6667	0.3333				
BC1F1-4	0.3333	0.6667	0.0000	0.0000	0.3333	0.3333	0.6667	0.3333	0.6667	0.6667	0.0000	0.0000	0.6667	0.3333	0.3333	0.3333	0.0000	0.3333			
BC1F1-41	0.6667	1.0000	0.3333	0.3333	0.6667	0.6667	1.0000	0.6667	0.3333	1.0000	0.3333	0.3333	1.0000	0.6667	0.6667	0.6667	0.3333	0.6667	0.3333		
BC1F1-43	0.0000	0.3333	0.3333	0.3333	0.0000	0.6667	0.3333	0.6667	1.0000	0.3333	0.3333	0.3333	0.3333	0.6667	0.6667	0.0000	0.3333	0.6667	0.3333	0.6667	
BC1F1-47	0.3333	0.0000	0.6667	0.6667	0.3333	0.3333	0.0000	0.3333	0.6667	0.0000	0.6667	0.6667	0.0000	0.3333	0.3333	0.3333	0.6667	0.3333	0.6667	1.0000	0.3333

Table 5. Summary of Nei's (1973) genetic distance (below diagonal) value for 32 BC₁F₁ populations along with their parents of rice

Table 5 continued.

BC ₁ F ₁ populations	BCIF1-47	BC1F1-49	BC1F1-5	BCIF1-50	BCIF1-55	BC1F1-57	BC1F1-59	BC1F1-60	BCIF1-7	BC1F1-8	BCIF1-9	BINA 7	FL-478
BC1F1-47													
BC1F1-49	0.0000												
BC1F1-5	0.3333	0.3333											
BC1F1-50	0.6667	0.6667	0.3333										
BC1F1-55	0.6667	0.6667	0.3333	0.0000									
BC1F1-57	0.0000	0.0000	0.3333	0.6667	0.6667								
BC1F1-59	0.6667	0.6667	0.3333	0.0000	0.0000	0.6667							
BC1F1-60	0.3333	0.3333	0.6667	0.3333	0.3333	0.3333	0.3333						
BC1F1-7	0.6667	0.6667	0.3333	0.0000	0.0000	0.6667	0.0000	0.3333					
BC1F1-8	0.3333	0.3333	0.6667	0.3333	0.3333	0.3333	0.3333	0.0000	0.3333				
BC1F1-9	0.3333	0.3333	0.6667	0.3333	0.3333	0.3333	0.3333	0.0000	0.3333	0.0000			
Binadhan- 7	0.3333	0.3333	0.0000	0.3333	0.3333	0.3333	0.3333	0.6667	0.3333	0.6667	0.6667		
FL 478	0.3333	0.3333	0.6667	0.3333	0.3333	0.3333	0.3333	0.0000	0.3333	0.0000	0.0000	0.6667	



Fig. 4. Dendrogram for 32 germplasm along with their parents derived from a UPGMA cluster

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MANAGEMENT OF FUSAURIM WILT OF TOMATO WITH SOIL AMENDMENTS

H. A. Begum¹ and M. B. Meah²

Abstract

The study was conducted with seven treatments viz. (i) mustard oil cake (ii) groundnut oil cake (iii) soybean oil cake (iv) cow dung (v) their integration (vi) lime and (vii) untreated inoculated control as preventive and curative measure separately for the management of fusarium wilt of tomato during the winter season of 2010-11. The treatments were applied on two tomato varieties viz. Binatomato-2 and Pusaruby as preventive and curative measure. Among the treatments, groundnut oil cake was the most effective one with 10.04% and 22.08% disease index in pre (preventive) and post (curative) inoculation application of soil amendments, respectively. Application of integrated treatment as preventive (41.33%) and curative (38.62%) measure was the most effective one in increasing yield over control but was inferior to groundnut oil cake and mustard oil cake in reducing disease index and severity. Plant growths in terms of shoot length, root volume and root weight was benefited in all the treatments as compared to inoculated control.

Key words: Soil amendments, Fusarium oxysporum f.sp. lycopersici, Tomato

Introduction

Management of wilt of tomato incited by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Synder and Hasen is difficult because of the soil borne nature of the pathogen. Modification of soil environments with organic amendments can prevent many devasting soil borne diseases (Raj and Kapoor, 1996 and Singh, 2002).

Soil amendments through addition of oil cakes, plant debris, green manure, farm yard manure, compost are known to improve crop productivity by improving nutrient status and soil tilt, besides increasing the microbial activity in the rhizosphere to suppress certain soil borne diseases (Begum, 2007; Mili, *et al.*, 2008; Begum *et al.*, 2008). As these organic substances are easily available in Bangladesh, there is an ample scope to utilize them and provide an economical means to dispose such waste and control plant pathogenic fungi and bacteria (Singh, 2002). The present study was undertaken to evaluate the effect of oil cakes, limes, cow dung and their integration on fusarium wilt of tomato.

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Materials and Methods

A field trial was conducted during the winter season of 2010-11 at Bangladesh Institute of Nuclear Agriculture (BINA). The land was medium high land with loamy soil texture, sonatala soil series, pH range 5.5-6.8, low in organic matter content and medium K-bearing minerals (FAO, 1998). Two separate experiments were set up as preventive and curative measure to find out the effect of soil amendments like oil cakes, cow dung, lime and their integration on the index and severity of fusarium wilt of tomato. The experiments were laid out following two factors randomized complete block design with three replications. The factorial experiments consisted of two factors such as (a) soil amendments (b) varieties. (a) Seven soil oil amendments including control were as follows (i) lime (ii) soybean oil cake (SOC) (iii) groundnut oil cake (GOC) (iv) mustard oil cake (MOC) (v) cow dung (CD) (vi) integration of SOC, GOC, MOC and CD and (vii) control (untreated inoculated control). (b) Two varieties were Binatomato-2 and Pusaruby. The treatments were incorporated into the soil as powder at the rate of mustard oil cake 200 kg, groundnut oil cake 150 kg and soybean oil cake 200 kg and cow dung 5 ton per hectare. To raise the soil p^{H} to 7.0 required quantity of lime added to the soil was calculated as per guide of Agrivarsity Humbold Soil Testing Laboratory of Bangladesh Agricultural University (Anonymous, 2001). Urea (358 kg ha⁻¹), TSP (250 kg ha⁻¹), MP (200 kg ha⁻¹) and cow dung (5 t ha⁻¹) were applied during the final land preparation. In both the experiments, plants were inoculated with seven days old culture of F. oxysporum f.sp. lycopersici following soil drenching method (Gongopadhyay, 1984).

As pre-inoculation application (preventive measure) of treatments, (three oil cakes powder, cow dung and lime) were mixed in the upper 15 cm of the soil at 10 days before transplanting. Four week old nine seedlings of tolerant tomato variety Binatomato-2 or susceptible variety Pusaruby were transplanted in each plot. Vigorously grown conidial suspension (10⁷ CFU ml⁻¹) of the pathogen (150 ml plant⁻¹) was sprinkled around the seedlings (wounding roots) so that the suspension can reach at a depth of at least 15 cm of soil (Gangopadhyay, 1984) at 20 DAT.

As post-inoculation application (curative measure) of treatments, the soils were first infested with the same concentration and dose of conidial suspension of the pathogen of preventive measure at 20 days before transplanting of tomato seedling. Soil amendments were applied to the rhizosphere soil of the tomato seedlings at 10 DAT.

The symptoms were noticed regularly up to 85 days after transplanting to observe the development of the disease. The disease severity was computed by adopting 0-4 scale to cover all the broad symptoms (Kapoor, 1987). The percent disease index (PDI) and percent increase in shoot length, root volume and root weight were calculated using the following formulae.

Percent disease indx (PDI) = $\frac{\text{Sum of total scores}}{\text{Maximum grade} \times \text{Total number of plant assessed}} \times 100$

Percent increase in shoot length, root volume and root weight =

MSL/MRV/MRW (Healthy)–MSL/MRV/MRW (Infected) MSL/MRV/MRW (Infected) ×100

Where,

MSL = Mean shoot length MRV = Mean root volume MRW = Mean root weight

Results and Discussion

Preventive measure

Percent disease index (PDI) and disease severity (DS) were recorded at 40, 55, 70 and 85 days after transplanting (DAT) and the summarized results are presented in Fig. 1 and 2. PDI and DS of fusarium wilt were conspicuously reduced due to application of soil amendments before inoculation over untreated inoculated control. Significantly the least disease index and severity were observed in treatments receiving groundnut oil cake at all the dates of data collection. Integrated treatment was the next best in reducing the PDI and DS at 40, 55, and 70 DAT whereas the scenario was different at 85 DAT. Mustard oil cake ranked 2nd and integrated treatment, mustard oil cake, soybean oil cake, cow dung, and lime were at par with each other in reducing the PDI and DS of fusarium wilt. On the other hand, the untreated control plants always showed the highest PDI and DS in tomato plants.

In general, a progressive decrease in disease index and severity was evident with increase in plant growth in all the treatments except untreated control. However, the groundnut oil cake showed significantly the highest decrease in disease index and severity at all the dates of data recording. Similar trend of decrease in disease index and severity were also observed in mustard oil cake, cow dung, integrated treatment, soybean oil cake and lime with increase in growth of the plants.

Between the two varieties, Pusaruby exhibited significantly the higher disease index and severity than that of Binatomato-2 irrespective of plant growth (Fig. 3 and 4).

Application of all the treatments significantly increased the shoot length, root volume, shade dry root weight and yield over control (Table 1). However, the groundnut oil cake was the most effective and lime the least effective treatment for improving shoot length, root volume and root weight. Two oil cakes viz. groundnut oil cake and mustard oil cake treated plants showed almost similar shoot length. The untreated control showed the least shoot length, root volume, root weight and yield. Though the highest plant growth parameters were observed in groundnut oil cake treated plots, the highest yield was recorded in integrated treatment applied plots.



Fig 1. Effect of pre-inoculation application of soil amendments on the index of fusarium wilt of tomato.



Fig. 3. Effect of pre-inoculation application of soil amendments on the cultivars of tomato against fusarium wilt index.



Fig. 2. Effect of pre-inoculation application of soil amendments on the severity of fusarium wilt of tomato.



Fig. 4. Effect of pre-inoculation application of soil amendments on the cultivars of tomato against fusarium wilt severity.

	Shoot length	Root volume	Shade dry root	Fruit yield	Perc	ent plant g over c	rowth inco ontrol	rease
Treatments	plant ⁻¹ (cm)	plant ⁻¹ (cc)	weight plant ⁻¹ (g)	plant ⁻¹ (kg)	Shoot length plant ⁻¹	Root volume plant ⁻¹	Root weight plant ⁻¹	Yield plant ⁻¹
Lime	65.8d	10.5e	3.07d	1.60d	9.1	289	287	7.33
Soybean oil cake (SOC)	76.9c	11.2de	3.25c	1.70cd	28	315	311	13.33
Groundnut oil cake (GOC)	93.6a	16.8a	4.89a	1.86b	55	522	519	24.0
Mustard oil cake (MOC)	94.0a	13.3b	3.19b	1.84bc	56	393	430	22.67
Cow dung (CD)	84.5b	13.0bc	3.81b	1.74bcd	40	381	382	16.0
Integration of	84.5b	12.0cd	3.53bc	2.12a	40	344	346	41.33
(SOC, GOC, MOC & CD)								
Control	60.3e	2.7f	0.79e	1.50e				
Level of significance	0.05	0.05	0.05	0.05				
Varieties								
Binatomato-2	77.8b	11.5a	3.42a	1.80a		2.7	3	4.65
Pusaruby	81.9a	11.2b	3.32b	1.72b	5.0			
$S(\overline{X})$	0.05	0.05	0.05	0.01				

Table 1. Effect of application of soil amendments before inoculation of Fusarium oxysporum f.sp. lycopersici on growth and yield of tomato plants (average of two varieties)

In a column figures having common letter (s) do not differ significantly

Curative measure

Like preventive methods of disease control with various soil amendments, disease index and severity significantly decreased with time by post inoculation application of soil amendments except soybean oil cake (Fig. 5 and 6). All the treatments as curative measure were effective in managing fusarium wilt of tomato at 40, 55, 70 and 85 DAT. Groundnut oil cake caused the highest reduction of the disease index and severity among the other treatments. Soybean oil cake was the least effective while mustard oil cake, cow dung, lime and integrated treatment showed intermediate effect in reducing PDI and DS. Nearly 100% plants were affected with the disease in untreated control plots in the field.

Obviously, the lower disease index and severity were recorded in the variety Binatomato-2 compared to Pusaruby (Fig. 7 and 8).

Considerably the highest shoot height (77.3 cm) was recorded in groundnut oil cake applied plots after inoculation of plants by F. oxysporum f. sp. lycopersici (Table 2). The effect of mustard oil cake, cow dung and integrated treatment was statistically similar in response to shoot height. The lower response in shoot length increase was recorded in soybean oil cake treated plot having 57.2 cm while the untreated control gave the lowest (52.3 cm) shoot height. Significantly the highest root volume and shade dry root weight were observed in groundnut oil cake treated plots followed by mustard oil cake and cow dung treated ones. Moderately higher root volume and root weight were found to be

recorded in the plots where mixed treatments were applied followed by lime. Significantly the highest fruit yield was observed in the integrated treatment. The second highest yield was observed in groundnut applied plot whereas the least root volume, dry root weight and yield were observed in untreated control plot.



Fig 5. Effect of post-inoculation application of soil amendments on the index of fusarium wilt of tomato.



Fig. 7. Effect of post-inoculation application of soil amendments on the cultivars of tomato against fusarium wilt incidence.



Fig. 6. Effect of post-inoculation application of soil amendments on the severity of fusarium wilt of tomato.



Fig. 8. Effect of post-inoculation application of soil amendments on the cultivars of tomato against fusarium wilt severity.

	Shoot length	Root volume	Dry root weight	Yield plant ⁻¹	Perc	ent plant g over c	rowth inc ontrol	rease
Treatments	plant ¹ (cm)	plant ¹ (cc)	plant ⁻¹ (g)	(g)	Shoot length plant ⁻¹	Root volume plant ⁻¹	Root weight plant ⁻¹	Yield plant ⁻¹
Lime	65.7c	9.12 bc	2.65 bc	1.48b	26	248	263	2.06
Soybean oil cake (SOC)	57.2d	8.33 c	2.44 c	0.78c	9	218	134	-46.0
Groundnut oil cake (GOC)	77.3a	15.08 a	4.41a	1.75ab	48	476	504	20.68
Mustard oil cake (MOC)	75.3ab	13.58 a	3.98 a	1.65ab	45	445	448	13.73
Cow dung (CD)	72.5b	13.50 a	3.94 a	1.60b	39	438	439	10.97
Integration of	73.4b	10.50 b	3.07 b	2.01a	40	301	321	38.62
(SOC, GOC, MOC & CD)								
Control	52.3e	2.62 d	0.73 d	1.45b				
Level of significance	0.05	0.05	0.05	0.05				
Variety								
Binatomato-2	64.33b	10.36	3.03	1.66a				11.4
Pusaruby	71.05a	10.41	3.03	1.49b	10.4	0.48		
$S(\overline{X})$	0.05	-	-	0.05				

 Table 2. Effect of application of soil amendments after inoculation of Fusarium oxysporum f. sp. lycopersici on growth of tomato plants (average of two varieties)

In a column figures having common letter (s) do not differ significantly

Obviously higher plant height was recorded in the variety Pusaruby than the variety Binatomato-2 in both the experiments of preventive and curative methods (Table 1 and 2). Both the varieties performed good in developing root volume and dry root weight. However, higher root volume and root weight were observed in Binatomato-2 as preventive methods while higher root volume was observed in Pusaruby as curative method. Significant difference was observed between the two varieties in yield in both the experiments. However, higher yield was observed in Binatomato-2 in both the cases.

The present study indicated that soil amendments including oil cakes reduced the index and severity of fusarium wilt of tomato as compared to inoculated control. Many investigators reported about effectiveness of organic amendments to the soil against different plant pathogens and parasites including fungi and bacteria (Yadvinder *et al.*, 2004; Singh *et al.*, 2007; Begum *et al.*, 2008; Mili *et al.*, 2008). Groundnut oil cake was more effective than mustard oil cake in reducing fusarium wilt. Chakrabarti and Sen (1996) reported that mustard oil cake was more effective in managing musk melon wilt caused by *F. oxysporum f. sp. melonis* while Raj and Kapoor (1996) and Begum (2007) observed that groundnut oil cake was superior to mustard oil cake as it not only reduced disease index of tomato but also improved plant growth also. The action of organic amendments in reducing plant pathogen has been attributed due to release of decomposition product like formic, acetic, propionic and butyric acids, H₂, phenol and ammonia which are directly toxic to fungi, bacteria and nematodes (Padmodaya, 2003).

Organic amendments might favor the build up of antagonistic microbes that can decrease the inoculum potential of root pathogens (Linderman, 1999). The plant growth in terms of shoot length, root volume and root weight was more in all the cakes, lime and cow dung applied plot as compared to inoculated control. Similar types of results were reported by Raj and Kapoor, (1996) who observed that beneficial effect of groundnut on plant growth was quite visible as the shoot and root length were improved by 84.0 and 86.2%, respectively over inoculated control. In both the experiments, the index and severity of fusarium wilt of tomato were reduced due to soil treatment wilt lime in comparison as compare to control. The present finding correlated with the findings obtained by Kushal et al (2008) who reported that F. oxysporum f. sp. lycopersici favored by a acid soil. In fungal wilt of tomato raising the pH of the field soil to 7.0 by adding lime provided good control of the disease. Application of soil amendments as preventive measure resulted comparatively better disease control than the curative measure. Application of soil amendments as preventive measure was done at 10 days before transplanting and the soils were inoculated 30 days after transplanting. The method of application of soil amendments was in line with the report of Raj and Kapoor (1996) who reported that decomposition of compost and oil cakes in soil for a longer period was the most appropriate for bringing down the viable propagules of the pathogen in soil. Our observation got further support from the finding of Yao et al. (1994) who suggested that the heat generated to cake application which might affect plant survival in curative method of disease control.

Thus it could be concluded from the present study that groundnut oil cake (150 kg ha⁻¹) and mustard oil cake (200 kg ha⁻¹) effectively reduced percent disease index and severity and showed a substantial improvement in plant heath. Therefore, groundnut and mustard oil cake might be considered as possible amendments for the control of tomato wilt.

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EVALUATION OF SUBMERGENCE TOLERANT RICE GENOTYPES FOR FLOOD PRONE AREAS OF BANGLADESH

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Abstract

Flood in Bangladesh is almost an annual feature and the second most climate problem or stress. Eight rice genotypes including one mutant variety were tested for submergence. Two genotypes viz., Ciherang-*sub 1* and Samba Mahsuri-*sub 1* performed better with regard to complete submergence of 20-25 days. These two submergence tolerant genotypes showed better performance with respect to earliness, long slender grain and intermediate amylose content than submergence tolerant rice varieties, BRRI dhan51 and BRRI dhan52. Ciherang-*sub 1* possessed long and medium grain, milling yield was 71%, chalkiness less than 10%, and 23.74% amylose content and milling yield of Samba Mahsuri-*sub 1* was 72%, chalkiness less than 10%, size of the dehuled grain was short and medium and amylose content 24.36%.

Key words: Rice, Submergence tolerance, Grain quality

Introduction

Rice is the staple food for Bangladeshi (Dhar *et al.*, 2012), it provides 75% of total calorie supply and 66% of the protein intake of human diet in Bangladesh (BBS, 2012). Rice production is reducing by prolonged flooding. On an average about a quarter of the country's landmass is currently flood prone (STRASA, 2013). The climate of the country is strongly influenced by monsoon. Monsoon rainfall may increase by 11% by 2030 and 27% by 2070 (Climate Change Cell, 2006). Over 20% of rice land in Bangladesh is prone to floods which occur every year (IRRI, 2008). At present, flood is the second most problem in rice growing countries after drought and is considered as a serious constraint to increase rice production worldwide (Plett *et al.*, 2010).

In recent years, increasing incidences of moderate to severe storms were noticed in the Philippines and Bangladesh, such as cyclone Nargis in Myanmar in May 2008, with a surge of over 4 m, damaging more than 1.7 million ha of rice crop (USDA-FAS, 2008). Similarly, Cyclone Sidr in southern Bangladesh resulted in a loss of more than 640,000 ha of cropland in 2007, and rice as the main crop during the aman season when the cyclone

¹Bitechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh ²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh happened was the most seriously damaged (Hossain *et al.*, 2008). Developing rice cultivars with tolerance to submergence and with agronomic and quality traits acceptable to farmers is a feasible approach to address this problem.

Performance of rice mainly depends on grain yield, stress tolerance (i.e., submergence tolerance) and its grain quality (grain size, shape, amylose content). The objectives of this study were to (a) assess the submergence tolerance ability and yield performance (b) select the submergence tolerant genotypes as varieties.

Materials and Methods

A total of eight rice genotypes including one mutant variety, Binadhna-7 were tested for submergence at aman season from 2008-12. The 25-30 days old seedlings were transplanted at 15 cm distances within rows of 20 cm apart during last week of June to 1st week of July. Fertilizer application was as like other T. aman rice but when if flooded the dose was changed. Under submergence condition the fertilizer recommendation dose was TSP 125 kg ha⁻¹, MP 47 kg ha⁻¹ (2/3 was applied during field preparation), Gypsum 60 ha⁻¹, Zinc-Sulfate 10 kg ha⁻¹. Ten days after flooding 45 kg ha⁻¹ and after 20-25 days 45 kg ha⁻¹ urea was added. One third of MP 23 kg ha⁻¹ was applied with urea after 10 days of flood. Moreover recommended insecticides, fungicide and nematocides were applied as necessary. After recession of flood water Furadan 5G 50 g decimal⁻¹ was applied with Urea to kill the nematode and fungus. Marshal 20 EC was applied to recovery the Tungro diseases as 30 ml in 10 L water decimal⁻¹ after 7 and 25 days sowing seed.

Evaluation of some submergence tolerant rice lines

Eight rice genotypes along with one mutant variety, Binadhan-7 were screened for submergence tolerance at Dhubaura, Mymensingh district in aman (wet) season during 2008. The experiment was laid out in RCBD with three replications. Unit plot size was 5 m \times 2.2 m and spacing between hills and rows were 15 cm \times 20 cm. The experimental genotypes were submerged for 14 days. Participatory varietal selection was done to select the best lines. The selected two lines were evaluated in the subsequent years (2009-2012) for their agronomic and yield performance.

Grain Characters

The size and shape of dehulled grain/kernel was measured according to USDA scale (Dela Cruz and Khush, 2000). Standard procedure for estimating amylose content was followed (Gongshe *et al.*, 2010).

Results and Discussion

Evaluation of some submergence tolerant rice lines in T. aman 2008

Eight rice genotypes including one mutant variety were tested under submergence condition at Dhobaura, Mymensingh. Two lines namely Ciherang-Sub 1 and Samba Mahsuri-Sub1 were selected based on farmer's preference and the characters viz., good yield, long panicle, more number of tillers in a hill, attractive color of grain, fine grain etc. were considered (Table 1).

Entry name	Total Farm	lers $(n = 30)$	Preference	Yield
	Positive	Negative	score	$(t ha^{-1})$
Ciherang -Sub 1 (Ciherang-Sub 1)	30	0	+0.25	5.2
Samba Mahsuri-Sub1 (Samba Mahsuri-Sub 1)	30	0	+0.232	4.8
IR85260-391-1192	0	0	-	2.7
BRRI dhan51	0	10	-0.083	4.2
BRRI dhan52	27	0	+0.23	4.7
BRRI dhan33	1	0	+0.008	3.8
Binadhan-7	25	1	+0.201	4.1
BR 11 Sub-1	0	1	-0.008	4.0

Table 1.	Preference analysis of	f submergence	tolerant rice	genotypes a	t Dhobaura in	T. aman,
	2008					

Morphological and agronomical characters of Ciherang-Sub 1 and Samba Mahsuri-Sub1 were evaluated in aman season 2009 in non-submerged and submerged condition. In non-submerged condition Ciherang-Sub 1 was early (120 days) and Samba Mahsuri-Sub1 was medium duration variety (135 days) where as BRRI released variety BRRI dhan51 needed long duration to mature. Yield performance was the best in Ciherang-Sub 1. On the other hand, in submergence condition, the duration became longer because after flood new tiller was appeared and they need extra time to mature. In this case Ciherang-Sub 1 was best in terms of duration and yield. These tested lines can tolerate 20-25 days submerge condition (data generated by DAE people) whereas the flood tolerant or "scuba" versions of popular rice varieties can withstand about 17 days of complete water submergence (Singh *et al.*, 2010). These two submergence tolerant genotypes were more yielder and short duration than other popular aman rice, especially it can 25-30 days earlier then BRRI released submergence variety BRRI dhan-51.

Submergence tolerant rice contains the submergence 1 (*Sub1*) gene that allows it to survive 10-14 days of complete submergence and to renew growth when the water subsides (IRRI, 2013). The duration of survival is also influenced by environmental factors such as water turbidity, temperature, and light, and other factors such as seedling age.

Proposed lines	Plant height	Days to	Days to	1000-grain	Yield
r toposeu nines	(cm)	flowering	maturity	wt. (gm)	$(t ha^{-1})$
Non-submerged condi	tion				
Ciherang -Sub 1	95	98	120	28.2	5.4
Samba Mahsuri-Sub 1	89	102	135	17.0	4.2
BRRI dhan51(Check)	87	112	145	20.4	4.2
Average	90.3	104	133.3	21.4	4.6
SE(±)	4.2	7.2	12.6	5.7	0.7
Submerged condition					
Ciherang -Sub 1	92	110	135	28.1	4.1
Samba Mahsuri-Sub 1	84	120	151	16.0	3.8
BRRI dhan51 (Check)	82	130	165	20.3	3.9
Average	86	120	150.3	21.5	3.9
SE(±)	5.3	10.0	15.0	6.1	0.2

 Table 2.
 Morphological and agronomical characters of the Ciherang-Sub 1 and Samba Mahsuri-Sub 1 in aman season at Non-submerged and Submerged condition

Yield performance of Ciherang-*Sub 1* and Samba Mahsuri-*Sub 1* was evaluated with a chack variety BRRI dhan51 in aman season 2009 and 2010 in four flood prone regions. In the year of 2009 the mean performanc was 4.0, 3.4 and 3.8 t ha⁻¹, respectively for Ciherang -*Sub 1*, Samba Mahsuri-*Sub 1* and BRRI dhan51. In the following year, 2010 the mean performanc was 4.1, 3.5 and 3.6 t ha⁻¹, respectively for Ciherang-*Sub 1* and BRRI dhan51. The yield performance of Ciherang-*Sub 1* and Samba Mahsuri-*Sub1* and BRRI dhan51. The yield performance of Ciherang-*Sub 1* and Samba Mahsuri-*Sub1* became higher in 2010 than 2009 but it became lower in case of BRRI variety. According to the IRRI servay, Bangladesh and India alone paddy loss to flooding amounts to an estimated 4 million tons of rice per year- enough to feed 30 million people, In Bangladesh, sowing of the flash-flood tolerant varieties could produce an additional 1 million tones of paddy annually, making the country more food secure and creating export potential (IRRI, 2013).

Table 3. Performance (yield, t ha⁻¹) under submerged condition in Aman season, 2009 and
Aman season, 2010

Designation		Aı	nan season, 2	.009	
Designation	Rangpur	Sherpur	Jamalpur	Mymensingh	Mean
Ciherang-Sub 1	3.9	4.0	3.9	4.2	4.0
Samba Mahsuri-Sub 1	3.3	3.7	3.4	3.2	3.4
BRRI dhan51 (Check)	3.6	3.8	3.7	4.0	3.8
Average	3.6	3.8	3.7	3.8	3.7
SE(±)	0.30	0.15	0.25	0.53	0.31
		Aı	nan season, 2	010	
Ciherang -Sub 1	4.0	3.9	4.1	4.4	4.1
Samba Mahsuri-Sub 1	3.4	3.8	3.5	3.2	3.5
BRRI dhan51 (Check)	3.7	3.6	3.7	3.5	3.6
Average	3.7	3.8	3.8	3.7	3.7
SE(±)	0.30	0.15	0.31	0.61	0.31

5	2
J	4

Ciherang-Sub 1 and Samba Mahsuri-Sub1 were evaluated with BRRI dhan51 in multi location during the aman season 2011. The average plant height of Ciherang-Sub 1, Samba Mahsuri-Sub 1 and BRRI dhan51 was 92.1, 83.2 and 80.6 cm, respectively. Ciherang -Sub 1 maturity date is 25 days earlier than check variety and in case of Samba Mahsuri-Sub1 it is 12 days earlier. Ciherang-Sub 1 showed highest yield 4.0 t ha⁻¹ (Table 4).

Proposed lines	Plant height (cm)	Days to maturity	Grain yield (t ha ⁻¹)
Nokla, Sherpur			
Ciherang -Sub 1	90.5	129	3.5
Samba Mahsuri-Sub1	85.6	147	3.3
BRRI dhan51 (Check)	81.2	159	3.4
Nalitabari, Sherpur			
Ciherang -Sub 1	95.4	133	5.1
Samba Mahsuri-Sub1	86.4	144	4.9
BRRI dhan51 (Check)	81.2	151	3.3
Dewangonj, Jamalpur			
Ciherang-Sub 1	92.4	125	3.8
Samba Mahsuri-Sub 1	76.5	138	3.1
BRRI dhan51 (Check)	75.3	154	3.6
Kawnia, Rangpur			
Ciherang -Sub 1	90.0	116	3.2
Samba Mahsuri-Sub1	82.3	131	3.5
BRRI dhan51 (Check)	80.3	146	3.8
Pirgonj, Rangpur			
Ciherang-Sub 1	92.0	117	4.3
Samba Mahsuri-Sub1	85.3	134	3.8
BRRI dhan51 (Check)	84.8	149	4.9
Average of 5 locations			
Ciherang-Sub 1	92.1	124	4.0
Samba Mahsuri-Sub 1	83.2	139	3.7
BRRI dhan51 (Check)	80.6	152	3.8

Table 4. Yield trial at different locations under submerged condition in aman season 2011

On-farm trial

Ciherang - Sub 1 was 5-8 days higher submergence tolerant, 1 month earlier and high yield (20%) more than BRRI dhan51 (Table 5). Samba Mahsuri- Sub 1 was found to be 5 days more submergence tolerant, 10 days earlier and high yielder.

Tolerant varieties like the Indian landrace FR13A can survive submergence for 2 weeks or more, whereas susceptible varieties are severely damaged or killed within a week (Mackill D. J. *et al.*, 2012). Ciherang-*Sub 1* and Samba Mahsuri-Sub1 can survive one week more than Indian submergence tolerant variety, FR13A.

Four indica rice varieties were evaluated in the four provinces Vietnam during the 2008-'09. IR64-*Sub 1* was preferred for its high yield (5 t ha⁻¹) and shorter maturity (Lang, N. T *et al.*, 2011). Swarna-*Sub 1* is good for both seasons with a yield of 4.6 t ha⁻¹. Though BINA released submergence tolerant varieties yield is lower than IR64-Sub1 but they are earlier than IR64-*Sub 1*. Compare with Swarna-*Sub 1*, Ciherang-*Sub 1* showed higher yield in farmer filed at Rangpur. The Dehulled grain quality of Samba Mahsuri-*Sub 1* is much better than other submergence tolerant rice due to its natural miniket shape and shininess.

	Plant	Days	Days	1000 grain	Grain	
Proposed lines	height	to	to	weight	yield	Remarks
	(cm)	flowering	maturity	(gm)	$(t ha^{-1})$	
Ciherang-Sub 1	92	110	135	28.1	4.1	Ciherang-Sub 1
Samba Mahsuri-Sub 1	84	120	151	16.0	3.8	Best yield 4.8
BRRI dhan51(Check)	82	130	165	20.3	3.9	t ha ⁻¹
Designation		Moon				
Designation	Rangpur	Sherpur	Jamalpur	Mymensingh	Rangpur	Ivicali
Ciherang-Sub 1	4.0	3.9	4.1	4.4	4.8	4.2
Samba Mahsuri-Sub 1	3.4	3.8	3.5	3.2	3.1	3.4
BRRI dhan51(Check)	3.7	3.6	3.7	3.5	3.5	3.6
Average	3.7	3.8	3.8	3.7	3.8	3.7
SED	0.30	0.15	0.31	0.62	0.89	0.42

 Table 5. On-farm trial of Ciherang-Sub 1 and Samba Mahsuri-Sub 1 in aman season-2012 under submerged condition

At the beginning of aman season the nuclear seeds 120 kg of Ciherang-Sub 1 and 112 kg of Samba Mahsuri-Sub 1 were distribute. 22.20 tons and 13.03 tons of Ciherang-Sub 1 and Samba Mahsuri-Sub 1 were produced, respectively.

Grain characteristics

With respect to grain quality, milling yield of Ciherang-Sub 1 was 71%, chalkiness less than 10%, size of the dehuled grain is long and medium and 23.74% amylose content (Table 6). Milling yield of Samba Mahsuri-Sub 1 was 72%, chalkiness less than 10%, size of the dehuled grain is short and medium and 24.36% amylase.

Ashish Jain *et al.* (2012) found rice amylose in the range of 17.35% to 25.99% when he was working with amylose contain of cooking rice for diabetic patients. The more amylose indicate rapidly breakdown of food and quickly increase of blood sugar. Both the tested lines are intermediate amylose content.

Plant scientists of the University of California have complained the gene responsible for flood or submergence tolerance in rice which allows it to conserve energy

until the floodwaters recede is transferred into high-yielding, good grain quality and pest and disease resistant rice (The Daily Star. 2012). In this context Ciherang-Sub 1 and Samba Mahsuri-Sub1 are pest and diseases resistance.

	Milling	Head rice		Whole	I	Dehulled g	rain ker	nel ⁻¹	Amylose
Proposed lines with std. checks	yield (%)	yield (%)	Chalkiness*	grain length (mm)	length (mm)	Breadth (mm)	L/B ratio	Size and shape	(%)
Ciherang-Sub 1	71	90	Wb1	9.0	7.1	2.4	2.96	Long Medium	23.74
Samba Mahsuri-Sub1	72	90	Wb1	7.1	5.3	2.0	2.65	Short Medium	24.36
BRRI dhan51(Check)	71	90	Wb1	7.5	6.0	2.3	2.60	Medium bold	28.12

Table 6. Grain characteristics of submergence tolerant rice lines

^{*}Wb1 = Less than 10% chalkiness

BINA introduced two submergence tolerant rice varieties Ciherang-Sub 1 and Samba Mahsuri-Sub1 by marker assisted selection (MAS). Two genotypes viz., Ciherang-Sub 1 and Samba Mahsuri-sub1 performed better with regard to complete submergence of 20-25 days. These two lines were evaluated in different location from 2009 to 2012 and finally in the year of 2013 they got the recognition as variety from national seed board. Ciherang-Sub 1 and Ciherang-Sub 1 are more submergence tolerant than IRRI and BRRI released varieties. Ciherang-Sub 1 is 20-25 days earlier than BRRI released popular submergence tolerant variety BRRI dhan51. Samba Mahsuri-Sub1 got highly preference due to its natural miniket shape and shininess. Both submergence tolerant rice contain medium amount of amylose which is highly preferred by the consumers.



Submerged (19 days)

After relese of flood water

Fig.1: Binadhan-11 at Aman season 2011, Sherpur Submergence 18-08-2011 to 01-09-2011



Submerged (25 Days)

After relese of flood water

Fig 2: Binadhan-12 at Aman season 2011, Sherpur Sebmerge 04-08-2011 to 19-08-2011 and 22-08-2011 to 30-08-2011

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SCREENING OF DIFFERENT AROMATIC AND NON-AROMATIC FINE RICE VARIETIES IN AMAN SEASON IN TERMS OF YIELD

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Abstract

A field experiment was carried out at Bangladesh Institute of Nuclear Agriculture (BINA) farm, Mymensingh during July to December 2007 to study the growth and yield performance of 33 aromatic and non-aromatic fine rice varieties in aman season. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Among the aromatic and non-aromatic rice varieties, plant height, total dry matter (TDM) and chlorophyll content varied significantly at 60 and 90 DAT while the leaf area hill⁻¹ varied at all sampling dates i.e. at 30, 60 and 90 DAT. The results showed that aromatic and non-aromatic fine rice variety Kataribhogh produced the maximum grain yield (3.86 t ha⁻¹) which was statistically identical to that of non-aromatic fine rice Basmati (3.73 t ha⁻¹). On the other hand, aromatic rice variety Ukunimadhu produced the highest grain yield (2.6 t ha⁻¹) which was statistically identical with that of aromatic rice variety BRRI dhan34 (2.57 t ha⁻¹). From the present study, it might be conclueded that Aromatic and non-aromatic fine rice Ukunimadhu, BRRI dhan34. Basmati and Kataribhogh performed the best regarding grain yield in aman season.

Key Words: Aromatic and non-aromatic fine rice varieties, Yield, Aman season

Introduction

Bangladesh is an agro-based country. Most of her economic activates depend on agriculture. Agriculture in Bangladesh is dominated by intensive rice cultivation. The humid tropical climate of this country provides an excellent habitat for rice culture. Rice as such evolved as the staple food for the people of Bangladesh and intrinsically associated with their culture, rites and rituals. Rice (*Oryza sativa* L.) is one of the most extensively cultivated cereals of the world and it is feeding one-half of the world's total population. The total area and production of rice in Bangladesh is about 11.65 million hectare and 33.5 million M. ton, respectively (BBS, 2011). There are, two types of aman rice viz. coarse and fine rice and most of the fine rices are aromatic in nature e.g., Kataribhough, Badshahough and Kalizira. The productivity of aromatic fine rice in Bangladesh is very low because of lack of fertilizer management, optimum spacing and date of transplanting. Although the geographical, climatic and ethmic conditions of Bangladesh are favourable

¹Agronomy Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh ²Department of Agronomy, Bangladesh Agricultural University, Mymensingh, Bangladesh ³Director (Research), Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh for year-round rice cultivation. But histrorically the aromatic rice has been growing in transplant aman season due to climatic reasons the T. aman season produced the lesser grain yield. Therefore, effort should be made to improve the productivity of aromatic fine rice through agronomic manipulation with suitable cultivars for *Boro* season. There is a huge demand of Bangladeshi aromatic fine rice in the Middle-East countries, Malaysia, Korea, Japan, Australia, U.S.A, Canada, U.K, Italy and Sweden. There are several special dishes like *polau, khir, firney; paish, chira, khoi, briany, jurda*, etc. are prepared from this kind of milled rice. Thus milled rice of such type are used as a luxurious special food. Moreover, the price of 1 kg aromatic fine milled rice is about 90 TK whereas 1 kg HYV coarse milled rice is about 35 TK. So, it indicates that the production of aromatic and fine rice in the country is economically profitable (Raju, 2000). Hence the objective of this study was to identify the best performance of aromatic and non-aromatic fine rice varieties.

Materials and Methods

The experiment was conducted at the Agronomy farm of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during July to December 2007 in aman season. The experimental land was sandy loam in texture having soil pH 6.8. The experimental materials such as seeds for experiment were collected from the Agronomy Division and Plant Breeding Division of BINA, Germplasm Centre of BRRI, and Agronomy Field laboratory of Bangladesh Agricultural University. The unit plot size was 4.0 m x 2.5 m. The plots of aromatic and non-aromatic fine rice were fertilized with N, P, K, S, and Zn at the rate of 126.9, 19.81, 65, 43.3 and 1.8 kg ha⁻¹, respectively in the form of urea, TSP, MP, gypsum and zinc sulphate according to the Fertilized Recommendation Guide of (BARC-2005). The whole amounts of triple super phosphate, muriate of potash, gypsum and zine sulphate were applied to the soil at the time of final land preparation. Urea was applied in three equal splits. One split of urea was applied with other fertilizers as basal dose and the other two splits were applied 21 and 45 days after transplanting (DAT). The experiment was laid out in a randomized complete block design with three replications. The average value of daily maximum temperatures during the period of July 2007 to December 2007 were 28.36, 28.46, 29.33, 28.96, 37.35 and 24.10°C, respectively. A total of 33 aromatic and non-aromatic fine rice varieties were evaluated in this study. The varieties were as follows:

Awn less Minicat, Awn Minicat, BRRI dhan38, BRRI dhan34, BRRI dhan37, Binadhan-4, Binasail, Basmati, Banajira, Chinisagar, Chiniatab, Chikonlal, Chinikahai, Dudhsar, Jasmain, Jumadhuma, Kalobhog, Kalizira, Kasmiribasmati, Kala manik, Kataribhog, Kamonisail, Lalmoi, Lalgilona, Minikichi, Maharani, Marich bati, Pulashi, Sharisaful, Surjamukhi, Sonamukhi, Tripunachinal, Ukunimadhu.

Only selected healthy seedlings were transplanted in the experimental plots in July in 20 cm apart line maintaining a distance of 15 cm from hill to hill with three seedlings hill⁻¹. Intercultural operations and pesticide application were done as and when necessary. From each plot ten hills (excluding border hills) were collected for collecting data on 30, 60 and 90 DAT. Each plant sample was separated into leaf as their area was measured with the help of a protable leaf area meter. During harvest, 10 hills were again randomly seclected from each plots excluding border hills and from the hills, data on yield contributing characters were collected. Grain and straw yields were sun dried and the weight of grains was adjusted to 12% moisture content. Grain and straw yields were then converted to t ha⁻¹. Data were analyzed statistically using "Analysis of Variance" technique and differences among treatments means were adjudged by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Results and Discussion

Plant height significantly varied among the varieties under study at 60 and 90 DAT. Total dry matter (TDM) varied significantly due to variety at 60 and 90 DAT. Chlorophyll content significantly varied among the varieties at 60 and 90 DAT leaf area hill⁻¹ was differed significantly among varieties at all sampling stage. All the variety under the study differed significantly for plant height, total tillers hill⁻¹, effective tillers hill⁻¹, grains panicle⁻¹, unfilled spikelets panicle⁻¹, 1000 grain wt, grain yield, biological yield and harvest index except non-effective tillers hill⁻¹ and straw yield had been shown for all the crop characters.

Growth parameters

Plant height states the stature of any kind of plants, its plant type and nature of leaf/canopy arrangement. It determines the nature of lodging, light and air penetration inside the canopy. The increasing patterns of plant height were almost the same for all varieties. At 30 DAT, although plant height was not significantly affected by variety numerically the tallest plant (62.0 cm) was recorded in Kamonisail (Table 1). On the other hand, BRRI dhan37 produced the shortest plant (43.0 cm). Similar trend of plant height growth was recorded at 60 and 90 DAT. However, at tillering stage (60 DAT) Kamonisail and Surjamukhi had the tallest plant (95.0 cm) and the shortest one (70.6 cm) was in Pulashi. At 90 DAT the tallest plant height (150.6 cm) was recorded in Sharisaful and Kamonisail. On the other hand, Basmati produced the shortest plant (97.6 cm) (Table 1). From Table 1, it can be seen that at 30 DAT, numerically the highest TDM hill⁻¹ (7.1g) was produced by Kalizira which was followed by Basmati (6.9 g). At 60 DAT, the highest TDM hill⁻¹ (20.6 g) was found in Kataribogh and the lowest (10.1 g) was observed in Sharisaful. At 90 DAT, TDM of Dudhsar was the highest (43.0 g) and the lowest (14.1 g) was observed in Chikonlal (Table 1). The variety Binasail showed high amount of

chlorophyll content (42.7 SPAD value) at 30 DAT. Chlorophylll content was highest (42.6 SPAD) at 60 DAT in Minikichi variety whereas the variety BRRI dhan34 showed the lowest (29.5 SPAD value) at 60 DAT. At 90 DAT Lalgilona gave the highest amount of chlorophyll content (42.2 SPAD value). However, the lowest amount of chlorophyll content (28.4 SPAD value) was recorded in Jasmin (Table 1). At 30 DAT, Ukunimadhu gave the higher leaf area hill⁻¹ (746.0 cm²). However, the lowest value (269.1 cm²) was recorded in Basmati. At 60 DAT, the highest leaf area hill⁻¹ was found in Dudhsar (2776.0 cm²) and the lowest leaf area hill⁻¹ (785.2 cm²) was produced in Basmati. At 90 DAT, Awned Minicat produced the highest leaf area hill⁻¹ (1394.3 cm²) and the lowest leaf area hill⁻¹ (451.0 cm²) was produced in Basmati (Table 1).

Yield and yield contributing characters

The result reveals that the aromatic and non-aromatic fine rice had different plant height (Table 2). Table 2 shows that the tallest plant was produced by Chinikahai (168.2 cm), which, was identically followed by the varieties Kalizira (156.0 cm) and Kalobhog (155.6 cm). The shortest plant was produced by aromatic and non-aromatic fine rice Awned Minicat (98.6cm) which, was identically followed by Awnless Minicat (103.2 cm) and Minikichi (103.3 cm). The significant variation may be associated mainly with the genetic make-up among the aromatic and non-aromatic fine rice. Table 2 showed that the number of total tillers hill⁻¹ ranged from 8.1 to 17.2. The highest number of total tillers hill⁻¹ ¹ was observed in aromatic and non-aromatic fine rice Kataribhog (17.2), which, was statistically similar to BRRI dhan34 (15.8). The lowest number of total tillers hill⁻¹ was produced by aromatic and non-aromatic fine rice Jumadhuma (8.1), which was identically followed by Lalgilona (8.2) and Sonamukhi (8.2) (Table 2). This variation might be due to genetic characteristics among the aromatic and non-aromatic fine rice varieties. The highest number of effective tillers hill⁻¹ (16.1) was produced by Kataribhog. The second highest number of effective tillers hill⁻¹ (13.2) was observed by Chiniatab, which was statistically identical with Basmati (12.6) and Chinisagar (11.6). The lowest number of effective tillers hill⁻¹ was produced by Jumadhuma (6.5), which, was statistically similar to Binadhan-4 (7.0), Lalgilona (7.3), Marichbat (6.8), Sharisaful (7.4) and Sonamukhi (7.1) (Table 2). Varietal differences regarding the number of effective tillers hill⁻¹ might be due to their differences in genetic constituents. The variability in number of effective tillers hill⁻¹ among different variety was also reported by Om et al. (1998) and BINA (1998). Ukunimadhu produced maximum number of grains panicle⁻¹ (162.3) (Table 2). The second highest one (150.3) was observed by variety BRRI dhan34. The non-aromatic fine rice Kataribogh produced medium number of grains panicle⁻¹ (144.0), which, was identically

Variation	Plant height (cm)			TDM (g) hill ⁻¹ .			Chlorophyll contents leaf ¹			Leaf area hill ⁻¹		
varieties	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
Awned Minicat	57.0	84.0j	108.3lm	6.0	15.6def	27.9fg	36.6	34.1kl	42.1a	596.6efg	1791.0i	1394.3a
AwnlessMinicat	55.3	90.0de	124.6jk	5.5	12.5k-n	30.3de	34.2	37.0f-i	40.5cd	515.6hij	1938.0e	904.3if
BRRI dhan38	54.0	86.3hi	99.30	6.3	12.7j-m	14.8o	34.4	34.1kl	40.1d	507.4hij	1289.5nop	-
BRRI dhan34	48.0	92.0cd	106.3mn	5.7	18.6b	24.0jk	39.6	29.50	34.5kl	350.9opq	1165.0s	1037.6ef
BRRI dhan37	43.6	86.3hi	101.6no	5.0	16.1cde	21.2ľm	36.6	32.8lm	33.9kl	325.3qr	1174.3rs	1067.3d
Binadhan-4	59.6	71.6mn	98.0o	5.1	18.8b	34.3b	35.8	34.9o	38.2fg	246.6s	1959.1e	540.0n
Binasail	58.6	86.3hi	98.0o	5.7	20.4a	23.3jk	42.7	30.9n	34.3kl	286.1rs	1849.0gh	1296.0ab
Basmati	60.0	76.61	97.60	6.9	15.9cde	17.1n	36.2	41.9a	34.7jhl	269.1s	785.2v	451.0o
Banajira	59.6	93.6ab	131.6e-i	6.7	19.3b	31.3cd	35.9	35.8ij	40.7bcd	688.3bc	1487.0m	1169.0c
Chinisagar	54.6	90.0de	130.6f-j	5.7	14.2ghi	30.6d	35.6	31.7mn	31.9m	466.1jkl	2153.1d	624.6m
Chiniatab	48.6	92.0cd	110.0lm	5.9	11.4n	30.7kl	38.5	33.31	36.5h	434.3klm	919.2v	812.0j
Chikonlal	49.3	86.0hij	121.6k	5.1	11.7mn	14.1o	33.3	36.9ghi	41.7abc	406.6mno	800.0v	560.0n
Chinikahai	55.6	93.3abc	114.31	5.4	12.3k-l	24.9n	37.0	39.5bcd	40.5cd	438.2klm	1248.3pq	794.0jk
Dudhsar	52.0	94.0abc	141.3bc	6.1	15.2efg	43.0a	41.3	40.1b	35.2ijk	360.3n-q	2776.0a	1106.0d
Jasmin	45.0	80.6k	106.6mn	6.1	13.0jkĪ	22.8kl	41.4	38.4def	28.40	398.4m-p	1862.5fg	975.0g
Jumadhuma	53.6	93.0bc	123.3k	6.3	13.8hij	14.60	39.4	38.7cde	39.7de	609.2d-f	943.5v	905.3j
Kalobhog	49.0	93.3abc	114.6l	6.4	18.9b	24.8ij	36.4	39.2b-e	38.5efg	564.8fgh	1648.3k	1056.3dc
Kalizira	53.3	89.0cfg	122.3k	7.1	13.5ijk	30.3de	42.1	33.9kl	33.81	609.2d-f	2265.3c	723.21
Kasmiribasmati	57.6	87.0ghi	132.6d-h	6.3	17.0c	23.3jk	36.9	39.9bc	29.2o	606.3def	1582.01	1263.3b
Kal <i>aman</i> il	50.0	86.0hij	125.3ijk	5.0	12.7j-m	15.10	36.8	36.5hi	37.9g	544.7ghi	1033.6t	871.1hi
Kataribogk	56.3	85.0ij	110.3lm	6.2	20.6a	28.8ef	35.8	37.1f-i	40.3d	732.0ab	2331.0b	1297.3ab
Kamonisail	62.0	95.0a	150.6a	6.4	13.4i-l	32.9bc	39.1	40.2b	36.4hi	487.2ijk	1855.0g	1108.0d
Lalmoi	53.3	85.6hij	137.3cde	6.2	13.8hij	20.7m	41.1	33.41	38.3fg	500.9ij	1222.0qr	750.5kl
Lalgilona	55.0	87.3fgh	127.3h-k	5.9	15.6def	28.0fg	35.8	38.0efg	42.2a	4100lmn	1797.3hi	914.3h
Minikichi	47.3	73.3m	107.0mn	5.4	14.6fgh	25.9hi	38.7	42.6a	38.6efg	510.6hij	1708.0j	911.0h
Maharani	58.6	89.3ef	131.6e-f	5.9	13.3j-1	26.8gh	38.9	33.41	35.8hij	341.5pqr	1329.0n	844.3ij
Marichbati	50.6	90.6de	146.6ab	5.9	16.6cd	26.4ghi	35.3	40.2b	39.5def	488.6ijk	1309.0no	670.3m
Pulashi	47.0	70.6n	136.3c-g	5.1	12.6j-m	31.5cd	32.9	39.0b-e	38.6efg	391.6m-p	1912.0ef	1178.3c
Sharisaful	51.3	93.3abc	151.0a	6.1	10.10	26.5ghi	32.9	38.3d-g	42.0ab	532.0hi	1265.0opq	999.0fg
Surjamukhi	65.0	95.3a	138.3cd	5.5	16.9c	24.0jk	39.0	37.2fgh	41.6abc	503.6ij	1583.01	888.0hi
Sonamukhi	59.0	93.6abc	142.6bc	6.5	16.2cde	26.4ghi	36.8	33.9kl	28.50	628.0de	1236.0pq	1201.0c
Tripunachinal	52.6	90.6de	137.0c-f	6.5	12.2lmn	21.1Īm	34.3	37.9e-h	30.6n	663.0cd	1561.01	805.6j
Ukunimadhu	47.0	90.0de	130.0g-j	6.3	15.2efg	27.2fgh	33.7	35.0jk	33.41	7460a	1534.0lm	1075.3dc
LSD _{0.05}	NS	1.926	5.802	NS	1.034	1.595	NS	1.221	11.205	53.18	53.32	50.37
CV (%)	15.67	5.82	12.57	19.92	18.23	16.48	10.91	11.83	8.65	28.78	9.09	14.13

Table 1. Screening of different growth parameters of aromatic and non-aromatic fine rice varieties in aman season in terms of yield potentiality

In a column, figures with same letters or without letters do not differ significantly whereas figures with dissimilar letter differ significantly as per DMRT. NS = Not significant

Varieties	Plant	Total tillers	Effective	Non-	Grains	Unfilled	1000-grain	Grain	Straw	Biological	Harvest
	height	hill $^{-1}$	tillers	effective	panicle ⁻¹	spikelets	weight	Yield	yield	yield	index
	(cm)	(No.)	hil ⁻¹	tillers hil ⁻¹	(no.)	panicle ⁻¹	(g)	$(t ha^{-1})$	$(t ha^{-1})$	$(t ha^{-1})$	(%)
			(no.)	(no.)		(no.)		. ,			
Awnlee Minicat	126.8 c-h	17.2a	16.1a	1.1	162.3 a	72.1	15.53 h-l	3.86 a	4.96	8.82 ab	43.76 a
Awn Minicat	103.2 ii	10.7b-g	9.5b-e	1.2	83.2 c-i	35.5	21.20 c-f	1.99 c-g	5.20	7.19 a-l	27.67abc
BRRI dhan38	121.2 e-i	11.7b-g	8.4b-e	3.3	118.3 a-f	47.0	18.30 d-h	2.41 b-e	5.00	7.41 a-k	32.52 ab
BRRI dhan34	142.5 b-e	15.8ab	11.9а-е	3.9	139.3 a-e	51.0	12.25 lm	2.57 a-d	5.51	8.08 a-g	31.80 ab
BRRI dhan37	127.3 d-h	12.5a-g	10.5b-e	2.3	124.3 a-f	50.5	14.93 h-m	2.18 c-f	5.10	7.28 a-1	29.94 abc
Binadhan-4	115.3 g-j	8.6fg	7.0de	1.6	103.0 a-h	36.0	24.90 bc	3.30 abc	5.40	8.70 abc	37.93 ab
Binasail	136.4 b-g	9.6d-g	8.5b-e	1.1	101.0 a-h	30.3	14.53 h-m	2.82 a-d	4.86	7.68 a-i	36.71 ab
Basmati	117.6 f-g	13.6a-f	12.6a-d	1.0	144.0 abc	53.5	19.96 d-g	3.73 ab	5.25	8.98 a	41.53 a
Banajira	128.7 c-h	8.9fg	7.7cde	1.2	37.3 ijk	40.5	20.30 d-g	0.27 ij	5.20	5.47 lm	4.93 e
Chinisagar	138.5 b-f	13.1a-g	11.6а-е	1.5	74.6 f-k	31.0	13.10 klm	2.58 a-d	5.90	8.48 a-d	30.42abc
Chiniatab	139.6 b-f	14.2a-e	13.2abc	1.2	140.3 a-d	48.0	11.23 m	2.36 b-c	4.86	7.22 a-l	32.68 ab
Chikonlal	122.4 e-i	9.4efg	7.7cde	1.7	88.3 b-i	67.5	12.73 lm	0.42 ij	5.50	5.92 i-m	7.09 e
Chinikahai	168.2 a	12.7a-g	11.4а-е	1.3	100.6 a-h	43.0	13.30 klm	1.95 c-h	4.86	6.81 d-m	28.63 abc
Dudhsar	146.5 bcd	9.8efg	8.1cde	1.7	68.3 f-k	47.3	29.50 a	2.82 a-d	5.40	8.22 a-f	34.30 ab
Jasmin	110.0 hij	11.7b-g	10.7а-е	1.0	101.3 a-h	31.8	26.30 ab	2.81 a-d	4.84	7.65 a-j	36.73ab
Jumadhuma	125.1 d-i	8.1g	6.5e	1.6	18.6jk	115.3	13.90 i-m	0.17 j	5.36	5.53 lm	3.07 e
Kalobhog	155.6 ab	10.4a-g	8.5b-e	1.9	107.6 a-g	61.3	13.53 j-m	2.40 b-e	5.26	7.66 a-j	31.31 ab
Kalizira	156.0 ab	11.5b-g	10.4b-e	1.1	105.0 a-h	54.0	15.53 h-l	2.50 a-d	4.40	6.90 c-m	36.31 ab
Kasmiribasmati	135.6 b-g	11.8b-g	9.5b-e	2.3	38.0 ijk	82.0	22.13 cd	1.66 d-i	5.13	6.79 d-m	24.47 bcd
Kalamanik	114.1 g-j	15.1abc	11.1a-e	4.0	30.0 ijk	37.8	21.60 cde	0.35 ij	5.40	5.75 j-m	6.08 e
Kamonisail	98.6 j	9.6d-g	8.6b-e	1.0	107.0 a-h	53.7	18.24 d-h	2.59 a-d	5.23	7.82 a-h	33.12 ab
Lalmoi	122.0 e-i	11.0b-g	9.9b-e	1.1	15.0 k	67.7	15.83 h-l	0.69 g-j	5.04	5.73 klm	12.04 de
Lalgilona	113.5 g-j	8.2g	7.3de	0.9	34.0 ijk	45.8	16.90 g-k	0.81 f-j	5.63	6.44 e-m	12.57 de
Minikichi	103.3 ij	11.5b-g	9.2b-e	2.3	63.3 f-k	58.3	21.93 cd	1.95 c-h	5.03	6.98 b-m	27.93abc
Maharani	141.3 b-e	15.2abc	11.8a-e	3.3	79.3 d-j	39.2	20.40 d-g	2.23 cde	4.46	6.69 d-m	33.33 ab
Marichbati	128.1 d-h	8.9fg	6.8e	2.1	72.3 f-k	33.9	17.03 g-k	1.01 e-j	5.36	6.37 f-m	15.85 cde
Pulashi	117.6 f-j	12.9a-g	11.2а-е	1.7	51.3 g-k	71.0	15.13 h-m	0.45 ij	5.26	5.71 klm	7.88 e
Sharisaful	115.4 g-j	8.5fg	7.4de	1.1	77.3 e-j	58.6	18.53 d-h	0.75 a-j	5.30	6.05 h-m	12.39 de
Surjamukhi	154.2 ab	9.3efg	7.6cde	1.7	86.3 c-i	99.0	25.03 bc	3.17 abc	4.76	7.93 a-l	39.97 ab
Sonamukhi	129.7 c-h	8.2g	7.1de	1.1	44.3 h-k	61.3	17.50 f-j	0.57 hij	5.56	6.13 g-m	9.29 e
Tripunachinal	125.4 d-i	10.2c-g	9.0b-e	1.2	65.3 f-k	82.3	17.90 e-i	0.32 ij	4.96	5.28 m	6.06 e
Ukunimadhu	151.1 abc	14.5a-d	13.8ab	0.7	150.3 ab	39.0	11.23 m	2.60 a-d	5.80	8.40а-е	30.95 ab
LSD _{0.05}	141.1 b-e	10.0d-g	8.3b-e	1.7	109.0 a-g	25.3	15.88 h-l	2.23 cde	5.00	7.23 a-l	30.84 ab
Level of significance	0.01	0.01	0.01	NS	0.01	NS	0.01	0.01	NS	0.01	0.01
LSD _{0.05}	19.32	4.203	4.741	-	52.69	-	3.478	1.222	-	1.554	13.50
CV (%)	6.88	22.95	22.62	81.78	28.24	31.18	9.03	29.35	11.52	10.10	24.76

Table 2. Varietal performance of yield and yield contributing characters of aromatic rice varieties in aman season

In a column, figures with same letters or without letters do not differ significantly whereas figures with dissimilar letter differ significantly as per DMRT. NS = Not significant. followed by Basmati (140.0). The aromatic and non-aromatic fine rice Lalmoi (15.0) produced the lowest number of grains panicle⁻¹ was statistically identical to the aromatic and non-aromatic fine rice Jumadhuma (18.6). In the cropping season *kharif* II, the sunshine duration and light intensity usually remain low due to cloudy sky and monsoon rainfall. This might have caused the plants to be short of required assimilates for fulfilling the grain in panicle with the consequence of a poor grain production panicle⁻¹. Varietal variation is also responsible for the difference in grains panicle⁻¹ is identical to the observance of Singh and Gangwer (1989) and Devaraju *et al.* (1998). The aromatic and non- aromatic fine rice variety Jumadhuma produced the highest number of unfilled spikelets panicle⁻¹ (115.3) followed by Surjamukhi (99.0) (Table 2). Kamonisail produced the lowest number of unfilled spikelets panicle⁻¹ (25.3), which was identical with Chinisagar (31.0) Jasmin (31.8) and Binasail (30.3) (Table 2).

The heaviest grains (29.50 g) were produced by Dudhsar (Table 2). The second heaviest grains (26.30 g) were observed by Jasmin followed by Binadhann 4 and Kasmirbasmati (22.13 g). The lowest 1000-grain weight (10.08 g) was found in Chinisagar, which was statistically identical to BRRI dhan 34 (10.91 g). This variation was perhaps due to the variation in the genetic make-up and the flow of current assimilates toward grains among the variety studied. The results (Table 2) shows that aromatic and non-aromatic fine rice variety Kataribogh produced the maximum grain yield (3.86 t ha⁻¹), which, was statistically identical to those of aromatic and non-aromatic fine rice Basmati (3.73 t ha⁻¹), Binadhan 4 (3.30 t ha⁻¹), Surjamukhi (3.17 t ha⁻¹), Ukunimahu (2.60 t ha⁻¹) and BRRI dhan34 (2.57 t ha⁻¹). The highest grain yield obtained in Kataribhog might be due to highest number of effective tillers hill⁻¹ and highest number of grains panicle⁻¹. The lowest grains observed in aromatic and non-aromatic fine rice variety Jumadhuma (0.17 t ha⁻¹) and other five varieties such as Chikonlal (0.42 t ha⁻¹), Kalamani (0.32 t ha⁻¹), Pulashi (0.45 t ha⁻¹), Tripunachinal (0.32 tha⁻¹) and Banajira (0.27 t ha⁻¹) were statistically identical to variety Jumadhuma. Biswas et al, (1998), Guowei et al. (1998) and Chandra et al. (2000) recorded variable grain yields among varieties due to varieties. The highest straw yield was produced by Chinisagar (5.90 t ha⁻¹). Ukunimahu gave the second highest straw yield (5.80 t ha⁻¹) which, was similar to those of Lalgilona (5.63 t ha⁻¹), BRRI dhan34 (5.51 t ha⁻¹), Chikonlal (5.50 t ha⁻¹), Basmati (5.25 t ha⁻¹), Binadhan-4 (5.40 t ha⁻¹), Kalamanil (5.40 t ha⁻¹), (5.20tha⁻¹), Sonamukhi (5.56 t ha⁻¹), Banajira (5.20 t ha⁻¹), Awned Minicat (5.23 t ha^{-1}) and Awnless Minicat (5.20 t ha^{-1}). The lowest straw yield (4.40 t ha^{-1}) was produced by Kasmaribasmati, which was identically followed by Maharani (4.46 t ha ¹) (Table 2). Table 2 shows that the highest biological yield (8.98 t ha^{-1}) was recorded from variety Basmati, which, was statistically identical to Kataribogh (8.82 t ha⁻¹). The lowest biological yield (5.28 t ha⁻¹) was obtained in aromatic and non-aromatic fine rice Tripunachinal. The results were consistent with those of Singh and Gangwer (1989) who recorded variable biological yields among varieties. The highest harvest index was

recorded from aromatic and non-aromatic fine rice Kataribogh (43.76%). Banajira gave the lowest harvest index (4.93%) (Table 2). From the present study, it might be concluded that Aromatic and non-aromatic fine rice Kataribhogh, Ukunimadhu, BRRI dhan34 and Basmati performed the best regarding grain yield in aman season.

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PERFORMANCE OF TOMATO MUTANTS AND VARIETIES AGAINST BACTERIAL WILT

H. A. Begum¹ and M. B. Meah²

Abstract

Ten induced mutants/advanced lines/varieties of tomato of Bangladesh Institute of Nuclear Agriculture (BINA) along with eleven cultivars of exotic and local origin were screened under artificially inoculated conditions against bacterial wilt in the field of BINA, Mymensingh. Among them eleven mutants/cultivars were evaluated in the farmer's field under natural condition during the winter season of 2007. Field survey under natural conditions revealed that four mutants/cultivars viz. King Kong, TM-12, TM-135, and Manik were graded as highly resistant to bacterial wilt. Binatomato-2, Binatomato-3, Ratan and TM-130 were resistant, Bahar was moderately resistant and the cultivated varieties Pusaruby and Roma VF were moderately susceptible to the disease. Under artificially inoculated conditions, the induced mutant TM-15 and the exotic variety King Kong showed highly resistant reaction. The mutants TM-18 and TM-12 were graded as moderately resistant while, the mutants/varieties TM-16, Manik, BARItomato-5 and Binatomato-3 showed moderately susceptible reaction to the disease.

Key words: Tomato, Mutants and varieties, Resistance, Bacterial wilt.

Introduction

Tomato (*lycopersicon esculentum*), the most important vegetable in Bangladesh suffers heairly from bacterial wilt caused by *Ralstonia solanacearum*. It is the most destructive disease of tomato wherever tomatoes are grown intensively. Bacterial wilt may result 100% yield loss of tomato under severe attack (Bari *et al.*, 2001). The disease has economic impotence and world wide spread occurrence (Bari *et al.*, 2001). Management of this disease using chemicals on the field is difficult, costly and hazardous. Varietal resistance is a type of biological control in which the host itself plays the role of an antagonist (Singh, 2002). Using of resistant varieties can be the most simple, practical, effective and economical method of plant disease management.

They not only ensure protection against disease but also save time, energy and money spent on other measures of control. So, growing of resistant variety is the best way of managing a disease problem. It is therefore, wise to explore the possible sources of

¹Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh ²Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh natural resistance in existing mutants/varieties against the disease. But such variety is not available in Bangladesh. Therefore, the present study was undertaken to find out suitable source of resistance of the crop against the pathogen. With this end in view an attempt has been made to test the available mutants/cultivars against *R. solanacearum* under both natural and inoculated conditions.

Materials and Methods

The seeds of twenty two mutants/cultivars were collected from Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural Research Institute, Asian Vegetable Research Development Centre, Taiwan and seed store of Natoon Bazar, Mymensingh.

Test bacteria

An isolate of *R. solanacearum* the causal organism of bacterial wilt of tomato was isolated from the fresh tomato diseased tissues following dilution plating technique. Hundred microlitre of the bacterial suspension was poured on the surface of the solidified triphenyle tetrazolium chloride salt containing agar medium (Kelman, 1954), in sterilized petrideshes. The bacterial suspension was spread by rotating the plate and incubated at 32° C for 48 hours. Well separated colonies of *R. solanacearum* (having irregular, dull white, fluidal, with pink centre) were picked up and streaked on the surface of casimino acid peptone glucose (CPG) agar (Kelman, 1954) in petridishes. Then the petridishes were incubated at 32° C for 48 hours. The well separated colonies were isolated and sub cultured on CPG agar slants. Three to four loop-full of the virulent colonies were suspended in sterile water taken in screw cap tubes. The tubes were stored at 5° C for stock culture of *R. solanacearum*.

Field survey under natural conditions

Six induced mutants/varieties of tomato of BINA along with five check varieties viz. King Kong, Manik, Ratan, Roma VF and Pusaruby were screened against bacterial wilt under natural condition during the winter season of 2007 in the farmer's field at Boyra, Mymensingh. The experiment was carried out in a randomized block design with three replications. Thirty days old healthy seedlings grown with intensive care in aluminum trays were transplanted in 2.0 m \times 1.5 m plot maintaining 50 cm space both for row to row and plant to plant. Data on percent wilted plants/plot and disease severity of each plant was recorded following (0-5) scale for the disease (Ahmed and Haque, 1986).

Prevalence of bacterial wilt under artificially inoculated conditions:

The experiment was set up in the field of Bangladesh Institute of Nuclear Agriculture, Mymensingh during the winter season of 2007. Screening of 20 mutants/ cultivars was done through artificial inoculation of plants with 48 hours old culture of *R*.

solanacearum following soil drenching method. Experimental design, plot size, spacing and data recording were same as described in former experiment.

Results and Discussion

Cultivar reaction to bacterial wilt under natural field conditions

Among the tested mutants/cultivars King Kong and TM-12 were graded as highly resistant having nil infection of bacterial wilt under natural field conditions. Manik and TM-135 also showed highly resistant reaction having 2.4 and 4.0% of wilted plants (Table 1). The percentage of wilted plants varied from 6.0-9.0% in Binatomato-3, Ratan, Binatomato-2 and TM-130 and the mutants/varieties were graded as resistant while the variety Bahar was identified as moderately resistant. However, the highest percentage of wilted plants (31.6-41.1%) was found in Roma VF and Pusaruby and these two varieties were graded as moderately susceptible to the disease.

 Table 1. Prevalence of bacterial wilt under natural conditions at farmer's field of Boyra, Mymensingh

Mutants/varieties	% wilted plants	Disease reaction
King Kong and TM-12	0	ЦD
Manik and TM-135	2.2-4.0	шк
Binatomato-2, Binatomato-3, Ratan, and TM-130	6.0-9.0	R
Bahar	17.3	MR
Roma VF and Pusaruby	31.6-41.1	MS

HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible

Prevalence of bacterial wilt under artificially inoculated condition

The mutant TM-15 and the cultivar King Kong showed nil wilted plants indicating these two testing materials as highly resistant (Table 2). The percent wilted plants (26.7%) and disease severity (2 and 2.3) of the two mutant lines TM-12 and TM-18 placed them in moderately resistant category. Four mutants/varieties such as Manik, BARI tomato-5, TM-16, Binatomato-3 showed moderately susceptible reaction and other eight mutants/ cultivars showed comparatively higher percentage of wilted plant (53.3-76.7) and higher disease severity (3.7-4.3) resulting in susceptible disease reaction. The bacterial wilt caused significantly the highest mortality percentage (96.7%) of the tomato plants in the variety Apurba. Pusaruby also experienced the second highest mortality percentage (93.3%). The disease severity of the four cultivars (Bahar, Shila, Pusaruby and Apurba) was higher (4.6-5.0) and the disease reaction was rated as highly susceptible.

S1.	Mutant lines/variation	% wilted plants	Disease severity	Disease
No.	Mutant miles/varieties	% writed plants	(0-5)	reaction
1	TM-15	0 (0.37)	0	HR
2	King Kong	0 (0.37)	0	HR
3	TM-18	26.7 (30.3)	2.0	MR
4	TM-12	26.7 (30.8)	2.3	MR
5	Manik	46.7 (43.1) de	3.0	MS
6	BARItomato-5	46.7 (43.0) d	3.0	MS
7	TM-16	46.7 (43.0)	3.0	MS
8	Binatomato-3	50.0 (45.0) cd	3.3	MS
9	Roma VF	53.3 (47.0) cd	3.7	S
10	TM-19	66.7 (54.8)	4.0	S
11	E 6	66.7 (55.1) cd	4.0	S
12	BARI tomato-4	66.7 (55.1) cd	4.0	S
13	Binatomato-2	66.7 (55.1) cd	4.3	S
14	Ratan	70.0 (57.0) cd	4.3	S
15	TM-14	76.7 (61.7) bcd	4.3	S
16	Marglobe	76.7 (61.9) bcd	4.3	S
17	Bahar	73.0 (59.2) cd	4.6	HS
18	Shila	83.3 (70.1) b	4.7	HS
19	Pusaruby	93.3 (81.2) ab	5.0	HS
20	Apurba	96.7 (83.9)	5.0	HS

Table 2. Reaction of twenty tomato mutants/cultivars to Bacterial wilt

Means within the same column with a common letter don't differ significantly (p > 0.01)

HR = Highly resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, HS = Highly susceptible, Figures in parenthesis indicate transformed values

The findings of the present study revealed that the tested materials showed different types of reactions to bacterial wilt of tomato under both natural field conditions and artificially inoculated conditions. The plants with same age, having same dose of nutrients, same nursing within the same environment were inoculated by the same pathogen with equal inoculum load but still they showed differences in wilted plants and severity. Variation in percent wilted plants and severity of bacterial wilt recorded in the present study is in consistence with the observation of other workers (Meah *et al.*, 1998, Begum, 2007, Wicker *et al.*, 2007 and Begum *et al.*, 2008). It was reported that resistant crops showed hypersensitive reactions in which reduced number of colony were recorded. The concentration of toxic substances like phenolic compounds and chlorogenic acid and phytoalexine were higher in resistant tomatoes than susceptible ones (Shanshoury *et al.*, 1996). Mandavia (2000) reported the genetic variation among the varieties that govern the resistance mechanism of plant against *F. oxysporum* f. sp. *lycopersici* or *R. solanacearum*.

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EVALUATION OF PHOSPHATE SOLUBILIZING BACTERIA IN RELATION TO PHOSPHORUS SOLUBILIZATION AND PHOSPHATASE ACTIVITY

M. A. Haque¹, M. A. Sattar², M. R. Islam³, M. A. Hashem³ and M. K. Khan¹

Abstract

This study aimed to investigate the phosphorus (P) solubilization capacity and phosphatase activity of phosphate solubilizing bacterial (PSB) cultures on different insoluble minerals under laboratory condition at Microbiology laboratory, Soil Science Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh. Eight PSB cultures were isolated from different crop rhizospheres and evaluated their P solubilization performance on three insoluble minerals such as tricalcium phosphate (TCP), ferric phosphate (Fe-P) and aluminium phosphate (Al-P). The PSB cultures showed large variation in producing halo zone (P solubilization zone) around their colonies and P solubilization index (20 to 100%) on TCP based solid medium. The PSB isolates widely varied in terms of pH change, increasing P solubilization and acid phosphatase activity in liquid media containing three insoluble minerals. The PSB isolate MR1 significantly solubilized maximum amount of P (479.2 µg P mL⁻¹) with the highest P solubilization efficiency of 45.1% from aluminium phosphate (Al-P) followed by the isolate MW1. The second highest P was solubilized by all the PSB isolates from TCP. Al-P was easily solubilized by all the PSB isolates than TCP or Fe-P substrates. The PSB isolate MR1 showed the highest acid phosphatase activity $(31.1 \ \mu g \ pNP \ mL^{-1} \ h^{-1})$ from Fe-P substrates followed by the isolate MW1 (28.0 μg $pNP mL^{-1} h^{-1}$) at two days after incubation. All the PSB isolates decreased the pH of the media and negatively correlated (r = -0.98, -0.86 and -0.94 for TCP, Fe-P and Al-P, respectively) with the quantity of P solubilized. Decreasing of pH by the PSB isolates was an important mechanism of P solubilization in all three liquid media. The results revealed that all the PSB cultures have good ability to solubilize P from three minerals. Among the PSB cultures, MR1 and MW1 can be used as potential phospho bioinoculant to increase available P for diiferent crops.

Key words: PSB, Insoluble minerals, P solubilization, Phosphatase activity, pH decrease

Introduction

In most of the soils, inorganic phosphorus occurs in low concentrations while a large proportion of this held by diverse soil minerals in combination with metals such as Ca, Fe and Al. In acid soils, free oxides and hydroxides of Al and Fe fix P, while in alkaline soils it is fixed by Ca causing a low efficiency of soluble P fertilizers. Greater part

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of soil phosphorus exists in the form of insoluble phosphates which is often unavailable to the plants (Khiari and Parent, 2005). These forms of P are important aspects for P mineralization/mobilization through microbial mediated process to make available for the crop plants (Chen et al., 2005). Many soils of Bangladesh are deficient in P but some reports claimed that total P exist at high level (900 to 1300 mg P kg⁻¹ soil) in many soils (Ali and Wakatsuki, 2008). Long term application of P fertilizer to the soil resulted in a build-up of residual P and inorganic P fractions (Thanh Vu et al., 2006). These fractions and high proportion of organic P fractions could be a potential source for plant available P. Therefore, sustainable management of P in agriculture requires strategies to enhance its acquisition or uptake by plants. In this context room is available for conducting research on P mobilization in soil through microbial mediated process. Biological means of recovering nutrients in the available form offer an environment friendly sustainable system to support plant and soil health. The principal mechanism for mineral phosphate solubilization is the production of organic acids and acid phosphatases play a major role by phosphate solubilizing bacteria (Panhwar et al., 2009). Therefore, among the P management practices, phosphate solubilizing biofertilizer may be a good option to mobilize P in soils (Sattar and Gaur, 1989; Panhwar et al., 2012) but information of PSB as biofertilizer in Bangladesh is meager due to lack of effective bacterial strains. Keeping the above facts in mind, the present investigation was undertaken with the objectives to evaluate the phosphate solubilizing potentiality and phosphatase activity of PSB isolates on different insoluble minerals.

Materials and Methods

Isolation of phosphate solubilizing bacteria (PSB) and Determination of phosphate solubilization index on solid medium

Rhizosphere soils were collected (viz. rice, wheat, lentil and chickpea) from four agro-ecological zones (AEZs) such as High Ganges River Floodplain (AEZ 11, Ishurdi and Magura), Old Brahmaputra Floodplain (AEZ 9, Mymensingh), Madhupur Tract (AEZ 28, BADC farm, Madhupur) and High Barind Tract (AEZ 24, Rajabari, Rajshahi). Phosphate solubilizing bacteria (PSB) were isolated through serial dilution techniques (Subba Rao, 1999) using Pikovskaya's solid medium (Pikovskaya, 1948) containing tricalcium phosphate (TCP). The bacterial colonies that appeared with discrete clear zones (halo zones) indicated the dissolution of TCP, was assumed to be phosphate solubilizers. PSB isolates were purified through repeated cultures and finally maintained on Pikovskaya's medium slants in a refrigerator at 4°C for subsequent study. Phosphate solubilization index was calculated according to Edi Premono *et al.* (1997).

Determination of pH, P solubilization and phosphatase activity

With a view to determine the relative efficiency of PSB strains, laboratory incubation experiments were carried out for determination of pH, P solubilization and assay of phosphatase activity by the PSB isolates in PVK's liquid medium amended with different insoluble minerals such as tricalcium phosphate (TCP), ferric phosphate (Fe-P) and aluminium phosphate (Al-P). In order to determine the P solubilization from different substrates, fifty millilitre of PVK's liquid medium was taken into 250 mL Erlenmeyer flasks which contained 50 mg P (1 g P L^{-1}) from respective substrates (Pikovskaya, 1948). About 0.2 mililitre of 48 h old PSB cultures were inoculated in each flask containing sterile medium with three replications for each PSB isolate followed by Completely Randomized Design (CRD). Uninoculated flasks, containing same media were also used as control. All the flasks were incubated at 30°C for 2, 4, 8 and 16 days. End of each incubation interval, bacterial suspension were centrifuged at 10,000 rpm for 15 minutes to remove bacterial cells and other insoluble materials to get clear supernatants. Bacterial supernatants were divided into two portions. One portion was used for determination of pH by Glass Electrode pH Meter (pH ep^R S321610, Hanna instruments). Other portions were stored in refrigerator at 4^oC until the determination of available P and extracellular acid phosphatase activity (APA). Available P from bacterial supernatant was determined by ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962). Extracellular acid phosphatase activity (APA) was assayed according to Tabatabai and Bremner (1969).

Results and Discussion

P solubilization index of PSB on solid medium

Eight PSB were isolated from different rhizosphere soils (Table 1). P solubilization index of eight PSB isolates on TCP based solid medium varied from 20 to 100% (Table 1 and Plate 1). The isolate MW1 gave maximum (100%) P solubilization index which obtained from wheat rhizosphere at Magura followed by the isolate MdR3 (92.3%) from rice rhizosphere at Madhupur. Minimum P solubilization index was recorded with the isolate IW1 (20%) which obtained from wheat rhizosphere of Ishurdi (Table 1). The results indicated that different PSB isolates have good ability to solubilize phosphate from TCP on solid medium. Any microorganism which is capable to produce a halo/clear zone on TCP medium is selected as a potential phosphate solubilizer. P solubilization or production of halo zone on solid medium depends on production of organic acids into the surrounding medium by the PSB (Vikram et al., 2007). Solubilization of TCP in agar medium is used as initial criterion for isolation and enumeration of P solubilizing microorganisms (Pikovskaya, 1948). However, the formation of halo zone on solid medium is not the only criterion of the ability of an organism to solubilize P due to the varying diffusion rates of different organic acids secreted by an organism (Panhwar et al., 2009). Therefore these isolates were again examined for P solubilization in liquid medium.

Changes of pH and P solubilization in liquid media

All the PSB isolates decreased the pH of TCP, Fe-P and Al-P containing liquid media up to 8 days of incubation (Table 2). Irrespective of the PSB isolates, maximum decrease of pH was observed with 8 days of incubation in all the media followed by 4 days of incubation. Irrespective of the incubation days, the PSB isolate MR1 showed maximum decrease of pH (2.8, 3.0 and 2.6) in all the liquid media, respectively. With the interaction between PSB isolates and incubation days, maximum decrease of pH (2.35 and 2.9) was observed with the PSB isolate MR1 in TCP and Fe-P media, respectively while the PSB isolates MdR3 gave maximum decrease of pH (2.4) in Al-P based medium after 8 days of incubation (Fig. 1). Phosphorus solubilizing microorganisms have been reported to dissolve insoluble phosphates by the production of inorganic or organic acids and hence by the decrease of the pH (Barroso *et al.*, 2006).

Posphorus solubilization by different PSB isolates increased significantly up to 8 days of incubation from TCP, Fe-P and Al-P based media (Table 3). Among the three substrates, the highest P solubilization was found from Al-P followed by TCP and Fe-P. Among the eight PSB isolates, the MR1 showed the highest P solubilization from all the substrates followed by MW1 (Table 3). The PSB isolate MR1 solubilized $364.2 \mu g P mL^{-1}$ from Al-P, 230.4 μ g P mL⁻¹ from TCP and 62.3 μ g P mL⁻¹ from Fe-P. It appeared that the phosphate from Al-P was easily solubilized by PSB and phosphate from Fe-P was least solubilized. With the interaction between PSB isolates and inocubation days, the isolate MR1 showed significantly highest P solubilization (479.2, 438.5 and 89.3 μ g P mL⁻¹) from Al-P, TCP and Fe-P, respectively at 8 days of incubation (Fig. 2) followed by the isolate MW1. The lowest P solubilization was recorded with the isolate IL1 (53.1 μ g P ml⁻¹) from TCP while the isolate RC1 (24.6 and 140.7 µg P mL⁻¹) showed the lowest soluble P from Fe-P and Al-P, respectively at 2 days of incubation. P solubilization efficiency increased up to eight days of incubation with the different PSB isolates in all the media (Fig. 3). The isolate MR1 showed the highest P solubilization efficiency (45.1, 43.8 and 8.9%) in Al-P, TCP and Fe-P based media at 8 days of incubation, respectively followed by the isolate MW1 (44.4, 36.2 and 8.0% P solubilization from Al-P, TCP and Fe-P, respectively). There is a large variability in P solubilization among the isolates. These variations might be due to the variations in their metabolic activity as also reported by many workers (Mahesh et al.1999; Barroso et al., 2006). Hesham and Komy (2005) found that Pseudomonas fluorescens and Bacillus megaterium strains were the most powerful phosphate solubilizers on solid and liquid medium containing tricalcium phosphate. The extent of P solubilization was low from Fe-P by the different PSB isolates, probably due to complex nature of Fe-P (Mishra et al., 1983). Increase of pH and decrease the P solubilization from different compounds were observed by all the PSB isolates after 16 days of incubation. It might be occurred due to cease the secretion of organic acids by the bacteria and soluble P might be converted into insoluble form with time (Gaur and Gaind, 1983). The present study was well corroborated with those findings.

Acid phosphatase activity of PSB isolates in liquid media

Acid phosphatase acitivity (APA) of PSB isolates was significantly highest at 2 days of incubation in TCP (15.5 μ g pNP mL⁻¹ h⁻¹), Fe-P (20.9 μ g pNP mL⁻¹ h⁻¹) and Al-P $(5.8 \ \mu g \ pNP \ mL^{-1} \ h^{-1})$ based meida and then decreased significantly over time (Table 4 and Fig. 3). The lowest APA was recorded at 16 days of incubation with all the isolates. Among the PSB isolates, MR1 showed maximum APA (12.9, 18.8 and 4.0 μ g pNP mL⁻¹ h⁻¹ ¹ on TCP, Fe-P and Al-P, respectively) on three substrates followed by the MW1. The lowest APA was found with the isolate MdR3 on all the substrates. Uninoculated control treatment showed no APA through out the incubation periods on all the substrates. With the interaction between PSB isolates and incubation days, the isolate MR1 showed the highest APA (31.1 µg pNP mL⁻¹ h⁻¹) followed by MW1 (28.0 µg pNP mL⁻¹ h⁻¹) in Fe-P medium at 2 days after incubation (Fig. 3). The lowest APA was observed with the isolate MW1 (0.6 μ g pNP mL⁻¹ h⁻¹) on Al-P based medium at 16 days of incubation. In the present study, all the PSB isolates showed higher APA at two days of incubation than subsequent incubation period in all the media which might be happened due to lesser soluble P in the media. APA decreased after 16 days of incubation in all the media might be due to increased the soluble P in all the media by the action of bacteria which repressed the phosphatase activity of PSB in the media. Inorganic phosphate in the assay medium might be a competitive of the phosphatase activity (Prasanna et al., 2011). However, the efficiency of the released phosphatase varied from strain to strain resulting in variation of P released into the medium (Ponmurugan and Gopi, 2006; Prasanna et al., 2011). The present results are well supported with earlier findings.

Relationships between changes of pH, acid phosphatase activity (APA) and P solubilization by PSB isolates in liquid media

P solubilization was exponentially increased with the decrease in pH (r = -0.98, -0.86 and -0.94 for TCP, Fe-P and Al-P, respectively) of all the culture media (Table 5). The PSB isolates decreased the pH of the media and negatively correlated with quantity of soluble P were reported by many workers (Chen *et al.*, 2005; Kpomblekou and Tabatabai, 1994). Polynomial relationships were found between phosphatase activity and soluble P in all the media except in TCP medium where an exponential relationship was observed. Significant (P_{0.01}) correlations were also observed between phosphatase activity and P solubilization by the PSB isolates in TCP (r = 0.71) medium while insignificant relationships were found in other two media (Table 5). pH and phosphatase activity showed polynomial relationships in all the media but significant relationship was observed only in TCP medium (r = 0.71). The relationships between pH, phosphatase activity and soluble P indicated that P solubilization was greater influenced with decreasing of pH than phosphatase activity of PSB isolates in all liquid media. Therefore, the present study revealed that decreasing pH was an important mechanism of P solubilization by the PSB isolates in all three liquid media (Chen *et al.*, 2005).

From this study, it can be concluded that the eight PSB isolates have the ability to solubilize a good amount of P from three insoluble compounds. Among the PSB isolates, MR1 was found as highly efficient P solubilizer from different P substrates followed by MW1. The PSB isolates MR1 and MW1 can be used as promising phospho bioinoculants for mobilization of phosphate in soil.

Table 1.	Phosphate	solubilizing	bacteria	(PSB)	isolated	from	rhizosphere	soils	and	P
	solubilizati	on index on t	ricalcium	phosph	nate based	l solid i	medium			

PSB Isolates	Locations	Rhizosphere of crops	P solubilisation index (%)
IL1	Ishurdi	Lentil	70.0
IW1	Ishurdi	Wheat	20.0
IC2	Isurdi	Chickpea	90.0
MR1	Magura	Rice	81.8
MW1	Magura	Wheat	100.0
RC1	Rajshahi	Chickpea	50.0
MdR3	Madhupur	Rice	92.0
MyR2	Mymensingh	Rice	50.0



Plate 1. Some promising PSB isolates (MW1, MR1, IC2 and MdR3) produced halo zone (P solubilization zone) on Pikovskaya solid medium

Factors	ТСР	Fe-P	Al-P
Incubation days			
2	3.7a	4.5	3.3ab
4	3.5ab	4.2	3.1bc
8	3.1c	4.1	3.0c
16	3.2bc	4.3	3.4a
SE(±)	0.007	NS	0.068
PSB isolates			
Control	6.4a	6.9a	7.0a
IL1	3.0cd	4.0d	2.7bc
IW1	3.0cd	3.6e	2.6cd
MR1	2.8d	3.0f	2.6cd
RC1	3.1bc	5.0b	2.8b
IC2	3.2b	4.4c	2.9b
MyR2	3.1bc	4.8bc	2.9b
MdR3	3.0cd	3.6e	2.6cd
MW1	2.8d	3.2f	2.6cd
SE(±)	0.025	0.031	0.063

Table 2.	Changes in pH with PSB isolates in tricalcium phosphate (TCP), ferric phosphate (Fe-P)
	and aluminium phosphate (Al-P) based liquid media at different days after incubation

Initial pH: 7.2; SE = Standard error; NS = Non significant

In a column figures having common letter(s) do not differ significantly at 1% level of probability.



Fig. 1. Interaction effects of PSB isolates and incubation days on changes in pH in various liquid media

Factors	ТСР	Fe-P	Al-P
Incubation days			
2	35.3d	35.3c	175.7d
4	93.54c	40.9b	280.2b
8	257.7a	64.1a	348.9a
16	170.9b	33.9c	221.8c
SE(±)	2.38	0.53	4.10
PSB isolates			
Control	0.3f	0.2g	23.9h
IL1	129.0e	51.4c	325.3c
IW1	178.3c	47.1d	263.1e
MR1	230.4a	62.3a	364.2a
RC1	124.6e	35.9f	243.2f
IC2	136.9d	49.4c	220.8g
MyR2	125.4e	38.8e	218.1g
MdR3	130.4e	51.0c	309.9d
MW1	199.3b	56.1b	341.3b
SE (±)	1.72	0.52	3.87

Table 3. Effects of PSB on P solubilization (µg P mL⁻¹) in TCP, Fe-P and Al-P based liquid media at different days after incubation

SE = Standard error

In a column figures having common letter(s) do not differ significantly at 5% level of probability.



Fig. 2. Interaction effects of PSB isolates and incubation days on P solubilization in various liquid media (Vertical bars indicate standard error)



Fig. 3. Interaction effects of PSB isolates and different incubation periods on % added P solubilization in various liquid media (Vertical bars indicate standard error)

Table 4.	Effects of PSB on acid phosphatase activity (µg pNP mL ⁻¹ h ⁻¹) in TCP, Fe-P and Al-J
	based liquid media at different days after incubation

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Factors	TCP	Fe-P	Al-P
Incubation days			
2	15.5a	20.9a	5.8a
4	6.2b	13.6b	2.2b
8	5.8b	9.1b	1.6c
16	5.5b	2.6c	0.7d
SE(±)	0.16	0.46	0.05
PSB isolates			
Control	Og	0.0f	0 f
IL1	10.1c	14.6c	2.4e
IW1	9.1d	10.4d	3.1c
MR1	12.9a	18.8a	4.0a
RC1	7.9e	13.8c	2.6de
IC2	9.2d	10.6d	2.8cd
MyR2	8.3e	11.8d	2.5de
MdR3	5.4f	7.3e	2.2e
MW1	11.2b	16.9b	3.5b
SE(±)	0.17	0.40	0.08

SE = Standard error

In a column figures having common letter(s) do not differ significantly at 5% level of probability.



Fig. 4. Interaction effects of PSB isolates and incubation days on acid phosphatase activity (APA) in various liquid media (Vertical bars indicate standard error)

Table 5. Correlation	matrix (r)	between	changes	of	pН,	Р	solubilization	and	phosphatase
activity of Pa	SB in differ	ent liquid	media						

		TCP		Fe-P	Al-P		
Variables	pН	Acid phosphatase	pН	Acid phosphatase	pН	Acid phosphatase	
		activity		activity		activity	
pH	-	0.71**	-	0.34 ^{ns}	-	0.39 ^{ns}	
P solubilization	-0.98**	0.71**	-0.86**	0.50^{ns}	-0.94**	0.68^{**}	

**Significant at 1% level of probability.

NS = Non significant

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EFFECT OF CULTIVAR AND ROW SPACING ON THE YIELD AND YIELD CONTRIBUTING ATTRIBUTES OF CHICKPEA

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Abstract

A field experiment was conducted at Rajshahi (Godagari) during November 2007 to April 2008 to find out the effect of population densities on the yield and yield contributing characters of chickpea varieties. Among different row spacing's, Binasola-4, Binasola-3 and Hyprosola produced higher seed yield of 2367, 2376 and 2328 kgha⁻¹, respectively in closer spacing (40 cm row). Higher yield in closer spacing was due to the production of higher population stands than wider spacing. In medium spacing, second highest seed yield of 2251, 2170, and 2139 kgha⁻¹ were produced by Binasola-4, Binasola-3 and Hyprosola, respectively.

Key words: Chickpea cultivar, Row spacing, Seed yield

Introduction

Yield variability is a global problem in grain legumes, which can lead to low yields. This variability can be reduced by manipulating population density and sowing pattern. More uniform sowing may give rise to less variable populations with reduced plant to plant variation and increased yields. Plant population density is a key component in optimizing the productivity of chickpea. The use of high Plant Population Density (PPD) usually increase seed yield of chickpea in areas with a short growing season (Gan et al., 2003 and Regan et al., 2003), but the magnitude of the yield increased depends on environmental conditions (Lamb and Podder, 1988; Singh and Saxena, 1999). In areas where the growing season is short, the increased PPD is largely due to increased light interception of the crop canopy (Li, 2006). The use of high PPD in chickpea production decreases soil water evaporation early in the growing season when plant canopy closer is low (Turner et al., 2001). In contrast, low PPD may allow weeds to develop more aggressively and limit crop yield potential. Plants grown at lower PPD are usually shorter and branchy, which increases losses during combine harvest (Jettner et al., 1999). An experiment was conducted by Ayaz et al. (2001) on legumes and concluded that as plant density increases, intensity of interplant competition increases and yield plant⁻¹ declines,

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although total yield unit⁻¹ area may increase. They also reported that yield total dry matter (TDM) and harvest index (HI) of lentils, chickpeas, peas and faba beans increase as plant populations increased. Optimum yields (e.g. in *Phaseolus*) have been found at equidistant inter, and intra-row spacing's. Many components of seed yield are inversely related to population density. Based on above information an experiment was therefore, made to evaluate the effect a of plant spacing on the yield and yield contributing characters of chickpea under sub-tropical condition.

Materials and Methods

A field experiment was conducted at Rajshahi (Godagari) during November 2007 to April 2008 with the objective to find out the optimum population for yield of chickpea varieties. The land was opened with a power tiller plough. Subsequently, ploughing, crossploughing, laddering and leveling were done with the help of power tiller followed by cleaning and removal of weeds. The land was left as such for a few days for operation and conditioning. The land was again ploughed, laddered and cleaned of remaining stubbles and weeds. Two sets of treatments namely three varieties (Binasola-4, Binasola-3 and Hyprosola) and three row spacing's (80 cm, 60 cm and 40 cm) were used. The experiment was laid out in a split plot design with three replications. The entire experimental field was divided into three blocks and each block was divided into three main plots and each main plot was divided into three sub plots. The total plots of the experiment were $27 (3 \times 3 \times 3)$. The variety was placed in the main plot and the spacing was placed in the sub plot. After making lay out of the experiment, the lands were fertilized. Chemical fertilizers were applied @ 20.0, 25.0, 28.0, 10.0, and 1.0 ha⁻¹ as N, P, K, S and Zn from urea, triple super phosphate, muriate of potash, gypsum and zinc sulphate, respectively. The fertilizers were used in the experimental plots following Fertilizer Recommendation Guide (BARC, 2005). The Full dose of N, P, K, S, and Zn were applied as basal at the time of final land preparation. Line to line distance was maintained 80 cm, 60 cm and 40 cm in the sub plot. The cultural practice such as weeding, insect and disease control were done as and when necessary. The cleaned seeds were dried in the sun and the dried seeds were cooled and measured for each plot and converted to kg ha⁻¹. The plant characters were recorded from ten randomly selected plants and were analyzed statistically and the mean values were adjudged using DMRT.

Results and Discussion

The varieties showed significant difference for plant height, population/m², pods plant⁻¹, 1000 seed weight, seed yield and harvest index and spacing found to be significant for all the characters except stover yield (Table 1). Interaction between variety and spacing were found to be significant for plant height, seeds pod⁻¹, 1000 seed weight, seed yield and harvest index (Table 2). The different plant characters are discussed below. Highest

population/m² (26.46) was obtained by Hyprosola and other two varieties Binasola-4 and Binasola-3 produced 24.88 and 24.03 plants per metre square area which was statistically identical. The wider row spacing (80 cm) gave lowest number of plants (17.78) and the closer spacing (40 cm row) produced highest plants per metre square area. Again the interactions of closer spacing (40 cm row) and varieties produced higher plant stands (31.63, 31.0 and 34.30).

Traatmonto	Popula-	Plant	Branches	Pods	Seeds	1000-seed	Yield $(\log \log^{-1})$	Stover	Harvest
Treatments	(no)	(cm)	(no.)	(no.)	(no.)	(g)	(kg lia)	$(t ha^{-1})$	(%)
T T 1 (1	(110.)	(CIII)	(110.)	(110.)	(110.)	(g)		(t lia)	(70)
Varieties:									
Binasola-4	24.88 b	38.28 b	5.43	73.78 b	1.72	123.21 b	2208 a	4.13	34.82 a
Binasola-3	24.03 b	40.90 a	5.34	77.72 a	1.74	167.37 a	2199 a	4.07	35.08 a
Hyprosola	26.46 a	36.33 c	5.42	77.18 a	1.77	103.76 c	2103 b	4.25	33.66 b
LSD _{0.05}	1.06	1.25	ns	2.62	ns	0.88	40.93	ns	0.72
Row spacing	gs:								
80cm	17.78 c	35.63 c	6.59 a	79.56 a	1.85 a	133.32 a	1967 c	4.27	31.54 c
60cm	25.28 b	38.23 b	5.23 b	76.11 b	1.75 b	131.36 b	2187 b	4.06	35.02 b
40cm	32.31 a	41.64 a	4.38 c	73.01 c	1.63 c	129.65 c	2357 a	4.12	37.01 a
LSD _{0.05}	1.06	1.25	0.41	2.62	.04	0.88	40.93	ns	0.72
CV (%)	2.94	2.26	5.26	2.39	1.82	0.47	1.31	5.01	1.44

 Table 1. Effect of cultivars and row spacing on the yield and yield contributing characters of chickpea

The highest plant height (40.90 cm) was obtained by Binasola-3 followed by Binasola-4 (38.28 cm). The wider spacing (80 cm row) produced shortest plant (35.63 cm) than closer (40 cm row) spacing (41.64 cm). On the other hand, interaction showed (Table 2) highest plant height in Binasola-3, Binasola-4 and Hyprosola (45.0 cm, 42.07 cm and 37.87 cm, respectively). Spacing had significant effect on branches plant⁻¹ (Table 1) e.g. higher row spacing gave higher number of branches plant⁻¹ but no significant effect was observed on variety and interaction between variety \times spacing (Table 1 and 2). Trait pods plant⁻¹ is important for variety and spacing (Table 1) which is statistically significant but no significant effect was found on interaction between them (Table 2). Highest number of pods was produced in Binasola-3 and Hyprosola (77.72 and 77.18) and lowest was in Binasola-4. Wider row spacing (80 cm) reported to be highest (79.56) than narrow row spacing (76.11 by 60 cm and 73.01 by 40 cm). In all three varieties, seeds pod⁻¹ was not statistically significant but spacing and interaction were found very low level of significant differences. Wider row spacing (80 cm) showed highest no. of seeds pod⁻¹ followed by 60 cm and 40 cm (Table 1). In interaction (Table 2), wider spacing exhibited highest no. of seeds for Hyprosola, Binasola-3 and Binasola-4 (1.88, 1.84 and 1.82, respectively). Among the varieties, Binasola-3 produced maximum 1000 seed weight (167.37 g) and minimum in Hyprosola (103.76 g). Among different row spacing's, highest 1000 seed

weight (133.32 g) was obtained in wider spacing (80 cm) and lowest (129.65 g) in 40 cm row. The interaction effect (Table 2) showed that all the three varieties (Binasola-4, Binasola-3 and Hyprosola) produced maximum 1000 seed weight 124.5 g, 169.2 g and 106.3 g, respectively in wider spacing (80 cm row) and showed lowest 1000 seed weight in closer spacing (40 cm row).

Treatments	Popula-	Plant	Branch	Pods	Seeds	1000 seed	Yield	Stover	Harvest
Interaction	(no.)	(cm)	(no.)	(no.)	(no.)	(g)	(kg lla)	$(t ha^{-1})$	(%)
Binasola-4									
80 cm	17.70	34.87 d	6.83	76.66	1.82 abc	124.5 c	2008 d	4.33	31.69 d
60 cm	25.30	37.90 c	5.40	74.30	1.75 cde	122.9 cd	2251 b	4.07	35.57 b
40 cm	31.63	42.07 b	4.06	70.31	1.60 f	122.2 d	2367 a	3.99	37.19 a
Binasola-3									
80 cm	16.80	37.17 c	6.56	81.65	1.84 ab	169.2 a	2051 b	4.34	32.08 d
60 cm	24.30	40.53 b	5.10	76.67	1.78 bcd	167.9 a	2170 c	3.84	36.10 ab
40 cm	31.00	45.00 a	4.37	74.82	1.59 f	164.9 b	2376 a	4.03	37.06 a
Hyprosola									
80 cm	18.83	34.87 d	6.36	80.33	1.88 a	106.3 e	1844 e	4.13	30.84 d
60 cm	26.23	36.26cd	5.20	77.32	1.72 de	103.1 f	2139 с	4.26	33.37 c
40 cm	34.30	37.87	4.70	73.86	1.68 e	101.8 f	2328 a	4.33	36.78 ab
LSD _{0.05}	ns	2.17	ns	ns	.07	1.54	70.89	ns	1.24
CV (%)	2.94	2.26	5.26	2.39	1.82	0.47	1.31	5.01	1.44

 Table 2. Interaction effect of spacing and cultivars on the yield and yield contributing characters of chickpea seed yield

Seed yield was highest (2208 kg ha⁻¹) by Binasola-4 and 2199 kg ha⁻¹ by Binasola-3 which was statistically insignificant. Seed yield increased (2357 kg ha⁻¹) in closer spacing and in wider spacing, seed yield decreased (1967 kg ha⁻¹). Interaction effect showed that, in the closer spacing, Binasola-3, Binasola-4 and Hyprosola produced higher seed yield of 2376, 2367 and 2328 kg ha⁻¹, respectively. Higher seed yield in closer spacing might be due to the higher population stands than wider spacing. In medium (60 cm row) spacing, Second highest seed yield (2251, 2170, and 2139 kg ha⁻¹ by Binasola-4, Binasola-3 and Hyprosola, respectively) was obtained by the varieties. Highest stover yield (4.25 t ha⁻¹) was obtained by Hyprosola and lowest (4.07 t ha⁻¹) was by Binasola-3. Different spacing performed equal amount of stover yield (4.27, 4.06 and 4.12 t ha⁻¹). Interaction effect showed that higher stover yield was obtained by Binasola-4 (4.33 t ha⁻¹) and Binasola-3 (4.34 t ha⁻¹) in wider spacing (80 cm row). Hyprosola produced highest stover in closer spacing (4.33 t ha⁻¹). Stover yield was not affected by the variety, spacing and their interaction. Harvest index of 35.08% obtained by Binasola-3, 34.82% by Binasola-4 and 33.66% by Hyprosola and closer spacing showed highest harvest index of 37.01% (Table 1). Interaction of variety with spacing showed higher harvest index in closer spacing by all the

variety (37.19%, 37.06% and 36.78% by Binasola-4, Binasola-3 and Hyprosola, respectively). Higher value of harvest index in closer spacing (Table 1 and 2) might be due to the contribution of higher seed yield by different variety.

The results indicated that seed yield was increased in closer spacing (2357 kg ha⁻¹). In wider spacing, seed yield was lowest (1967 kg ha⁻¹). Interaction effect showed that, in the closer spacing (40 cm) Binasola-4, Binasola-3 and Hyprosola produced higher seed yield of 2367, 2376 and 2328 kg ha⁻¹, respectively. On the other hand, wider row spacing (80 cm) showed higher branching, pods plant⁻¹, seed plant⁻¹ and 1000 seed weight. Higher yield in closer spacing might be due to the establishment of higher population stands than wider spacing. The results were in agreement with the findings of Ayaz *et al.* (2001). They conducted an experiment on legumes and concluded that as plant density increases, intensity of interplant competition increases and yield plant⁻¹ declines, although total yield unit⁻¹ area may increase. They also reported that yield, total dry matter and harvest index of lentils, chickpeas, peas and faba beans increase as plant populations increased.

Conclusion

From the above results and discussion, it may be concluded that, for obtaining higher yield of chickpea closer spacing (40 cm row) may be recommend for the end users. Although closer spacing accommodates more plants and interplant competition increases and yield decreased, but increased total yield. So, Binasola-3, Binasola-4 and Hyprosola can be grown in Rajshahi region at closer spacing of 40 cm row.

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ASSESSMENT OF DIFFERENT CHEMICAL PROPERTIES IN BALU RIVER WATER DURING WET AND DRY SEASON

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Abstract

An extensive study was carried out in the Balu river water during the year of 2011-2012 with a view to identify the water quality regarding different chemical contamination. A total of 72 surface water samples were collected from four different locations adjacent to the Balu river area of Dhaka-Narayangonj-Demra area, during wet (July to September 2011) and dry season (November to January 2011-'12). To determine the spatial distribution and seasonal variation of physico-chemical properties (pH, EC, NO₃-N, NH₄-N, PO₄, Ca and Mg) details laboratory analyses were performed in the Laboratory of Soil Science Division of Bangladesh Institute of Nuclear Agriculture (BINA) following standard procedures. Among the different locations, the maximum mean concentration of EC, NO₃-N, NH₄-N, PO₄, Ca and Mg were observed at Termuni area as 1.44 mScm^{-1} and 2.79, 4.13, 19.14, 53.29 and 51.15 mgL⁻¹, respectively. Considering locations and seasons, the highest concentration of the analyzed parameters wee found at Termuni area during dry season as EC (1.54 mScm⁻¹), NO₃-N (3.95 mgL⁻¹), NH₄-N (5.29 mgL⁻¹), PO₄ (25.45 mgL⁻¹), Ca (61.08 mgL⁻¹) and Mg (72.57 mgL⁻¹). The minimum concentration of EC, NO₃-N and Mg was noted at Demra area during wet season as 1.05 mScm⁻¹, 1.02 and 14.26 mgL⁻¹, respectively. The NH₄-N, PO₄ and Ca concentrations were observed as 1.78, 3.19 and 23.20 mgL⁻¹ at Nagarpara area and pH value of 8.04 was found at Itakhola area. The study revealed that the concentrations of EC, PO₄ and Mg exceeded the standard level for drinking irrigation and aquaculturre.

Key words: Balu river, Chemical properties of water, Wet and dry season

Introduction

Water is becoming an increasing scarce resource for agriculture. Wherever good quality water is limited, marginal quality water like sewage and other waste water is used to supplement irrigation needs, particularly in the peri-urban zones. River plays a major role in assimilating or carrying off industrial and municipal waste water, manure discharges and run off from agricultural fields, roadways and streets, which are responsible for river pollution (Stroomberg *et al.*, 1995; Word and Elliot, 1995). Among the polluted areas of Bangladesh, the worst problem is in the area surrounding to the river

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Balu where the most significance source of pollution appears from different industries in the Dhaka city area. Although waste water is an important source of plant nutrients and helps in improving crop yields, its likely adverse impact on human and soil health warrants constant monitoring. Most of the industries have no effluent treatment plant (ETP) and recycle plant in Dhaka city. Every year in monsoon, most of the crop fields under river catchments are flooded by the river. During winter, the river water and also waste water are extensively pumped for irrigation. In Bangladesh, there is a progressive increase in industrial wastes and due to the rapid industrialization such waste products have been causing severe contamination to the air, water and soils, thus polluting the environment. Amount of different constituents that have been identified in the polluted environment include pH, EC, NO₃⁻-N, NH₄⁺-N, PO₄⁻³, Ca⁺² and Mg⁺² etc. These chemical agents enter into the environment through various industrial processes. Water resource, the prominent component of the environment is getting polluted over the years. Wastewater is mainly used for irrigation purpose, because this contains nutrients that enhance the growth of crop plants but it also known to have significant contribution to different chemical properties of soils. About 80% of the diseases in developing countries are related to contaminated water and the resulting death toll is as much as 10 million per year (Annonymous, 2004). Considering the above facts, the present study was conducted to assess toxic metals contamination, the spatial distribution and seasonal variation of Balu different chemical properties and evaluate the river water quality with irrigation standard for crop production during wet and dry season.

Materials and Methods

The study was conducted at Balu river, Dhaka-Narayangonj-Demra area. Balu river is located at 23°43′51′′ (23.7308°) north latitude and 90°30′5′′ (90.5014°) east longitude. A total of seventy two water samples were collected (from 3 replications) from different locations during July 2011 to January 2012. For the river Balu, samples were taken from 4 locations namely- Termuni, Itakhola, Nagarpara and Demra Bridge to cover the maximum pollution hazards due to industrial activities, municipal sewage sludge and urban runoff including agricultural activities. All samplings were performed through water sampler using country boat. After collection, the bottles containing samples were sealed immediately to avoid exposure to air. The samples were taken from the midstream. Three (3) samples were collected in same location within 0-50 cm depth. To provide necessary information for each sample such as data collection, location, depth etc. were noted and each sample collected in a plastic bottle was labeled separately with a unique identification number. After collection, all the water samples were carried to BINA, Mymensingh for chemical analysis. Samples bringing to the laboratory in bottles were kept in a clean, cool and dry place. For collection and preservation of samples, care was taken to obtain a sample that was truly representative of existing conditions and to handle it in such a way that it did not deteriorate or become uncontaminated before it reached the laboratory. All materials coming into contact with the sample namely glass or plastic and a sample

container was thoroughly washed and rinsed with distilled water. The samples were stored at 4°C temperature in a refrigerator. From the collected water samples, the following chemical properties such as; pH (Singh and Parwana, 1999), EC (Ghosh *et al.*, 1983), NO₃ -N and NH ₄-N (Bremner, 1965), PO4 (Jackson, 1958), Ca (Page *et al.*, 1982) and Mg (Clesceri *et al.*, 1989) were analyzed in the Soil Science Laboratory, BINA. The obtained data were subject subjected to statistical analysis using MSTAT-C programme and significant spatial and temporal variations were assessed using analysis of variance (ANOVA) at 5% level.

Results and Discussion

pH (H⁺ concentration)

The pH value of the Balu river water significantly varied due to different locations and seasons (Table 1 and Fig. 1). During the wet season (i.e., July to September 2011) on average, the pH value of the Balu river water ranged from 7.24 to 7.51, whereas, it varied from 7.46 to 8.04 during dry season (November 2011 to January 2012). Among the different locations, the maximum pH value was found at Nagarpara (7.74). The minimum pH value was noted from the water sample at Demra as 7.43. From previous study, Sultana (2005) found the average value of pH for river of Dhaka metropolitan city was 6.61. Tareq (2010) found the average value of pH for river of Dhaka metropolitan city was 7.40. Considering both location and season the maximum pH value was found at Itakhola (8.04) during dry season and the minimum value was noted at Termuni (7.24) during wet season. These results might be due to the presence of ions such as Ca, Mg and Na in water (Rao et al., 1982). So, the measured pH of all samples under the investigation area was not problematic for long-term drinking and irrigation. The pH of Balu river water samples ranged from 7.24 to 8.04 indicating alkaline. Mokaddes (2008) found the average pH value of river water was 7.03. The existing value of pH showed an increasing trend from the previous research results (Sultana, 2005; Mokaddes, 2008; Tareq, 2010) because of addition of basic materials in water.

Table 1. H⁺ - ion concentration (pH) of Balu river water (0-50 cm) at different locations and
seasons during the year, 2011-'12

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	7.24f	7.36c-f	7.51cd	7.39c-f	7.39
Dry season	7.88ab	8.04a	7.96a	7.46с-е	7.76
Mean (Location)	7.56	7.72	7.74	7.43	

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.

⊞ Wet season ■ Dry season



Location

Fig. 1. Average pH in Balu river water varied at different locations and seasons during 2011-'12

Electrical Conductivity (EC)

The EC value of the Balu river water significantly varied due to different locations and seasons (Table 2 and Fig. 2). During the wet season (i.e., July to September 2011) on average, EC value of the Balu river water ranged from 1.05 to 1.33 mScm⁻¹, whereas, it varied from 1.10 to 1.54 mScm⁻¹ during dry season (November 2011 to January 2012). Among the different locations, the maximum EC value of 1.44 mScm⁻¹ was found at Termuni area. The minimum EC value of as 1.08 mScm⁻¹ was noted from the water sample at Demra location. Rahman and Rahman (2007) observed from a study on Sherpur upazila under Bogra district that the EC values of water samples varied from 0.36 to 0.67 mScm⁻¹ showing medium salinity hazards class and could be used for moderately salt tolerant plants with moderate level of permeability and leaching. Sumi (2010) from Gazipur districts observed that the obtained EC values of samples were within the range of 0.01 to 2.10 mScm⁻¹. The high salinity content caused high EC in this contaminated sample, so it should be controlled or minimized immediately. Considering both locations and seasons the maximum EC value was found at Termuni area (1.54 mScm^{-1}) during dry season and the minimum value was noted at Demra ghat (1.05 mScm⁻¹) during wet season. Higher EC value reflected the higher amount of salt concentration which affected irrigation water quality related to salinity hazard (Agarwal et al., 1982). From our study, the EC value of Balu River was 1.14 and 1.30 mScm⁻¹ during wet and dry season respectively, i.e., comparetively, the EC value was higher in dry season than in wet season.

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	1.33c	1.17e	1.14f	1.05h	1.14
Dry season	1.54a	1.40b	1.30d	1.10g	1.30
Mean (Location)	1.44	1.28	1.22	1.08	

 Table 2. Electrical Conductivity (EC mScm⁻¹) of Balu river water (0-50 cm) at different locations and seasons during the year, 2011-'12

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

⊞ Wet season B Dry season

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.

1.6 1.4 1.2 1 0.8 0.6 0.4 0.2 0 Termuni Itakhola Nagarpara Demra

Location

Fig. 2. Average EC in Balu river water at different locations and seasons during 2011-'12

Nitrate Nitrogen (NO₃ - N)

The NO₃ – N concentration of the Balu river water significantly varied due to different locations and seasons (Table 3 and Fig. 3). During the wet season (i.e., July to September 2011) on average, the NO₃ – N value of the Balu river water ranged from 1.02 to 1.63 mgL⁻¹, whereas, it varied from 1.26 to 3.95 mgL⁻¹ during dry season (November 2011 to January 2012). On average the maximum NO₃ – N was recorded as 2.12 mgL⁻¹ in dry season. Among the different locations, the highest NO₃ – N value was found at Termuni area (2.79 mgL⁻¹).

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	1.63d	1.23ef	1.19fg	1.02h	1.20
Dry season	3.95a	2.31b	1.74c	1.26e	2.12
Mean (Location)	2.79	1.78	1.47	1.14	

Table 3. NO₃–N (mgL⁻¹) concentration in Balu river water (0-50 cm) at different locations and seasons during the year, 2011-'12

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.

⊞ Wet season
 ■ Dry season



Location

Fig. 3. Average NO₃ - N in Balu river water at different locations and seasons during 2011-'12

The minimum NO₃-N value was noticed from the water sample at Demra (1.14 mgL⁻¹) area. Ahiarakwem *et al.* (2011) found the average value of NO₃-N in Southeastern Nigeria was 0.22 mgL⁻¹. Considering both location and season the highest NO₃-N value was noted at Termuni area during dry season (3.95 mgL⁻¹) and the minimum value was found at Demra ghat during wet season (1.02 mgL⁻¹). According to Shameem (2006), the highest NO₃-N value was 0.76 mgL⁻¹ at Narikhal whereas at Noripur and Demra ghat the NO₃-N concentrations were 0.40 mgL⁻¹ and 0.30 mgL⁻¹, respectively in the wet season.

Ammonium Nitrogen NH₄ - N (mgL⁻¹)

The NH_4 -N concentration of the Balu river water significantly varied due to different locations and seasons (Table 4 and Fig. 4). During the wet season (i.e., July to September 2011) on average, the concentration of NH_4 -N of the Balu river water ranged

from 1.77 to 2.97 mgL⁻¹, whereas, it varied from 2.19 to 5.29 mgL⁻¹ during dry season (November 2011 to January 2012). Among the different locations, the highest NH_4^+ -N concentration was noted as 4.13 mgL⁻¹ in Termuni area. The minimum NH_4 -N concentration was noticed as 1.99 mgL⁻¹ from the water sample at Nagarpara location. Considering both location and season the maximum NH_4 -N concentration was recorded as 5.29 mgL⁻¹ at Termuni area during dry season whereas; the minimum value was noted as 1.78 mgL⁻¹ at Nagarpara during wet season. According to Shameem (2006), during dry season, the values of NH_4 -N ranged from 0.1 to 0.34 mgL⁻¹ in the upstream sites of Balu river and its downstream sites were between 2.18 to 3.95 mgL⁻¹. Narikhal showed very high concentration of NH_4 -N, 11.09 mgL⁻¹.

Table 4. NH4-N (mgL⁻¹) concentration in Balu river water (0-50 cm) at different location and
season during the year, 2011-'12

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	2.973cd	2.528de	1.777e	2.040de	2.340
Dry season	5.290a	5.153a	2.193de	3.633bc	3.037
Mean (Location)	4.132	3.841	1.985	2.837	

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.

⊞ Wet season
 B Dry season

Location

Fig. 4. Average NH₄-N in Balu river water at different locations and seasons during 2011-'12

Phosphate concentration (mgL⁻¹)

The phosphate concentration of the Balu river water significantly varied due to different locations and seasons (Table 5 and Fig. 5). During the wet season (i.e., July to September 2011) the mean phosphate value of the Balu river water ranged from 3.193 to 12.827 mgL⁻¹, whereas, it varied from 9.41 to 25.447 mgL⁻¹ during dry season (November 2011 to January 2012). Among the different locations, the maximum phosphate concentration was found at Termuni area (19.137 mgL⁻¹). The minimum phosphate value was noted from the water sample at Nagarpara (6.302 mgL⁻¹). Considering the variation both locations and seasons the highest phosphate concentration was found as 25.447 mgL⁻¹ at Termuni during dry season and the minimum value was noted at Nagarpara (3.193 mgL⁻¹) during wet season. According to Shameem (2006), in the dry season, Norikhal had a phosphate concentration of 1.07 mgL⁻¹ which is almost 2.5 times higher than that observed in the wet season.

Table 5. PO₄ (mgL⁻¹) concentration in Balu river water (0-50 cm) varied at different location and season during the year, 2011-'12

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	12.827cd	9.757e	3.193h	4.693g	7.485B
Dry season	25.447a	20.493b	9.410ef	12.633cd	17.293A
Mean (Location)	19.137A	15.125B	6.302D	8.663C	

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.



Location

Fig. 5. Average PO₄ concentration in Balu river water varied at different locations and seasons during 2011-'12

Calcium concentration (mgL⁻¹)

The calcium concentration of the Balu river water significantly varied due to different locations and seasons (Table 6 and Fig. 6). During the wet season (i.e., July-Sept' 2011) on average calcium concentration of the Balu river water ranged from 23.20 to 45.50 mgL⁻¹, whereas, it varied from 39.22 to 61.08 mgL⁻¹ during dry season (Nov'11 to Jan' 12). Among the different locations, the maximum calcium concentration was found as 53.29 mgL⁻¹ at Termuni area. The minimum calcium concentration was noted as 31.21 mgL⁻¹ from the water sample at Nagarpara area. Considering both locations and seasons the maximum calcium concentration was noted as 31.21 mgL⁻¹ and the minimum value was noted at Nagarpara during wet season (23.20 mgL⁻¹). More or less similar results were found from the research results of Kotlhao *et al.* (2005), who conducted an experiment with secondary treated sewage water in Gaborone, Bostwana.

 Table 6. Ca concentration (mgL⁻¹) in Balu river water (0-50 cm) at different location and season during the year, 2011-'12

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	45.50cd	38.73ef	23.20h	25.75g	33.57
Dry season	61.08a	55.50b	39.22e	42.63d	50.54
Mean (Location)	53.29	47.11	31.21	34.19	

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.



Location

Fig. 6. Average Ca concentration in Balu river water at different locations and seasons during 2011-'12

From their result, it was observed that the Ca⁺⁺ concentration of sewage water which was 3.7 and 5.5 μ g L⁻¹ used for irrigation during monsoon and winter, respectively. Sumi (2010) also reported that Ca concentration of water samples collected from Gazipur districts were 20.44 μ gmL⁻¹.

Magnesium concentration (mgL⁻¹)

The magnesium (Mg) concentration of the Balu river water significantly varied due to different locations and seasons (Table 7 and Fig. 7). During the wet season (i.e., July to September 2011) on average, the Mg concentration of the Balu river water ranged from 14.25 to 29.72 mgL⁻¹, whereas, it varied from 35.69 to 72.57 mgL⁻¹ during dry season (November 2011 to January 2012). Among the different locations, the maximum magnesium concentration was as 51.15 mgL⁻¹ found at Termuni area. The minimum magnesium concentration was noticed from the water sample at Nagarpara area (26.05 mgL⁻¹). Considering both locations and seasons the maximum magnesium concentration sand seasons the maximum magnesium concentration was noticed at Demra ghat area (14.26 mgL⁻¹) during wet season.

Table 6. Mg concentration (mgL⁻¹) in Balu river water (0-50 cm) at different location and season during the year, 2011-'12

Location/season	Termuni	Itakhola	Nagarpara	Demra	Mean (season)
Wet season	29.72e	23.85f	16.41g	14.26h	20.64
Dry season	72.57a	64.61b	35.69d	52.25c	58.46
Mean (Location)	51.15	44.23	26.05	33.25	

Note: i. Mean followed by same letter(s) is not significantly different at 5% level of probability. Capital letters were used for the mean variation for locations and seasons; small letters were used for the interaction mean of both i.e. Season × Location.

ii. Wet season indicates the average value of three months: July to September 2011 and Dry season indicates the average value of three months: November to January 2011-'12.



🖽 Wet season 🛛 📾 Dry season

Location

Fig. 7. Average Mg in Balu river water at different locations and seasons during 2011-'12

From a study with secondary treated sewage water in Gaborone, Bostwana during the year of 2005 Kotlhao *et al.* found that the Mg^{++} concentration of the irrigation water (sewage water) was 0.003 and 0.005 mgL⁻¹ during monsoon and winter, respectively. During 2011, Ramesh *et al.* (2011) conducted an experiment on the physiochemical properties of river Ram Ganga at Bareilly. From their study they conclude that due to difference of two locations (station I and station II) the maximum difference of Mg^{++} concentration was occurred (32.7%).

Sl. No.	Parameter	Drinking Standard	Irrigation Standard
1	pН	6.5 - 8.5	6.5 - 8.5
2	EC	$0.6 - 1.0 \text{ mScm}^{-1}$	1.2 mScm^{-1}
3	Nitrate	10 mgL ⁻¹	10 ppm as N_2
4	Calcium	75 mgL^{-1}	-
5	Magnesium	$30-35 \text{ mgL}^{-1}$	-
6	Phosphate	6 mgL^{-1}	10 mgL^{-1}

Table 8. Standard for drinking and irrigation water (DoE, 1997; GOB, 1997)

Conclusion

From the study, it may be concluded that the Balu river water contains acceptable value/amount of pH, $NO_3 - N$, $NH_4 - N$ and Ca whereas, the EC, PO_4 and Mg exceeded the recommended limit for drinking and irrigation water. Therefore, it is hazardous for human health, crops and aquaculture. From the results of the experiment it is clearly evident that the concentrations of studied pollutants were higher during dry season particularly in the month of November to January, when the rainfall was comparatively low or absent and water current is not sufficient to remove this pollutants. But during the wet season the values were generally low and fall within various standard levels. The excess toxic metal load of river water could be attributed to the discharge of industrial effluents and municipal wastes, geology of river bed and catchment area. Adoption of adequate measures to remove the chemical load from the industrial waste water and renovation of sewage treatment plants are suggested to avoid further deterioration of the river water quality. Routine research work with wide public awareness, government participation and government regulations can save the water of Balu river and thus safe and sound water environment can be made for future.

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EVALUATION OF FUNGICIDES ON THE GROWTH OF RHIZOCTONIA SOLANI, RHIZOCTONIA ORYZAE AND RHIZOCTONIA ORYZAE- SATIVAE

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Abstract

The sensitivity of five fungicides, Tilt 250 EC, Bavistin 50 WP, Folicur 250 EW, Contaf 5 EC and Forastin 50 WP were evaluated against the mycelial growth and production of sclerotia of *Rhizoctonia solani*, *Rhizoctonia oryzae* and *Rhizoctonia oryzae-sativae* on PDA plates. The concentrations of each fungicide were 0, 10, 25, 50, 100 ppm. The tested fungicides significantly inhibited the mycelial growth of the pathogens. The fungicide Bavistin and Folicur were the most effective against *R. solani* and *R. oryzae*, respectively for inhibiting the mycelial growth. The pathogen *R. oryzae-sativae* was very sensitive to all the fungicides tested even at 10 ppm concentration.

Keywords : Fungicides, Rhizoctonia spp., Sheath blight complex

Introduction

Rice (Oryzae sativa L.) is the staple food for about 130 million people of Bangladesh. The total area and production of rice in Bangladesh is about 11.359 million hectares and 31.975 million metric tons, respectively (BBS, 2011). Among the factors of low yield, diseases are considered to be the most important one. In Bangladesh 31 rice diseases have been identified of which ten are considered as major including sheath blight (Miah and Shahjahan, 1987; Anon., 1995). Sheath blight prevails in almost all rice growing areas and in all seasons of Bangladesh and is one of the major constraints to rice production in the country (Miah et al., 1985). The Rhizoctonia sheath disease complex, caused by Rhizoctonia solani, Rhizoctonia oryzae and Rhizoctonia oryzae-sativae is responsible for significant yield losses in rice in Asia (Ou, 1985). R. solani, a ubiquitous pathogen, incites rice sheath blight, which is one of the two most serious fungal diseases in all rice growing countries while R. oryzae and R. oryzae-sativae are not considered as important like R. solani. R. oryzae is reported on rice in regions where sheath blight frequently occurs and both pathogen produce lesions on leaf sheath very similar to those of leaf blight. In Bangladesh until 1988 there was no report of R. oryzae and R. oryzaesativae except R. solani, which is the most common pathogen of rice sheath blight disease (Shahjahan et al., 1988). The disease may cause substantial yield losses in the severely

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infected fields. The extent of yield loss depends mainly on the severity of the disease in the fields (Kozaka, 1970; Hori and Anraku, 1971). The yield loss was recorded about 25% when severe infection occurred in the leaf sheath and leaf blade (Kozaka, 1970). To manage sheath blight of rice, several techniques such as modification of agronomic practice, use of resistant varieties, application of fungicides and to some extent biological control have been practiced. No resistant cultivar is available for practical use and the intensive rice cultivation practices offer a favorable condition for disease development. Under these circumstances the control of disease is difficult. Therefore, the information available on the sheath blight disease complex is not enough to develop effective management strategies to combat the disease. The present study was undertaken to assess the sensitivity of the pathogens to some important fungicides.

Materials and Methods

Pure culture of *Rhizoctonia solani*, *Rhizoctonia oryzae* and *Rhizoctonia oryzae*sativae were collected from Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA). The pathogens were purified by hyphal tip method. The pathogens were set to grow in the petridishes containing 2% water agar medium. These petridishes were observed under sterio-microscope and tip of the hyphae along with a small block of medium cut by a thin and sharp sterilized needle. The blocks were immediately transferred in to PDA slants and incubated at $28 \pm 2^{\circ}$ C for seven days. *R. solani*, *R. oryzae* and *R. oryzae-sativae* were characterized based on the appearance or colour of the colony and the formation pattern, size and shape of the sclerotia or sclerotial crusts. The purified culture thus obtained were kept in a refrigerator (5-6°C) for subsequent uses.

Evaluation of fungicides on the growth of R. solani, R. oryzae and R. oryza sativae

The effectiveness of five fungicides namely Tilt 250 EC (Propiconazole), Bavistin 50 WP (Carbendazim), Folicure 250 EW (Tebuconazole), Contaf 5 EC (Hexaconazole) and Forastin 50 WP (Carbendazim), collected from different agro-chemical companies of Bangladesh were evaluated against *R. solani, R. oryzae* and *R. oryzae-sativae*. The experiment was conducted at the laboratory of Plant Pathology Division, BINA, Mymensingh during February to May, 2007.

The fungicides were used to evaluate the sensitivity among the *Rhizoctonia* spp. in terms of responses to chemicals. The concentrations of each fungicide were 0, 10, 25, 50, 100 ppm. Requisite amount of individual fungicide was mixed with PDA before sterilization. Autoclaved PDA was poured in to petridishes (90 mm) at 20 ml per petridish. After solidification of the medium, the plates were inoculated with 5 mm mycelium blocks taken from the edge of three days old colony of each of the *R. solani*, *R. oryzae* and *R. oryzae-sativae* strain grown on PDA. In case of control treatments (0 ppm) water was used

instead of chemical. Each treatment was replicated five times. The plates were incubated at $28 \pm 2C^{\circ}$. Data on radial mycelial growth of the fungi were recorded at 24 hours interval.

The percent growth inhibitions of the pathogens were calculated by using the following formula (Bashar, 1990).

Percent growth inhibition (I) = $\frac{\text{Radial growth in control (C) - Radial growth in treatments (T)}}{\text{Radial growth in control (C)}} \times 100$

Where,

I, C and T denote percent growth inhibition, radial growth in control and radial growth in treatment, respectively.

Results and Discussion

The results of mycelial growth inhibition test indicated that all the test fungicides possessed inhibitory effect against the test fungi.

Effect of fungicides on mycelial growth of R. solani

Growth was affected by *R. solani* at different concentrations of Tilt 250 EC (Fig. 1). The per cent inhibition of mycelial growth of *R. solani* at 10, 25, 50 and 100 ppm concentrations of Tilt after 72 hours observations were 51, 69, 86 and 88, respectively. The highest per cent inhibition was recorded in 100 ppm and the lowest in 10 ppm concentration of Tilt.

Different concentrations of Bavistin inhibited the mycelial growth of *R. solani*. There was no growth of the fungus in PDA media. It was observed that Bavistin completely inhibited (100%) the mycelial growth of *R. solani* even at 10 ppm concentration (Fig. 1).

After 72 hours observation, the highest inhibition (100%) of mycelial growth was found at 100 ppm concentration and the lowest (50%) inhibition was recorded at 10 ppm concentration when Folicur amended with the media (Fig. 1).

It was found that Contaf completely inhibited (100%) the mycelial growth of *R*. *solani* at 50 ppm concentration after 72 hours observation (Fig. 1). But the highest inhibition (89%) was found at 100 ppm concentration and the lowest inhibition (63%) at 10 ppm concentration was recorded after 72 hours of growth in case of Forastin. Among the fungicides tested the Forastin was observed as less effective.



Fig. 1. Per cent growth inhibition of *Rhizoctonia solani* on PDA plates amended with various concentration of five fungicides

However, considering their efficacy, Bavistin was noted as the suitable fungicide followed by Tilt, Folicur, Contaf and Forastin against *R. solani*. Excellent performance of Bavistin in controlling the mycelial growth of *R. solani* exhibited at 10 ppm where growth of the fungus was totally inhibited. Tilt, Folicur, Contaf and Forastin also showed inhibitory effect on the growth of *R. solani*. Bavistin also has been reported very effective against *R. solani* (Behera *et al.*, 1982; Anon, 1999).

Effect of fungicides on mycelial growth of R. oryzae

The effects of different concentrations of five fungicides in inhibiting the mycelial growth of *R. oryzae* are presented in Fig. 2. After 72 hours observation, the highest inhibition (85%) was recorded at 100ppm and the lowest (78%) at 10 ppm of Tilt.

The fungicide Bavistin showed less effect in inhibiting the mycelial growth of *R*. *oryzae* at different concentrations (Fig. 2). Among the tested fungicides, Bavistin was found to be least effective in inhibiting the mycelial growth of *R*. *oryzae*, whereas Folicur completely inhibited (100%) the mycelial growth at 100 ppm concentration after 24 hours of incubation. The fungicide was also effective even at 10 ppm.

In case of Contaf the highest inhibition was observed at 72 hours grown. The lowest inhibition was recorded at 10 ppm concentration. The fungus *R. oryzae* could grow on medium containing 10, 25, 50, and 100 ppm concentrations of Forastin. After 72 hours observations, the highest inhibition was found at 100 ppm and the lowest inhibition (49.72%) at 10 ppm.



Fig. 2. Per cent growth inhibition of *Rhizoctonia oryzae* on PDA plates amended with various concentrations of five fungicides

On the basis of the efficacy, Folicur was noted as the suitable fungicides followed by Tilt 250EC, Bavistin, Contaf 5EC and Forastin against *Rhizoctonia oryzae*. Excellent performance of Folicur in controlling the mycelial growth was exhibited at 100 ppm where growth of fungus was totally inhibited. Tilt was better than Contaf in inhibiting the growth of *R. oryzae*. Contaf 5EC was better than Forastin in inhibiting the growth of *R. oryzae*. Bavistin was least effective in inhibiting the growth of *R. oryzae*. Anon (1999) reported that nine isolates of *R. oryzae* tested, exhibited the normal rate of growth at all Carbendazim concentration up to 100 ppm.

Effect of fungicides on mycelial growth of R. oryzae-sativae

There was no growth of fungal mycelia of *R. oryzae-sativae* in the study (Table 1). After 24 hours observation, it was found that all the fungicides completely inhibited the mycelial growth of *R. oryzae-sativae* even at 10 ppm concentration.
Tilt 250EC, Bavistin, Folicur, Contaf 5EC and Forastin significantly reduced the mycelial growth of *R. oryzae-sativae* even at 10ppm concentration. Anon (1999) reported differential growth of *R. oryzae-sativae* on agar medium amended with Bavistin and the growth was completely inhibited at 1ppm concentration. So, the present study is agreement with the study of Anon. (1999).

Treatment	Concentration	% growth inh	ibition at different i	ntervals (hrs.)
	(ppm)	24	48	72
Tilt 250EC	0	0.00	0.00	0.00
	10	100.00	100.00	100.00
Bavistin 50WP	0	0.00	0.00	0.00
	10	100.00	100.00	100.00
Folicur	0	0.00	0.00	0.00
	10	100.00	100.00	100.00
Contaf 5EC	0	0.00	0.00	0.00
	10	100.00	100.00	100.00
Forastin	0	0.00	0.00	0.00
	10	100.00	100.00	100.00

 Table 1. Per cent growth inhibition of *Rhizoctonia oryzae-sativae* at various concentrations of five fungicides amended with PDA media

Based on the findings of the present study, it might be concluded that Bavistin was effective in inhibiting the growth of *R. solani*. Folicur was effective in inhibiting the growth of *R. oryzae*. The fungus *R. oryzae-sativae* was very sensitive to the tested fungicides even at low concentration.

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STUDIES ON AGRO-CLIMATIC PARAMETERS OF GANGES RIVER FLOODPLAIN AND ITS LONG-TERM TREND ANALYSIS USING "MAKESENS" MODEL

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Abstract

An agro-climatic study was conducted at Magura district of Ganges River Floodplain area with 30 (1981-2010) years climatic data (relative humidity, temperature, rainfall, sunshine hour and wind speed) to evaluate long-term trend and to predict the climatic variability for the year 2020, 2025 and 2030. Maximum relative humidity increased at the rate of 0.14% year⁻¹ at third decade in the month of February. The predicted scenarios of maximum relative humidity will be maximum in January. Minimum relative humidity decreased at the rate of 0.152% year⁻¹ in the month of August. The predicted scenarios of minimum relative humidity will be minimum in February. Maximum temperature decreased at the rate of 0.02°C year⁻¹ and changed 0.51°C in the month of January from 1981 to 2010. Maximum temperature increased at the rate of 0.05°C year⁻¹ and changed 1.50°C in the month of August from 1981 to 2010. Maximum temperature was observed 36.25°C at third decade in April 2012. Maximum temperature will be 35.40, 35.59, 35.78°C in May in the year 2020, 2025 and 2030, respectively. Minimum temperature decreased at the rate of 0.036°C year⁻¹ and changed 1.04°C in the of month January from 1981 to 2010. Minimum temperature increased at the rate of 0.07°C year-1 and changed 1.91°C in the month of April from 1981 to 2010. Minimum temperature will be minimum in January for the prediction year 2020, 2025 and 2030. Rainfall decreased at the rate of 0.09 cm year⁻¹ in May and increased at the rate 0.02 cm year⁻¹ in July. Maximum rainfall will occur in July for the prediction year 2020, 2025 and 2030. Sunshine duration decreased at the rate of 0.071 hr year⁻¹ in October and increased at the rate of 0.077 hr year⁻¹ in June. Maximum sunshine duration will occur in April. Wind speed decreased significantly in every month over the years. It was observed that the maximum decreased rate of wind speed was 0.073 m⁻¹ sec⁻¹ year⁻¹ in July. Minimum wind speed will occur in November.

Keywords Agro-climatic parameters, Climate change, Trend, Prediction, Magura

Introduction

Bangladesh is the most vulnerable to global climate change in the world which has a complex influence on economic and social aspects, mainly for its geographic location and physiographic condition. According to current scientific understanding, the state of well

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being and survival of the people in Bangladesh will be under serious threat from climate change over the coming decades. Hazards like floods, droughts, cyclones and others, which are aggravated by climate change and its variability being experienced more frequently in Bangladesh than ever before. Uncertainty of rainfall and uneven temporal and spatial distribution in one hand, creating flooding and on the other hand longer dry spells evoking droughty conditions (Lai, *et al.*, 1998).

Climatic change situation may influence land and crop productivity and resources use pattern. Therefore, water management for enhancing crop production under climate change will be required for better utilization of land and water resources of the country. Water availability of the country makes water management a critical issue (Ghani, 2005), especially for about two to two and half months (Mid February to April).

Since agricultural experimentation is expensive and time consuming, the climatological information may help a great deal to consider extrapolation of research results to a particular region- having 'climatic analogues'. Climatic analogues are "areas sufficiently alike with respect to certain major weather characteristics that techniques and materials developed for one area have applications chance of success when transferred to its climatic counterpart" (Chang, 1981).

The study has been undertaken in Magura district with a view to generate information for planners and professional working people in the field of agro-climatic parameter development. The main objectives of this study are (i) to evaluate long-term trend of agro-climatic parameter and (ii) to predict the agro-climatic parameter for the future.

Materials and Methods

Experimental Site

Magura is a district in South-Western Bangladesh which includes the eastern part of the High Ganges River Floodplain (AEZ-11) and the western part of the Low Ganges River Floodplain (AEZ-12) in Agro-Ecological Zone (AEZ) of Bangladesh (BBS, 2011). It is a part of the khulna division. It is bounded by Rajbari district to the north, Jessore and Narail districts to the south, Faridpur district to the east and Jhenaidah district to the west. This region is situated between 23°16′ to 23°40′ North and 89°15′ to 89°37′ East. It has four upazilas. They are Magura sadar upazila, Mohammadpur upazila, Shalikha upazila and Sreepur upazila. The study area covers about 1048 Km². The map of study area is shown in Fig. 1.

Data collection

Daily agro-climatic parameters like rainfall, maximum and minimum temperatures, maximum and minimum relative humidity, wind speed and sunshine duration of Faridpur and Jessore for a period of 30 years (1981-2010) were collected from the Bangladesh Meteorological Department. Daily agro-climatic parameters of Magura were prepared by the average value of Faridpur and Jessore data. Daily agro-climatic parameters were arranged month wise and then reduced to mean.



Fig. 1. Location map of the study area

Studies on agro-climatic parameters trend

The "MAKESENS" software was used to detect and estimate trends. This software is based on the non-parametric Mann-Kendall test for trends and the non-parametric Sen's method for the magnitude of the trend (Salmi *et al.*, 2002). The advantage of the non-parametric method is that it is applicable for both monotonic and non-monotonic trends, and it can operate with missing data.

The software utilizes *S* statistics and *Z* statistics given in Gilbert (1987). For data series with <10, the *S* test is used and for ≥ 10 , the *Z* test is used. When the number of data points <10, the test statistic *S* is computed as:

$$s = \sum_{k=1}^{n-1} \sum_{j=1}^{n} \operatorname{sgn}(x_j - x_k)$$
(1)

Here, x_j and x_k are the annual values in years j and k, respectively, $(j \ k)$ and

$$sgn(x_{j} - x_{k}) = \begin{bmatrix} 1 \text{ if } x_{j} - x_{k} > 0\\ 0 \text{ if } x_{j} - x_{k} = 0\\ -1 \text{ if } x_{j} - x_{k} < 0 \end{bmatrix}$$
(2)

A very high positive value of S is an indicator of an increasing trend, and a very low negative value indicates a decreasing trend. However, it is necessary to compute the probability associated with S and the sample size, n, to statistically quantify the significance of the trend. The two-tailed test is used for four different significance levels (α): 0.1, 0.05, 0.01 and 0.001. Details regarding the MAKESENS model can be found in Salmi *et al.* (2002). The agro-climatic parameter conditions are predicted as:

Agro-climatic parameter =
$$B + Q \times (Simulation year-Base year)$$

where,

B is the intercept and Q is the slope of the trend line, which were found from model output. The simulation years were selected as 2020, 2025, and 2030. The base year was 1981 (the first year of the data set).

Results

Agro-climatic parameters changed due to changes of climate and rate of changes and increase or decrease of changes of agro-climatic parameters differed at different times in Magura district of Ganges River Floodplain area. Maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity, rainfall, sunshine duration and wind speed monthly increase and decrease and long time prediction showed in Table 1 to Table 7.

Relative humidity

It was observed that monthly maximum relative humidity changed significantly in the month of February, March, June and July. It was increased at the rate of 0.109% year⁻¹ of month February and decreasing at the rate of 0.003% year⁻¹ of month September in Magura (Table 1). At decade wise, maximum relative humidity increased at the rate of 0.14% year⁻¹ at third decade of month February and decreasing at the rate of 0.006% year⁻¹ at first decade of month June. The change of monthly maximum relative humidity from 1981 to 2010 was observed 3.17% in the month February. The predicted scenarios of maximum relative humidity will be 98.08% in February in the year 2030 for Magura compared to the present maximum relative humidity 96.11% in the month of February.

It was also observed that monthly minimum relative humidity increased at the rate of 0.14% year⁻¹ of month April and decreasing at the rate of 0.252% year⁻¹ of month May in Magura (Table 2). At decade wise, minimum relative humidity increased at the rate of 0.484% year⁻¹ at first decade of month April and decreasing at the rate of 0.25% year⁻¹ at third decade of month June. The rate of changes of monthly minimum relative humidity decries significantly in the month of May to August. The predicted scenarios of minimum relative humidity will be 70.41% in July in the year 2030 while in 2012 it was found 72.86%.

Temperature

It was observed that monthly maximum temperature changed significantly in the month of May, June, July, August, September and November. It was found that monthly maximum temperature increased at the rate of 0.052 °C year⁻¹ of month August and decreased at the rate of 0.018% year⁻¹ of month January in Magura (Table 3). At decade wise, maximum temperature increased at the rate of 0.073 °C year⁻¹ and decreased at the rate of 0.05° C year⁻¹ at third decade of month February and January, respectively. The change of monthly maximum temperature from 1981 to 2010 was observed maximum (1.50°C) in the month August. Maximum temperature in 2012 was observed 36.25 °C at third decade of month April (Table 3). Maximum temperature will be predicted 35.40, 35.59, 35.78 °C of month May in the year 2020, 2025 and 2030, respectively.

It was observed that monthly maximum temperature increased at the rate of 0.066 $^{\circ}$ C year⁻¹ of month April and decreased at the rate of 0.036% year⁻¹ of month January in Magura (Table 4). At decade wise, minimum temperature increased at the rate of 0.096 $^{\circ}$ C year⁻¹ at third decade and decreased at the rate of 0.037 $^{\circ}$ C year⁻¹ at first decade of month February and January, respectively. The change of monthly minimum temperature from 1981 to 2010 was observed (1.40 $^{\circ}$ C) in the month November.

Rainfall

Long term (1981-2010) average annual rainfall are about 1801 mm in Magura while the national average is 2030 mm (BBS, 2011). It is observed that the distribution of rainfall over the months of the year is quite uneven. Approximately 78% rainfall in Magura occurs during the months from May to September which is noted as monsoon season. The period between May to September is surplus period. It was observed that monthly rainfall decreased at the rate of 0.09 cm year⁻¹ in May and increased at the rate 0.02 cm year⁻¹ in July (Table 5). At decade wise, rainfall decreased at the rate of 2.17 cm year⁻¹ at third decade in April and increased at the rate 3.31 cm year⁻¹ at first decade in July. Maximum and minimum monthly rainfall in 2012 was observed 404.86 cm and 5.00 cm in the month July and January, respectively. Maximum rainfall will occur in July for Magura from prediction year 2020, 2025 and 2030.

Sunshine duration

It was observed that monthly sunshine hour increased at the rate 0.05 hr year⁻¹ in July and decreased at the rate of 0.06 hr year⁻¹ in the month of October (Table 6). At decade wise, sunshine duration decreased at the rate of 0.068 hr year⁻¹ at second decade and increased at the rate of 0.074 hr year⁻¹ at first decade in the month of August. Maximum and minimum sunshine hour in 2012 was observed 8.29 hour and 4.24 hour in the month April and July, respectively. Maximum sunshine duration 8.45, 8.54 and 8.64 hour will predicted in April for Magura in the year year 2020, 2025 and 2030, respectively.

Wind speed

Wind speed decreased significantly in every month over the year. It was observed that monthly maximum wind speed decreased at the rate 0.073 m sec⁻¹ year⁻¹ in July (Table 7). At decade wise, maximum decreased rate was 0.092 m sec⁻¹year⁻¹ at first decade in May. Minimum wind speed 0.56, 0.21 m sec⁻¹ and almost zero will occur in November for Magura from prediction year 2020, 2025 and 2030, respectively.

Month	Ra	te of char	nge (% year	⁻¹)	Ch	ange from	1981 to 2	010		Present val	lue in 201	2
Wontin	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	0.068	0.073^{+}	0.070	0.069	1.97	2.11	2.04	2.00	96.63	96.91	96.44	96.44
February	0.100^{+}	0.077	0.135^{+}	0.109^{+}	2.90	2.24	3.93	3.17	96.53	95.75	96.29	96.11
March	0.079^{+}	0.042	0.080	0.057^{+}	2.28	1.21	2.31	1.66	94.95	93.76	94.48	94.35
April	0.111^{**}	0.090^{+}	0.000	0.052	3.21	2.61	0.00	1.52	95.06	94.69	93.25	94.14
May	-0.042	-0.015	0.023	-0.012	-1.21	-0.43	0.66	-0.35	93.40	93.55	94.38	93.68
June	-0.006	-0.040	-0.050^{**}	-0.034^{+}	-0.17	-1.16	-1.45	-0.99	94.73	94.91	95.33	95.01
July	-0.037*	-0.011	-0.070***	-0.046**	-1.09	-0.31	-2.04	-1.33	95.70	95.86	95.01	95.21
August	-0.037	0.006	-0.032^{+}	-0.020	-1.07	0.18	-0.92	-0.58	95.02	95.68	95.08	95.24
September	-0.035^{+}	-0.007	-0.021	-0.003	-1.00	-0.19	-0.60	-0.09	95.44	96.22	96.08	96.15
October	0.017	0.009	0.030	0.023	0.48	0.26	0.88	0.67	96.77	96.35	96.64	96.54
November	0.023	0.002	0.042	0.010	0.67	0.07	1.23	0.30	96.19	96.34	96.62	96.33
December	0.038	0.021	0.050	0.042	1.09	0.62	1.44	1.22	96.89	96.31	96.86	96.63

 Table 1. Rate of change (% year-1), change from 1981 to 2010 and prediction of maximum relative humidity (%) for different months and decades in Magura district

Prediction of maximum relative humidity (%) for different year

		2020				20	25			20	30	
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	97.17	97.49	97.00	96.99	97.51	97.85	97.35	97.33	97.85	98.22	97.70	97.68
February	97.33	96.37	97.38	96.99	97.83	96.76	98.05	97.53	98.33	97.14	98.73	98.08
March	95.58	94.09	95.11	94.80	95.98	94.30	95.51	95.09	96.37	94.51	95.91	95.38
April	95.94	95.41	93.25	94.56	96.49	95.86	93.25	94.82	97.05	96.31	93.25	95.08
May	93.07	93.44	94.56	93.58	92.86	93.36	94.67	93.52	92.65	93.29	94.78	93.46
June	94.69	94.59	94.93	94.74	94.66	94.39	94.68	94.57	94.63	94.19	94.43	94.40
July	95.40	95.78	94.45	94.84	95.21	95.72	94.10	94.62	95.03	95.67	93.74	94.39
August	94.72	95.73	94.83	95.08	94.54	95.77	94.67	94.99	94.35	95.80	94.51	94.89
September	95.17	96.16	95.91	96.12	94.99	96.13	95.81	96.10	94.82	96.10	95.70	96.09
October	96.90	96.42	96.88	96.73	96.98	96.47	97.03	96.84	97.07	96.51	97.18	96.96
November	96.37	96.36	96.96	96.42	96.49	96.38	97.17	96.47	96.60	96.39	97.38	96.52
December	97.19	96.49	97.26	96.97	97.38	96.59	97.51	97.18	97.56	96.70	97.76	97.39

Month	Ra	ate of chan	ge (% year	·-1)	Ch	ange from	1981 to 2	010		Present val	lue in 201	2
Wontin	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	-0.006	0.154	0.133	0.081	-0.17	4.46	3.85	2.34	50.07	50.87	47.82	49.35
February	0.050	-0.053	0.002	-0.023	1.45	-1.55	0.06	-0.68	43.60	40.29	38.03	41.93
March	0.019	0.006	0.273	0.102	0.54	0.16	7.91	2.96	35.96	38.12	47.61	42.21
April	0.484^*	0.230	-0.205	0.142	14.04	6.67	-5.95	4.11	54.85	52.55	51.28	50.32
May	-0.358*	-0.347**	-0.086	-0.252*	-10.39	-10.06	-2.49	-7.31	51.12	54.19	61.85	55.57
June	-0.104	-0.154	-0.250**	-0.125*	-3.01	-4.47	-7.25	-3.63	65.45	69.88	70.53	69.20
July	-0.100	-0.061	-0.221**	-0.136**	-2.90	-1.76	-6.40	-3.95	74.80	73.92	71.02	72.86
August	-0.250***	0.033	-0.234***	-0.152**	-7.25	0.97	-6.78	-4.41	69.73	74.45	69.86	71.41
September	-0.250**	-0.071	-0.064	-0.108^{+}	-7.25	-2.06	-1.86	-3.14	69.60	69.57	69.60	70.08
October	0.186	-0.017	0.287	0.072	5.40	-0.48	8.32	2.08	71.08	63.12	61.62	63.70
November	0.077	-0.041	0.035	0.027	2.23	-1.18	1.03	0.77	55.40	49.91	48.05	51.76
December	0.075	0.169	-0.009	0.043	2.18	4.90	-0.26	1.23	47.54	51.16	47.39	48.45

 Table 2.
 Rate of change (% year⁻¹), change from 1981 to 2010 and prediction of minimum relative humidity (%) for different months and decades in Magura district

Prediction of maximum relative humidity (%) for different year

		20	20			20	25			20	030	
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	50.03	52.10	48.88	50.00	50.00	52.87	49.55	50.40	49.97	53.64	50.21	50.81
February	44.00	39.87	38.05	41.75	44.25	39.60	38.06	41.63	44.50	39.33	38.07	41.51
March	36.11	38.17	49.80	43.03	36.21	38.19	51.16	43.54	36.30	38.22	52.52	44.05
April	58.73	54.39	49.64	51.45	61.15	55.54	48.61	52.16	63.57	56.69	47.59	52.87
May	48.25	51.42	61.16	53.55	46.46	49.68	60.73	52.29	44.67	47.95	60.30	51.03
June	64.62	68.65	68.53	68.20	64.10	67.88	67.28	67.58	63.58	67.11	66.03	66.95
July	74.00	73.44	69.25	71.77	73.50	73.13	68.15	71.09	73.00	72.83	67.05	70.41
August	67.73	74.72	67.99	70.19	66.48	74.88	66.82	69.43	65.23	75.05	65.66	68.67
September	67.60	69.00	69.08	69.21	66.35	68.65	68.76	68.67	65.10	68.29	68.44	68.13
October	72.57	62.98	63.91	64.27	73.50	62.90	65.35	64.63	74.43	62.82	66.78	64.98
November	56.01	49.58	48.33	51.97	56.40	49.38	48.51	52.10	56.78	49.18	48.68	52.24
December	48.14	52.51	47.31	48.79	48.51	53.36	47.27	49.01	48.89	54.20	47.22	49.22

Month	Ra	te of chan	ge (% yeai	⁻¹)	Ch	ange from	1981 to 2	010		Present val	lue in 201	2
Wonu	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	-0.004	0.018	-0.050^{+}	-0.018	-0.10	0.53	-1.45	-0.51	24.40	25.04	24.50	24.85
February	0.015	0.039	0.073^{*}	0.042	0.44	1.12	2.11	1.21	27.20	29.33	31.18	29.10
March	0.037	-0.035	0.021	0.018	1.09	-1.02	0.62	0.53	32.13	32.73	34.46	33.35
April	-0.073^{+}	-0.033	0.058^{*}	0.004	-2.11	-0.97	1.68	0.11	33.67	35.14	36.26	34.97
May	0.044	0.065^{**}	0.005	0.038^{+}	1.28	1.88	0.13	1.09	35.52	35.53	34.54	35.10
June	0.016	0.038	0.048^{**}	0.037^{*}	0.47	1.12	1.38	1.07	34.52	33.42	33.10	33.75
July	0.029^{+}	0.029	0.045^{***}	0.036^{***}	0.85	0.85	1.31	1.05	32.46	32.56	32.71	32.60
August	0.092^{***}	0.004	0.060^{***}	0.052^{***}	2.67	0.13	1.75	1.50	33.90	32.23	33.38	33.12
September	0.065^{***}	0.029	0.010	0.036**	1.89	0.84	0.28	1.05	33.45	33.18	32.68	32.95
October	0.001	0.023	-0.001	0.013	0.04	0.67	-0.02	0.37	32.52	32.74	31.46	32.29
November	0.035	0.037^{+}	0.033^{*}	0.036**	1.02	1.08	0.96	1.05	31.53	30.45	29.13	30.38
December	0.025	-0.001	0.009	0.012	0.72	-0.03	0.26	0.34	27.88	26.40	25.56	26.61

Table 3. Rate of change (°C year⁻¹), change from 1981 to 2010 and prediction of maximum temperature (°C) for different months and decades in Magura district

Prediction of maximum temperature (°C) for different year

		2020				20	25			20	30	
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	24.37	25.19	24.10	24.71	24.35	25.28	23.85	24.62	24.33	25.37	23.60	24.53
February	27.32	29.64	31.76	29.44	27.39	29.84	32.13	29.65	27.47	30.03	32.49	29.85
March	32.43	32.45	34.63	33.49	32.62	32.27	34.74	33.59	32.81	32.10	34.84	33.68
April	33.09	34.87	36.72	35.00	32.73	34.70	37.01	35.02	32.36	34.53	37.30	35.04
May	35.87	36.05	34.57	35.40	36.09	36.38	34.60	35.59	36.31	36.70	34.62	35.78
June	34.65	33.72	33.48	34.05	34.73	33.91	33.72	34.23	34.81	34.11	33.96	34.42
July	32.69	32.79	33.07	32.89	32.84	32.94	33.30	33.07	32.98	33.08	33.53	33.25
August	34.64	32.27	33.86	33.53	35.10	32.29	34.17	33.79	35.56	32.31	34.47	34.05
September	33.97	33.41	32.76	33.24	34.30	33.56	32.81	33.43	34.63	33.70	32.85	33.61
October	32.53	32.93	31.46	32.39	32.53	33.04	31.45	32.46	32.54	33.16	31.45	32.52
November	31.81	30.75	29.40	30.67	31.99	30.93	29.57	30.85	32.16	31.12	29.73	31.03
December	28.07	26.39	25.63	26.71	28.20	26.39	25.68	26.77	28.32	26.38	25.72	26.83

Month	Ra	ate of chan	ge (% yea	r ⁻¹)	Ch	ange from	1981 to 2	010		Present va	lue in 201	2
Wonu	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	-0.037	-0.020	-0.002	-0.036	-1.07	-0.58	-0.05	-1.04	11.09	11.37	12.29	11.16
February	-0.009	0.026	0.096^{*}	0.029^{+}	-0.27	0.77	2.78	0.84	13.35	15.69	18.01	15.26
March	0.050	-0.012	0.061	0.032	1.46	-0.34	1.78	0.94	18.33	19.57	22.30	20.08
April	0.053	0.096^{*}	0.075^{**}	0.066^{**}	1.53	2.78	2.19	1.91	23.78	25.56	24.88	24.57
May	0.063^{*}	0.057^{*}	0.018	0.037^{**}	1.82	1.66	0.53	1.06	25.12	25.88	25.88	25.45
June	-0.004	0.027^{+}	0.034^{*}	0.016	-0.11	0.78	1.00	0.46	25.84	26.33	26.60	26.12
July	0.033^{*}	0.023^{+}	0.017^{+}	0.026^{*}	0.96	0.65	0.49	0.76	26.48	26.52	26.41	26.49
August	0.035^{**}	0.008	0.017	0.017^{*}	1.02	0.22	0.48	0.48	26.57	26.33	26.44	26.38
September	0.021^{+}	0.016^{+}	0.019	0.018^{+}	0.62	0.47	0.54	0.52	26.26	26.00	25.84	25.99
October	0.011	0.031	0.032	0.016	0.32	0.89	0.92	0.46	25.16	24.59	22.63	23.88
November	0.048	0.044	0.042	0.040	1.40	1.28	1.23	1.15	21.33	19.28	17.16	19.30
December	0.024	0.043	0.018	0.010	0.69	1.26	0.51	0.28	14.97	14.05	12.72	13.33
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 Table 4.
 Rate of change (°C year⁻¹), change from 1981 to 2010 and prediction of minimum temperature (°C) for different months and decades in Magura district

Prediction of minimum temperature (°C) for different year

	2020					20	25			20	030	
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	10.79	11.21	12.28	10.87	10.61	11.11	12.27	10.69	10.42	11.01	12.26	10.52
February	13.28	15.90	18.78	15.49	13.23	16.03	19.26	15.64	13.18	16.17	19.74	15.78
March	18.73	19.47	22.79	20.34	18.99	19.42	23.10	20.50	19.24	19.36	23.41	20.66
April	24.21	26.32	25.48	25.10	24.47	26.80	25.86	25.43	24.74	27.28	26.24	25.76
May	25.62	26.33	26.02	25.74	25.93	26.62	26.11	25.93	26.25	26.91	26.20	26.11
June	25.81	26.54	26.87	26.25	25.80	26.68	27.04	26.33	25.78	26.81	27.22	26.41
July	26.74	26.70	26.54	26.69	26.90	26.81	26.63	26.82	27.07	26.93	26.71	26.95
August	26.85	26.39	26.58	26.51	27.03	26.43	26.66	26.60	27.21	26.47	26.74	26.68
September	26.43	26.13	25.99	26.13	26.54	26.21	26.08	26.22	26.65	26.29	26.18	26.31
October	25.25	24.83	22.88	24.01	25.31	24.99	23.04	24.09	25.36	25.14	23.20	24.17
November	21.71	19.63	17.50	19.62	21.95	19.85	17.71	19.81	22.20	20.07	17.92	20.01
December	15 16	14 39	12.86	13 41	15 28	14 61	12.95	13 46	15 40	14 83	13 04	13 51

Month	Ra	ate of char	1ge (% yea	r ⁻¹)	Ch	ange from	1981 to 2	010		Present val	ue in 201	2
WIOIIII	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.50	0.25	0.00	5.00
February	0.000	0.000	0.000	-0.002	0.00	0.00	0.00	-6.77	1.00	2.00	1.50	14.15
March	0.000	-0.100	-0.239	-0.032*	0.00	-2.90	-6.93	-32.28	2.00	0.45	12.14	22.40
April	-0.036	-0.857*	-2.167*	-0.078^{*}	-1.04	-24.86	-62.83	-101.50	19.41	3.93	15.58	33.25
May	-1.453^{+}	-1.368+	0.250	-0.086*	-42.14	-39.68	7.25	-104.06	23.21	47.54	78.50	136.53
June	1.457	-0.806	-0.813	0.001	42.24	-23.36	-23.56	-14.50	99.82	60.91	97.59	288.00
July	3.306^{*}	1.538^{+}	-1.700	0.022	95.86	44.62	-49.30	142.28	183.28	97.15	75.10	404.86
August	-2.000^{*}	1.750^{*}	-1.667^{+}	-0.063	-58.00	50.75	-48.33	-89.90	55.25	101.63	44.33	215.05
September	-0.318	0.056	0.500	-0.027	-9.23	1.61	14.50	-11.35	62.77	57.47	89.50	217.68
October	1.375	-0.900	0.231	0.015	39.88	-26.10	6.69	46.72	74.38	16.50	15.62	169.91
November	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	6.00	0.50	0.00	8.33
December	0.000	0.000	0.000	0.000	0.00	0.00	0.00	0.00	6.00	0.00	0.00	6.25
				Predictio	on of rain	fall (cm) f	or differe	nt year				
		2020				20	25			20	30	

Table 5. Rate of change (cm year⁻¹), change from 1981 to 2010 and prediction of rainfall (cm) for different months and decades in Magura district

SD FD TD Monthly FD SD TD Monthly FD SD TD Monthly 0.50 0.25 5.00 0.50 0.25 5.00 0.25 5.00 0.00 0.00 0.50 0.00 January February 1.00 2.00 1.50 12.28 1.00 2.00 1.50 11.12 1.00 2.00 1.50 9.95 10.23 7.93 7.84 March 2.00 -0.35 13.50 2.00 -0.85 9.03 2.00 -1.35 2.37 19.13 -2.93 -1.75 5.25 18.95 -7.21 -12.58 -12.25 18.77 -11.50 -23.42 -29.75 April 11.58 36.59 80.50 107.82 4.32 29.75 89.88 -2.95 22.91 83.00 71.94 May 81.75 111.47 54.47 91.09 284.00 118.75 50.44 87.03 281.50 126.03 46.41 82.97 279.00 June July 209.72 109.46 61.50 444.11 226.25 117.15 53.00 468.64 242.78 124.85 44.50 493.17 August 39.25 115.63 31.00 190.25 29.25 124.38 22.67 174.75 19.25 133.13 14.33 159.25 September 60.23 57.92 93.50 214.55 58.64 58.19 212.60 57.05 58.47 98.50 210.64 96.00 October 85.38 9.30 182.80 92.25 190.86 99.13 0.30 198.91 17.46 4.80 18.62 19.77 November 8.33 6.00 0.50 0.00 8.33 6.00 0.50 0.00 8.33 6.00 0.50 0.00 December 6.25 6.25 6.00 0.00 0.00 6.00 0.00 0.00 6.25 6.00 0.00 0.00

Month	Ra	ate of chan	ge (% year	r ⁻¹)	Ch	ange from	1981 to 2	010		Present val	lue in 201	2
Wonui	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	-0.022	-0.017	-0.036	-0.036	-0.63	-0.48	-1.05	-1.01	6.36	6.63	6.80	6.08
February	-0.001	-0.040	-0.017	-0.017	-0.03	-1.16	-0.49	0.18	7.54	6.10	7.76	7.80
March	0.014	-0.005	-0.023	-0.023	0.41	-0.14	-0.66	-0.16	8.17	7.99	7.08	7.56
April	-0.012	0.017	0.042^{*}	0.042^{*}	-0.35	0.49	1.21	0.57	7.79	8.31	8.66	8.29
May	0.008	-0.011	-0.023	-0.023	0.24	-0.31	-0.66	0.01	7.49	7.90	6.61	7.16
June	-0.046	0.077^{*}	0.039	0.039	-1.34	2.22	1.13	-0.23	5.18	8.59	4.49	4.53
July	0.008	-0.002	0.050	0.050	0.24	-0.06	1.44	0.53	3.46	4.37	5.41	4.24
August	0.074^{*}	-0.068^{+}	0.035	0.035	2.15	-1.97	1.03	0.31	5.98	3.40	5.16	4.85
September	0.057^{+}	-0.045	-0.033	-0.033	1.66	-1.31	-0.94	-0.03	5.37	3.58	4.40	4.60
October	-0.061	-0.071**	-0.060^{+}	-0.060^{+}	-1.76	-2.06	-1.74	-1.31	4.84	4.01	6.32	5.94
November	-0.010	-0.053^{+}	-0.034^{+}	-0.034^{+}	-0.29	-1.54	-0.99	-0.66	7.39	6.14	7.12	7.15
December	-0.035	-0.006	-0.011	-0.011	-1.02	-0.17	-0.33	-0.89	6.85	7.20	6.74	6.48

 Table 6.
 Rate of change (hr year⁻¹), change from 1981 to 2010 and prediction of sunshine duration (hr) for different months and decades in Magura district

Prediction of sunshine duration (hr) for different year

				curcuon or a	Sumstinite	uuranon (m) for u	mici chi y ca	L			
		20	20			20	25	-		20	030	
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	6.18	6.50	6.51	5.81	6.07	6.42	6.33	5.63	5.97	6.33	6.15	5.46
February	7.53	5.78	7.63	7.86	7.52	5.58	7.54	7.89	7.51	5.38	7.46	7.92
March	8.28	7.95	6.90	7.51	8.35	7.93	6.78	7.49	8.42	7.90	6.67	7.46
April	7.69	8.44	8.99	8.45	7.63	8.53	9.20	8.54	7.57	8.62	9.41	8.64
May	7.55	7.82	6.43	7.16	7.60	7.76	6.31	7.16	7.64	7.71	6.20	7.16
June	4.82	9.20	4.80	4.47	4.58	9.58	5.00	4.43	4.35	9.96	5.19	4.39
July	3.52	4.36	5.81	4.39	3.57	4.35	6.06	4.48	3.61	4.34	6.31	4.57
August	6.57	2.85	5.44	4.93	6.94	2.52	5.62	4.98	7.31	2.18	5.80	5.04
September	5.83	3.22	4.14	4.59	6.12	2.99	3.97	4.59	6.40	2.77	3.81	4.58
October	4.35	3.45	5.84	5.58	4.05	3.09	5.54	5.35	3.75	2.74	5.24	5.13
November	7.31	5.72	6.85	6.97	7.26	5.45	6.68	6.85	7.21	5.19	6.51	6.74
December	6.57	7.15	6.65	6.23	6.40	7.12	6.60	6.08	6.22	7.09	6.54	5.93

Month	Rate of change (% year ⁻¹)				Change from 1981 to 2010				Present value in 2012			
WOIIII	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly
January	-0.025	-0.052***	-0.013	-0.027*	-0.74	-1.51	-0.39	-0.80	2.67	2.25	3.14	2.67
February	-0.034^{+}	-0.019	-0.044^{+}	-0.036**	-0.98	-0.55	-1.27	-1.05	2.78	3.39	3.28	3.07
March	-0.028^{+}	-0.075***	-0.037	-0.038**	-0.81	-2.17	-1.08	-1.11	3.77	3.36	4.57	3.79
April	-0.064^{+}	-0.071	-0.052^{+}	-0.061 [*]	-1.85	-2.07	-1.49	-1.77	4.67	4.66	5.46	5.19
May	-0.092**	-0.036	-0.036	-0.047*	-2.67	-1.04	-1.05	-1.36	4.44	5.37	5.23	4.99
June	-0.064*	-0.062^{+}	-0.073**	-0.070***	-1.86	-1.80	-2.11	-2.03	4.26	4.56	4.08	4.37
July	-0.076***	-0.063*	-0.057**	-0.073***	-2.21	-1.82	-1.66	-2.13	3.81	4.35	4.26	4.20
August	-0.071***	-0.075***	-0.052***	-0.068***	-2.07	-2.16	-1.52	-1.96	3.92	3.94	4.03	3.86
September	-0.060***	-0.068^{*}	-0.052^{+}	-0.053**	-1.74	-1.96	-1.51	-1.54	3.70	3.20	3.21	3.50
October	-0.046	-0.063**	-0.035^{+}	-0.047**	-1.34	-1.82	-1.01	-1.37	2.63	1.89	2.14	2.18
November	-0.072	-0.058**	-0.075***	-0.069***	-2.10	-1.67	-2.19	-2.00	1.27	1.52	1.06	1.11
December	-0.088	-0.075***	-0.037*	-0.064***	-2.55	-2.18	-1.07	-1.85	0.65	1.23	2.05	1.44

 Table 7. Rate of change (m sec⁻¹ year⁻¹), change from 1981 to 2010 and prediction of wind speed (m sec⁻¹) for different months and decades in Magura district

Prediction of wind speed (m sec⁻¹) for different year

	2020					2025				2030			
-	FD	SD	TD	Monthly	FD	SD	TD	Monthly	FD	SD	TD	Monthly	
January	2.47	1.83	3.04	2.45	2.34	1.57	2.97	2.31	2.21	1.31	2.90	2.18	
February	2.51	3.24	2.93	2.78	2.34	3.14	2.71	2.60	2.18	3.05	2.49	2.42	
March	3.54	2.76	4.27	3.49	3.40	2.38	4.09	3.29	3.26	2.01	3.90	3.10	
April	4.16	4.09	5.04	4.71	3.84	3.74	4.79	4.40	3.52	3.38	4.53	4.10	
May	3.70	5.09	4.94	4.61	3.24	4.91	4.76	4.38	2.78	4.73	4.58	4.15	
June	3.75	4.07	3.50	3.80	3.43	3.76	3.14	3.45	3.11	3.45	2.78	3.10	
July	3.20	3.84	3.80	3.61	2.82	3.53	3.52	3.25	2.44	3.21	3.23	2.88	
August	3.35	3.34	3.61	3.32	2.99	2.97	3.35	2.98	2.63	2.59	3.09	2.64	
September	3.22	2.66	2.80	3.07	2.92	2.32	2.54	2.81	2.62	1.98	2.28	2.54	
October	2.27	1.39	1.86	1.80	2.03	1.07	1.69	1.57	1.80	0.76	1.51	1.33	
November	0.70	1.05	0.45	0.56	0.33	0.77	0.08	0.21	0.00	0.48	0.00	0.00	
December	0.00	0.63	1.75	0.93	0.00	0.25	1.57	0.61	0.00	0.00	1.38	0.29	

 $\frac{1.75}{1.5} = 0.00 = 0.00 = 1.75 = 0.95 = 0.00 = 0.25 = 1.57 = 0.61 = 0.00 = 0.00 = 1.38 = 0.29$ The symbol ***, **, * and + indicate significant at levels a were 0.001, 0.01, 0.05 and 0.10, respectively level of probability. FD, SD, TD, means first decade second decade and third decade, respectively.

Conclusion

Using the MEKESENS software, long-term projections were made by assuming the present trend of weather parameter. The agro-climatic information is most useful for the solution of practical agricultural problems. Without such analysis, the adoption of farming system or an agronomic technology to an area might be unsuccessful. Climatic information should be used for all aspects of crop production. From the study, it can be observed that the rate of change of relative humidity will be maximum in January and minimum in August. The temperature showed decreasing trend in January and increasing trend in May. Maximum rainfall occurs in July. Decreasing trends of sunshine were recorded for all regions. Sunshine duration decreased in October and increased in June. Wind speed decreased significantly in every month over the years.

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EFFECT OF TRANSPLANTING DATE ON THE GROWTH AND YIELD OF MODERN AROMATIC FINE RICE VARIETIES IN AMAN SEASON

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Abstract

The experiment was done at the Bangladesh Rice Research Institute (BARI) Farm, Gazipur, during the aman season. Modern aromatic rice BR5, BRRI dhan34, BRRI dhan37 and BRRI dhan38 were transplanted with 30 day old seedlings from 22 July and continued up to 7 October at an interval of 15 days in 2008 and 2009. The plant height, number of tiller and dry matter increased with the advancement of planting dates up to 7 September and thereafter decreased gradually. The growth duration and straw yield decreased gradually with the advancement of planting dates. The maximum number of panicles and grains panicle⁻¹ were found on 22 August planted crop that reflected to produce higher grains yield. Compared to the 22 August planting, grain yield of rice decreased by 14, 7, 8, 34 and 74 percent, respectively, when planted on 22 July, 7 August, 7 September, 22 September and 7 October. The BRRI dhan37 and BRRI dhan38 gave higher grain yield due to higher number of panicles and heavier individual grain weight irrespective of planting date. BRRI dhan34 contained the highest number of grains panicle⁻¹ and matured 3-5 days earlier than BRRI dhan37 and BRRI dhan38 but exhibited lower grain yield for lower number of panicles and lighter individual grain weight. Thus, the optimum planting time of modern aromatic high yield potential rice BRRI dhan37 and BRRI dhan38 is 17-21 August to obtain maximum grain yield during the aman season.

Key words: Date of transplanting planting, Modern aromatic rice, Aman season

Introduction

Rice is the most important cereal crop in the world and widely consumed grain, plays a unique role in combating global hunger (IRRI, 2004). Farmers cultivate the coarse rice to meet up their daily food requirements, but the aromatic fine grain rice is used in special occasion or in festivals. However, the choice of grain types depends on the consumers' demand and their income. Most of the urban people or rich people preferred long, slender aromatic fine grain rice and rural people who works in the field likes coarse grain. But the price of fine rices specially the aromatic one is 2–3 times higher than that of

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coarse rice (BRRI, 2003). Thus, there is a substantial demand of fine aromatic rice for national consumption and to export in the international markets. Recently aromatic rice had gained a wider acceptance in Asia and even in Middle East and Europe (Yoshihashi, 2005). At present, there is enough scope to increase production of fine rice by growing varieties having high yield potential and adjusting appropriate date of planting. The technological information for growing modern coarse rice is available but in case of aromatic fine rice it is still to be studied. Based on the above findings, the present experiment was undertaken to determine the optimum date of planting and to find out the modern aromatic fine rice varieties having high yield potential for growing in Aman season.

Materials and Methods

The experiment was conducted at the Bangladesh Rice Research Institute, Farm, Gazipur in Aman season. The modern aromatic rice BR5, BRRI dhan34, BRRI dhan37 and BRRI dhan38 were transplanted on 22 July (Julian date 203), 7 August (Julian date 218), 22August (Julian date 233), 7 September (Julian date 248), 22 September (Julian date 263) and 7 October (Julian date 278) with 30-day-old seedlings spaced at 20 cm \times 15 cm both in 2008 and 2009. The treatments were distributed in a split-plot design, placing planting date in the main plot and variety in the sub plot and replicated thrice. Fertilizers were applied @ 60-40-40-10-4 kg N, P₂O₅, K₂ O, S and Zn ha⁻¹, respectively during final land preparation except nitrogen. Nitrogen was top-dressed in three equal splits at 15 dates after transplanting (DAT), 30 DAT and before panicle emergence (BRRI, 2004). Five destructive sample hills were collected from each individual plot to measure the plant height and tiller number at different growth stages. The samples were oven dried for 72 hours in a constant temperature for dry matter determination. For grain and straw yields five square meter areas was harvested from the center of the plot and yield was adjusted to 14% moisture content and expressed in ton ha⁻¹. The straw was dried in the sun until complete drying and the weight was expressed in ton ha⁻¹. The degree of photoperiod sensitivity was calculated by using the following formula (Ghosh and Saran, 1982).

[Degree of photoperiod sensitivity = $\frac{X - Y}{X} \times 100$

Where, X = Flowering duration due to long day effect (Seeding on 22 July)

Y = Flowering duration due to short day effect (Seeding on 7 September)

Regression analysis was done to determine optimum planting time. Optimum planting date of the test varieties was determined by regressing the grain yield with the transplanting dates. Functional relationship between planting dates and rice yields was described by the following quadratic equation (Eqn.):

$$Y = a + bP + cP^2 \tag{1}$$

Where Y is rice yield (kg ha⁻¹), P is transplanting date (Julian date), a is intercept (estimated yield) initial planting time, b and c are coefficients, respectively. Differentiating Y with respect to planting date P of the Eqn. (1) gives planting date for maximum yield. The estimated planting date for highest yield would be at the point of

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

$$or, b + 2cP = 0$$

$$or\frac{dY}{dP} = 0 = b + 2cP$$

$$or, P = -\frac{b}{2c}$$
(2)

Results and Discussion

Plant height, tiller number and dry matter

The early planted on 22 July crop showed short plant, less number of tillers and low dry matter but these increased successively until 7-22 September transplanting and decreased thereafter irrespective of varieties (Table 1 & 2). Probably, the cloud in sky and bright sunshine hours in July and August impaired vegetative growth. As the weather became cloud-free in September the height and tillers increased faster resulting higher dry matter production. Similar findings were also reported by Salam et al. (2004). On the contrary, late planted on 7 October crop suffered due to cold spell at night, and the growth became slow, resulting shorter plant, less number of tillers and dry matter production. However, the plant height at maturity was reversed than that of vegetative growth stages and early planted crop showed taller plant and the height gradually decreased with the advancement of planting dates. The possible causes of tallness of early-planted crop at maturity might be due to the longer vegetative growth period that enhanced plant height. The vegetative period progressively decreased with the delay of planting and late planted crop became shorter. The results confirmed the findings of Salam et al. (2004). Among the test varieties the BRRI dhan34 was taller irrespective of growth stages and showed intermediate height (about 118 cm). It indicated that this variety is suitable for growing in low laying areas to avoid crop damage by flash flood in Aman season.

The BRRI dhan38 produced maximum number of tillers and dry matter followed by BRRI dhan37 while the less number of tillers and dry matter was found in BR5 and BRRI dhan34 irrespective of growth stages (Table 2). The relationship between dry matter production and planting date was quadratic irrespective of variety. Almost similar trend was observed by Angrish *et al.* (1997).

Planting		Plant	height	Nur	nber of ti	llers	Dry matter				
date		(0	cm)			(m-2)			(t ha-1)		
	30 DAT	45 DAT	60 DAT	Maturity	30 DAT	45 DAT	60 DAT	30 DAT	45 DAT	60 DAT	
22-July	51.85d	74.74d	88.24d	134.09a	183c	311e	357c	0.45d	2.40d	4.30c	
7-Aug.	55.43c	85.85b	97.66c	129.85b	229b	350c	378bc	0.68c	3.26bc	5.10b	
22-Aug.	68.43ab	89.61a	105.41a	123.91c	252a	372b	392ab	0.77b	3.56a	5.66a	
7-Sept.	69.10ab	90.46a	107.37a	112.86d	260a	395a	404a	0.89a	3.66a	5.84a	
22-Sept.	69.67a	86.26b	101.22b	102.04e	253a	381b	359c	0.87a	3.32b	5.23b	
7-Oct.	65.72b	78.83c	84.53d	83.19f	236b	324d	315d	0.75b	3.14c	4.47c	
Mean	63.78	84.29	97.40	114.32	236	355	367	0.73	3.22	5.1	
CV (%)	4.3	3.9	3.2	2.1	7.8	6.3	6.8	11.1	5.5	5.3	

 Table 1. Effect of planting date on plant height, number of tillers and dry matter of modern aromatic fine rice in aman season (average of 1999 and 2000)

Figures in a column followed by different letters differ significantly but with common letter do not differ significantly at 0.01% level of probability.

DAT = Days after transplanting

 Table 2. Plant height, number of tillers and dry matter of modern aromatic fine rice varieties in Aman season (average of 1999 and 2000)

Varieties		Plant	height		Nur	nber of ti	llers	Dry matter		
		(0	cm)			(m-2)		(t ha-1)		
	30 DAT	45 DAT	60 DAT	Maturity	30 DAT	45 DAT	60 DAT	30 DAT	45 DAT	60 DAT
BR5	63.0b	82.1c	95.9b	112.6c	221c	332c	342c	0.67c	3.1c	5.0c
BRRI dhan34	63.4a	86.5a	991a	117.8a	225bc	344bc	357bc	0.64c	3.0d	4.8c
BRRI dhan37	64.5a	84.6ab	97.3ab	112.6c	241b	356b	371b	0.78b	3.3b	5.2b
BRRI dhan38	64.2a	83.9b	96.4b	114.2b	254a	390a	398a	0.85a	3.5a	5.5a
Mean	63.8	84.39	97.40	114.3	235	355	367	0.73	3.2	5.1
CV (%)	4.1	2.9	3.7	2.5	8.8	8.2	7.8	14.2	6.7	5.5

Figures in a column followed by different letters differ significantly but with common letter do not differ significantly at 0.01% level of probability..

DAT = Days after transplanting

Yield components

The 22 August planted crop produced more number of panicles than those planted earlier or later than 22 August (Table. 3). With the advancement of planting dates beyond 22 August the number of panicles gradually decreased and late planted on 7 October showed the least number of panicles. Singh *et al.* (2002) also observed less number of panicles in late-planted crop. The test variety BRRI dhan38 produced the highest number of panicles while BRRI dhan34 exhibited least number of panicles (Table 3). Early planted crop produced the longest panicles and higher number of grains panicle⁻¹ and these decreased successively in late planted crop (Table 3). These findings are in conformity with the results obtained by Chopra *et al.* (2003). The longest panicle and more number of

grains panicle⁻¹ was found in BRRI dhan34 while, the shortest panicle was found in BR5 and the least number of grains panicle⁻¹ was observed in BRRI dhan38. Probably, these variations were due to the interaction of higher solar radiation associated with optimum temperature, which markedly influenced the increase panicle length and grains panicle⁻¹. The early-planted crop in July became taller and lodged at grain filling stage that caused decreased grains panicle⁻¹, resulted higher percentage of spikelet sterility. On the other hand, late planted crop in October, the grain development was badly affected by low temperature and resulted increase percentage of spikelet sterility. The results are in agreement with those of BRRI (2003). The higher percentage of sterility was found in taller variety BRRI dhan34 irrespective of planting dates. The quadratic relationship was found between planting date and spikelet sterility irrespective of varieties (Fig. 1). The lowest spikelet sterility was observed in the crops which planted in late August and in early September. The findings confirmed the results of Islam *et al.* (1999).

Rice planted in August exhibited heavier grain weight while late planted crop gave lighter grain due to hampering of grain filling in late planting situation (Table 3). The heaviest individual grain was found in BRRI dahn38 while the lightest grain was observed in BRRI dhan34.



Fig. 1. Sterility (%) of modern rice as affected by planting date (Aman season)

Grain yield

Early planted crop of 22 July showed lower grain yield and the yield progressively increased with the advancement of planting dates up to 22 August and decreased thereafter. Compared to the 22 August planting, grain yield decreased by 14, 7, 8, 34 and 75 percent, respectively, when the crop planted on 22 July, 7 August, 7 September, 22 September and 7 October. The more grain yield on 22 August planted crop might be due

to the availability of more sunshine hours and favorable temperature, which helped to produce more number of panicles⁻¹ unit area, grains panicle⁻¹ and low spikelet sterility percentage. However, the optimum planting date 9-12 August (220-223 Julian date) was estimated through regression analysis (Fig. 2). The finding of this study is an agreement with the observations made by Reddy (2002) for cultivation of aromatic fine rice. The early-planted crop required longer period to complete their life cycle and enhanced to increase plant height that proned to lodge during grain filling stage which increased spikelet sterility and ultimately decreased grain yield. These results are in agreement with the findings of Kropff and Spitters (1991).



Fig. 2. Grain yield of modern fine rice as affected by planting date (Aman season)

Planting	No of	Panicle	Grains	Sterility	1000-grain	Grain	Straw	Growth
time	panicle	length	panicle ⁻¹	(%)	weight	vield	vield	duration
	(m^{-2})	(cm)	(no.)		(g)	$(t ha^{-1})$	$(t ha^{-1})$	(day)
22-July	235 bc	25.61 a	96 ab	35 c	14.26 a	2.66 b	5.25 a	152 a
7-Aug.	243 ab	25.31 a	99 a	31 c	14.21 ab	2.88 b	5.09 ab	139 b
22-Aug.	265 a	24.30 b	96 ab	31 c	14.20 ab	3.11 a	4.81 b	137 c
7-Sept.	249 ab	23.52 b	92 b	33 c	13.99 bc	2.87 b	4.19 c	119 de
22-Sept.	219 с	22.60 c	74 c	44 b	13.91 b	2.04 c	3.64 d	118 e
7-Oct.	170 d	21.56 d	45 d	64 a	13.69 c	0.78 d	3.17 e	120 d
Mean	230	23.82	84	40	14.05	2.39	4.36	130
CV (%)	10.6	3.7	10.1	12.3	2.3	6.4	6.3	0.9

 Table 3. Effect of planting dates on yield components, grain and straw yields and growth duration of modern aromatic fine rice during aman season (average of 1999 and 2000)

Figures in a column followed by different letters differ significantly but with common letter do not differ significantly at 0.01% level of probability.

132

The BRRI dhan37 and BRRI dhan38 produced significantly the highest grain yield over the rest of the varieties due to heavier individual grain weight. The taller variety BRRI dhan34 showed significantly low grain yield among the test varieties due to light individual grain weight. These findings are in conformity with the results obtained by Ghosh and Ganguly (1994). The grain yield variation due to transplanting of rice in different dates was identified through step up regression analysis (Fig. 3). The test varieties gave the highest grain yield when transplanted from 7 August to 7 September. Delay transplanting beyond 22 August (up to 7 September) the grain yield declined @ 16 kg ha⁻¹ day⁻¹, 7 September to 22 September is 55 kg ha⁻¹ day⁻¹ and further delaying in transplanting beyond 22 September to 7 October the grain yield reduced drastically and is 84 kg ha⁻¹ day⁻¹. The grain yield of BRRI dhan38 gave 3%, 10% higher grain yield than BRRI dhan37 and BR5, respectively. Late planted crop adversely affected by low temperature during panicle emergence and grain filling period resulted lower grain yield. These findings are in conformity with the findings obtained by Salam *et al.*, (1992).

 Table 4. Yield components, grain and straw yields, and growth duration of modern aromatic fine rice varieties during the aman season (average of 1999 and 2000)

Varieties	Number of	Panicle	Grains	Sterility	1000-grain	Grain	Straw	Growth
	panicles	length	panicle ⁻¹	(%)	weight	yield	yield	duration
	(m^{-2})	(cm)	(no.)		(g)	$(t ha^{-1})$	$(t ha^{-1})$	(day)
BR5	230 ab	22.58 d	90 b	41 a	12.06 c	2.41 b	4.34 b	132 a
BRRI dhan34	220 b	25.49 a	99 a	40 a	9.60 d	1.88 c	3.92 c	125 d
BRRIdhan37	229 ab	23.02 c	84 b	41 a	15.13 b	2.59 a	4.44 b	128 c
BRRIdhan38	242 a	24.18 b	61 c	36 b	19.37 a	2.67 a	4.74 a	130 b
BR5	230 ab	22.58 d	90 b	41 a	12.06 c	2.41 b	4.34 b	132 a
BRRI dhan34	220 b	25.49 a	99 a	40 a	9.60 d	1.88 c	3.92 c	125 d
Mean	230	23.82	84	40	14.05	2.39	4.36	130
CV (%)	10.6	3.7	10.1	12.3	2.3	6.4	6.3	0.9

Figures in a column followed by different letters differ significantly but with common letter do not differ significantly at 0.01% level of probability.

The optimum planting date of BR5 is 19 August; BRRI dhan34 is 21 August; BRRI dhan37 is 29 Augus and BRRI dhan38 is 8 August. The estimated date of planting had the similarity with the predicted time and most of the variety responded well when transplanted within August. The late planted crop could not emerge panicle and decreased the number of grains panicle⁻¹. However, the reduction of yield of late planted crops depends on the genotypic potentiality. Similar trend also observed by Mannan and Siddique (1991) in aman season.

Thus, by adjusting planting dates, farmers can take advantage of natural conditions favorable for higher grain yield or reduce risks in aman season.



Fig. 3. Grain yield of modern rice as affected by planting dates (aman season)

Straw yield

Early planted (22 July) crop had longer vegetative growth period that produced higher amount of biomass and resulted more straw yield (>5.0 t ha⁻¹) and are declined progressively in the subsequent plantings dates (Table 3). The lowest straw yield was recorded in the late planted crop of 7 October. This finding supports the results of Paliwal *et al.* (1996). The BRRI dhan38 produced the highest straw yield followed by BRRI dhan37 irrespective of planting dates due to high number of tillers and more dry matter production while the BRRI dhan34 gave the lowest straw yield (Table 4).

Flowering behavior and growth duration

Early planted (22 July) crop flowered within 22 October to 30 October. On the other hand late planted (7 October) crop flowered 10 November to 26 November. However, the growth duration pattern was related with flowering time. The early-planted crop completed the active vegetative phase and passed the lag vegetative phase and wait up to optimum short day length for floral induction and matured within 152 days (Table 3). While in late planted crop, floral initiation occurred just after a minimum period required for the active vegetative phase due to short day length and matured within 120 days. The growth duration of BR5 and BRRI dhan38 was 130-132 and BRRI dhan34 and BRRI dhan37 was 125-128 days.

The flowering and growth duration of rice varied due to the influenced of planting dates and the genetic variability. These results are agreed with those of Maiti and Sen (2003).

Photoperiod sensitivity

The degree of photoperiod sensitivity varied from 25.0 to 27.1. The degree of sensitivity was the highest in BR5 followed by BRRI dhan37 while it was the lowest in BRRI dhan34 (Table 5). This fact well agrees with the results obtained by Ghosh and Saran (1982).

Varieties	Flowering du	ration (days)	Difference	Degree of sensitivity	
	Preceded by long	Preceded by short	(days)	$\underline{X}-\underline{Y} \times 100$	
	day (X)	day (Y)	(X-Y)	X	
BR5	129	94	35	27.1	
BRRI dhan34	123	91	32	26.0	
BRRI dhan37	128	94	34	26.6	
BRRI dhan38	128	96	32	25.0	

 Table 5. Degree of photoperiod sensitivity of modern aromatic fine rice in Aman season at Gazipur

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