

ASSESSMENT OF PHENOTYPIC AND GENOTYPIC DIVERSITY IN RICE FOR SALINITY TOLERANCE AT REPRODUCTIVE STAGE

C. Saha¹, M.S.R. Khanom^{2*}, S. Razia³, A.C. Sharma⁴, K.K. Saha⁵, S. Yasmin¹ and S.N. Begum²

Abstract

Soil salinity has turned out to be an important global concern, which affects tenable rice production in many agricultural countries like Bangladesh. A cross was made between Binadhan-7 with FL-478 for developing salt tolerant advance lines to assess phenotypic and genotypic diversity in rice for salinity tolerance. Twenty advance rice lines (Binadhan-7/FL-478) along with check varieties were screened for salinity tolerance at reproductive stage using sea site saline water having electrical conductivity (EC) 10 dS m⁻¹. Among the twenty rice lines, seven lines were tolerant, three were moderately tolerant, six were susceptible, four were highly susceptible on the basis of phenotypic evaluation. Seven simple sequence repeat (SSR) markers linked with salt tolerance quantitative trait loci were used for investigation of salt tolerant rice lines. The result revealed that an average number of 6.286 alleles per locus were detected, with polymorphism information content (PIC) values ranging from 0.672 (RM490) to 0.838 (RM562). The highest gene diversity value (0.812) was found in RM562 and the lowest (0.684) was in RM490. A dendrogram constructed from the genetic distance of the genotypes produced four distinct clusters of twenty rice genotypes. Considering both phenotypic and genotypic observation, seven genotypes viz., Binadhan-10, FL-478, SL-51, SL-56, SL-77, EFSD-59 and IZSD-45 were identified as salt-tolerant; on the other hand, EFSD-21, SL-28, SL-32, SL-10, BRRI dhan28, and Binadhan-7 were identified as salt-susceptible. The identified salt-tolerant rice genotypes could be used in the improvement of rice breeding programs.

Keywords: Rice, Salinity Tolerance, Phenotypic and Genotypic Diversity, Reproductive Stage

Introduction

Rice (*Oryza sativa* L.) is the staple food of an estimated 3.5 billion people worldwide (Amagliani *et al.*, 2016). It is also the most extensively cultivated cereal crop and a vital food for about 156 million people in Bangladesh (Shelley *et al.*, 2016) which

¹ Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, ²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh, ³Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh, ⁴Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, ⁵Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

*Corresponding author's email: sifabau@gmail.com

inevitably meets most of the nutritional demand of the majority of its people. Rice production in the world has become stagnant due to different biotic and abiotic causes. Abiotic stresses as like floods, drought, cold and saline excesses in precipitation increasingly limit food, fiber, and forest production worldwide (Collard *et al.*, 2008). Among them salinity is a major abiotic stress which bring negative effect in crop production worldwide (Abdallah *et al.*, 2016). Bangladesh is not beyond the change. Southern part of Bangladesh is facing this salinity problem which affects the rice crop production in those areas. Soil salinity affects 1.06 million hectares of arable land of Bangladesh (SRDI, 2010). Khulna, Satkhira, Bagerhat, Barguna, Patuakhali, Noakhali, Chittagong and some other districts are the coastal areas of Bangladesh where crop yields are drastically decreases due to salinity problem.

In Bangladesh, existing released varieties for salt tolerance named BRRI dhan40, BRRI dhan41, BRRI dhan47, BRRI dhan53, BRRI dhan54, BRRI dhan55, BRRI dhan61, BRRI dhan67, BRRI dhan73, Binadhan-8 and Binadhan-10 within the last ten years, which are not up to the mark in term of grain quality and tolerance as well. However, none of them are fine grained variety which is a good option for the farmers in the coastal regions. So, for the farmer's choice, development of salt tolerant short duration and fine grained rice variety is a prime need in the coastal region. That's why a cross was made between Binadhan-7 with FL-478 to introgress salt tolerant genes into a potential popular fine grained early rice variety.

Soil salinity affects all stages of growth and development of rice plant, but salinity at reproductive stage declines grain yield much more than salinity at the vegetative stages. (Singh *et al.*, 2021). Therefore, screening for salt tolerance at reproductive stages has been considered to be more useful. In order to manage rice productivity in the field and to develop salt-tolerant rice varieties, tolerance during the reproductive stage of the crop is essential (Ahmadizadeh *et al.*, 2016; Hussain *et al.*, 2017). Breeding for salinity tolerance in rice requires suitable screening techniques and appropriate molecular marker technology (Gregorio *et al.*, 2002).

Genetic diversity is a pre-requisite for any crop improvement program since it facilitates the development of improved recombinants. For assessing changes in genetic diversity over time and space molecular marker based genetic diversity analysis has potential process (Duvick, 1984). In order to create segregating progenies with maximum genetic variability for further selection and introgression of desirable genes from diverse germplasm into the available genetic base, genetic diversity analysis is used to estimate and establish genetic relationships in germplasm collections. It also identifies diverse parental combinations (Thompson *et al.*, 1998; Islam *et al.*, 2012). Many crop species including rice for which SSR markers have been developed. These markers are becoming more popular because of their technical simplicity, the small amount of starting DNA required, relatively

low cost, the level of allelic diversity and high power of resolution (Panaud *et al.*, 1995 and McCouch *et al.*, 1997). For an efficient selection of salt-tolerant genotypes both at the seedling and the reproductive stages, SSR markers can be widely used. The current study set out to assess the diversity of twenty rice genotypes using phenotypic and molecular markers.

Materials and Methods

Plant materials

Experiments were conducted at the glasshouse and Molecular Plant Breeding Laboratory of Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during October 2020 to June 2021. A total of twenty rice genotypes (BC₂F₇ developed from crossing between Binadhan-7 and FL-478) along with three check varieties (BRRI dhan28, BRRI dhan58 and Binadhan-10) were used in this experiment (Table 1).

Table 1. List of rice genotypes used in the experiment

Sl. No.	Name of the rice lines/variety	Type	Origin
1	SL-10		BINA
2	SL-28		BINA
3	SL-32		BINA
4	SL-39		BINA
5	SL-44		BINA
6	SL-51		BINA
7	SL-56		BINA
8	SL-57	Advanced line	BINA
9	SL-77		BINA
10	EFSD-21		BINA
11	EFSD-58		BINA
12	EFSD-59		BINA
13	IZSD-10		BINA
14	IZSD-45		BINA
15	IZSD-44		BINA
16	FL-478	Salt-tolerant parent	BINA
17	Binadhan-7	Salt-susceptible parent	BINA
18	BRRI dhan28	Susceptible check	BRRI
19	BRRI dhan58	Susceptible check	BRRI
20	Binadhan-10	Tolerant check	BINA

BINA= Bangladesh Institute of Nuclear Agriculture, BRRI = Bangladesh Rice Research Institute

Phenotypic screening

The genotypes were evaluated for their tolerance to salinity using International Rice Research Institute (IRRI) developed standard protocol (Gregorio *et al.*, 1997) at

reproductive stage. The experimental design was completely randomized design (CRD) with three replications. Two conditions were maintained: control (without salt stress) and salinized at EC 10 dS m⁻¹. Rice genotypes were scored based on standard evaluation system (SES) (IRRI, 2002) on flag leaf symptoms at reproductive stage (Table 2).

Table 2. Modified standard evaluation system (SES) of visual salt injury at reproductive stage

Score	Observation (Flag-leaf damage percentage)	Tolerance
1	No flag leaf symptoms	Highly tolerant
3	1-10% damage	Tolerant
5	11-25% damage	Moderately tolerant
7	26-50% damage	Susceptible
9	More than 51% damage	Highly Susceptible

Agronomic parameters and data visualization

Rice yield component data were recorded from the reproductive stage in both control and salinized conditions. Data were recorded on days to first flowering, days to maturity, plant height (cm), number of total tillers plant⁻¹, number of effective tillers plant⁻¹, panicle length (cm), number of filled grains plant⁻¹, number of unfilled grains plant⁻¹, thousand seed weight (g) and yield (t ha⁻¹). The reduction (%) of plant character was calculated with the following equation:

$$\text{Reduction percentage} = \frac{\text{Traits in normal condition} - \text{Traits in saline condition}}{\text{Traits in normal condition}} \times 100$$

Genotyping of rice genotypes for salinity tolerance through SSR markers

Seven SSR markers were selected for polymorphisms, clear DNA band to use in final polymerase chain reaction (PCR) by amplifying template DNA for this study of the rice genotypes (Table 3). Genomic DNA was extracted from healthy 21-day old leaf samples of the twenty rice genotypes using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method. The extracted DNA was quantified using a Nanodrop and diluted to a final concentration of 50 ng µl⁻¹. A 10 µl PCR reaction mixture containing 2 µl of genomic DNA template, 3.2 µl of PCR master mix, 1 µl of forward primer, 1 µl of reverse primer and 2.8 µl nuclease free water was prepared. PCR program was maintained as initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation for 30 seconds at 94 °C, annealing at (55-60) °C for 45 seconds, extension at 72 °C for 2 minutes and final extension at 72 °C for 7 minutes. PCR amplified products were separated in 8% polyacrylamide gel at 90 V for 1.5 h in 1X TBE buffer and stained with ethidium bromide

(0.5 µg ml⁻¹). DNA banding patterns were visualized using Alpha Imager documentation system. The amplified DNA fragment size was determined by comparing the migration distance of 100-base pair DNA Ladder.

Table 3. Details of the SSR markers used for genotyping

Marker Name	Location on Chromosome	Forward (F) and Reverse (R)	Primer Sequence (5' – 3')	Annealing Temperature (°C)	Expected PCR Product Size
RM3412b	1	F R	TCATGATGGATCTCTGAGGTG GGGAGGATGCACTAATCTTTC	55	211
RM493	1	F R	GTACGTAAACGCGGAAGGTGACG CGACGTACGAGATGCCGATCC	55	211
RM1287	1	F R	GGAAGCATCATGCAATAGCC GGCCGTAGTTTTGCTACTGC	55	162
RM490	1	F R	ATCTGCACACTGCAAACACC AGCAAGCAGTGCTTTCAGAG	55	101
RM10793	1	F R	GACTTGCCAACTCCTTCAATTCG TCGTCGAGTAGCTTCCCTCTCTACC	60	123
RM562	1	F R	CACAACCCACAAACAGCAAG CTTCCCCCAAAGTTTTAGCC	55	243
AP3206	1	F R	TTCTCATCGCACCATTCTG GGAGGAGGAGAGGAAGAAG	55	375

Analysis of SSR markers

Molecular weight for each amplified allele was measured in base pair using AlphaEaseFC 4.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and polymorphism information content (PIC) values were analyzed using PowerMarker 3.25 (Liu and Muse, 2005). The Nei's genetic distance (Nei *et al.*, 1983), coefficient and a dendrogram representing the genetic relationships between genotypes based on the unweighted pair group method with arithmetic mean (UPGMA), which as constructed using the program of MEGA-X software.

Results

Screening based on variation in visual injury of flag leaf

The degree of flag leaf injury and necrosis caused by salinity were ascertained by SES score values on the basis of growth symptoms. Among the 20 rice genotypes, 7 genotypes scored 3 (tolerant), 3 genotypes scored 5 (moderately tolerant), 6 genotypes scored 7 (susceptible), 4 genotypes scored 9 (highly susceptible) (Fig. 1 and Table 4).

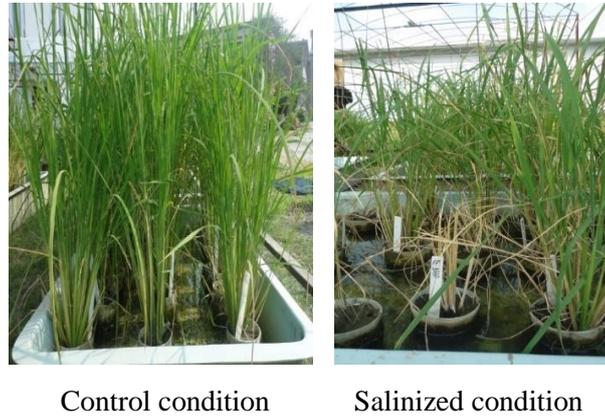


Fig 1. Performance of rice genotypes under control and salinized setup

Table 4. Performance of twenty rice genotypes under salinized condition (EC: 10 dS m⁻¹) grown in sustained saline water condition at reproductive stage

Sl. No.	Genotypes	SES Score	Tolerance	Sl. No.	Genotypes	SES Score	Tolerance
1	SL- 10	7	S	11	EFSD- 58	7	S
2	SL- 28	9	HS	12	EFSD- 59	3	T
3	SL- 32	9	HS	13	IZSD- 10	7	S
4	SL- 39	5	MT	14	IZSD- 44	3	T
5	SL- 44	5	MT	15	IZSD- 45	7	S
6	SL- 51	3	T	16	FL- 478	3	T
7	SL- 56	3	T	17	Binadhan- 7	7	S
8	SL- 57	5	MT	18	BRRi dhan28	9	HS
9	SL- 77	3	T	19	BRRi dhan58	7	S
10	EFSD- 21	9	HS	20	Binadhan-10	3	T

In 1-9 scale, where 1 = highly tolerant (HT), 3 = tolerant (T), 5 = moderately tolerant (MT), 7 = susceptible (S) and 9 = highly susceptible (HS)

Days to maturity

Due to salt stress, salinized plant mature earlier than the non-salinized plant. Considering the days to maturity, SL-51, SL-10, SL-57, EFSD-58, IZSD-10, IZSD-44 and SL-28 showed higher (>5.0%) reduction. But SL-44, FL-478, BRRI dhan28, Binadhan-10, Binadhan-7, EFSD-21, SL-56, SL-32, EFSD-59, BRRI dhan58, SL-39, IZSD-45 and SL-77 showed lower reduction (<5.0%) (Fig. 2).

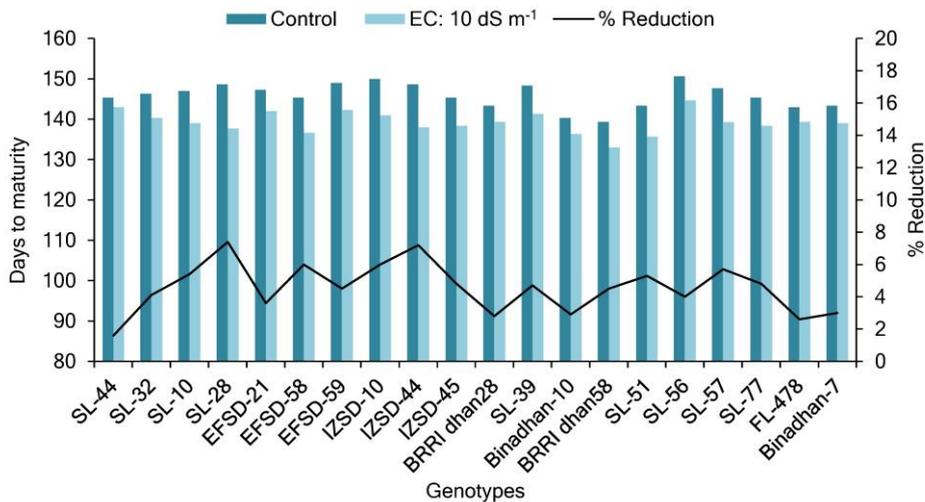


Fig. 2. Days to maturity reduction of rice genotypes under the salinity level at EC 10 dS m⁻¹

Plant height

Plant height reduction percent of different rice genotypes was influenced by salinity. At 10 dS m⁻¹ salinity level, EFSD-59, EFSD-58, IZSD-10, FL-478, SL-10, SL-56 and SL-32 showed higher (>5.0 %) reduction. On the other hand, SL-77, SL-57, SL-51, IZSD-44, SL-39, BRRI dhan58 and BRRI dhan28 showed lower reduction (<5.0 %) (Fig. 3).

Number of effective tillers plant⁻¹

Salinity level had significant effect on number of effective tillers plant⁻¹. The maximum number of effective tillers plant⁻¹ of all genotypes was found in control condition and the minimum was at 10 dS m⁻¹ salinity levels. Due to salinity the effective tillers reduction was varied from 10.2 to 35.8%. At 10 dS m⁻¹ salinity levels, other than check variety, the lowest effective tillers reduction percent were found in SL-57, SL-56 and IZSD-10 and the highest was found in BRRI dhan28, SL-44, EFSD-58 and IZSD-45 (Fig. 4). The decrease in number of tillers might be due to the toxic effect of salt on plant growth.

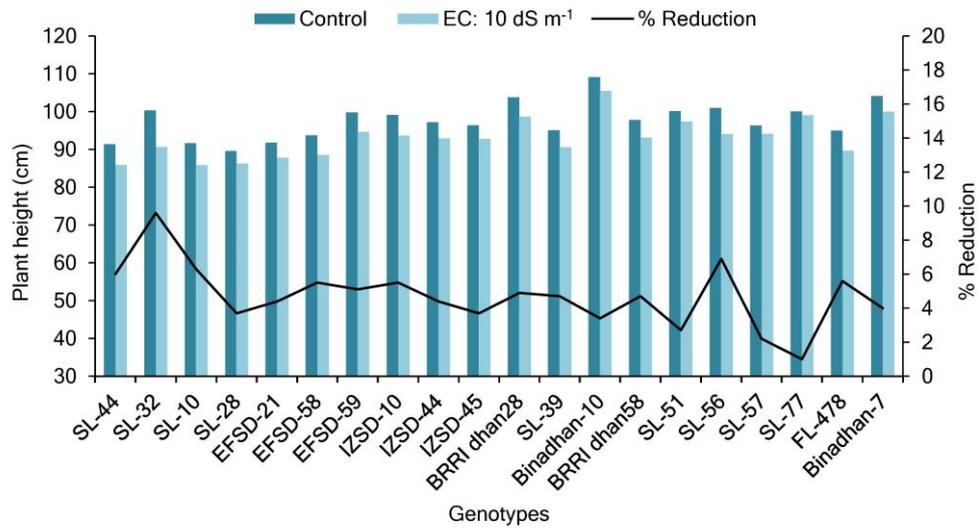


Fig. 3. Plant height reduction (%) of rice genotypes under the salinity level at EC 10 dS m⁻¹.

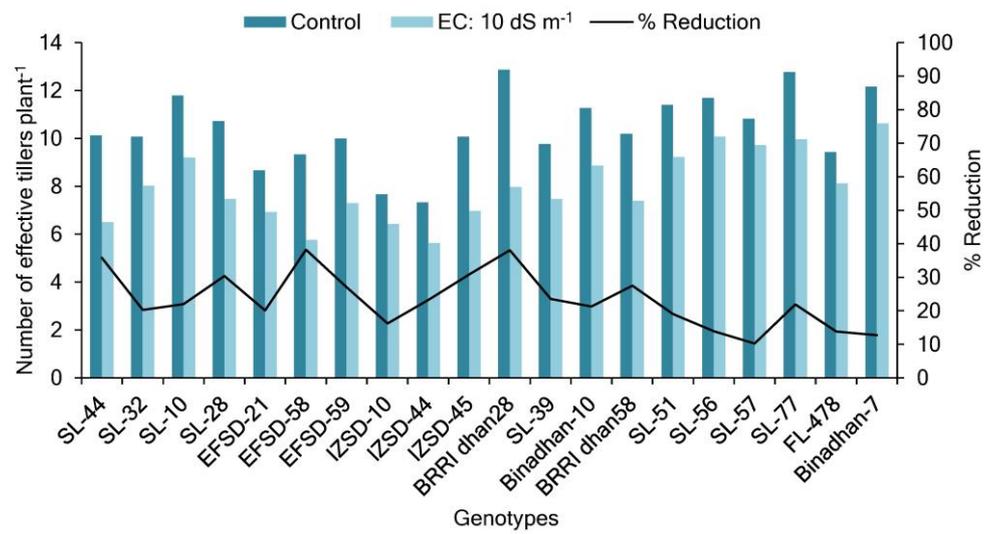


Fig. 4. Number of effective tillers reduction (%) of rice genotypes under the salinity level at EC 10 dS m⁻¹.

Number of filled grain plant⁻¹

The number of filled grain plant⁻¹ is the most influential yield component, and most closely correlated with seed yield. Data regarding filled grain plant⁻¹ of rice as influenced by different salinity levels. Results showed that the highest number of filled grains plant⁻¹ (610.9) was found in Binadhan-7 at control and the lowest (350) was found in SL-39 at 10 dS m⁻¹ salinity which has 23.60% decrease in compare with control treatment. At 10 dS m⁻¹ salinity, the minimum filled grain reduction was found in SL-51, SL-56 and SL-77 (20.40%, 20.90% and 20.70% respectively) and the maximum (38.30%) was found in SL-10 compared to that of check variety (Fig. 5). Considering no. of filled grains, FL-478, SL-51, Binadhan-10, SL-77, SL-56 and SL-57 showed lower reduction (<2.60%) but SL-39, EFSD-59, EFSD-58, IZSD-45, SL-32, BRRI dhan58 and SL-10 showed higher (>23.60 %) reduction.

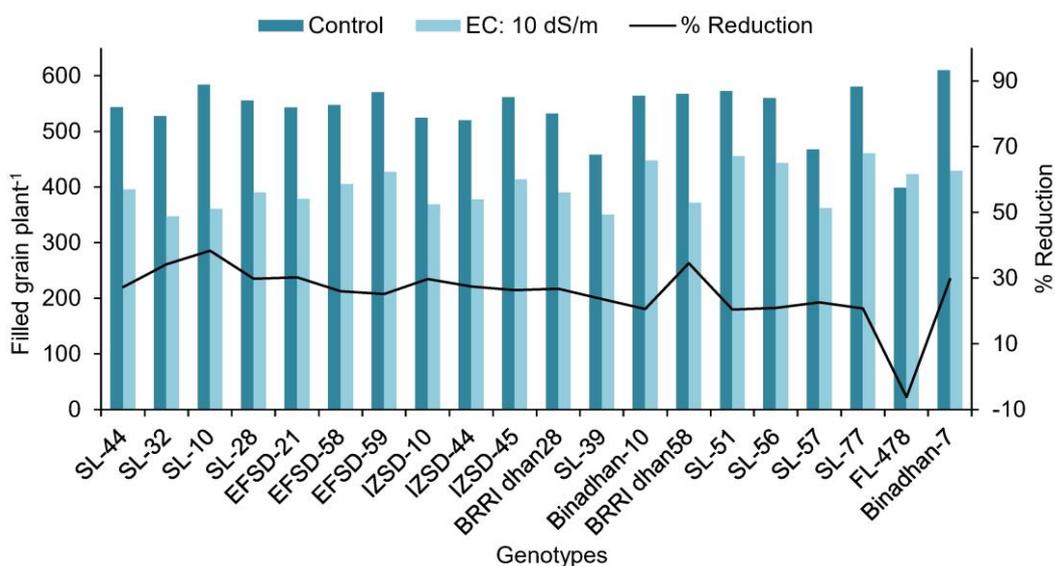


Fig. 5. Number of filled grain reduction (%) of rice genotypes under the salinity level at EC 10 dS m⁻¹.

Grain yield (t ha⁻¹)

The results indicated that seed yield of rice was significantly influenced by salinity level. The maximum seed yields of all genotypes were found in control and the minimum were at 10 dS m⁻¹ salinity. At control, the maximum seed yield (5.72 t ha⁻¹) was found in

SL-51 while at 10 dS m⁻¹ salinity, the maximum seed yield (5.85 t ha⁻¹) was found in SL-77 and the minimum (1.7 t ha⁻¹) was found in SL-32 other than check variety. SL-51 (4.09 t ha⁻¹) and SL-56 (3.56 t ha⁻¹) also showed better seed yield at 10 dS m⁻¹ salinity (Fig. 6). Other than the check variety, the minimum seed yield reduction (22.7%) was found in SL-77 and the maximum (57.8%) was found in SL-32 at 10 dS m⁻¹ salinity level. SL-51 (28.5%) and SL-56 (33.6%) also showed better result compared to other genotypes. Considering yield (t ha⁻¹), SL-77, SL-51, FL-478, SL-56, Binadhan-10 and EFSD-59 showed reduction less than (<38.5%). On the other hand, SL-44, IZSD-45, IZSD-10, SL-28, BRRI dhan28, Binadhan-7, SL-32 and SL-10 showed reduction more than (>43.5%).

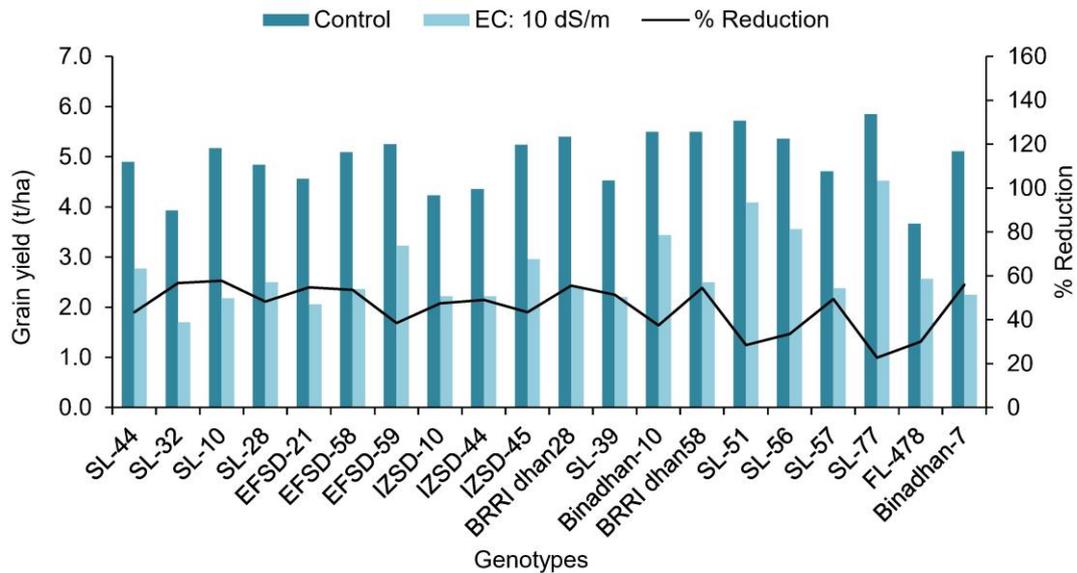


Fig. 6. Grain yield reduction (%) of rice genotypes under the salinity level at EC 10 dS m⁻¹.

Screening of rice genotypes for salinity tolerance through SSR markers

Using seven markers across twenty genotypes, 44 alleles were identified. The loci RM562 and RM1287 had the highest number of alleles (8), whereas the loci RM490 had the lowest number of alleles (4). The average value of the allele was 6.286 (Table 5). The highest genetic diversity was found for RM562 (0.812) and lowest for RM490 (0.684) (Table 5). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity. Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. On an average 32.10% of the twenty genotypes

shared a common major allele ranging from 20% (RM562) to 40% (RM490 and RM3412b) at each locus (Table 5). PIC value is a reflection of allele diversity and frequency among the varieties that can be evaluated on the basis of its alleles. It varied significantly for all the studied SSR loci. In the present study, the level of polymorphism among the 20 genotypes was evaluated by calculating PIC values for each of the 7 loci. The PIC value ranged from 0.672 (RM490) to 0.838 (RM562) with an average of 0.749 per locus (Table 5).

Table 5. Allele number, allele size, frequency, gene diversity and PIC of twenty rice genotypes for seven SSR markers

Locus name	No. of Allele	Major Allele Frequency	Gene Diversity	PIC
RM493	6	0.35	0.732	0.736
RM490	4	0.4	0.684	0.672
RM10793	6	0.3	0.746	0.753
RM3412b	6	0.4	0.694	0.688
RM1287	8	0.3	0.789	0.811
RM562	8	0.2	0.812	0.838
AP3206	6	0.3	0.741	0.746
Mean	6.286	0.321	0.742	0.749

Banding pattern of twenty rice genotypes using seven SSR markers

Figures of banding patterns of twenty rice genotypes for molecular analysis using seven SSR markers are presented (Fig. 7 - 9). The banding patterns were compared with reference to those of Binadhan-7 and Binadhan-10. According to the phenotypic performance, Binadhan-10 was considered as tolerant and Binadhan-7 was considered as susceptible. The genotypes which gave bands with same position or near with salinity tolerant Binadhan-10 were supposed to be tolerant to salinity and those similar to Binadhan-7 were considered as salt susceptible.

Genetic distance based analysis

The values of pair-wise comparisons of Nei's (Nei *et al.*, 1983) genetic distance between genotypes were computed from combined data for the seven markers, ranged from 0.00 to 1.00 (Table 6). Comparatively higher genetic distance (1.00) was observed between a number of genotypes or genotypes pair. Among them Binadhan-10 vs. Binadhan-7, Binadhan-10 vs. BRR1 dhan28, Binadhan-10 vs. FL-478, Binadhan-10 vs. IZSD-44, Binadhan-10 vs. IZSD-45, Binadhan-10 vs. SL-32 and FL-478 vs. SL-51, FL-478 vs. SL-57 were important. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.29) was found SL-10 vs. SL-28 and SL-28 vs. SL-32 etc.

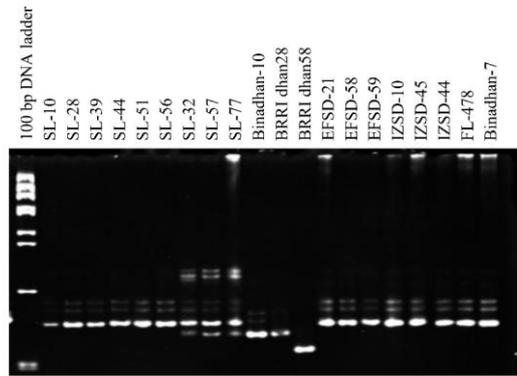


Fig 7: Banding pattern of 20 rice genotypes for SSR marker RM562.

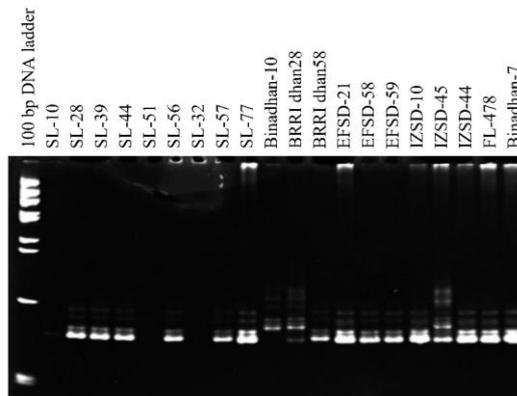


Fig 8: Banding pattern of 20 rice genotypes for SSR marker RM1287.

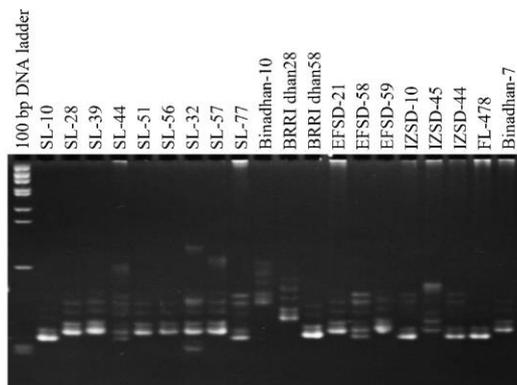


Fig 9: Banding pattern of 20 rice genotypes for SSR marker RM3412b

Table 6: Pair-wise comparisons of Nei's genetic distance for twenty rice genotypes

Genotypes	Binadhan-10	Binadhan-7	BRR1 dhan28	BRR1 dhan58	EFSD-21	EFSD-58	EFSD-59	FL-478	IZSD-10	IZSD-44	IZSD-45	SL-10	SL-28	SL-32	SL-39	SL-44	SL-51	SL-56	SL-57
Binadhan-7	1																		
BRR1 dhan28	1	1																	
BRR1 dhan58	0.57	1	0.86																
EFSD-21	0.86	0.86	1	1															
EFSD-58	0.71	0.86	0.86	1	0.71														
EFSD-59	0.86	0.86	0.86	1	0.57	0.71													
FL-478	1	0.71	0.86	1	1	1	0.57												
IZSD-10	0.71	0.71	1	0.86	1	0.71	0.71	0.86											
IZSD-44	1	0.86	0.43	1	1	0.71	0.86	0.71	0.71										
IZSD-45	1	0.71	1	1	0.86	0.86	0.57	0.57	0.86	1									
SL-10	0.86	0.71	0.86	1	0.86	0.57	0.86	0.71	0.71	0.57	0.71								
SL-28	0.86	1	0.86	1	0.57	0.71	1	0.86	0.86	0.57	1	0.29							
SL-32	1	1	0.71	0.86	0.86	0.86	1	0.86	0.86	0.57	1	0.43	0.29						
SL-39	0.86	1	0.57	0.86	1	1	0.86	0.71	0.86	0.57	1	0.86	0.86	0.71					
SL-44	1	0.86	1	1	0.43	0.86	0.57	0.86	0.86	0.71	0.86	0.86	0.71	0.57	0.86				
SL-51	0.43	0.71	0.86	0.57	0.71	0.86	1	1	1	0.86	1	0.71	0.71	0.86	1	1			
SL-56	0.71	0.71	1	0.86	1	0.86	0.86	0.71	0.86	0.86	0.71	0.71	0.86	0.86	0.71	0.86	0.71		
SL-57	0.57	0.86	0.86	0.71	0.71	0.86	1	1	0.71	1	1	0.86	0.86	1	0.86	1	0.57	0.86	
SL-77	0.71	0.86	0.86	1	0.86	0.71	0.57	0.43	0.71	0.86	0.71	0.71	0.86	1	0.86	1	0.86	0.86	0.86

Cluster analysis

The UPGMA cluster analysis led to the grouping of the twenty genotypes in four major clusters (Fig. 10). Except for BRRI dhan58, SL-57 was moderately tolerant and SL-51, Binadhan-10 were tolerant but BRRI dhan58 was found susceptible in SES scoring. Cluster II was the biggest group which contained seven genotypes. All of them are tolerant in SES score. Cluster III was divided into two sub clusters and consists of SL-39 which were moderately tolerant whereas BRRI dhan28, IZSD-44, EFSD-21, SL-44, EFSD-58, SL-32, SL-10 and SL-28 were found susceptible. The dendrogram revealed that the genotypes that found genetically similar type were clustered together.

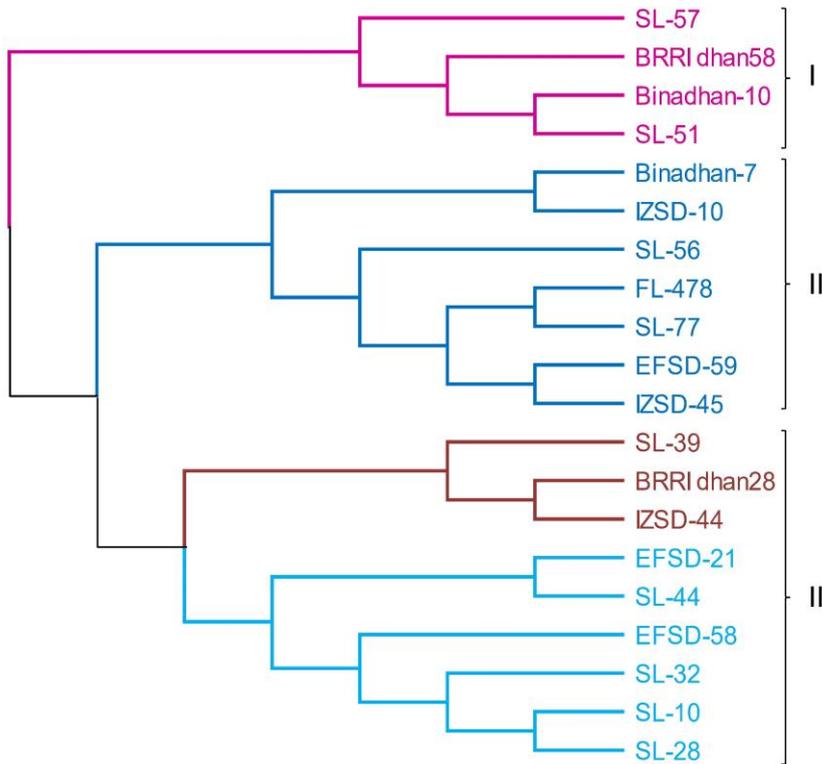


Fig. 10. A dendrogram showing the genetic relationships between twenty rice genotypes based on the alleles detected by seven SSR markers.

Discussion

The genotypes of the salt-tolerant lines showed relatively better growth and lower symptoms than susceptible genotypes after salinization (Bhuiyan, 2005) (Fig. 1). Plant height is the most significant morpho-physiological characteristic, which also determines shoot yield and overall biomass production. Salt stress might inhibit cell division or cell enlargement so that plant height was reduced. It has been reported that plant height decreased progressively with increase in salinity levels. Therefore, the reduction might be occurred due to salt stress during growth and development (Rad *et al.*, 2011). These results indicate that high salinity decreased filled grains plant⁻¹ of rice. This is because of loss of biomass production was lower in tolerant genotypes which increased the assimilation and ultimately produced the higher number of grains. Therefore, increased salinity resulted in increased total number of empty grains plant⁻¹ and finally it decreases yield. Increased number of empty grains might be a result of assimilate shortage during grain filling, brought about by early leaf senescence caused in this case by salinity (Aref and Ebrahimi-Rad, 2012). Grain yield production was also reduced due to salt stress. The same result was reported by Asch *et al.*, 1998 where eighty rice cultivars were used. This result suggests that the salt tolerant cultivars are different from susceptible in up taking salt and yield production. Rice has been reported as being salt-sensitive at reproductive stage (Moradi and Ismail, 2007), leading to a reduction in productivity of more than 50% when exposed to 6.65 dS m⁻¹ salinity (Fig. 6) (Chaum and Kirdmanee, 2010).

The results of SSR markers also consistent with previous work done by Heenan *et al.*, (1988), who observed that the gene diversity at each SSR locus was significantly correlated with the number of alleles detected, number of repeat motif and with the allele size range. Nearly similar observation was found by Dhar *et al.*, (2012), where they got that average number of allele per locus was 10, with a range of 8 (RM152) to as many as 12 (RM7075 and RM10701) among the 26 rice germplasm by using 6 SSR markers. It also observed that the highest level of gene diversity value (0.899) was in loci RM10701 and the lowest level of gene diversity value (0.774) was observed in loci RM152 with a mean diversity of 0.854, the frequency of the most common allele at each locus ranged from 15.38% (RM10701) to 37.51% (RM152) with a mean frequency of 24.15. PIC values showed a significant, positive correlation with the number of alleles and allele size range for SSR markers evaluated in this study. The allele size range and the number of alleles were themselves also highly correlated. PIC values ranged from a low of 0.746 (RM152) to a high of 0.891 (RM10701) and averaged 0.857 was observed by Dhar *et al.*, (2012). Mohammadi-Nejad *et al.* (2010) also found that PIC value varied from 0.56 to 0.88, the highest value belonged to RM8094, while RM8095 showed the lowest PIC value (0.56). The SSR marker RM8094 was found to be superior for analysis of genetic diversity among the markers in the region. Genotypic pair indicating that they are genetically much closer among the genotype tested. Hence SSR marker based molecular fingerprinting could serve as a potential basis for the identification of genetically distance genotypes as well as sorting

of morphologically closer genotypes. The cluster analysis based on pair-wise comparison of Nei's genetic distance agreed with the allelic diversity observed among Basmati and Non-basmati long grain *indica* rice varieties (Chakravarthi and Naravaneni, 2006; Siwach *et al.* 2004; Ren *et al.*, 2003).

Conclusion

Salinity is major constraint to cereal production worldwide and has become huge challenge for agriculture and food security in developing countries. In fighting against this problem development of salt-tolerant rice line is realized as the most promising, less resource consuming, economically viable and socially acceptable approach. Salinity tolerance of rice could noticeably be enhanced if superior alleles for all useful mechanisms are combined into popular rice varieties using molecular technique. Considering both phenotypic and genotypic observation, seven genotypes viz. Binadhan-10, FL- 478, SL-51, SL-56, SL-77, EFSD-59 and IZSD-45 were identified as salt-tolerant and on the other hand, EFSD-21, SL-28, SL-32, SL-10, BRR1 dhan28, and Binadhan-7 were identified as salt-susceptible. The markers (RM490, RM493, RM562, RM1287, AP3206, RM3412b and RM10793) were used in marker-assisted selection, and identified salt-tolerant rice genotypes could be used in the improvement of rice breeding program.

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MUTAGENIC EFFECT OF ETHYL METHANE SULFONATE ON SOME FIBER SPECIES

N.B. Atiq*, M.S. Haque, M.M.A. Noor, S.A. Sammy, M. Perves and S. Khanam

Abstract

Jute, kenaf and mesta are prominent fibrous plants with significant industrial and nutritional value for optimization of their cultivation process can have substantial agricultural and economic implications. The study was conducted on exploring the impact of ethyl methane sulfonate (EMS) presoaking treatments on the germination and the sensitivity of fiber crops to mutagens. This research aimed to evaluate the germination potential of jute, kenaf and mesta variety using various concentrations of EMS presoaking treatments, and analyze the sensitivity and induced variability in the M_1 generation. Seeds were presoaked in four different concentrations of EMS. The subsequent parameters, including germination, root length, and shoot length, were measured at three crucial growth stages: 12, 24, and 36 days after sowing (DAS). A probit analysis was conducted to determine the LD_{50} value, which signifies the concentration at which 50% of the population exhibits an adverse effect. Moreover, significant variances were detected in germination rates, root elongation, and shoot growth across the different presoaking treatments. The findings emphasize the sensitivity of these fibrous plants to EMS concentrations, with increasing concentrations having a detrimental effect on growth parameters. This information can be instrumental for breeders and agriculturists looking to optimize germination in these crops.

Key words: Mutation, Mutagen, Probit analysis, LD_{50}

Introduction

Jute, a significant bast fiber in the world of fibrous crops, stands out not just for its economic value but also for its commendable eco-friendly attributes. Often hailed as the green counterpart to cotton, it occupies a coveted position in terms of usage, global consumption, production and availability (Ahmed and Nizam, 2008). The adaptability of jute is evident in its manifold applications spanning textiles, construction, packaging, agriculture, and beyond. As a natural fiber, it possesses superior tensile strength, alongside a low extensibility factor, making it an ideal choice for crafting a multitude of everyday items including ropes, twines, hessian fabrics, carpets, and rugs (Mir *et al.*, 2008). Its eco-centric attributes, like recyclability and 100% biodegradability, further endorse its environmental credentials.

Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202

*Corresponding authors' email: nusratamia@gmail.com

However, the glory days of jute, especially during the 1970-80s when it covered a sprawling 9 lakh hectares of Bangladesh's cultivable land, have witnessed a decline. By 2017-22, these numbers plummeted to a mere 6-7 lakh hectares, producing roughly 77-84 lakh bales of fiber (BBS, 2022). This reduction is partially attributed to the pressing need to allocate land for food crops, catering to the escalating demands of a booming population. But the challenge doesn't end here. Jute's progress is hampered by intrinsic biological constraints such as its high sensitivity to photoperiodism and cross-breeding issues between cultivated and wild species. These hurdles underline the urgency to innovate and develop adaptable jute genotypes, which not only have a shorter life span but are also versatile in varied cropping patterns (Islam *et al.*, 2017; Sarker *et al.*, 2007).

In light of these challenges, there emerges a beacon of hope: chemical mutagenesis, with a special focus on Ethyl Methane Sulfonate (EMS) treatments. This method promises an efficient alternative, as mutation breeding offers plant breeders a swift mechanism to enhance crop qualities ranging from yield and quality to resilience (Oladosu *et al.* 2016; Bolbhat and Dhumal, 2009). The introduction of genetic variability via mutation breeding can pave the way for unveiling mutant lines enriched with sought-after traits (Mei *et al.*, 2007). With these insights, our study takes the helm, aiming to scrutinize the germination potential of not just jute, but also kenaf and mesta, when subjected to the chemical mutagen, EMS.

Materials and Methods

Seed Selection and Preparation

For this study, we sourced high-quality seeds from distinct fibrous plants: jute varieties (O-9897, O-795, O-72 and BJRI deshi patshak), kenaf (HC-95) and Mestapat-1. Careful scrutiny ensured only the healthiest seeds were retained for experimentation, ensuring consistency in our observations.

Seed Treatment Procedure

A batch of two hundred seeds for each specified treatment underwent a thorough cleansing process. This involved washing the seeds thrice using a 0.1% (v/v) Tween-20 solution prepared in distilled water. This step ensured removal of any contaminants from the seed surface.

With precision, seeds were subsequently exposed to EMS concentrations of 0.5%, 0.75%, 1.0%, and 1.5%. Each concentration was applied for specific durations: 2, 3, 4, and 6 hours, respectively. To ensure uniform EMS penetration, the seeds were subjected to continuous agitation in an electric shaker set at 80 rpm.

Control seeds were incubated in distilled water maintaining the same incubation condition. To ensure no residual EMS remained, the treated seeds underwent eight consecutive washes using distilled water.

Post-cleansing, seeds were air-dried under ambient room conditions. Once adequately dried, seeds were sown under two distinct settings: standard Petri dishes and field conditions. All field-based experiments were meticulously executed at the Bangladesh Institute of Nuclear Agriculture, located in Mymensingh, Bangladesh.

Data Collection and Analysis

LD₅₀ value for EMS was calculated based on probit analysis (Finney, 1978). Probit analysis was carried out in excel by following procedure

Mortality percentage of seeds was calculated for all the doses and the value was rounded to the nearest whole number. Corrected mortality percentage was calculated using Abbott's formula.

$$\text{Corrected mortality} = \frac{M_{\text{observed}} - M_{\text{control}}}{100 - M_{\text{control}}} \times 100$$

All the corrected values were rounded to the nearest whole number. Probit value was worked for the corresponding corrected mortality percentage value. Probit graph was drawn using probit values on Y-axis against treatment concentration on X-axis. EMS dose at corresponding probit 5 values was estimated as LD₅₀ for the mutagen.

Results and Discussion

Effects of EMS on germination in fibrous plants

As displayed in *Fig. 1*, the data elucidates the sensitivity of different varieties of fibrous plants to varying EMS levels. At control stage (0% EMS) presented an impressive germination rate for kenaf (HC-95) and jute (O-9897) with a full 100% germination. As we introduce EMS, we observe a predictable reduction in germination rates. The most profound dip in germination was witnessed in Mestapat-1 at 1.5% EMS, settling at just 30%. This decremental trend with escalating EMS doses echoes the patterns noted in prior studies on crops like rice, reinforcing the established knowledge on mutation breeding (Mohamad *et al.*, 2006; Manneh *et al.*, 2007).

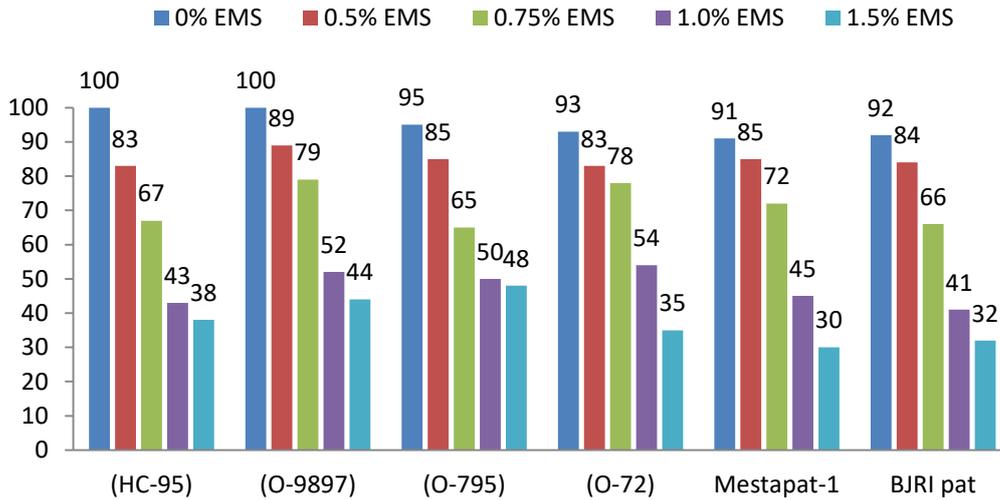


Fig1. Effect of different doses of EMS on germination% of different varieties of jute, kenaf and mesta.

LD₅₀ values from probit analysis

These values essentially capture the EMS concentration at which half the sampled population of seeds failed to germinate. Moreover, different varieties exhibited varied sensitivities. HC-95 had the most resistance with the lowest LD₅₀ value of 1.09%, whereas both O-795 and O-72 showed heightened sensitivity with LD₅₀ values peaking at 1.17%. Such differential responses across varieties resonate with observations by Ramchander, *et al.*, 2015 and Rajarajan, *et al.*, 2016, emphasizing that even under uniform treatment conditions, the genetic makeup of the plant significantly influences the outcomes.

Table1. Probit Analysis for Calculating LD₅₀ Doses

Varieties	Mutagenic doses (%)	Observed mortality (%)	Corrected mortality (%)	Probit table value	LD ₅₀ value (%)
(HC-95)	Control	0	0	0	1.09
	0.5	17	17	4.05	
	0.75	33	33	4.56	
	1.0	57	57	5.18	
	1.5	62	62	5.28	
(O-9897)	Control	0	0	0	1.16
	0.5	11	11	3.77	
	0.75	21	21	4.19	
	1.0	48	48	4.95	
	1.5	56	56	5.15	
(O-795)	Control	5	0	0	1.17
	0.5	15	11	3.77	
	0.75	35	32	4.53	
	1.0	50	47	4.92	
	1.5	52	49	4.97	
(O-72)	Control	7	0	0	1.17
	0.5	17	11	3.77	
	0.75	22	16	4.01	
	1.0	46	42	4.8	
	1.5	65	62	5.28	
Mestapat-1	Control	9	0	0	1.13
	0.5	15	7	3.52	
	0.75	28	21	4.19	
	1.0	55	51	5.03	
	1.5	70	67	5.44	
BJRI deshi patshak	Control	8	0	0	1.12
	0.5	16	9	3.66	
	0.75	34	28	4.42	
	1.0	59	55	5.15	
	1.5	68	65	5.3	

Based on mutagenic doses, observed mortality (%), corrected mortality (%) and probit values were calculated (Table1) and also the LD₅₀ was determined for different varieties (Fig.2)

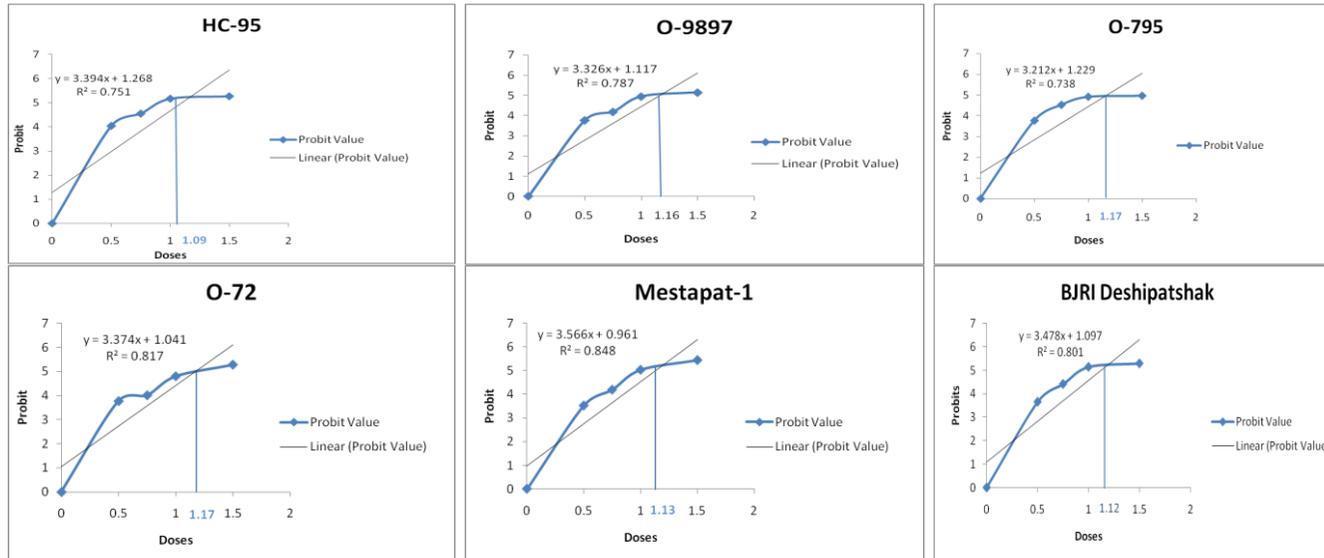


Fig. 2. Plots of mutagenic doses vs. probits for calculation of LD₅₀ of EMS.

Root growth patterns in response to EMS doses

As we navigate through Table 2, a noteworthy trend surfaces. The root length in Mestapat-1 (36 DAS) stood out, reaching a substantial 14 cm in the control group. On the contrary, Jute (O-9897) recorded a mere 6.2 cm at 36 DAS with a 0.75% EMS dosage. Such disparities underscore the variable resilience and responses of different varieties to EMS treatments. Moreover, certain varieties, like the BJRI deshi patshak, exhibited notable fluctuations in root length across various EMS doses, highlighting the intricate ways in which EMS interacts with the genetic makeup of each variety.

Table 2. Effect of different doses of EMS on Root length of different varieties of jute, kenaf and mesta

Treatments EMS level	Kenaf			Jute												Mestapat-1		
	(HC-95)			(O-9897)			(O-795)			(O-72)			BJRI deshi patshak					
	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS									
0% EMS	7.7	10.3	10.75	2.88	5.4	7.8	3.2	6.0	7.75	3.2	7.0	7.25	3.7	7.08	7.0	2.42	9.0	14.0
0.5% EMS	7.3	10.1	11.6	2.1	5.9	8.0	4.4	6.3	8.5	4.6	6.2	8.2	5.0	6.5	9.75	6.38	9.0	10.0
0.75% EMS	9.1	9.9	11.0	3.5	4.5	6.2	2.5	7.0	8.5	4.0	6.4	8.4	3.6	8.2	8.8	5.7	10.0	10.0
1.0% EMS	7.4	8.8	10.2	3.2	5.1	7.0	4.0	8.3	8.4	3.5	6.0	7.25	4.6	7.9	8.0	5.5	9.0	12.0
1.5% EMS	5.3	8.7	10.3	2.2	5.2	6.4	3.2	5.8	7.0	2.6	5.6	6.5	2.9	4.5	6.6	6.75	7.6	8.0
Mean	7.36	9.56	10.77	2.78	5.22	7.08	3.46	6.68	8.03	3.58	6.24	7.52	3.96	6.84	8.03	5.35	8.92	10.8
SE	0.61	0.34	0.25	0.27	0.23	0.36	0.33	0.45	0.29	0.34	0.23	0.35	0.37	0.66	0.58	0.77	0.38	1.02

Shoot growth trends in the wake of EMS treatments

Table 3. Effect of different doses of EMS on shoot length of different varieties of jute, kenaf and mesta

Treatments EMS level	Kenaf			Jute												Mestapat-1		
	(HC-95)			(O-9897)			(O-795)			(O-72)			BJRI deshi patshak					
	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	12 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS
0% EMS	22.7	25.6	44.3	6.13	13.3	28	7.7	15.6	27.3	6.4	17.8	24.5	7.6	16.2	34.3	13.6	19.0	33.0
0.5% EMS	20.9	24.9	45.4	5.5	16.4	26.5	8.0	18.6	27.0	9.9	16.0	22.6	10.0	13.0	29.5	15.0	19.0	32.0
0.75% EMS	22.3	24.8	41.3	5.2	13.5	26.4	7.0	17.3	23.8	4.8	18.4	26.4	6.6	18.7	25.0	18.1	22.5	31.0
1.0% EMS	18.5	23.1	40.0	4.0	15.3	25.6	6.3	17.6	21.2	5.9	16.6	23.0	7.5	19.8	25.6	16.8	22.7	28.2
1.5% EMS	15.1	22.4	11.5	3.3	12.8	22.6	3.2	15.3	20.2	4.4	12.8	17.5	7.1	14.4	24.6	7.5	20.0	26.0
Mean	19.9	24.16	36.5	4.82	14.26	25.82	6.44	16.88	23.9	6.28	16.32	22.8	7.76	16.42	27.8	14.2	20.64	30.04
SE	1.4	0.6	6.32	0.51	0.68	0.89	0.86	0.62	1.45	0.97	0.97	1.48	0.58	1.27	1.84	1.84	0.82	1.28

As portrayed in Table 3, shoot growth, much like root growth, exhibited a decremental trend with increasing EMS doses. The kenaf (HC-95) variety towered over others with a striking shoot length of 44.3 cm at 36 DAS in the control setup. Yet, as we introduce EMS, this length experiences a significant reduction. For instance, in the presence of a 1.5% EMS dose, the shoot length of the same variety plunged to a mere 11.5 cm at 36 DAS. Such observations spotlight the profound influence of EMS on plant growth, emphasizing the need for judicious utilization of such mutagenic agents in breeding programs.

Conclusion

The meticulous examination of the influence of Ethyl Methane Sulfonate (EMS) on fibrous plants such as jute, kenaf and mesta has yielded noteworthy insights. The data clearly underscores the decremental impact of EMS on germination and growth rates across the studied plant varieties. At optimal conditions without EMS interference, these plants exhibit their maximum potential in both germination and growth. However, as the EMS concentration increases, there's a pronounced reduction in germination percentage, root length, and shoot length. Some plants showcase heightened resilience to EMS, while others are more susceptible. This emphasizes the importance of understanding each plant's mutagenic effects and doses before introducing mutagenic agents in breeding programs. Based on the results of the study, the LD₅₀ value of EMS treated kenaf (HC-95) was found to be 1.09%, jute varieties (O-9897, O-795, O-72 and BJRI deshi patshak) were 1.16%, 1.17%, 1.17% and 1.12%, respectively and mesta was 1.13%. By this, we can easily determine the mutation doses for fibrous crops. The study's findings are crucial for plant breeders, geneticists and agriculturists. Since there is very less literature of lethal doses in fibrous crop, determination of optimum mutagen doses could be useful while formulating fiber crop mutation breeding programme for improvement of specific traits in fibrous crops.

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EFFECT OF GAMMA IRRADIATION ON EMBRYOGENIC CALLI OF RICE AND SUBSEQUENT SHOOT AND REGENERATION

M.M. Islam¹, M. Akter², M.K.A. Nadim^{1*}, M.S. Reza³, M.I. Uddin¹ and F. Khatun²

ABSTRACT

This study was carried out during May 2021 to January 2022 at the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh for callus induction and regeneration ability of plantlet from low doses irradiated embryogenic calli of Binadhan-13 and BR5 (Dulabhog). The effect of low gamma rays such as 4, 6 and 8 Gy were observed *in vitro* shoot, root and plantlet formation. Two different incubation conditions such as light and dark incubation were maintained. Between two varieties, Binadhan-13 showed the highest ability of callus induction in both conditions. Callus induction was significantly higher under dark condition (77.14%) than light condition (67.14%) at 21 days. Data revealed that gamma rays affect both shoot and root regeneration ability of embryogenic callus. Shoot regeneration ability was the highest at 4 Gy in Binadhan-13 (60%) and gradually decreased with the increased doses of gamma rays. Among the treatments, shoot regeneration ability (60% in Binadhan-13 and 50% in BR5) was higher at 4 Gy dose of gamma ray followed by 6 Gy where shoot regeneration ability was 55% and 45% in Binadhan-13 and BR5 respectively. In root induction, the highest root induction ability (80%) was observed in the cultivar Binadhan-13 at control condition. Like shoot regeneration, 4 Gy gamma ray showed better root induction in both the varieties (70% in Binadhan-13, 60% in BR5). The *in vitro* regenerated plantlets from irradiated embryogenic calli were successfully transferred to soil in pots. The regeneration protocol could be further used for varietal improvement of rice varieties using nuclear technique (⁶⁰Co gamma ray).

Keywords: Embryogenic callus, Irradiation, Regeneration protocol, Rice.

Introduction

Rice (*Oryza sativa* L.) is the most important human food crops and directly supplies food of more than three billion people in the world (Tyagi *et al.*, 2004). It is also the staple food around Asia where half of the world people live and is

¹Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, ²Department of Biotechnology, Bangladesh Agricultural University (BAU), Mymensingh, ³Planning and Development Section, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh

*Corresponding author: nadim.kawsaralam@gmail.com

becoming increasingly important Latin America and Africa (Muthayya *et al.*, 2014). Compare to other crop in the world, rice is grown by more people than any other crops. Bhuiyan *et al.* (2002) reported that rice supplies 92.85% of food requirement, 55% of protein and 75% of daily calorie intake where these have led overall improvement in health, literacy and life expectancy and more importantly declined the poverty level in Bangladesh. Rice belongs to Gramineae family and the genus *Oryza* and believed to have originated 130 million years ago (Khush, 1997). Cultivated rice (*Oryza sativa* L.) is divided into 3 subspecies namely *indica*, *japonica*, and *javanica* (Datta *et al.*, 2003). Indica type rice feeds more than two billion people, predominately in developing countries.

In Bangladesh, rice is the most dominant and main food crop, which grows in all the three crop growing seasons of the year. The climate and geographical conditions of Bangladesh are favorable for year-round production during Aus, Amon and Boro seasons. Bangladesh ranks 3rd in area and production (FAO, 2021) and 39th in yield of rice among the rice growing countries (Calpa, 2004). Rice is grown over 11.5 million hectares of land with total production of 35.65 million metric tons in Bangladesh (BBS, 2022). It occupies about 77% of the total cropped area of about 13.9 million hectares. A modest estimate suggests that the demand for rice in Bangladesh will increase by over 80% in the next 20 years to feed the growing population (Zaman, 1996). Furthermore, the agriculture production is challenged by numerous natural disasters due to the impact of climate change. Therefore, at present plant biotechnology (Hoque *et al.*, 2007) or various tissue culture techniques (Zapata *et al.*, 1987), widely recognized breeding tools, are being used for the genetic improvement of rice plant throughout the world (Raina, 1989).

Embryogenesis is a process in which bipolar structures resembling a zygotic embryo develop from a non-zygotic cell without vascular connections with the original tissue (Arnold *et al.*, 2002). It is a promising method for the establishment of protocols reaching rapid multiplication of new and elite genotypes (Kamle *et al.*, 2011). Biotechnology, while making use of both mutagenesis and conventional methods, can assist in overcoming hurdles during the development of new and improved cultivars for sustainable crop production (Jain and Swennen, 2004). The most commonly used mutagens so far are physical mutagens such as gamma rays (Roux *et al.*, 2004). The use of whole plantlet or part of plant organs and tissues as irradiation treatment materials is generally considered to be prone to chimera. In contrast, the use of callus as irradiation treatment material is theoretically a single cell mutation, which is conducive to the acquisition of stable mutants, and shortens the breeding cycle (Bai *et al.*, 2022). The following ranges of doses are recommended: 10–20 Gy for diploid cultivars (AA and BB), 30–40 Gy for triploid cultivars (AAA and AAB), and 40–50 Gy for triploid cultivars (ABB) (Roux *et al.*, 2004).

However, immature embryo culture and matured seed culture are important in rice to create additional variation and novel rice varieties (Sathish *et al.*, 1995). The use of matured seed embryos has distinct advantage over other explants as starting material for *in vitro* regeneration. Callus induction and subsequently plant regeneration is dependent on

the type of explants, growth conditions and plant species (Feng *et al.*, 1995). So, the best growth condition, suitable explants and varieties are needed to be identified for large scale utilization in rice improvement program through biotechnology. Nowadays, callus initiation and use of mutagen for induction of mutation in the callus is one of the proven tools employed by plant breeders for creating variability in crop plants. This technique is known as *in vitro* mutagenesis that is a sudden change in heritable characters of an organism which serves as a source of creating variability for better selection in short time. Tissue culture as well as callus production followed by irradiation is an important tool for creating variants and could play an important role in crop improvement (Bansal *et al.*, 1990). The application of ionizing radiation in optimal dose to plant tissue culture is to investigate the culture response and *in vitro* culture, in combination with induced mutation could open new ways in which exploit somaclonal variations for plant improvement. The present piece of research work was undertaken to study the callus induction and regeneration ability of Binadhan-13 and BR5 and to study the effect of gamma irradiation on embryogenic calli of Binadhan-13 and BR5, and their subsequent regeneration potential.

Materials and Methods

The experiment was conducted during the period from May 2021 to January 2022 at the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh to establish efficient shoot and root regeneration protocol from mature grains of selected two rice varieties i.e. Binadhan13 and BR5 (*Oryza sativa* L.). MS medium was used for callus induction, shoot differentiation and half strength for root initiation (Murashige and Skoog, 1962, Ducefha, Netherlands). When the regenerated plants were established, plantlets were transferred to the pot containing 50% soil and 50% cowdung.

Preparation of the MS media

To prepare one liter of MS media about 500ml of distilled water was added in the flask to dissolve all the ingredients. After that, MS powder (4.4g) and Sucrose (30g) was added to this solution and gently agitated to dissolve completely. pH of the medium was adjusted to 5.8 with a digital pH meter with the help of 0.1N NaOH or 0.1N HCl as necessary. The whole mixture was then made up to 1000 ml with further addition of distilled water and mixed well. After adjustment of the pH, 6g^l⁻¹ gelrite (Ducefha, Netherlands) was added to obtain semi-solid medium. The mixture then gently stirred to complete dissolution of gelrite. After MS media preparation, all the instruments, glassware were sterilized to ensure aseptic condition.

There are several methods that were applied during the course of culturing different explants. For the explant/embryo culture method, sterilized dehusked seeds were the main source of explants in the experiment. The plant growth regulators and their respective number of combinations of explant/embryo culture were used as callus

initiation, where MS medium (Murashige and Skoog Medium), 2.5 g l^{-1} 2,4-D growth regulators and sucrose (3%) were required as pretreatment. The shoot differentiation was done using MS medium, i.e., MS powder, sucrose (3%) and Gelrite (6%) supplemented with 1.0 g l^{-1} NAA + 2.0 g l^{-1} BAP + 2.0 g l^{-1} Kinetin. Half-strength MS medium was used for root initiation.

Irradiation effect on calli for regeneration of plants from mature seeds of Binadhan- 13 and BR5 when the calli attained at convenient size they were divided into four parts. Each part contains 40 calli. Four parts were irradiated with three different doses of gamma irradiation (4, 6, 8 Gy) from ^{60}Co source at the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Thus, the calli were prepared for subsequent regeneration. After that calli were removed aseptically from the test tube and placed on a sterilized petri-dish and were cut into small pieces, so that no contact of parental tissue remained and these were inoculated to a freshly prepared medium for the maintenance of the calli without root shoot differentiation. After 21 days, these were sub-cultured to freshly prepared medium containing different hormonal supplements for the maintenance of callus or for root-shoot differentiation. After 7 and 14 days, all cultures were examined, and whitish, compact and nodular embryogenic calli were separated and again sub-cultured for further growth. The dishes showing signs of contamination were discarded. Repeated sub-culturing was done at an interval of 15 days for maintenance of calli and organogenesis. Some of the sub-cultured calli continued to proliferate and differentiate into shoots. When these shoots grew upto 3-4 cm in height, they were separated aseptically from each other and transferred into freshly prepared rooting medium to induce roots. The conical flasks containing plantlets were incubated in same environment. Two types of incubation period were maintained in this study for callus initiation; i.e; dark incubation and light incubation. The culture vessels with inoculated explants were incubated in dark condition for 21 days in a temperature controlled growth chamber ($25\pm 1^\circ\text{C}$) less than 16-hour photoperiod with a light intensity of 2000-3000 lux. Day to day observations was made to note the response. Potting mixture containing garden soil and cow dung in the ratio of 1:1 was mixed properly and autoclaved for 1 hr. in 121°C at 1.16 kg/cm^2 . After cooling the soil mixture was taken into 10cm plastic pots for growing the plantlets at *in vivo* condition.

The regenerated plantlets with sufficient root system were ready for transfer in soil. The plantlets were brought out from the controlled environment of the incubation room and were kept in the room temperature for 2-3 days to be acclimatized in the normal environment. The plantlets with sufficient roots was then taken out from the culture vessels and thoroughly washed in running tap water to remove all adherent culture medium. The plantlets were then transplanted to small plastic pots containing sterilized ground soil mixed with cow-dung. After transplantation, the pots with the plantlets were covered immediately with polythene bag to prevent excessive evapotranspiration. The pots were kept in the controlled environment of glass house to reduce shock. Then the plants covered

by the polythene bags in the pot were sprayed with Hogland's solution (Hoagland and Arnon, 1938) . After 4 days the polythene bags were gradually perforated to expose the plantlets into natural environment and after 7 days it was removed completely. When the plantlets grew well, these were transferred to earthen pots where they developed into mature plants.

Recording of data

To investigate the effect of different treatments of the experiment, data were collected on the following parameters:

$$\text{Percent (\%)} \text{ callus induction} = \frac{\text{Number of explants induced calli}}{\text{Number of explants inoculated}} \times 100$$

$$\text{Percent (\%)} \text{ survival rate} = \frac{\text{Number of irradiated calli survived}}{\text{Number of explants inoculated}} \times 100$$

$$\text{Percent (\%)} \text{ establishment} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} \times 100$$

Statistical analysis

The experiment was conducted in growth room and arranged in Completely Randomized Design (CRD) with three replications. The analysis of variance for different characters were performed, and mean values were compared by the Duncan's Multiple Range Test (DMRT) by using statistical package MSTAT-C software (Russel, 1986).

Results and Discussion

A callus is largely unorganized, continued proliferation of undifferentiated parenchyma cells from parent tissue on clearly defined semi-solid media. Various numbers of explants were inoculated on semi-solid media containing 2.0 mg l⁻¹ 2, 4-D concentration for callus formation (Figure 1). To achieve the ultimate goal of plantlet regeneration via embryogenic calli, two rice varieties (Binadhan-13 and BR5) were cultured on MS medium supplemented with different combinations of plant growth regulators. Mature embryos of these varieties were used as explants. Callus initiation performance results of these varieties are presented on (Table 1). Significant difference was observed in callus initiation at light conditions between two varieties, Binadhan-13 and BR5. Among the varieties, in dark condition, the highest average callus initiation percentage was observed in Binadhan-13 (83.27%), while it was 73.65% for BR5. On the other hand, highest average callus initiation (%) in light condition was observed in Binadhan-13 (74.14%) followed by BR5 (61.78%) (Figure 2).

Different doses of gamma radiation (0, 4, 6, and 8 Gy) were applied in calli of two rice varieties Binadhan-13 and BR5 to know the effect of gamma rays on plant regeneration (Hossain and Alam, 2001). After irradiation, callus was transferred on MS sub culture medium supplement with 2,4-D 2.0 gl⁻¹, NAA 0.5gl⁻¹ for better shoot growth.

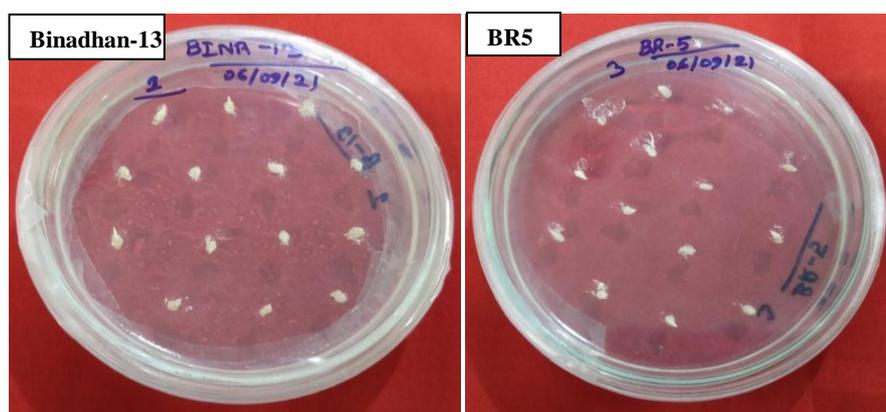


Figure 1. Inoculation of mature explant in MS medium.

Influence of light and dark condition on callus initiation from mature embryos of two rice varieties

Mature embryos of two rice varieties (Binadhan-13, BR5) were cultured in two different photoperiod's condition to observe callus response (24-hour under dark condition and 16/8-hour light using 3000 lux intensity). Calli grown under dark condition had higher cell mass than that under light condition, because the enhanced peroxidase activity and absence of oxidative stress might favor accumulation of polymers (Kevers *et al.*, 1995). Light is very important factor for callus induction, cell growth and production of plant secondary metabolites (Summart *et al.*, 2008). Among the conditions, average callus initiation efficiency was observed higher in dark condition followed by light condition (Table 1). From the present study, it was clear that calli induced and grew slightly better in dark than light condition.

Table 1. Effect of dark and light condition on callus initiation from mature embryos

Varieties	Replication	Average callus initiation in dark (%)	Average callus initiation in light (%)
Binadhan-13	R ₁ -R ₆	83.277	74.120
BR5	R ₁ -R ₆	73.650	61.785
Level of significance		*	*
LSD (0.05)		1.24	3.95
CV(%)		1.07	3.93

* = Significant at 5% level of probability

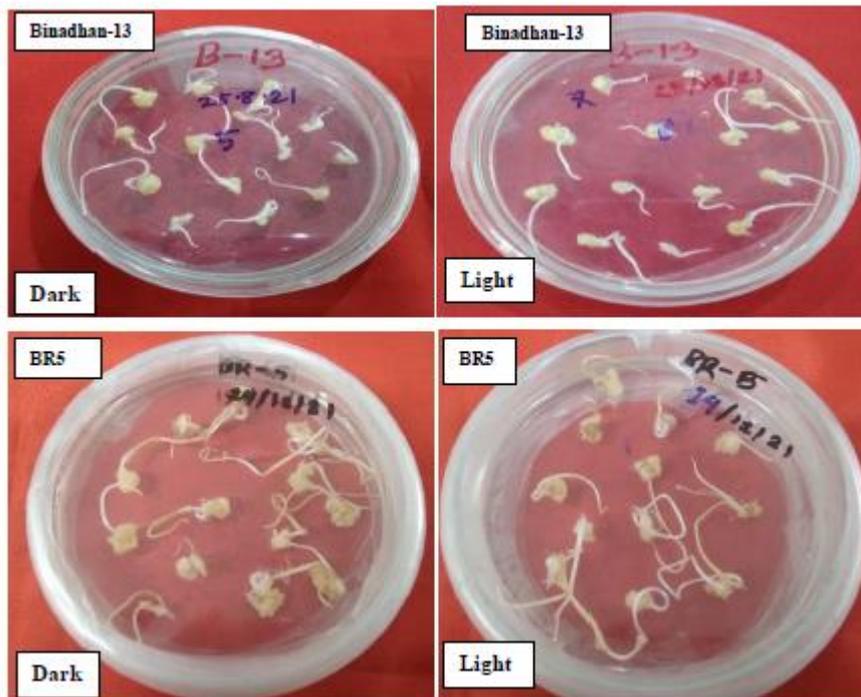


Figure 2. Callus initiation of two rice varieties cultured in MS medium supplemented with 2,4-D (2.0 mg/L) under dark and light condition at (25±3). Photo was taken after 15 days of inoculation.

Embryogenic callus initiation from mature embryos of rice varieties

The embryogenic callus is the cell with strong division ability and the potential of differentiation into somatic embryos. The mature embryos of two rice varieties were inoculated on the same media for callus initiation. Embryogenic callus formation invariably developed within 21 days. Results on embryogenic callus initiation performance of these varieties are presented in (Table 2). Among the varieties, high average embryogenic callus initiation (%) was observed in Binadhan-13 (77.14%) and BR5 (67.14%) in dark condition (Figure 3). There CV (%) value is (1.34) and LSD value (1.37) and there has significant difference in embryogenic callus initiation at dark condition between two varieties Binadhan-13 and BR5.

CV (%) value (5.39) found greater than LSD value (4.66); so, there has significant difference in embryogenic callus initiation at light condition between two varieties Binadhan-13 and BR5.

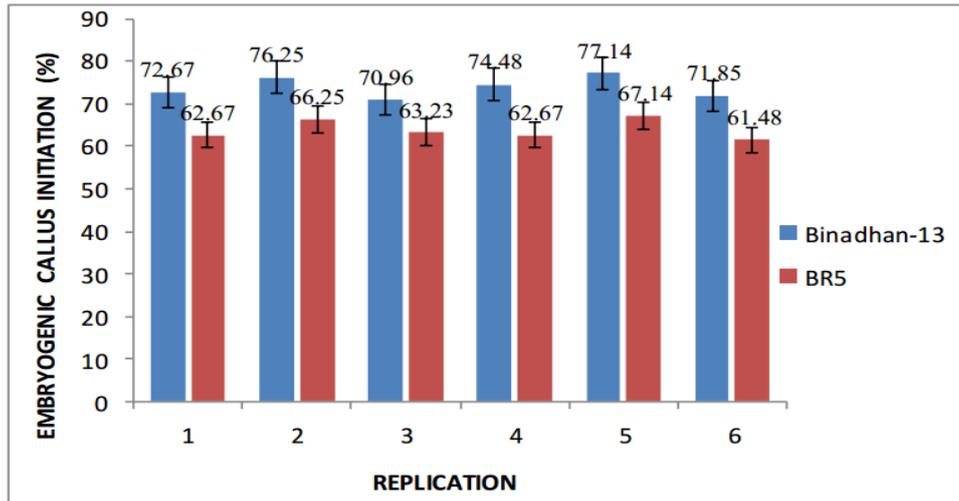


Figure 3. Embryogenic callus initiation response of mature embryos of two rice varieties in dark condition.

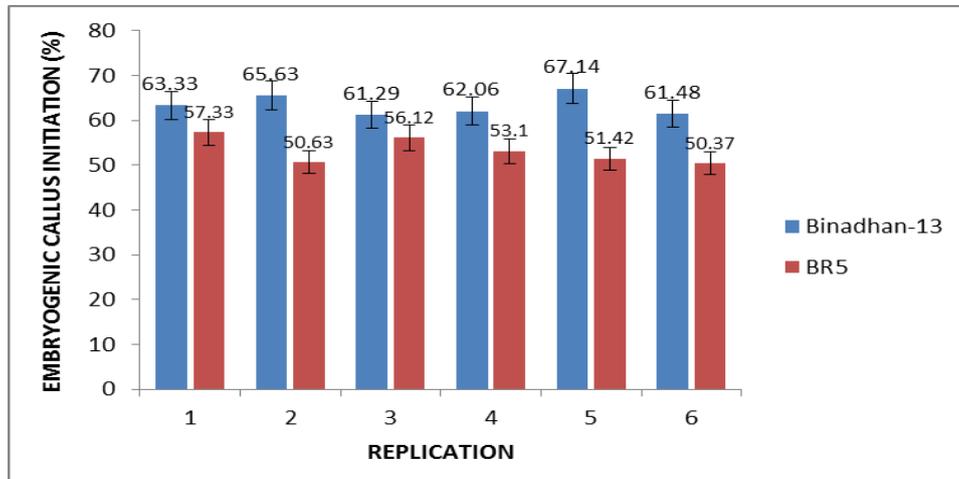


Figure 4. Embryogenic callus initiation response of mature embryos of two rice varieties in light condition.

Effect of irradiation on shoot regeneration of Binadhan-13 and BR5

Embryogenic calli of two rice varieties were cultured in MS medium with different plant growth regulators (NAA g l^{-1} + BAP g l^{-1} + Kn g l^{-1}). After 21 days of irradiation (0, 4, 6, and 8Gy), it was clearly observed (Table 2) that the extent of regeneration ability varied from different radiation doses. In both genotypes, regeneration percentage were found to be higher in the control and decreased with the increase of gamma ray dose. The high percentage of shoot induction from calli were in Binadhan-13 and BR5 in lower doses. After 4 weeks, these calli were turned into shoots and the shoot formation efficiency was measured on the basis of their vigor (Figure 5). This experiment was also conducted to check out root induction ability for first 3 replications for control and another 3 replications within different doses of gamma radiation. The reason was may be the tissues of these varieties were more sensitive to inhibition by gamma radiation because increase of high doses created low survivability of callus. Both growth and regeneration capacity decreased with increasing levels of gamma rays; however, plant regeneration capacity was more sensitive to gamma rays than growth (Hossain and Alam, 2001).

Table 2. Effect of irradiation on callus for shoot induction of Binadhan-13 and BR5 using MS media with growth regulators

Varieties	Dose (Gy)	No. of calli inoculated	No. of calli showing shoot induction	Shoot regeneration %
Binadhan-13	0	20	14	70
	4	20	12	60
	6	20	11	55
	8	20	9	45
BR5	0	20	12	60
	4	20	10	50
	6	20	9	45
	8	20	8	40

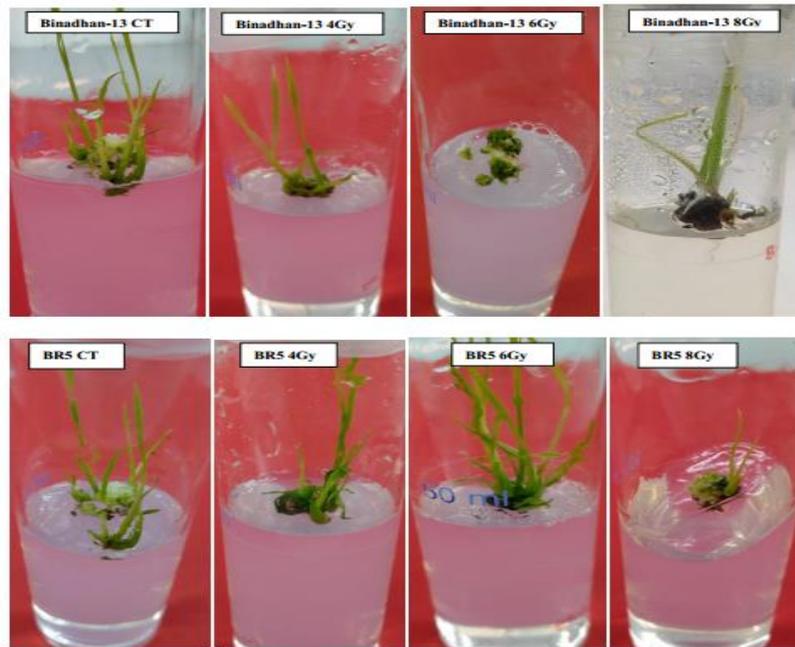


Figure 5. Effect of radiation on embryogenic calli of Binadhan-13 and BR5 at different doses of gamma ray (CT=Control). Photos were taken after 21 days of inoculation.

Effect of gamma irradiation on root induction from shoot

Half strength of MS media was used to see the rooting response of the regenerated shoot. Shoots were collected from irradiated calli exposed to the different doses (0, 4, 6 and 8Gy) of gamma rays. After 15 days, it was clearly observed that the extent of root induction ability varied from shoots that were differentiated due to different doses of irradiated calli. In both varieties, root induction percentage were found to be higher in the control and gradually decreased with the increasing doses of gamma rays (Figure 6). But in some cases, root induction prolificacy followed irregular trend in the higher doses of gamma rays. The root induction ability from shoot was higher in control treatment (0 Gy). Root induction frequency of two varieties under the various doses was observed and the results are presented in (Table 3). When the roots sprouted, these were turned into complete plant after 2-3 weeks later. This experiment was performed to check out root induction ability in control condition and within different doses of gamma irradiation. This finding is similar to Sarwar, (2003) who reported that root induction ability was decreased gradually with increase in the radiation dose.

Table 3. Effect of irradiation on root induction of Binadhan-13 and BR5

Varieties	Dose(Gy)	No. of shoot inoculated	No. of shoot showing root induction	Root induction %
Binadhan-13	0	10	8	80
	4	10	7	70
	6	10	6	60
	8	10	4	40
BR5	0	10	7	70
	4	10	6	60
	6	10	5	50
	8	10	3	30

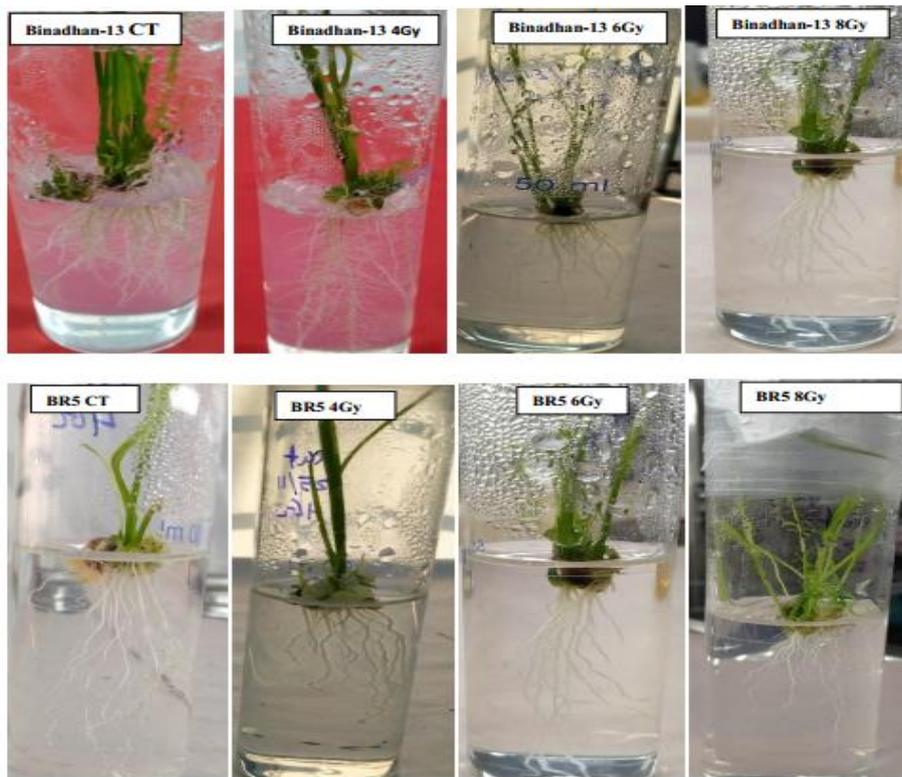


Figure 6. Root formation from regenerated shoots of Binadhan-13 and BR5 (CT= Control). Photos were taken after 28 days of inoculation.

Establishment of Plantlets

After sufficient development of root system, the small plantlets were taken out from the culture vessels without damaging roots. Excess agar around the roots was washed off by tap water to prevent microbial infection. Then the plantlets were transplanted in small pots. When the plantlets grew to a height of above 10 cm and sufficient roots were proliferated, those were transferred to earthen pots following the procedure described in materials and methods. The growth condition, tillering capacity of plantlets and survival rate of the plantlets in the pots were satisfactory.

Conclusion

This comparative study investigated optimal conditions for callus initiation and the impact of gamma rays on regeneration in Binadhan-13 and BR5 rice varieties. Callus initiation efficiency was influenced by genotypes, growth regulators, and light conditions, with 2.0 mg/L 2,4-D in dark conditions proving most effective. Gamma irradiation, particularly at 8 Gy, negatively affected *in vitro* regeneration, while 4 Gy demonstrated superior shoot and root regeneration. The study highlights the potential of gamma irradiation for inducing useful variability in rice varieties. The developed *in vitro* regeneration protocol, which successfully produced healthy plantlets, shows potential for improving different rice varieties in future breeding programs.

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EVALUATION OF CULTIVATED GLADIOLUS VARIETIES OF BANGLADESH AND RADIO SENSITIVITY TEST FOR VARIETAL IMPROVEMENT

N. Akhther^{1*}, M.R. Islam¹ and M.K. Hassan²

Abstract

The study was conducted at the research field of Nalitabari Sub-station, BINA, Sherpur during the period November 2019 to May 2020 to characterize, evaluate and select promising gladiolus varieties suitable for Bangladesh and to determine the optimum dose of radiation for LD₅₀ for varietal improvement. The parameters studied were plant height, days to 1st flowering, number of leaves per plant, leaf length, leaf breadth, length of spike, length of rachis, number of flower per spike, flower diameter, number of corms per plant, weight of single corm, vase life, per cent germination, per cent survivability, shoot length and root length. The longest plant (154.2 cm) was observed in White Prosperity, while the shortest plant (101.7 cm) was in Regency. The longest leaf (70.3 cm) was found in Red Cascade and the shortest leaf (48.0 cm) was found in Her Majesty. The highest number of floret per spike was produced by Red Cascade (17.3) followed by White Prosperity (17.0). The Regency produced the lowest number (8.0) of floret per spike. The longest vase life (9.3 days) was observed in American Beauty followed by (8.7 days) Red Cascade, while the shortest vase life (5.7 days) was recorded in Wine and Roses. In radio sensitivity test, 30-40 Gy of gamma irradiation would be the optimal dose for inducing useful mutation in gladiolus.

Key words: Gladiolus, Flower, Morphology, Gamma irradiation, Radio-sensitivity

Introduction

Gladiolus (*Gladiolus grandiflorus* L.) of Iridaceae family is one of the most important cut flowers in the world (Bai *et al.*, 2009) as well as in Bangladesh. Commercial cultivation of flowers in Bangladesh actually started during 80s, and 1991, flowers were listed as exportable products from Bangladesh (Mukul 2020). Gladiolus ranked the 1st in terms of production (9914 tons) with a market share of 31% (Hossen 2018). Today, floriculture has emerged as a lucrative profession in Bangladesh with a much higher potential for returns than most other field and horticultural crops (Sultana, 2003). Bangladesh is well suited for growing cut flowers and other ornamental plants due to favorable climatic and other conditions like cheap land, low labour cost, relatively low capital investment and high value addition (Dadlani, 2004). Now-a-days, farmers are

¹Horticulture Division, Bangladesh Institution of Nuclear Agriculture, Mymensingh-2202, Bangladesh;

²Department of Horticulture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh,

*Corresponding author's email: nakhther@yahoo.com

commercially growing gladiolus in Bangladesh (Islam and Haque, 2011). To enrich the genetic resources of gladiolus, available germplasm (varieties) from home and abroad are to be collected for evaluation with a view to its further improvement. Some progressive farmers, nurserymen and private entrepreneurs, have already collected different gladiolus varieties from abroad and other sources, and growing successfully. The exotic varieties are well-known for their better spike quality and multiplication rate of corms and cornels. However, their suitability under local conditions needs to be properly tested before recommendation as variety. Diverse genetic resources are the key for varietal improvement programme. Selection of ideal variety(ies) based on morphological traits and other postharvest quality attributes from the collected germplasm (varieties) may be of immense value for further improvement of gladiolus in Bangladesh.

Mutagenesis in ornamental plants represents a powerful tool, not only to clarify physiological mechanisms in plant functioning (Honda *et al.*, 2006), but also to obtain new varieties useful for the floriculture industry (Canul-Ku *et al.*, 2012). Changes in phenotypic traits, such as the color, shape or size of the flower and chlorophyll variegation in leaves, can easily be detected. Mutation induction has proven to be an efficient method for generating ornamental plant varieties that meet the quality standards as demanded by the international market. Many of the objectives of genetic improvement programs consist of achieving morphological changes and inducing resistance to biotic and abiotic stresses can be achieved through mutation induction. Mutation in the biosynthetic pathway of structural or regulatory genes may also cause a change in the color of the gentian (*Gentiana triflora*) flower (Nakatsuka *et al.*, 2005). Gamma ray is one of the most important mutagenic agents within ionizing radiation because they have been shown to be highly penetrating and potent in inducing variability in plants (Deshpande *et al.*, 2010). Radio sensitivity depends on the type of radiation and the dose used, as well as on the explant's traits; type of tissue and size; degree of development; and moisture content (Datta and Teixeira-da Silva, 2006) as these traits alter the cells' response to radiation. Sensitivity also depends on the genetic constitution of the plant material, such as the number and chromosomal size, the nucleotide, the heterochromatin, centromere number and position, degree of polyploidy, nuclear DNA content and replication time at initial stages, as well as cytoplasmic, biological, chemical and environmental factors (Deshpande *et al.*, 2010). Therefore, the present experiments were designed to characterize, evaluate and to select promising variety(ies) suitable for Bangladesh and to determine the optimum dose of gamma irradiation for LD₅₀ in gladiolus for varietal improvement through mutation induction.

Materials and Methods

Two experiments were conducted during the period from November 2019 to May 2020 at the research field of the Nalitabari Sub-station, Bangladesh Institute of Nuclear Agriculture (BINA), Sherpur. The experimental location was in high land, and the land was prepared by power tiller followed by laddering. All weeds and other stubbles were removed

prior to planting the corms. The design of the experiment was randomized block design consisting of 8 treatments with 3 replications, with a view to find out the overall performance of different exotic popular cultivars of *Gladiolus* viz. White Prosperity, Red Cascade, Novalux, American Beauty, Regency, Her Majesty, Jester and Wine and Roses. The germplasm were collected from farmers' field of Godkhali, Jashore and Savar, Dhaka, and exotic sources. The unit plot size was 1.5 m × 2 m. Medium sized (3.5-4.5 cm) corms of different gladiolus varieties were planted at about 7-8 cm depth in the plot maintaining a spacing of 30 cm × 25 cm. Manures and fertilizers were applied at the rate of cowdung- 5 t/ha, Urea- 180 kg/ha, TSP- 70 kg/ha, MoP-140kg/ha, Boron- 2.0 kg/ha and Zinc- 4.0 kg/ha and Sulphur 30 kg/ha (FRG, BARC, 2018). All manure and chemical fertilizers except urea were applied as basal dose during final land preparation and urea was applied in three splits at top-dressed at 30, 45 and 60 days after planting. Different intercultural operations like irrigation, weeding, earthing up, stacking, pesticide and fungicide application were performed as needed. The parameters studied were plant height (cm); days to 1st flower initiation; number of leaves per plant (NLPP), leaf length (cm) and leaf breadth (cm) at 1st flowering stage; length of spike (cm), spike diameter (cm), length of rachis (cm), number of flower per spikes, flower diameter (cm), number of corms per plant, weight of single corm (g), diameter of corm (cm), number of cormels per plant, diameter of cormels (cm) and shelf life (days). The spikes were cut when lower one or two florets showed color but still in tight bud stage. The cut spikes were kept into water to study the vase life. Corms and cormels were harvested only when the leaves turned into brown colour (Mukhopadhyay, 1995). For radio sensitivity test, fifty (50) corms were irradiated with gamma rays at the doses of 0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy by ⁶⁰Co gamma irradiator at Bangladesh Institute of Nuclear Agriculture, Mymensingh. Data were recorded on corm germination (%), corm survival (%), shoot length and root length. Data were analyzed using Analysis of Variance (ANOVA) by Statistix 10 (Version 10.0 Analytical Software USA). The means for all the treatments were calculated and analysis of variance (ANOVA) for all parameters was performed by F-test. Statistically significant differences among the different doses were identified by LSD at the 5% levels of significance as described by Gomez and Gomez (1984).

Results and Discussion

Plant height

Plant height is one of the most important characters as it contributes towards higher spike length with more number of florets and thereby enhances spike quality. Plant height among the genotypes ranged from 154.3 to 101.7 cm. The longest plant was observed in the White Prosperity (154.3 cm) while the shortest plant (101.7 cm) was observed in Regency (Table 1). The variation observed in plant height among the genotypes might be due to difference in genetically constituents as well as environmental effects. Wide variation in plant height amongst some genotypes of gladiolus was observed by Hossain *et al.* (2011)

and Swaroop (2010). Singh *et al.* (2017) recorded plant height from 80.3-134.7 cm with a mean of 112.0 cm in ten hybrids of gladiolus. In another study the longest plant (90.86 cm) also found in White Prosperity among 11 varieties examined (Solanki *et al.*, 2019).

Days to 1st spike initiation

The minimum days taken for spike initiation (69) was observed in Wine and Roses followed by Jester (70) maximum days in spike initiation (81.0) was observed in Regency (Table 1). Solanki *et al.* (2019) observed a range from 69.04 (White Prosperity) -83.73 (Candy Man) days required for 1st spike initiation of gladiolus. Variation in days to spike initiation and 1st floret opening in gladiolus seem to be genetically controlled as reported by Pragma *et al.* (2010).

Number of leaves, leaf length and leaf breadth

Significant variation was observed as to the number of leaves amongst the genotypes. The maximum number of leaves (9.0) was obtained from the White Prosperity followed by American Beauty (9.0) and the minimum number of leaves (7.7) was found from Regency (Table 1). The longest leaf (70.3 cm) was found in Red cascade and the shortest leaf (48.0 cm) was found in Regency. On the other hand, the largest leaf breadth (4.0 cm) was found American Beauty and the smaller leaf breadth (2.7 cm) was found in Wine and Roses (Table 1). This result was in agreement with the findings of Hossain *et al.* (2011), who found number of leaf ranged from 8.50-12.25. Hoque *et al.* (2019) found that number of leaf was varied significantly ranges from 11.7-8.0. Wide variation in leaf length amongst some genotypes of gladiolus was observed by Singh, and Dadlani (1990). This variation might be due to genotype as well as some known and/or unknown environmental factors. Plant produces food materials through the process of photosynthesis. With the increasing number of leaves, photosynthesis generally increases, and plant can produce more food that influences the growth and development of the plant.

Table 1. Morphological characters of eight gladiolus germplasm

Name of the germplasm	Plant height (cm)	Days of 1 st flower initiation	No. of leaves per plant	Leaves length (cm)	Leaves breadth (cm)	Spike length (cm)	Spike diameter (cm)
White Prosperity	154.3a	74.0bc	9.3a	57.4bc	3.3b	103.0a	6.8b
Red cascade	140.3ab	77.0b	8.0ab	70.3a	2.9bc	98.3ab	8.0a
American Beauty	127.3bc	71.0cd	9.0ab	59.0b	4.0a	87.3bc	6.1cd
Nova lux	125.3bc	75.0b	8.7ab	58.2bc	3.0bc	99.3ab	6.5bc
Her Majesty	102.3d	77.0b	8.0ab	58.0bc	3.3ab	64.7d	5.8d
Regency	101.7d	81.0a	7.7b	48.0c	2.9bc	68.3d	6.8b
Jester	122.3b-d	70.0d	8.7ab	53.7bc	3.0bc	78.0cd	6.2cd
Wine and roses	104.3cd	69.0d	8.0ab	53.3bc	2.7c	72.3d	6.9b
CV	11.2	2.5	9.17	10.47	12.11	9.7	4.3
Level of sig.	**	**	NS	*	*	**	**
LSD _(.05)	23.9	3.2	1.4	10.5	0.67	14.3	0.50

Spike length and diameter, and rachis length

The spike length, spike diameter and rachis length, number of varied significantly among the germplasms. The longest spike (103.0 cm) was produced by White Prosperity and the shortest spike (64.7cm) was found in Her Majesty. On the other hand, the thicker spike diameter (8.0 cm) was found in Red Cascade and thinner spike diameter (5.8 cm) was found in Her Majesty (Table 1). Bhagur (1989) found that spike length ranged from 61.60 to 137.97cm in varietal evaluation of gladiolus. Hossain *et al.* (2011) recorded significant variation in spike length which ranged from 92.05-59.63 cm. The highest rachis length was observed in White Prosperity (77.5cm), and the lowest rachis length (34.0 cm) was observed in Wine and Roses (Table 2). Rachis length differed from 19.0 cm to 53.7 cm with an average of 36.4 cm (Hoque *et al.* 2018). Tirkey *et al.* (2018) recorded a variable rachis length in six gladiolus genotypes that ranged from 37.3 cm to 62.7 cm. The variation in different characters among varieties might be due to variation of genetic traits and the effect of prevailing environmental conditions (Kumar, 2015).

Number of florets per spike and floret diameter

Variable flower characters were observed in the studied accessions (Table 2). Eight genotypes had different floret colour and marking (Figure 1). Variation in average number of floret per spike amongst the genotypes ranged from 8.0 to 17.3. The highest number of floret per spike was produced by Red Cascade (17.3) followed by White Prosperity (17.0). The Regency produced the lowest number (8.0) of floret per spike. White Prosperity and American Beauty produced the largest floret (10.4 cm) and the smallest floret (7.5 cm) was produced by Wine and Roses. The number of flowers per spike varied from 5.33 to 20.00 as reported by Negi *et al.*, (1982), .8.3 to 14.3 by Hossain *et al.* (2011); 11.2 to 15.0 by Tirkey *et al.* (2018), 7.0 to 15.7 by Hoque *et al.* (2019). Solanki *et al.* (2019) stated that maximum number of floret per spike (18.00) was found in White Prosperity followed by American Beauty (17.73) and minimum number of florets/spike (12.93) was found in Novalux.

Vase life

The vase life of flowers also differed among the cultivars, and the longest vase life (9.3 days) was observed in American Beauty followed by in Red Cascade (8.7 days) and the shortest longevity of flowers (5.7 days) was recorded in Wine and Roses (Table 2). Hoque *et al.* (2019) reported that vase life in the accessions varied and ranged from 8 to 11 days. The present findings are in conformity with the findings of Swaroop (2010) and Pandey *et al.* (2012) who studied morphological traits of various gladiolus genotypes and found wide variation among the studied genotypes. The variation in different characters among varieties may be due to genetic traits and the effect of prevailing environmental conditions.

Table 2. Floral properties and vase life of collected eight gladiolus germplasm

Name of the germplasm	Floret colour	Rachis length (cm)	No. of floret per spike	Flower diameter (cm)	Vase life (days)
White Prosperity	White in colour, ruffled	77.7a	17.0a	10.4a	8.0b-d
Red cascade	Bright red with white blotches.	68.0ab	17.3a	9.8a	8.7ab
American Beauty	Reddish pink in colour with whitish throat	48.3cd	12.3bc	10.4a	9.3a
Nova lux	Yellow with deep yellow throat	60.3bc	13.3b	10.2a	8.3a-c
Her Majesty	Deep violet	43.0d	12.0b-d	8.0bc	7.3cd
Regency	Deep maroon with velvety appearance and highly ruffled	44.3cd	8.0e	8.5b	8.0b-d
Jester	Deep yellow with red throat	50.0cd	9.3c-e	7.6bc	7.0d
Wine and roses	Soft rose-pink flowers with a red wine throat and a ruffled edge	34.0d	9.0de	7.5c	5.7e
CV		18.2	14.2	6.33	9.1
Level of sig.		**	**	**	**
LSD (.05)		17.0	1.0	1.0	1.2

Number, size and weight of corm and cormels

Different genotypes exhibited significant variation for corm characters (Tables 3, Figure 2). The number of corms produced per plant was the highest in White Prosperity (2.1) followed by American Beauty (1.9), Nova lux and Jester (1.7). The lowest number of corms (1.3) was produced by the Regency and Her Majesty. Variation in number of corm per plant amongst some genotypes of gladiolus was as 1.0-4.0 (Anuradha and Gowda, 1994) and 1.15-2.55 (Hossain *et al.*, 2011). Weight of individual corm was also found to be varied significantly among the genotypes. The highest corm weight was obtained from in American Beauty (76.1g) and the lowest in Her Majesty (29.0 g). The highest diameter (6.3 cm) of large corm was observed in White Prosperity and the lowest was (4.8 cm) in Jester. Sharma and Sharma (1984) reported that corm weight was the highest in Yellow genotype (67g) and lowest in GL-25(18g) genotype which was more or less in consonance with the present investigation. Hoque *et al.* (2019) also found that the corm size different among the varied from 6.6 to 3.6 cm. The number of cormel per plant differed significantly affected by genotypes (Table 3). The highest number of cormels per plant was obtained from the White Prosperity (34.0) followed by Nova Lux (30.0), and the Jester (13.3) produced the lowest number of cormels per plant.



Fig. 1. Variation in colour and forms of spikes of the collected eight gladiolus varieties.

Table 3. Properties of corms and cormels of eight gladiolus varieties

Name of the germplasm	No. of corm per plant	Size of single corm (cm)	Wt. of single corm (gm)	No. of cormels per plant	Size of cormels (cm)
White Prosperity	2.1	6.3a	61.6ab	34.0a	1.4ab
Red cascade	1.4	5.6ab	63.7ab	29.7ab	1.1ab
American Beauty	1.9	6.2a	76.1a	22.5a-c	1.6ab
Nova lux	1.7	6.0a	58.7ab	30.0a-b	1.2ab
Her Majesty	1.3	5.1bc	29.0c	19.7b-c	0.9b
Regency	1.3	4.9bc	42.7bc	19.3b-c	2.4a
Jester	1.7	4.8c	40.0bc	13.3c	1.3ab
Wine and Roses	1.6	5.9a	63.0ab	21.0bc	1.1ab
CV	-	7.98	28.4	31.1	59.5
Level of sign.	NS	**	*	NS	NS
LSD _(.05)	-	0.8	27.1	12.9	1.4

Radio sensitivity test

The maximum corm germination (98%) was found control (non irradiated) corms in Wine and Roses (Table 4). On the other hand, 10 Gy irradiated corms in American Beauty had maximum germination (95%), followed by 20 Gy irradiated corms White Prosperity (94%). The minimum corm germination (1%) was found in 80 Gy irradiated corms. Non irradiated control plants showed maximum survivability (95%) in Wine and Rose (Table 5). In case of irradiation, 10 Gy irradiated White Prosperity and American Beauty and 20 Gy irradiated Red Cascade showed 90% survivability. No plant was found to survive in the 80 Gy irradiated corms (Table 5). Among the doses of irradiation, 80 Gy showed lethal. The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). Seed germination with abnormalities in mitotic cycles and in metabolic pathways of the cells have correlated reported by Micco *et al.* (2011). The reduction in germination and survival may be due to absorption of ionizing radiation in biological materials, acting directly on critical targets in the cell (Kovacs and Keresztes, 2002). Bashir *et al.* (2013) also reported that the seed germination percentage and percent survival decreased with an increase in dose of the gamma irradiation. The present findings are in agreement with the above mentioned reports. Similar findings were also reported where in, higher doses of gamma radiation reduced germination percentage and survival in fennel (Verma *et al.*, 2017).

In case of shoot and root growth highly differences were observed among the lower and higher doses of irradiation. The longest shoot length (34 cm) was found 10 and 30 Gy

irradiated Nova Lux and shortest shoot length (10 cm) was found 70 Gy irradiated in Jester. In respect of root growth, longest root (16 cm) was found in 10 and 20 Gy irradiated in Nova Lux and White Prosperity and the minimum root length (3 cm) in 70 Gy irradiated in Regency (Table 6, Figure 2). Growth inhibition demonstrated in the case of high radiation dose could be due to cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome (Preussa and Britta, 2003). The results of the experiment indicated that high dose of gamma radiation reduced germination percentage, seedling survival, shoot length and root length. It was found that more than 30 Gy of gamma irradiation to cause near about 50% reduction in root length and shoot length (Tables 6 and 7). It is expected that 30 Gy of gamma ray would be the optimal dose for inducing useful mutation gladiolus which will help to develop desirable mutants.



Fig. 2. Corms of eight gladiolus varieties (A), and radio-sensitivity test of the selected gladiolus of variety, namely American Beauty (B).

Table 4. Effect of acute exposure of different doses of gamma rays on germination of gladiolus corms

Dose (Gy)	% Germination							
	White Prosperity	Red cascade	American Beauty	Nova Lux	Her Majesty	Regency	Jester	Wine and Roses
10	93	93	95	90	89	89	93	93
20	94	92	94	90	90	90	92	93
30	91	90	92	90	87	87	90	90
40	84	82	82	80	80	80	82	82
50	76	74	75	75	70	69	74	74
60	41	40	40	35	25	20	40	45
70	15	20	20	14	10	11	18	22
80	02	01	04	02	00	00	01	03
0	96	95	96	94	92	93	95	98

Table 5. Effect of acute exposure of different doses of gamma rays on survivability of gladiolus corms

Dose (Gy)	% Survivability							
	White Prosperity	Red Cascade	American Beauty	Nova Lux	Her Majesty	Regency	Jester	Wine and Roses
10	90	88	90	88	84	85	87	90
20	89	90	88	90	88	86	89	90
30	86	85	88	86	84	82	80	82
40	80	74	75	75	72	70	72	75
50	68	62	70	60	50	48	50	65
60	35	34	32	33	25	26	25	32
70	12	12	15	10	05	04	08	14
80	00	00	00	00	00	00	00	00
0	93	92	92	90	88	87	90	95

Table 6. Effect of acute exposure of different doses of gamma rays on shoot length of gladiolus seedlings

Dose (Gy)	Shoot length (cm) at 20 Days after planting								
	White Prosperity	Red Cascade	American Beauty	Nova Lux	Her Majesty	Regency	Jester	Wine and Roses	White Prosperity
10	39	39	37	40	35	36	37	38	14
20	38	36	36	38	36	33	32	38	16
30	38	39	36	40	34	35	36	36	15
40	27	26	25	26	20	21	23	25	08
50	24	23	23	22	20	20	21	24	06
60	16	14	16	14	15	14	13	12	05
70	15	12	14	12	12	13	10	13	05
80	00	00	00	00	00	00	00	00	00
0	40	37	37	41	35	35	35	35	14

Table 7. Effect of acute exposure of different doses of gamma rays on shoot and root length of gladiolus seedlings

Dose (Gy)	Root length (cm) at 20 Days after planting							
	White Prosperity	Red Cascade	American Beauty	Nova Lux	Her Majesty	Regency	Jester	Wine and Roses
10	14	12	13	16	12	13	13	14
20	16	11	16	14	12	12	14	15
30	15	14	14	15	13	14	12	14
40	08	09	07	08	09	09	08	10
50	06	06	07	06	05	05	06	08
60	05	04	05	06	05	04	06	05
70	05	04	07	04	04	03	04	06
80	00	00	00	00	00	00	00	00
0 (Control)	14	15	14	1	13	14	15	16

Conclusion

The study was undertaken to evaluate and select promising gladiolus lines and to determine the optimum dose of gamma irradiation for effective mutation. Considering overall performance, especially number of florets per spike and spike length, the American Beauty was the best followed by White Prosperity and Red Cascade. American Beauty was also the best in terms of longer vase life and attractive colour. Irradiation doses of 30-40 Gy were found to effectively induce mutation since the values for most the parameters were reduced by approximately 50% or more at the following doses from 40- 80 Gy.

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EVALUATION OF POSTHARVEST BEHAVIOUR OF BITTER GOURD AS INFLUENCED BY GAMMA IRRADIATION AND MODIFIED ATMOSPHERE PACKAGING

M.M. Rashid¹, N. Akhther^{2*}, M.K. Hassan¹, M.R. Islam² and L.B. Rafiq¹

Abstract

An experiment was conducted at the Postharvest Laboratory of Horticulture Division, Bangladesh Institute of Nuclear Agriculture during November to December 2022 to examine the effects of different doses of gamma irradiation and Modified atmosphere packaging (MAP) on shelf life and quality of bitter gourd at ambient condition. The experiment comprised ten postharvest treatment viz., T₀ =Control (untreated, unwrapped and non-irradiated); T₁ =Gourds wrapped in Polypropylene (PP) bag; T₂ =Gourds treated with 3% CaCl₂; T₃ = Irradiated at 1000 Gy, T₄ = Irradiated at 2000 Gy, T₅ =3% CaCl₂+ PP bag, T₆ = 1000 Gy+ PP bag, T₇ = 2000 Gy+ PP bag, T₈ =3% CaCl₂, + 1000 Gy+ PP bag and T₉ = 3% CaCl₂ + 2000 Gy+ PP bag. The single-factor experiment was carried out in a completely randomized design with three replications. Parameters investigated were fruit colour, shrinkage, moisture content, dry matter content, weight loss, disease incidence (DI), disease severity (DS) and shelf life. The minimum weight loss (3.49%), shrinkage (0.00%), DI (11.11%), DS (6.67%) and longest shelf life (10 days) were found in bitter gourds treated with T₈ treatment, whereas the maximum weight loss (52.31%), shrinkage (3.89%), DI (77.78%), DS (76.67%) and shortest shelf life (4 days) were T₀ treatment at 8 days after storage. The findings would have great impact in reducing enormous postharvest loss of bitter gourd and maintain their quality during postharvest handling and marketing under ambient condition.

Key words: Bitter gourd, Sanitizers, Gamma irradiation, MAP, Polypropylene

Introduction

Bitter gourd also known as bitter melon (*Momordica charantia*) is a member of cucurbitaceae family and it is one of the most popular vegetable in Bangladesh. The total production of bitter gourd in Bangladesh was about 65421.79 metric tons from 11639.22 hectares of land with an average yield of 5.62 t/ha (BBS, 2022). Emerald green young fruits are eaten as vegetables that turn to orange-yellow when ripe (Grover and Yadav, 2004). The bitter flavor is caused by the alkaloid momordicine generated in fruit and leaves (Din *et al.*, 2011). The fruits are used as antidiabetic, antitumorous, anticancer, anti-inflammatory, antiviral, and cholesterol lowering effects (Ahmed *et al.*, 2001). Bitter gourd is a good source of Vitamin A, Vitamin C, phosphorus and iron (Sultana and Bari, 2003; Paul *et al.*,

¹Department of Horticulture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

²Horticulture Division, Bangladesh Institution of Nuclear Agriculture, Mymensingh-2202, Bangladesh

*Corresponding author's email: nakhther@yahoo.com

2009). By delaying the ripening and senescence of vegetables, irradiation has been shown to be effective for reducing postharvest losses and extending the shelf life (Mostafavi *et al.*, 2010). Irradiation has been declared a safe and nonchemical technology for use on fruits and vegetables by the World Health Organization, the Food and Agriculture Organization, and the International Atomic Energy Agency (Ben-Fadhelet *et al.*, 2021). Nowadays, postharvest application of different calcium salts such as calcium chloride, calcium phosphate, calcium propionate and calcium gluconate have gained popularity in order to reduce various physiological disorders of fruits and vegetables (Aguayo *et al.*, 2008; Manganaris *et al.*, 2005). Calcium Chloride delayed fruit ripening, improved resistance to fungal attack and maintained structural integrity of cell walls (Mahajan *et al.*, 2004). Modified atmosphere packaging which is commonly known as MAP is used to enhance the shelf life of perishable horticultural products like fruits and vegetables (Kitinoja and Kader 2004). MAP effectively extends the postharvest shelf life of fresh commodities by delaying their enzymatic browning, reducing respiration rate, minimizing metabolic activity and by preserving their visual appearance (Waghmare and Annapure 2013). Recently the application of gamma irradiation on various foods has increased for the reason of its effect on insect disinfestations and improved food security. Ionizing irradiation reduces the spoilage and hence enhances the shelf life of many vegetables and spices (Akhther *et al.*, 2022). High postharvest loss is now a problem worldwide and it is to be minimized by 50% by 2030 to achieve SDG target 12.3. The recent report mentioned that 17-32% of fruits and vegetables are spoiled in Bangladesh (Hassan *et al.*, 2021). Hence, any attempt to reduce postharvest loss warrants investigation through reducing water loss, rates of respiration and ethylene production, chilling injury, and microbial activity. There is scanty of works which have been done regarding the preservation of bitter gourd under different storage conditions as mentioned above. In this regard, modified atmosphere packaging, sanitization and radiation technology were investigated to maintain postharvest quality and reduces losses of bitter gourd.

Materials and Methods

Experimental materials

The experiment was conducted at the Postharvest Laboratory of Horticulture Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh November-December, 2022 to study the standardization of sanitizer, gamma irradiation and modified atmosphere packaging for improving shelf life and quality of bitter gourd. A hybrid variety of bitter gourd, namely Tia, was used for conducting the present experiment. Fruits were harvested at the green stage of commercial maturity (approximately 20 days after pollination) from a farmers' field of Jamalpur District. The collected fruits were sorted into categories of uniform size, shape, and colour, checked to be free from defects or blemishes. After that the desired fruits were washed with tap water, and dried at room temperature for 15 min prior to treatment. The experimental bitter gourds' initial averages (5 gourds) of length, weight, breadth, and colour were 9.26 cm, 207.8 g, 4.33 cm and fully green,

respectively. The experiment comprised the treatments T_0 = Control, T_1 = Bitter gourds wrapped in 25 μ PP bag, T_2 = Bitter gourds treated with 3% CaCl_2 for 20 min., T_3 = Bitter gourds gamma irradiated at 1000 Gy, T_4 = Bitter gourds gamma irradiated at 2000 Gy, T_5 = Bitter gourds treated with 3% CaCl_2 for 20 min. and wrapped in 25 μ PP bag, T_6 = Bitter gourds gamma irradiated at 1000 Gy and wrapped in 25 μ PP bag, T_7 = Bitter gourds gamma irradiated at 2000 Gy and wrapped in 25 μ PP bag, T_8 = Bitter gourds treated with 3% CaCl_2 for 20 min., gamma irradiated at 1000 Gy and wrapped in 25 μ PP bag, T_9 = Bitter gourds treated with 3% CaCl_2 for 20 min., gamma irradiated at 2000 Gy and wrapped in 25 μ PP bag. The single-factor experiment was laid out in completely randomized design with three replications of three fruits per replication. Data were analyzed using analysis of variance (ANOVA) by Statistix 10 (Version 10.0 Analytical Software USA). The means for all the treatments were calculated and ANOVA for all parameters was performed by F-test. Statistically significant differences among the different doses were identified by LSD at the 1% and 5% levels of significance as described by Gomez and Gomez (1984).

Parameters investigated

Physiological weight loss:

For assessment of physiological weight loss of bitter gourd of each replication of each treatment was separately weighed using digital electronic balance at different days of observations (2, 4, 6 and 8 days after storage). Physiological weight loss was calculated using the following formula (Hassan 1998):

$$\text{Percent physiological weight loss (\%PWL)} = \frac{IW - FW}{IW} \times 100$$

Where, PWL = Physiological weight loss. IW = Initial weight (g) and FW = Final weight (g)

Estimation of moisture and dry matter content:

Ten grams of bitter gourd was taken in a petridish (which was previously cleaned, dried, and weighed) for each replication. The petridish was placed in an electric oven at 80°C for 72 hours until the constant weight attained. It was then cooled in a desiccator and weighed again. Percent moisture content of bitter gourd was calculated using the following formula (Hassan 1998):

$$\text{Moisture content (\%)} = \frac{IW - FW}{IW} \times 100$$

Where, IW = Initial weight of pulp (g), FW = Final weight of oven dried pulp (g)

Percent dry matter content of the bitter gourd was estimated from the data obtained during moisture estimation using the following formula:

Percent dry matter = (100 - percent moisture content).

Colour and Shrinkage:

The changes in colour of bitter gourd were determined using a numerical rating scale of 0-5, where 0 = fully green, 1= Breaker, 2 = Up to 25% light yellow, 3 = 26-50% yellow, 4 = 51-75% yellow and 5 = 76- 100% yellow. Shrinkage was recorded at the different days (2, 4, 6, and 8) using visual scale where 0 =No shrinkage, 1= 1-25% shrinkage, 2 = 26-50% shrinkage, 3= 51-75% shrinkage and 4 = 76-100% shrinkage. Similar scales were used by Jahan et al. (2020).

Disease incidence and Disease severity:

Diseases incidence means percentage of fruits infected with disease. This is measured by calculating the percentage of fruits infected in each replication of each treatment. The infected fruits of each replication of each treatment were selected to determine percent fruit area infected and was measured based on eye estimation.

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits in each replication}}{\text{Total number of fruits in each replication}} \times 100$$

Disease severity represents the percent diseased portion of the infected fruits. The infected fruits of each replication of each treatment were selected to determine percent fruit area diseased, and was assessed based on visual observation.

Shelf life:

Shelf life of fruits were calculated from daily estimation of disease severity on the same fruits from each replication and considered as ended when the fruits had little or no commercial viability as estimated by Rashid *et al.* (2015). Shelf life of bitter gourd as influenced by different postharvest storage treatments were calculated by counting the days required to the final stage having optimum marketing and eating qualities.

Results and Discussion

Physiological weight loss

The postharvest treatments used in the present study exhibited significant effect on physiological weight loss of bitter gourds during storage. The physiological weight loss increased with time and ranged between 0.74-53.91% (Table1). The minimum weight loss (3.49%) was found in bitter gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped gourds followed by 3% CaCl₂ treated, 2000 Gy gamma irradiated and PP bag wrapped gourds(5.43%) at 8th day after storage (Table1). Higher levels of weight losses (52.31%) were recorded in the unwrapped control gourds irrespective of treatments with CaCl₂ and gamma irradiation (Table1). For instance, the maximum weight loss (53.91%) was found in unwrapped CaCl₂ treated gourds which were statistically identical with those of unwrapped gamma irradiated gourds. The finding of the present study were in partial agreement with the findings reported by Bhattacharjee and Dhua (2017). They found that

weight loss decreased by creates a modified atmosphere that reduces the water vapor transmission and therefore slower respiration rates. Banu (2000) reported lower weight loss of pointed gourd and okra when stored in polythene bag.

Table 1. Effect of postharvest treatments on percent physiological weight loss at different days after storage of bitter gourd

Treatments	Physiological weight loss (%) at different days after storage			
	2	4	6	8
T ₀	8.41	23.90	38.36	52.31
T ₁	1.47	0.69	2.21	5.57
T ₂	6.19	25.98	40.83	53.91
T ₃	9.64	28.02	29.86	48.77
T ₄	9.71	18.88	25.65	38.65
T ₅	0.81	3.22	5.34	7.25
T ₆	4.67	6.10	11.79	13.31
T ₇	0.88	2.26	5.41	6.37
T ₈	0.74	0.90	2.12	3.49
T ₉	3.49	3.99	4.74	5.43
LSD _{0.05}	4.67	4.67	10.27	16.73

N. B. ** = Significant at 1% level of probability.

T₀ = Control, T₁ = wrapped in PP bag (25 μ), T₂ = treated with 3% CaCl₂, T₃ = Irradiated at 1000 Gy, T₄ = Irradiated at 2000 Gy, T₅ = 3% CaCl₂ + PP bag, T₆ = 1000 Gy + PP bag, T₇ = 2000 Gy + PP bag, T₈ = 3% CaCl₂ + PP bag, T₉ = 3% CaCl₂ + 2000 Gy + PP bag.

Colour and shrinkage

In case of perishable commodity, colour is one of the most important visual criteria for identifying their freshness quality. It is the important parameters for determination of quality of edible bitter gourd. Due to post harvest treatment the colour is changed from green to fully yellow. At the 2 days of storage, there were no significant changes of colour but with increasing storage time significant changes of colour were observed. The best visual colour (0.00) was observed in gourds wrapped in PP bag (T₁), gourds gamma irradiated at 2000 Gy and wrapped in PP bag (T₇) and also gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped (T₈) at 2, 4, 6, and 8 days of storage, respectively which were statistically identical with those of PP bag wrapped, CaCl₂ treated gourds (T₅). The worst visual colour (4.56) was observed in unsanitized, non-irradiated, unwrapped control at the 8th day after storage followed by gamma irradiated gourds at 1000 Gy (Figure1). The increasing in changes of colour with increasing storage period. Shrinkage is one of the most important aspects of fruit quality. It depends on the stages of maturity, environmental factors and different treatments. Similar result was reported by May *et al.* (2023) after 8 days storage of bitter gourd. When bitter gourds harvested, their colour was predominantly fully green, but as the storage days progressed the discolouration due to both

enzymatic reactions. Level of shrinkage increased with duration of storage. The result showed that there were no shrinkage occurred after 2 and 4 days of storage, respectively (Figure 1). The maximum levels of shrinkage (3.9) were occurred in unsanitized, non-irradiated, unwrapped control at the 8th day of storage followed by bitter gourds gamma irradiated at 1000 Gy. whereas minimum levels of shrinkage (0.00) were occurred in gourds wrapped in PP bag, sanitized with CaCl₂ and wrapped in PP bag, gamma irradiated at 1000 Gy and wrapped in PP bag, gamma irradiated at 2000 Gy and wrapped in PP bag and gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped gourds at the 8th day after storage (Table 2). Shrinkage scores slowly increased in the treatments with storage duration. Significantly lower and statistically identical shrinkage were also occurred in 3% CaCl₂, gamma irradiated 2000 Gy and wrapped in PP bag. Similar results were reported by Benitez *et al.* (2016).

Moisture content and dry matter

Significant variation was observed in respect of moisture content (%) in bitter gourd at the end of shelf life. It was found that during the whole storage period the % moisture content from the pulp of bitter gourd decreased with time and ranged between 94.63-92.10% (Table2). The maximum moisture content (93.87%) was found in bitter gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP wrapped gourd followed by 3% CaCl₂ treated and PP bag wrapped gourds at 8th day after storage those treatment (Table2). The minimum moisture content was recorded in unsanitized, non-irradiated and unwrapped control at the 2, 4, 6, 8 days, respectively (Table2). The decreased in moisture content were probably due to transpiration and evaporation loss and also starch hydrolysis. It also supported by Ashenafi and Tura (2001).

Dry matter contents of bitter gourd were calculated from percent moisture content. The result showed that there were significant differences among the treatment. During the whole storage period the percent dry matter content from the bitter gourds increased with time and ranged between 5.37-7.90% (Table2). The increase in dry matter content with increasing storage period may be due to osmotic withdrawal of water by transpiration and evaporation. The minimum dry matter content (6.13%) was found in bitter gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped gourds at the 8th day after storage followed by gourds sanitized with 3% CaCl₂. The maximum dry matter content (7.90%) was found in unsanitized, non-irradiated and unwrapped control gourds because weight loss was higher due to higher water loss in control at room temperature resulting in higher dry matter content (Table2). The higher levels of percent dry matter content (7.67%) was recorded in the unwrapped gamma irradiated at 2000 Gy and statistically identical with unwrapped 3% CaCl₂ treated (Table2). The results of the present investigation have got support of Debnath (2015) and Parvin (2004). The increase in dry matter content with increasing storage period may be due to osmotic withdrawal of water by transpiration and evaporation.

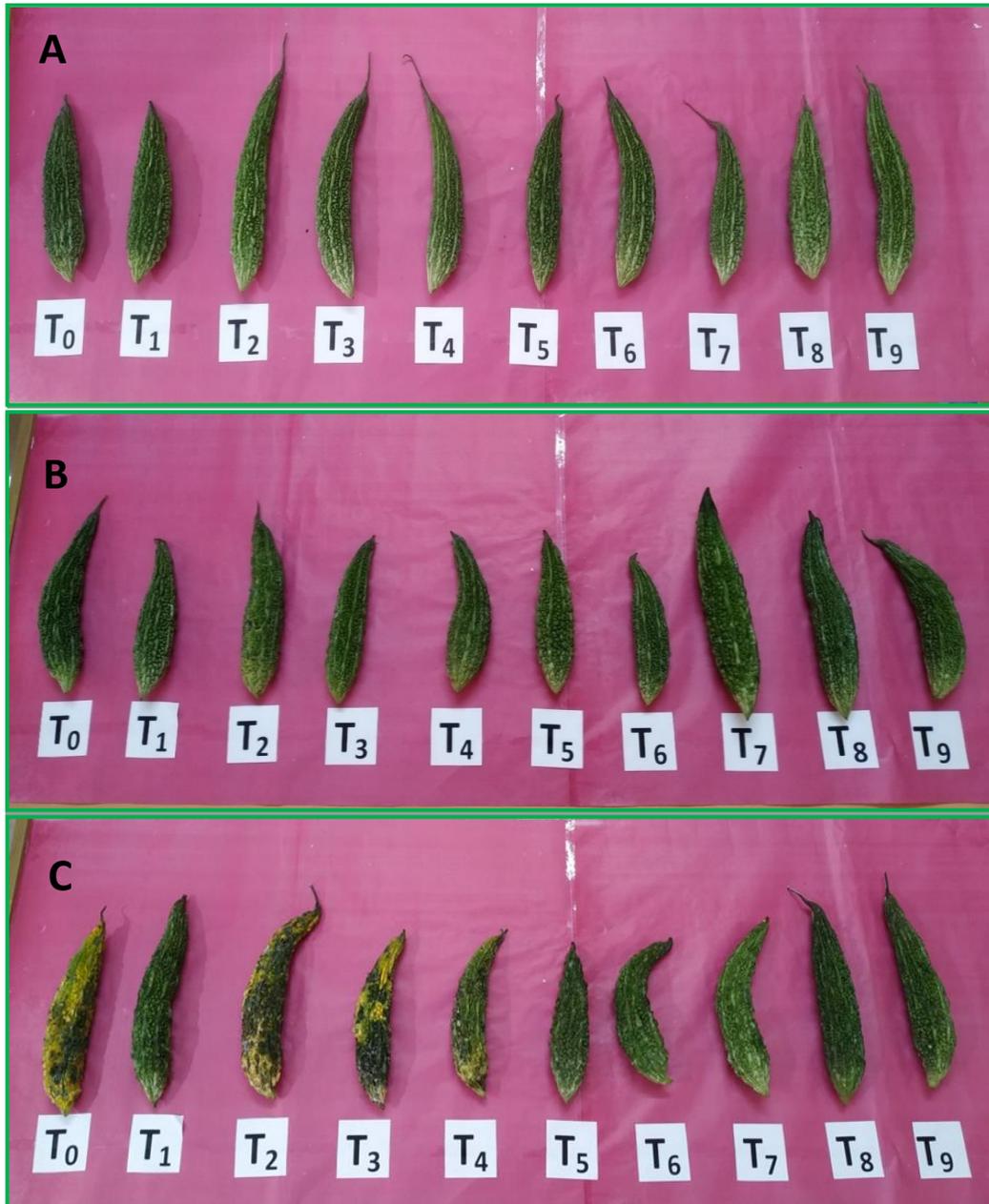


Fig. 1. Pictures showing of bitter gourd at A: 2; B: 4 and C: 8 days after treating.

T₀ = Control, T₁ = wrapped in PP bag (25 μ), T₂ = treated with 3% CaCl₂, T₃ =Irradiated at 1000 Gy, T₄ = Irradiated at 2000 Gy, T₅=3% CaCl₂ + PP bag, T₆= 1000 Gy+ PP bag, T₇=2000 Gy+ PP bag, T₈= 3% CaCl₂+ PP bag, T₉=3% CaCl₂ + 2000 Gy+ PP bag.

Table 2. Effect of postharvest treatments on percent moisture content and dry matter content at different days after storage of bitter gourd

Treatments	Moisture content (%) at different days after storage				Dry matter content (%) at different days after storage				Shrinkage at different days after storage			
	2	4	6	8	2	4	6	8	2	4	6	8
T ₀	92.9	92.6	92.4	92.1	7.1	7.4	7.6	7.9	0.0	0.0	1.2	3.9
T ₁	94.5	94.4	93.9	93.4	5.5	5.6	6.1	6.6	0.0	0.0	0.0	0.0
T ₂	94.5	94.1	93.8	93.5	5.5	5.9	6.2	6.5	0.0	0.0	1.1	2.3
T ₃	93.6	93.2	93.1	92.9	6.4	6.8	6.9	7.1	0.0	0.0	1.3	3.5
T ₄	93.3	93.1	92.7	92.3	6.7	7.0	7.3	7.7	0.0	0.0	0.2	2.83
T ₅	93.7	93.5	93.3	93.0	6.3	6.5	6.7	7.0	0.0	0.0	0.0	0.0
T ₆	94.0	93.8	93.5	93.3	6.0	6.2	6.5	6.7	0.0	0.0	0.0	0.0
T ₇	94.3	94.1	93.7	93.3	5.7	5.9	6.3	6.7	0.0	0.0	0.0	0.0
T ₈	94.6	94.4	94.2	93.9	5.4	5.6	5.8	6.1	0.0	0.0	0.0	0.0
T ₉	94.4	94.0	93.6	93.2	5.6	6.0	6.4	6.8	0.0	0.0	0.0	1.22
LSD _{0.05}	0.26	0.35	0.45	0.46	0.26	0.35	0.45	0.46	-	-	0.81	1.61

N. B. ** = Significant at 1% level of probability.

T₀ = Control, T₁ = wrapped in PP bag (25 μ), T₂ = treated with 3% CaCl₂, T₃ = Irradiated at 1000 Gy, T₄ = Irradiated at 2000 Gy, T₅ = 3% CaCl₂ + PP bag, T₆ = 1000 Gy + PP bag, T₇ = 2000 Gy + PP bag, T₈ = 3% CaCl₂ + PP bag, T₉ = 3% CaCl₂ + 2000 Gy + PP bag.

Disease incidence and severity

Variation in respect of percent disease incidence as influenced by postharvest treatments was found highly significant. Generally, the levels of disease incidence were found to gradually increased as the duration of storage progressed (Figure 2). The maximum disease incidence (77.78%) was recorded in untreated, non-irradiated, unwrapped control and gourds treated with 3% CaCl₂, respectively at 8 days after storage, whereas the minimum (11.11%) was observed in case of the treatment (gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped (T₈) at the 8 days after storage (Figure 2). Result showed that treatment remarkably suppressed disease levels during the entire period of investigation. Singh *et al.* (2016) stated that pathogen grows both aerobically and anaerobically, but generally grows more slowly under anaerobic conditions. Irradiation and MAP create anaerobic condition and reduce disease incidence which supports the observations of the present study. Variation in respect of disease severity was found to be significant in bitter gourd during storage. Disease severity increased with the advancement of storage period. The higher disease severity (35.43% and 76.67%) were recorded in unsanitized, non-irradiated, unwrapped control at the 6th, 8th day after storage, respectively followed by CaCl₂ treated gourds. The lower disease severity (3.33% and 6.67%) were found in gourds dipped in 3% CaCl₂, 1000 Gy gamma irradiated and PP bag wrapped gourds at the 6th, 8th day, respectively after storage which were statistically identical with those of gourds dipped in 3% CaCl₂, gamma irradiated at 2000 Gy and PP bag wrapped (Figure 2).

Shelf life

Shelf life is the basic quality index of fruit. Shelf life period begins from the time of harvesting and extends up to the start of rotting of fruit. In the present investigation significant variation was obtained on the shelf life of bitter gourd as influenced by the postharvest treatments. Significantly the longest shelf life (10 days) was recorded in gourds exposed to gourds dipped in 3% CaCl₂, gamma irradiated at 1000 Gy and wrapped in PP bag whereas the shortest shelf life (4 days) was recorded in the unsanitized, non-irradiated and unwrapped control (Figure3). Significantly longest and statistically identical shelf lives were also recorded in the treatments T₄, T₅, T₆, respectively (Figure 3). Akhther *et al.* (2023) found that the t shelf life of Himsagor mango increase by combined effects of gamma irradiation (400 Gy) and LDPE packaging. Devi *et al.* (2019) reported that shelf life of bitter gourd could be prolonged by combined effect of sanitizer and MAP which supports the observations of the present study.

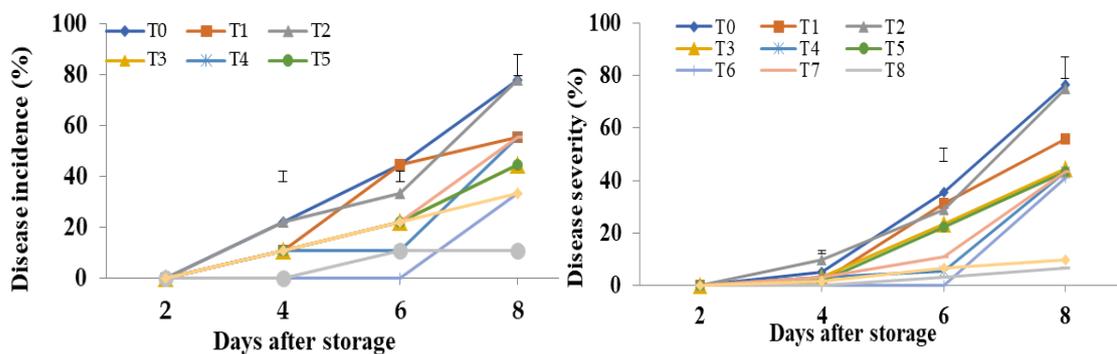


Fig. 2. Effect of postharvest treatments on Disease incidence and disease severity of bitter gourd. At each day, the vertical bar represents LSD at 5% level of significance.

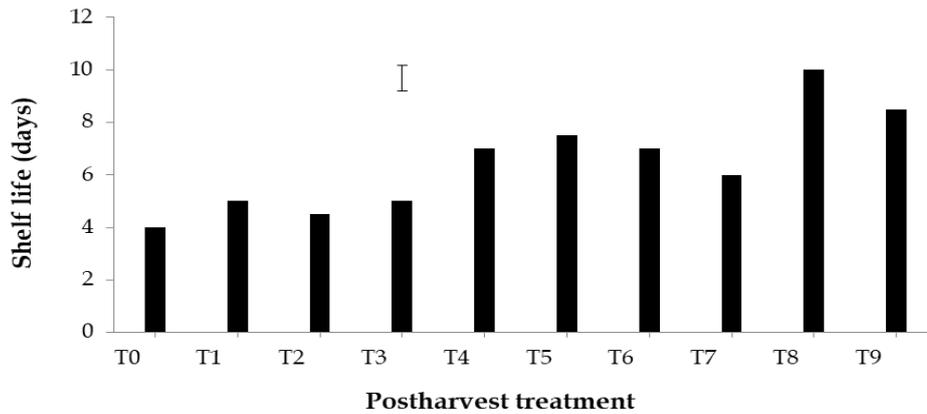


Fig. 3. Effect of postharvest treatments on shelf life of bitter gourd. The vertical bar represents LSD at 5% level.

(Vertical bar represents LSD at 5% level of significance. T₀ = Control, T₁ = wrapped in PP bag (25 μ), T₂ = treated with 3% CaCl₂, T₃ = Irradiated at 1000 Gy, T₄ = Irradiated at 2000 Gy, T₅ = 3% CaCl₂ + PP bag, T₆ = 1000 Gy + PP bag, T₇ = 2000 Gy + PP bag, T₈ = 3% CaCl₂ + PP bag, T₉ = 3% CaCl₂ + 2000 Gy + PP bag.)

Conclusion

The postharvest losses of bitter gourd can be reduced remarkably by applying irradiation and MAP. Bitter gourds sanitized with 3% CaCl₂ followed by gamma irradiation at 1000 Gy and wrapping in un-perforated polypropylene (PP) bags had marked effect on extending shelf life through minimizing disease incidence and disease severity and retaining postharvest quality attributes. However, further investigations with more promising postharvest treatments are suggested to carry out to confirm the results of the present study.

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EVALUATION OF TWO ADVANCED GENERATION *BORO* RICE MUTANTS BASED ON MORPHO-PHYSIOLOGICAL CRITERIA

M.A. Mondal^{1*}, S. Khanam² and M. Roknuzzaman³

Abstract

Experiments were performed at farmers' fields of five locations viz., Mymensingh, Sherpur, Jamalpur, Natore and Naogaon districts with two promising *Boro* rice mutants, RM-16(N)-8, RM-16(N)-10 and a check, BRRI dhan58 to evaluate some morpho-physiological features and its impact on grain yield. The experiment was setup in RCB design with three replications. Results revealed that high yielding genotypes, in general, showed superiority in morpho-physiological characters (leaf area, leaf area index, total dry mass production, absolute growth rate, relative growth rate, chlorophyll, total sugar and soluble protein content in leaves and harvest index) than the low yielding one. Results further indicated that genotype improvement efforts have achieved higher grain yield by higher growth rate at early growth stages and better assimilate partitioning to economic yield and short stature plants are unlikely to improve harvest index and resistant to lodging in rice. The mutant, RM-16(N)-10 had medium plant stature with greater biomass production capacity, superiority in growth and biochemical parameters and improve dry matter partitioning to economic yield which resulted higher number of grains panicle⁻¹, thereby grain yield. In contrast, RM-16(N)-8 showed inferiority in morpho-physiological characters and performed the lowest yield attributes and grain yield. This information may be used in future plant breeding programme.

Key Words: Growth, mutants, chlorophyll, photosynthesis, yield

Introduction

In Bangladesh, the yield potential of modern *Boro* rice varieties varies from 7.5 to 9.0 tons ha⁻¹. The domestic production of rice cannot entirely meet up the requirements of hungry millions of Bangladesh. To meet increasing demand of rice for the fast-growing population of Bangladesh, there need urgent attention to develop high yielding variety under sub-tropical environmental condition. To increase rice production, there is very little scope for horizontal expansion of rice area. Furthermore, the wide gap between achievable (8.4 t ha⁻¹) and average yield (4.96 t ha⁻¹) should be reduced. Now, the main strategy for development of rice in Bangladesh should be to develop lines having high yield potentials with short life span and high levels of resistance to major diseases. Due to the shortage of cultivable land, the scope of its extensive cultivation is very limited in this country.

¹Crop Physiology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202,

²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202, Bangladesh,

³Research Coordination office, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202,

*Corresponding author's email: mmamondal@gmail.com

That is why special attention should be given for increasing the yield per unit area by developing improved varieties/technology and management practices.

It is evident that grains per unit area are related to canopy photosynthesis during flowering and grain set. Furthermore, canopy photosynthesis rate determines through leaf area index and crop growth rate. Important physiological attributes such as leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), specific leaf weight (SLW) and photo-assimilate production capacity and its efficient partitioning to economic yield etc. can address various constraints of a variety for increasing its productivity (Mondal *et al.*, 2013). A plant with optimum LAI and NAR may produce higher biological yield. For optimum yield in rice, the LAI should be ranged from 6.0 to 7.0 (Asmamaw, 2017). Any reduction of leaf area or the amount/intensity of light may have an adverse effect on yield (Mondal *et al.*, 2011). The dry matter accumulation may be the highest if the LAI attains its maximum value within the shortest possible time (Mondal *et al.*, 2014). Not only TDM production, the capacity of efficient partitioning between the vegetative and reproductive parts may produce high economic yield (Mondal *et al.*, 2011). It is suggested that high partitioning efficiency (harvest index) would be advantageous for high yield. Probably a better understanding of crop growth and yield parameters and the partitioning of assimilates into seed formation would help to expedite yield improvement of this crop. Keeping all those above in mind, the current research was undertaken to investigate variations in some growth, biochemical parameters and yield attributes for selection of important sources and sinks in two elite mutants and one variety of *Boro* rice.

Materials and Methods

The field experiment was performed at farmers' field of five locations under different agro-ecological zones in Bangladesh viz., Mymensingh, Sherpur, Jamalpur, Natore and Naogaon districts during December 2021 to May 2022. Two advanced mutants (RM-16 (N)-8 and RM-16 (N)-10) and one check variety (BRRI dhan58) of *Boro* rice were used as treatment in the experiment. The experiment was laid out in a Randomized Complete Block Design with 3 replications. The size of the unit plot was 5 m x 6 m. Plant to plant distance was 15 cm and row to row distance was 20 cm. Thirty five days old seedlings were transplanted on January 20, 2022. The land was fertilized with 220, 150, 130, 60 and 5 kg ha⁻¹ of urea, triple superphosphate, muriate of potash, gypsum and zinc sulphate, respectively (BARC, 2018). Urea, triple super phosphate (TSP), muriate of potash (MP), gypsum and zinc sulphate were used as source of nitrogen, phosphorus, potassium, sulphur and zinc, respectively. One third urea and other fertilizers were incorporated with the soil at the final land preparation and rest of the urea was top dressed in two equal splits at 25 and 50 days after transplanting (DAT). Others standard cultural practices were followed to ensure the normal plant growth and development. The morpho-physiological parameters were recorded from the experiment that setup at farmer's field of Mymensingh district. For growth analysis, five hills were randomly sampled for growth analysis at Booting (60 DAT),

heading (90 DAT) and physiological maturity (120 DAT) stages. The uprooted plants were separated into stem, leaves and roots and the corresponding dry weight were recorded after oven drying at 80 ± 2 °C for 72 hours. Leaf area was measured by LICOR leaf area meter (LI 3000A, USA) before drying. The growth analyses like AGR and RGR were carried out following the formulae of Hunt (1978). Photosynthesis was measured at tillering, booting, heading and physiological maturity stages by portable photosynthesis meter (LI- 6800XT, USA). Leaf chlorophyll was determined at tillering, booting, heading and two weeks after heading stages by following the method of Yoshida., *et al.* (1976). Total sugar was determined at tillering, booting, heading and two weeks after heading stages following the method of Dubois., *et al.* (1956).

The yield contributing characters were recorded at harvest from ten competitive plants of each plot. The seed yield was recorded from five rows of each plot (2.50 m × 3.0 m) and converted into seed yield hectare⁻¹ and seed weight plant⁻¹ was determined by dividing the plant number. Harvest index was calculated from the collected data using formula: (economic yield/plot ÷ biological yield/plot) × 100. The collected data were analyzed statistically by using computer package program, MSTAT.

Results and Discussion

Morphological and phenological characters

Plant height, number of tillers hill⁻¹, leaf area (LA) and internode length varied significantly among the mutants/variety (Table 1). The tallest plant was recorded in BRRI dhan56 (109.1 cm) while the shortest plant was recorded in RM-16 (N)-8 (92.5 cm) followed by the mutant RM-16 (N)-10 with same statistical rank (94.2 cm). Results showed that two mutants, RM-16 (N)-8 and RM-16 (N)-10 were shorter than the control variety BRRI dhan58. There had no high difference for tiller production and leaf area among the mutants/variety (Table 1). However, two mutants, RM-16 (N)-8 and RM-16 (N)-10 produced higher number of tillers hill⁻¹ with being the highest in RM-16 (N)-8 (19.77). In contrast, the lowest tiller production was recorded in BRRI dhan58 (15.95 hill⁻¹). The highest leaf area was recorded in RM-16 (N)-10 (1182.4 cm² hill⁻¹) and the lowest was recorded in RM-16 (N)-8 (1088.9 cm² hill⁻¹). Genotypic variation in plant height, leaf area and tiller production was also reported by many workers (Rebetzke *et al.*, 2004; Das and Taliaferro, 2009; Sripathi *et al.* 2013; Kumar and Roy, 2015) that supported the present experimental results. Internode length was greater in the variety BRRI dhan58 than the two mutants that causes lodging of the variety, BRRI dhan58 which is not desirable character. The variety BRRI dhan58 matured earlier than the two mutants, RM--16 (N)-8 and RM--16 (N)-8. RM-16 (N)-10 took longest days to maturity (156 days) and this mutant also performed the highest grain yield. This result is agrees with Khare *et al.* (2015) and Atsedemariyam, (2018) who reported that days to maturity was positively correlated with grain yield in rice.

Table 1. Variation in morphological and phenological parameters of three *Boro* rice mutants/variety (mean over 5 locations)

Genotypes	Plant height (cm)	Leaf area (cm ² hill ⁻¹) [†]	Total tillers hill ⁻¹ (no.)	Internode length [†] (cm)	Lodging tendency (%)	Growth duration (days)	Days to maturity
RM-16 (N)-8	92.5 b	1088.9 b	19.77 a	14.84 c	0.00	123.3 b	153 b
RM-16 (N)-10	94.2 b	1182.4 a	17.31 ab	15.23 b	0.00	126.1 a	156 a
BRRI dhan58	109.1 a	1110.4 b	15.95 b	17.88 a	60.0	118.2 c	148 c
F-test	**	*	*	**		**	**
CV (%)	6.66	6.48	11.23	2.51		2.12	2.72

In a column, figure (s) with same letter do not differ significantly at $p \leq 0.05$; **, * significant at 1% and 5% level of probability, respectively; †: data collected at Mymensingh only.

Growth parameters

The effect of rice genotypes on growth characters like total dry mass (TDM) at three growth stages (booting, panicle initiation and mature), absolute growth rate (AGR) and relative growth rate (RGR) was significant (Table 2). Results indicated that TDM production increased with age until maturity whereas reverse trend was observed in AGR and RGR. At later growth stages (panicle initiation and mature stages), the highest TDM plant⁻¹ was recorded in BRRI dhan58. The mutant RM-16(N)-8 produced the lowest TDM at all growth stages. The mutant RM-16 (N)-10 produced the highest TDM plant⁻¹ at early growth stages (booting stage) followed by second highest TDM at later growth stages (panicle initiation and mature stages). Generally, high yielding genotypes produced higher TDM and LAI (Mondal *et al.*, 2013). In the present experiment, the variety BRRI dhan58 and the mutant RM-16(N)-10 were the yielder with highest/higher TDM and LAI producer variety/mutant. The value of AGR and RGR was greater early growth stages than later growth stages (Table 2). At later growth stages, AGR and RGR were higher in mutants than the variety, BRRI dhan58 but at early growth stages, reverse trend was in case of AGR and RGR. Results indicated that rice grain yield had no relation with AGR and RGR. It means selection of mutants based on superior growth parameters may be misleading. Plant growth and yield are represented by the crop's early ability to intercept solar radiation and its subsequent utilization for biomass production (Hanlan *et al.*, 2006). Increase interception of solar radiation at early seedling stages enable plant to make rapid early growth, resulting in high yield (Gautam *et al.*, 2018). In the present experiment, the mutant RM-16 (N)-10 showed early higher growth rate and also showed high yield potential. Similar result was also reported by Akther *et al.*, 2019 who observed that the genotypes which had capacity to early higher growth rate also showed higher seed yield in rice.

Table 2. Variation in total dry mass production and absolute growth rate at different growth stages of three *Boro* rice mutants/variety[†]

Genotypes	Total dry mass production at			Absolute growth rate at (g hill ⁻¹ day ⁻¹)		Relative growth rate at (mg g ⁻¹ day ⁻¹)	
	Bootin g stage	Panicle initiation stage	Mature stage	BS-PI stage	PI- MS stage	TS-PI stage	PI- MS stage
RM-16 (N)-8	40.4 b	90.90 c	138.3 b	1.68 c	1.58 a	27.0 ab	13.99 a
RM-16 (N)-10	48.6 a	104.2 b	148.0 a	1.85 b	1.46 a	24.4 b	11.76 ab
BRR1 dhan58	43.2 b	112.6 a	150.4 a	2.31 a	1.26 b	31.9 a	9.65 b
F-test	**	**	*	**	*	**	**
CV (%)	9.16	6.88	12.20	9.02	10.54	8.66	9.95

In a column, figure (s) with same letter do not differ significantly at $p \leq 0.05$; *, ** significant at 5% and 1% level of probability, respectively; BS, booting stage; PI, panicle initiation; MS, mature stage; †: data collected at Mymensingh only.

The effect of rice genotypes on leaf area index (LAI) at four growth stages like tillering, booting, panicle initiation and physiological maturity stages was significant (Table 3). Results indicated that LAI increased with age until heading stage followed by a decline due to leaf shading. The mutant RM-16(N)-10 showed the highest LAI at all growth stages and the lowest LAI at most growth stages was recorded in the mutant, RM-16(N)-8. Leaf area index (LAI) is a key variable used in field crops involving the relation between crop growth and yield. LAI is commonly used as an important structural and biophysical indicator of vegetation for crop photosynthesis (Brown *et al.*, 2020), productivity (Kanniah *et al.*, 2021), and water utilization (Gan *et al.*, 2018). This variable can be a very useful tool when looking to evaluate the performance of a crop. The LAI is correlated with yield (Rahman *et al.*, 2020). In this experiment, the high yielding mutant, RM-16(N)-10 showed superior LAI at all growth stages and also showed higher grain yield.

Biochemical parameters

Genotypes had no high variation on photosynthesis (Pn) in leaves at all growth stages of rice genotypes (Table 3). However, Pn increased with age until heading stage followed by a decline. The highest/higher Pn was recorded in RM-16(N)-10 and this mutant also showed higher seed yield which indicated seed yield is positively correlated with Pn. On the other hand, the mutant, RM-16(N)-8 showed inferiority in Pn and also showed lower yield performance. These results indicate that Pn rate is very much important for getting higher grain yield. These results are consistent with many workers (Long *et al.*, 2006; Islam, 2010; Makino, 2011;) who reported Pn was positively correlated with yield in rice. On the other hand, a lack of correlation between photosynthesis and plant yield has been frequently observed when different genotypes of a crop are compared (for wheat, Evans and Dunstone, 1970; for rice, Takano Y, Tsunoda S. 1971). This is also true because modern cultivars have been bred for various traits besides photosynthesis.

Table 3. Changes in leaf area index and leaf photosynthesis rate of three *Boro* rice mutants/variety[†]

Genotypes	Leaf area index at				Photosynthesis rate in leaves ($\mu\text{molCO}_2\text{m}^{-2}\text{S}^{-1}$) at			
	Tillering stage (30 DAT)	Booting stage (60 DAT)	Heading stage (90 DAT)	Three weeks after heading	Tillering stage	Booting stage	Heading stage	Three weeks after heading
RM-16 (N)-8	3.84 b	4.82 c	6.91 c	6.39 a	18.66 b	24.34	23.8 b	14.43
RM-16 (N)-10	4.16 a	5.90 a	7.90 a	6.54 a	21.30 a	24.15	26.40 a	15.22
BRRI dhan58	4.09 ab	5.58 b	7.36 b	5.41 b	19.58 ab	23.24	24.11 ab	14.01
F-test	*	**	**	**	**	NS	*	NS
CV (%)	8.45	6.40	9.12	6.92	6.66	9.80	9.14	11.34

In a column, figure (s) with same letter do not differ significantly at $p \leq 0.05$; *, ** significant at 5% and 1% level of probability, respectively; NS, not significant; †: data collected at Mymensingh only.

The variation in chlorophyll, total sugar and soluble protein content in leaves among the mutants/cultivar was assessed at four growth stages and presented in Tables 4 and 5. There was no significant variation in chlorophyll content of leaves at early growth stages (tillering and booting stages) and significant variation was also observed at later growth stages (Heading and mature stages). It was observed that chlorophyll content in leaves increased with age until heading stage followed by a rapid decline. On the other hand, total sugar and soluble protein content in leaves increased with age till booting stage followed by a decline (Table 5). It indicates that from heading to grain filling stages assimilates produce in leaves remobilized more from leaves to grains. The mutant RM-16 (N)-10 showed the highest chlorophyll, total sugar and soluble protein content in leaves at most growth stages and also showed the highest grain yield. In contrast, the lowest chlorophyll, total sugar and soluble protein content in leaves at most of the growth stages was observed in RM 16(N)-8 and also showed the lowest grain yield. Grain yield is positively correlated with chlorophyll content in leaves of rice as reported by many researchers (Xu *et al.*, 1997; Thomas *et al.*, 2005; Poshtmasari *et al.*, 2007; Nahakpam, 2017). Further, Caddell *et al.*, 2023 reported that quantum yield photosynthesis is closely related with chlorophyll density in leaves.

Table 4. Variation in chlorophyll content (mg g⁻¹ fw) in rice leaves at four growth stages of three *Boro* rice mutants/variety (data collected at Mymensingh only)

Genotypes	Growth stages			
	Tillering stage	Booting stage	Heading stage	Two weeks after heading
RM-16 (N)-8	2.22	2.61	3.16 a	1.82 b
RM-16 (N)-10	2.25	2.75	3.06 a	2.15 a
BRR1 dhan58	2.28	2.64	2.86 b	1.84 b
F-test	NS	NS	*	*
CV (%)	6.59	8.20	4.87	5.62

In a column, figure (s) with same letter does not differ significantly at $p \leq 0.05$; *, significant at 5% level of probability; NS, not significant.

Table 5. Changes in total sugar and soluble protein content in leaves of three *Boro* rice mutants/variety[†]

Genotypes	Total sugar (mg g ⁻¹ fw) at				Soluble protein (mg g ⁻¹ fw) at			
	Tillering stage	Booting stage	Heading stage	Two weeks after heading	Tillering stage	Booting stage	Heading stage	Two weeks after heading
RM-16 (N)-8	60.3	124.5 b	102.6 b	75.3	47.5	51.3 b	42.5 b	37.5
RM-16 (N)-10	58.2	138.4 a	117.3 a	73.0	44.3	56.1 a	47.0 a	39.6
BRR1 dhan58	64.0	116.8 b	103.5 b	79.0	44.6	52.0 b	44.3 ab	41.5
F-test	NS	**	**	NS	NS	*	*	NS
CV (%)	14.30	16.00	13.42	11.22	10.64	7.70	9.90	14.34

In a column, figure (s) with same letter do not differ significantly at $p \leq 0.05$; *, ** significant at 5% and 1% level of probability, respectively; NS, not significant; †: data collected at Mymensingh only.

Grain yield and yield attributes

Significant genotypic variation is also observed in case of grain yield and yield attributes (Tables 6 and 7). The highest grain yield both per hill and per hectare was observed in RM-16(N)-10 while the mutant RM-16(N)-8 produced lowest grain yield. The check variety BRR1 dhan58 showed its moderate yield performance. The yield was higher in RM-16(N)-10 due to production of higher number of effective tillers hill⁻¹ and grains panicle⁻¹. The variety BRR1 dhan58 produced the highest number of grains panicle⁻¹ with performing second highest grain yield might be due to production of fewer number of effective tillers hill⁻¹. Mondal *et al.* (2005) reported that the genotypes, which produced higher number of effective tillers hill⁻¹ and higher number of grains panicle⁻¹ also showed higher grain yield. Similar results were also reported by many previous report (Oladosu *et al.* 2014; Mondal, 2018; Paudel *et al.*,2021).

Results indicated that harvest index was higher in mutants than the check variety BRRI dhan58 (Table 6). The highest harvest index was recorded in RM-16(N)-8 followed by RM-16 (N)-10 with same statistical rank. The lower harvest index was recorded in BRRI dhan58 (40.84). Generally, high yielding genotypes produced higher TDM and LAI (Mondal *et al.*, 2013). In the present experiment, the mutant RM-16(N)-10 was the highest yielder with highest TDM and LAI producer variety. Further, the TDM's negative response to HI in BRRI dhan58 could be explained in way that high TDM producing capacity might have used assimilate for other vegetative sinks (taller plant) and that it deprived translocation of assimilates to economic sink. In other word, dry matter partitioning to economic yield was lower in BRRI dhan58 which is not desirable character. But dry matter partitioning to economic yield is more important than TDM production. However, the mutant RM-16(N)-10 produced intermediate TDM with highest dry matter partitioning to economic yield (49.26%) and resulting higher grain yield that is the desirable character.

Table 6. Variation in yield attributes and harvest index of three *Boro* rice mutants/variety (mean over 5 locations)

Genotypes	Effective tillers hill ⁻¹	Grains panicle ⁻¹	Unfilled grains panicle ⁻¹	1000-grain weight (g)	Grain weight 4m ² (kg)	Biological yield 4m ² (kg)	Harvest index (%)
RM-16 (N)-8	12.57 a	138.4 b	34.1 b	23.21 b	2.87 b	5.85 b	49.06 a
RM-16 (N)-10	12.95 a	151.7 a	31.2 b	24.06 a	3.32 a	6.74 a	49.26 a
BRRI dhan58	10.67 b	157.7 a	43.1 a	24.23 a	2.95 b	6.95 a	42.45 b
F-test	**	**	**	*	**	**	**
CV (%)	8.16	8.88	16.20	1.98	10.11	9.22	4.72

In a column, figure (s) with same letter do not differ significantly at $p \leq 0.05$; *, ** significant at 5% and 1% level of probability, respectively; †: data collected at Mymensingh only.

Considering grain yield over locations (Table 7), the mutant RM-16(N)-10 showed the highest grain yield (7.68 t ha⁻¹) at all 5 locations and showed 10.8% higher grain yield than check variety, BRRI dhan58 (6.93 t ha⁻¹). On the other hand, the mutant RM-16(N)-8 showed higher grain yield than the check variety, BRRI dhan58 at 3 locations with non-significant different to check variety. However, BRRI dhan58 showed lodging tendency in 3 locations out of five (Table 1).

Table 7. Variation in grain yield of two Boro rice mutants and one Boro rice variety at five locations during 2021-22

Mutants/ variety	Grain yield (t ha ⁻¹)					Mean (t ha ⁻¹)	Yield Changed over control (%)
	Mymensingh	Sherpur	Jamalpur	Natore	Naogaon		
RM-16(N)-8	6.73 b	6.10 b	6.23 b	7.14 b	8.00 c	6.84 b	- 1.3
RM-16(N)-10	7.23 a	7.06 a	6.79 a	7.54 a	9.79 a	7.68 a	10.8
BRR1 dhan58	6.58 b	5.82 b	6.33 b	7.33 a	8.70 b	6.93 b	--
F-test	**	**	**	*	**	**	
CV (%)	8.88	13.20	8.56	5.67	10.13	9.29	

Same letter (s) in a column indicates do not differ significantly at P≤ 0.05.

Conclusion

From the study, it is evident that out of three mutants/variety, RM 16(N)-10 showed superiority in respect of growth, biochemical parameters and grain yield that may be selected effectively to be a physiological superior mutant. This mutant may be released as improve variety after few more trails under farmers' field conditions at different agro-ecological zones of the country.

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EFFICACY OF ENTOMOPATHOGENS, BOTANICAL AND NEW GENERATION INSECTICIDES AGAINST PAPAYA MEALYBUG

A. Islam^{1*}, M. A. Haque², G. Das², A.A. Khan³, M.A.A. Topu¹, M.S. Rahman¹, M.J. Hasan¹, M.L.R. Mollah¹ and M. Roknuzzaman⁴

Abstract

The papaya mealybug is an invasive polyphagous pest species causing several economic damages to a wide range of agricultural crop. Five new generation insecticides of different groups, one botanical and one entomopathogenic fungus (*Beauveria bassiana*) were evaluated in laboratory for their effectiveness in reducing mealybug incidence on papaya. The present study was conducted to evaluate the toxic effect of different insecticides at different concentration against *P.marginatus*. Among the 7 insecticides, Imixam 70 WDG (Imidacloprid+Thiamethoxam) was the most effective insecticide followed by Ravjum 14.5 SC (Indoxacarb), Saka 25 SC (Abamectin 5% +Spirodiclofen 20%), Imixan and Biotrin 0.5% (Matrin), Tundra 20 SP (Acetameprid), Antario (*Bacillus thuringiensis*+Abamectin) and *Beauveria bassiana*, respectively. All the selected insecticides provided their highest efficacy at their maximum doses as well as within 7-9 DAT, among them two new generation insecticides e.g. Saka 25 SC (Abamectin 5% +Spirodiclofen 20%) @ 1.0 ml/L, Ravjum 14.5 SC (Indoxacarb) @ 1.0 ml/L and the botanical insecticide Biotrin 0.5% upto 7 DAT would be effective in controlling papaya mealybug considering the efficacy. To confirmation of the results, need to be tested under field condition.

Key words: Papaya mealybug, new generation insecticides, Botanical, Entomopathogenic fungus

Introduction

The papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink) affects a variety of host plants, including the economically significant tropical fruits and ornamentals (P. Sakthivel, 2012). Insects may inhibit papaya growth, particularly from fruit set until harvest. Mealybug activity is highest in warm, dry weather (MAM Khan, 2014). *Paracoccus marginatus* is a native of Central America and spread to the Caribbean region and South America in the 1990s; since then it has accidentally been introduced to some islands in the Pacific region and some countries in Africa and Asia. We recorded its presence in China for the first time in 2013 from Guangdong Province and Yunnan Province in southern and southwestern China, respectively. (Muhammad Z Ahmed, 2015).

Females can only move by short-distance crawling or by being carried by air currents because they lack wings and female crawlers have four instars, and depending on

¹Entomology Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202

²Department of Entomology, BAU, Mymensingh-2202, ³Maize Breeding Division, Bangladesh Wheat and Maize Research Institute, RS, Jamalpur, ⁴Research Coordination Office, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202, *Corresponding author's email: afsanabau201@gmail.com

the temperature, a generation lasts around one month. Males have five instars, the fourth of which is known as the pupa and is created in a cocoon (Amarasekare KG, 2010). The adult female is yellow and is covered with a white waxy coating. Adult females are approximately 2.2 mm long (1/16 inch) and 1.4 mm wide. A series of short waxy caudal filaments less than 1/4 the length of the body axis around the margin (Miller and Miller, 2002).

According to Tanwar *et al.* (2010) and Suganthy *et al.* (2012), the leaves become crinkled, yellowing, and withered. This mealybug excretes honeydew, which eventually grows into a sooty mold that covers the leaves, fruits, and stems, preventing photosynthesis and gas exchange. Chlorosis, plant stunting, deformed leaves, early leaf and fruit loss, significant honeydew accumulation, and host plant death are the consequences. Additionally, the mealybug leaves behind a thick, white, waxy substance that can render plants unusable (R.K. Tanwar, 2007).

It is thought that the papaya mealybug is a native of Mexico and/or Central America. *Paracoccus marginatus* was originally discovered in Mexico in 1995, according to (Williams and Granara de Willink, 1992). Papaya mealybug was discovered by IPM and CRSP (Collaborative Research Support Program) scientists in Joydebpur, Bangladesh, in May 2009. According to a recent assessment in Bangladesh, farmers are indiscriminately using various chemical insecticides that do not effectively manage the mealybug infestation that has affected roughly 40% of the papaya plants in the orchards (Khan *et al.*, 2014).

In the search for safer insecticides technologies, more selective mode of action and reduced risks for non-target organisms and the environment, progress has been made in the last few years with development of new generation insecticides and use of entomopathogens and botanicals. Entomopathogenic fungi and botanical extracts (neem or eucalyptus) caused significant reduction in survival and fecundity of *S. avenae*. Therefore, they may be used as promising natural alternatives to synthetic insecticides against the wheat aphid species (Ali, 2019). Entomopathogenic fungi infests the host insects via digestion, respiration and through integument. New generation insecticides are developed to replace earlier, more toxic chemicals in effort to clean up the environmental and human health impacts of these older agricultural insecticides.

So, five new generation insecticides, botanical and entomopathogens would be evaluated against papaya mealybug in the laboratory as the alternatives of conventional insecticides to combat resistances development, effective control, inhibition of growth and development, to keep the natural enemies safe and fit these molecules in IPM packages. Therefore, the present research work was planned to evaluate the efficacy of five new generation insecticides such Antario, Imixam 70 WDG, Ravjum 14.5 SC, Saka 25 SC, Tundra 20 SP on the mortality of papaya mealybug under laboratory conditions. The misuse of conventional pesticides leads to a variety of problems, including secondary pest outbreaks, environmental contamination, food poisoning, and health risks. This is the nation's current catchphrase for limiting the use of broad-spectrum insecticides and

promoting the use of substitute chemicals to create less poisonous fruits. Therefore, farmers may save the natural enemies and so save money on insecticidal expenditures if they use these compounds wisely.

The present study was therefore initiated with following objective:

- To elucidate the individual effects of selected new generation insecticides, entomopathogens and botanical against *Paracoccus marginatus* under laboratory condition.
- To find out the most effective treatment(s) in respect of mortality of *P. marginatus*.

Materials and Methods

The experiment was conducted in the laboratory, Department of Entomology, Bangladesh Agricultural University, Mymensingh from the period of July, 2021 to January, 2022.

Collection of papaya mealybug

Papaya mealybugs were collected from untreated papaya plant with leaves. Then ten insects were released on previously grown papaya seedlings until their settlements or adaptation in the laboratory condition. Regular checking was done for their movement as well as overall condition. Before conducting research, nymphs and adults were collected from the papaya seedlings and then kept in the petridish. Then collected mealybug was distributed in different petridish to know the effect of treatments according to the experimental objective. Populations of papaya mealybug were maintained in the laboratory until completion of the research work.

Insecticides

Commercial formulations of Antario (*Bacillus thuringiensis*+Abamectin) (recommended dose 2g/L) @ 0.5, 1.0, 1.5, 2.0 g/L, Imixam 70 WDG (Imidacloprid +Thiamethoxam) (recommended dose 0.15g/L) @ 0.1, 0.2, 0.3, 0.4 g/L, Ravjum 14.5SC (Indoxacarb) (recommended dose 1ml/L) @ 0.25, 0.50, 0.75, 1.0 ml/L, Saka 25 SC (Abamectin 5% +Spirodiclofen 20%) (recommended dose 2ml/L) @ 0.25, 0.50, 0.75, 1.0 ml/L, Tundra 20SP (Acetameprid) (recommended dose 0.25g/L) @ 0.2, 0.3, 0.4, 0.5 g/L were tested for their toxicity to *P.marginatus* under laboratory conditions. Besides one entomopathogens (*Beauveria bassiana*) @ 2.5, 5.0, 7.5, 10.0 g/L and one botanical, Biotrin 0.5 % (Matrin) (recommended dose 1.4 ml/L) @ 0.25, 0.50, 0.75, 1.0 ml/L were also evaluated against papaya mealybug. These insecticides were purchased from the local market and each insecticide was tested at four different concentrations.

Experimental procedure

To test the efficacy, insects were treated with selected entomopathogenic fungus, botanical and new generation insecticides using micro sprayer. At first, papaya seedlings were treated with selected insecticides with recommended specific concentrations using micro sprayer. The sprayer was done in such a way that the whole plant was thoroughly covered by spray material. After that, the treated plants were air-dried thoroughly using electric fan. Secondly, ten mealybugs were directly treated with selected insecticides using micropipette. Then, treated insects were carefully transferred on treated papaya plants using fine camel brush and special care was taken during transferring mealybugs to avoid and injury. After the release of mealybugs, each plant was placed individually on a special plastic tray with an inner and outer ring. The plant was placed on inner ring and the outer ring was filled with detergent water to prevent mealybugs escaping. Laboratory experiments was conducted with CRD consisted of seven treatments combinations. Four doses of each insecticide were provided as treatments. Each treatment was replicated thrice, and ten mealybugs were used for each replication. Care was taken to avoid spray drift on adjacent plants. The control plants were sprayed only with water.

Data collection parameter

Mortality of nymphs and adults of papaya mealybug.

For counting the dead insects, all the plants parts along with pot and soil surface was observed carefully. Small dead nymphs were counted using magnifying glass. The percentage of papaya mealybug mortality was calculated using the following formula:

$$\% \text{ mortality} = \text{Po}/\text{Pr} \times 100.$$

Where,

Po=Number of papaya mealybug died, Pr=Number of treated mealybugs provided.

Calculation of fold increase in mortality over control: This was calculated by using the following formula:

$$\text{Fold increase in mortality over control} = \text{Po}/\text{Pr} \times 100$$

Where,

Po= Mortality percentage in treated condition

Pr= Mortality percentage in control conditions.

Statistical analysis

The recorded data was compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done with the help of computer package MSTAT. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD).

Results and Discussions

The potency of five toxicants representing different chemical groups was assessed under laboratory conditions against *P.marginatus*. The result revealed that concentration of different insecticides had significant effect on the mortality of papaya mealybug and the effect was clearly time and dose dependent (Table 1).

Table 1. Percent mortality of papaya mealybug at different days after treatment (DAT) with different concentrations of chemical insecticides

Treatments	Percent mortality of papaya mealybug at different DAT			
	1 DAT	3 DAT	7 DAT	9 DAT
Antario @ 0.5 g/L	10.00 b	23.33 b	56.66 c	73.33 b
Antario @ 1.0 g/L	36.66a	46.66a	63.33 bc	73.33 b
Antario @ 1.5 g/L	43.33a	56.66a	70.00ab	76.66ab
Antario @ 2.0 g/L	36.66a	56.66a	73.33a	86.66a
Control	4.17b	8.33	12.50d	15.60c
CV (%)	20.31	14.65	8.79	9.85
F-test	**	**	**	**
Imixam @ 0.1 g/L	16.66 b	56.67 b	100.00a	100.00a
Imixam @ 0.2 g/L	20.00 b	60.00 b	100.00a	100.00a
Imixam @ 0.3 g/L	30.00a	100.00a	100.00a	100.00a
Imixam @ 0.4 g/L	33.33a	100.00a	100.00a	100.00a
Control	4.17c	4.17c	8.33b	12.50b
CV (%)	16.58	4.02	0.31	0.75
F-test	**	**	**	**
Tundra @ 0.2 g/L	43.33 c	56.66a	80.00a	86.66a
Tundra @ 0.3 g/L	50.00 b	60.00a	83.33a	90.00a
Tundra @ 0.4 g/L	56.66a	70.00a	83.33a	93.33a
Tundra @ 0.5 g/L	60.00a	70.00a	86.66a	96.66a
Control	4.17 d	4.17 b	8.33 b	12.50 b
CV (%)	7.89	16.28	11.58	8.38
F-test	**	**	**	**
Ravjum @ 0.25 ml/L	40.00 b	60.00 b	86.66 b	90.00 b
Ravjum @ 0.50 ml/L	46.66 b	70.00ab	90.00 b	100.00a
Ravjum @ 0.75 ml/L	50.00ab	76.66a	96.66a	100.00a
Ravjum @ 1.0 ml/L	60.00a	80.00a	100.00a	100.00a
Control	4.17c	4.17c	8.33c	12.50c
CV (%)	13.99	11.69	4.09	5.42
F-test	**	**	**	**
Saka @ 0.25 ml/L	33.33 c	50.00 c	80.00 c	100.00a
Saka @ 0.50 ml/L	50.00 b	56.66 b	83.33 bc	100.00a
Saka @ 0.75 ml/L	56.66a	60.00 b	86.67 b	100.00a
Saka @ 1.00 ml/L	60.00a	70.00a	100.00a	100.00a
control	4.17d	4.17d	8.33d	12.50b
CV (%)	8.46	5.43	4.68	0.75
F-test	**	**	**	**

Results indicated that mealybag mortality percent increased with time after insecticide sprayed. The results showed that Imixam 70 WDG (Imidacloprid +Thiamethoxam) was the most effective insecticide, achieving 100% mortality at the rate of 0.3 g/L after three days of treatment. Consequently, imidacloprid has a temporary but inhibitory effect on honey bees (Laura BORTOLOTTI, 2003) and it was found that imidacloprid was persistent in sample water and that it did not biodegrade rapidly in an aquatic environment (Tišler *et al.*, 2009). Ravjum 14.5 SC (Indoxacarb) and Saka 25 SC (Abamectin 5%+Spirodiclofen 20 %) were also effective against *Paracoccus marginatus*. At 9 days after treatment, all concentrations, except 0.25 ml/L of Ravjum 14.5 SC and 1.0 ml/L of Saka 25 SC, resulted in 100% mortality. It may be appropriate to include indoxacarb in integrated pest control plans for management ((Swarna Hewa-Kapuge, 2003). Tundra 20 SP showed a mortality rate of 96% at 9 days after treatment at 0.5 g/L, which was the highest mortality rate observed for this insecticide. However, the mortality rate was only 43.33% at 1 day after treatment at 0.2 g/L, which was the lowest observed for any insecticide tested. Antario (*Bacillus thuringiensis* + Abamectin) showed the lowest mortality rate against *P. marginatus*, with a maximum mortality rate of 63.33% observed at 9 days after treatment at 2.0 ml/L. the study shows that Ravjum 14.5 SC and Saka 25 SC were effective insecticides against *P. marginatus*, Imixam shows highest mortality. Tundra 20 SP also showed promising results but with a lower efficacy than the previously mentioned insecticides.

Significant mortality rates were observed at different days after treatment (DAT) for both *Beauveria bassiana* and Biotrin. For *Beauveria bassiana*, the highest mortality rate (68.33%) was observed at the rate of 10.0 g/L at 9 DAT. For Biotrin 0.5%, the highest mortality rate (100%) was attained with a 1.0 ml/L dose at 9 DAT. However, Biotrin was more effective than *Beauveria bassiana* in case of mealybag control. These results indicate the efficacy of these treatments in causing mortality in the studied organisms. Botanical compounds used for pest control are highly efficient in combating a wide range of harmful pests and diseases. Furthermore, they are readily accessible, cost-effective, easily degradable, and pose minimal risks to beneficial organisms (Ngegba *et al.*, 2022). Considering the above facts, the botanical Biotrin 0.5% can be effective against *P. marginatus* management.

Table 2. Mean percent mortality of papaya mealybug at different DAT with different concentrations of entomopathogen and botanical

Treatments	Mean percent mortality of papaya mealybug at different DAT			
	1	3	7	9
<i>Beauveria bassiana</i> @ 2.5 g/L	0.00	4.16bc	16.33cd	25.00c
<i>Beauveria bassiana</i> @ 5.0 g/L	0.00	9.52ab	23.80bc	43.22b
<i>Beauveria bassiana</i> @ 7.5 g/L	0.00	15.07a	30.11ab	65.33a
<i>Beauveria bassiana</i> @ 10.0 g/L	0.00	17.16a	38.33ac	68.33a
Control	0.00	4.16c	8.33e	8.33d
CV (%)	--	15.60	13.21	17.71
F-test	NS	*	*	**
Biotrin @ 0.25 ml/L	20.00 d	40.00 d	60.00a	83.33a
Biotrin @ 0.5 ml/L	30.00 c	50.00 c	70.00a	90.00a
Biotrin @ 0.75 ml/L	50.00 b	60.00 b	83.33a	93.33a
Biotrin @ 1.0 ml/L	60.00a	70.00a	90.00a	100.00a
Control	4.17e	4.17e	8.33b	12.50b
CV (%)	6.81	5.97	7.86	3.41
P-level	**	**	**	**

Conclusion

spraying of three new generation insecticides e.g. Imixan 70 WDG @ 0.1g/L, Saka 25 SC (Abamectin 5% +Spirodiclofen 20%) @ 0.25 ml/L, Ravjum 14.5 SC (Indoxacarb) @ 0.5 ml/L and the botanical Biotrin 0.5 @ 1.0 ml/L would be the most effective management approach against *P. marginatus*. However, botanical insecticide Biotrin 0.5 should be recommended for environment friendly as well. To confirmation of the results need to be tested under field condition.

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AREA COVERAGE OF BINA DEVELOPED RICE, PULSE, OILSEED AND HORTICULTURAL CROP VARIETIES IN BANGLADESH

M.H. Rahman*, M.M.A. Sarkar, M.R. Haque and S. Islam

Abstract

The study was conducted to assess the area coverage of BINA developed rice, pulse, oilseed and horticultural crop varieties during 2022-23. Field survey data were collected from 64 districts through concern DD, DAE office. Both tabular and descriptive statistical analysis was used. It was observed that the overall area coverage of BINA developed rice varieties were 8.91%. Among the three seasons; Aus, Aman and Boro the highest area coverage was found in Aman season that was 14.54% followed by Aus 5.95% and Boro 2.93%, respectively. Among the 14 agricultural regions the highest area coverage of rice was found 15.41% in Rajshahi region (Reg-7) and the lowest was found 1.01% in Dhaka region (Reg-11). The overall area coverage of BINA developed pulse varieties were 16.32% and among the 14 regions the highest area coverage for pulses was found in Barishal region 58.51% (Reg-6). The overall area coverage of BINA developed oilseed varieties were 23.46% and among the 14 regions the highest area coverage for oilseed was found in Jashore region 16.32% (Reg-13). Among the BINA developed horticultural varieties the highest area coverage was found 4.46% for Binalebu-1 followed by Binahalud-1 (0.93%) and Binatomato-10 (0.36%). It was also observed that, among the 14 regions the highest area coverage for Binalebu-1 and Binahalud-1 were found in Rangamati agricultural region (region-4) about 483 ha (87.26%) and 45 ha (18.19%), respectively. The study identified that the overall area coverage of BINA developed rice, pulse and oilseed crops for distributed seed were 0.93%, 1.41% and 3.45%, respectively. The study also observed some constraints such as non-availability of seed, lack of training, demonstrations, field day, collaboration etc. For continuation of variety expansion, the institute should ensure quality seed supply in proper time and training, demonstration as well as collaboration among other research institutes, DAE, BADC and NGOs should be emphasized.

Keywords: Area coverage, BINA crop variety.

Introduction

Bangladesh is predominantly an agrarian country. Due to its very fertile land and favorable weather, varieties of crop grow abundantly in this country. Agriculture sector contributes about 11.38 percent in 2022-23 to the country's Gross Domestic Product (GDP) and employs around 45.33 percent of total labor force (Yearbook of Agricultural Statistics, 2022). Due to natural calamities like flood, cyclone, drought, loss of production in both food and cash crops are almost a regular phenomenon. Agricultural holding in Bangladesh is generally small but use of modern machinery and equipment is gradually increasing.

Agricultural Economics Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

*Corresponding author: dr.mhr.habib@gmail.com

Rice, jute, sugarcane, potato, vegetables, wheat, tea and maize are the principal crops of Bangladesh. The country is now on the threshold of attaining self-sufficiency in food grain production (BBS, 2022). The current world population is over 6 billion and will reach 8 billion in 2030. Meanwhile, the annual loss of land to other use is 10 to 35 million ha, with half of this lost land coming from crop land.

The problem, however, is that the per capita land area is one of the lowest in the world, estimated at 0.12 ha. In addition, the mostly unplanned economic growth in the past has led to environmental degradation and adversely affected the resilience of society. The arable land in Bangladesh is 15.92 million hectares about 60 percent of the total land area which is contributing to feed 160 million people in Bangladesh. The country has a favorable natural environment for crop production. Of the arable land, 13.39 percent is under single cropping, 25.57 percent double cropping, 11.50 percent triple cropping, 0.10 quadruple cropping and 2.86 percent currently fallow land. Here, Cropping intensity increases up to 198% (BBS 2022). Among various issues such as agriculture, water, energy, climate change, disaster risk reduction and disaster management, food security considered as the key priority for sustainable development in Bangladesh. As population is increasing, cultivable land is decreasing day by day. Facing such severe situation of population growth pressure plus cropland reduction, it is obvious that the only way to solve food shortage problem is to greatly enhance the yield level of food crops per unit land area through advance of science and technology. Keeping this in mind to feed the growing population the scientists of Bangladesh Institute of Nuclear Agriculture (BINA) have developed 128 high yielding varieties (HYV), those are cultivating in all over Bangladesh.

Getting a new idea adopted, even when it has obvious advantages, is difficult (Rogers, 2003). It is a common experience that the adoption of an apparently useful agricultural technology is slower than predicted or desired by extension agents (Röling, 1988). Masangano and Miles (2004) pointed out “when an agricultural program introduces a new agricultural technology, the program must be able to evaluate whether the technology has been adopted. Of equal importance is the need to identify the factors that influence adoption”. The success of any variety depends on its dissemination among the potential users, which ultimately is measured by the level of adoption of the variety. A farmer who has access to agricultural extension service is approximately 39 times more likely to adopt the variety, and if yield increases by 100%, adoption of the varieties increases by 0.08% (Rahman et. al., 2022). Farmers level adoption study results suggested that increasing trend of farmers level adoption of BINA varieties will contribute country’s total production as well as will support in achieving food security (Rahman et. al., 2020). Although some research on the adoption of BINA varieties has been conducted in Bangladesh, no study has dealt with post adoption factors, i.e. continuing adopters or de-adopters (those who discontinue after having previously adopted).

After the release of varieties, due to the lack of information flow and experience with the new varieties, adoption was limited and slow. In order to prepare programs and

courses of action for wider adoption of varieties, it is important to know the current status of BINA varieties in Bangladesh in terms of area they brought under cultivation. The following specific objectives were set to guide the study: i) to examine the area coverage of BINA developed rice, pulse, oilseed and horticultural crop varieties; ii) to identify major constraints of cultivating BINA developed rice, pulse, oilseed and horticultural crop varieties; and iii) to suggest some policy guidelines.

Materials and Methods

Considering the variations of all these factors the total land area of Bangladesh, Department of Agricultural Extension (DAE) classified fourteen agricultural regions of Bangladesh (Fig.1).

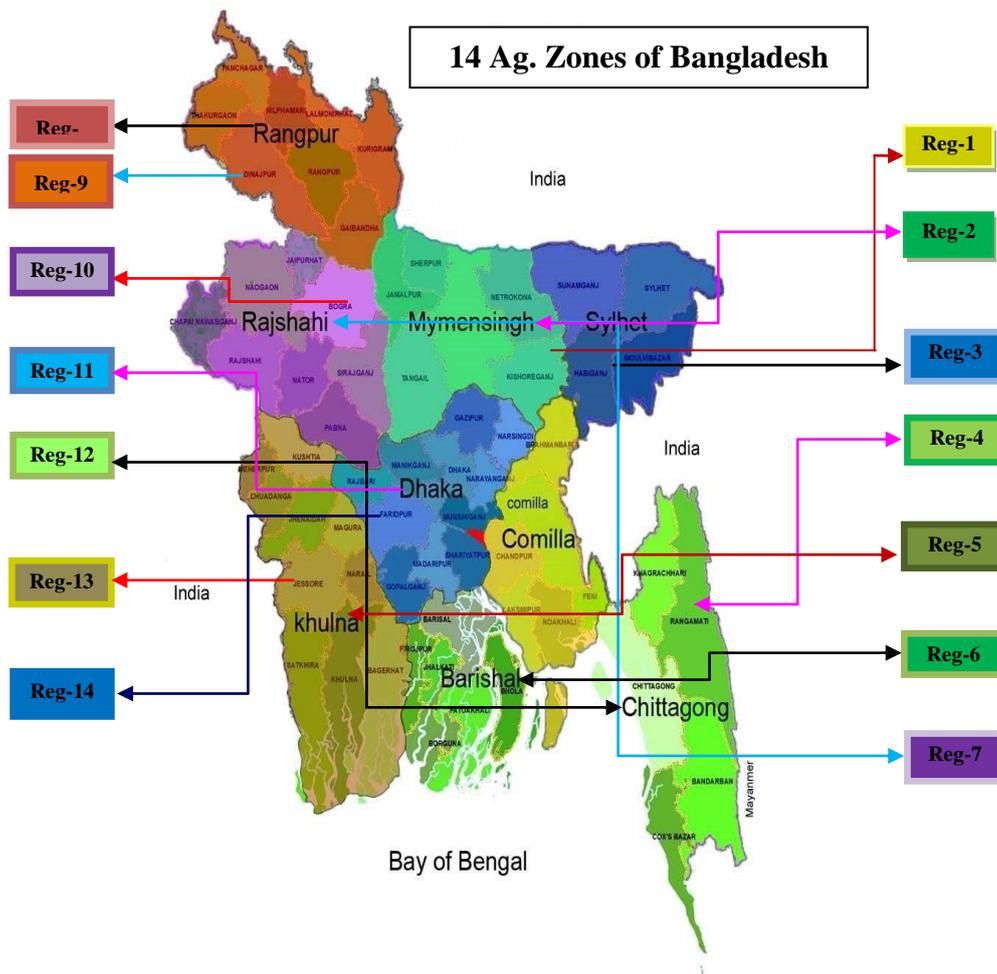


Fig. 1. Map of the 14 Agricultural regions of Bangladesh. Source: Department of Agricultural Extension (DAE).

The 14 agricultural regions were assigned such as Reg-1: Cumilla region (Cumilla, B. Baria, Chandpur), Reg-2: Mymensingh region (Mymensingh, Sherpur, Kishoregonj, Netrokona, Jamalpur), Reg-3: Sylhet region (Sylhet, Moulvibazar, Habiganj, Sunamganj), Reg-4: Rangamati region (Khagrachari, Bandarban, Rangamati) Reg-5: Khulna region (Khulna, Bagerhat, Meherpur, Kushtia, Chuadanga, Satkhira), Reg-6: Barishal (Potuakhali, Jhalokathi, Bhola, Borguna, Pirojpur, Barishal), Reg-7: Rajshahi region (Rajshahi, Pabna, Naogaon, Sirajganj, Natore, Chapainawabganj), Reg-8: Rangpur Region (Gaibandha, Lalmonirhat, Rangpur, Kurigram), Reg-9: Dinajpur region (Dinajpur, Panchagarh, Thakurgaon, Nilphamary), Reg10: Bogura region (Bogura, Joypurhat), Reg-11: Dhaka region (Narsingdi, Narayanganj, Gazipur, Tangail, Manikganj, Munshiganj), Reg-12: Chattagram region (Noakhali, Cox's Bazar, Feni, Lakshmipur, Chattagram), Reg-13: Jashore region (Jashore, Narail, Magura, Jhenaidah), Reg-14: Faridpur region (Rajbari, Madaripur, Faridpur, Sariatpur, Gopalganj). The study was conducted in 64 districts under fourteen agricultural regions of Bangladesh in collaboration with concerned sub-stations and regional station of BINA. In total sixty four data were collected through pre-designed interview schedule using structural questionnaire from concern Deputy Director, Department of Agricultural Extension (DD, DAE) of 64 districts. A stratified random sampling technique was employed in selecting the data. Data were collected using a pre-tested questionnaire. Through prior consultation, a six-page questionnaire was designed formatted with open and closed question items to obtain both quantitative and qualitative data. In the questionnaire per hectare area of BINA developed rice (Aus, Aman and Boro), pulses, oilseed and horticultural crop varieties were included to fulfil the objectives. Besides, secondary data from Bangladesh Bureau of Statistics (BBS) was also used. Tabular and descriptive statistics using mean, average and percentage were used to analyze the collected data. The period of data collection was 1 April to June 30, 2023.

Results and Discussion

It was observed that the overall area coverage of BINA developed rice varieties were 8.91% considering the whole Bangladesh (Table 1). Among the three seasons (Aus, Aman and Boro) the highest area coverage was found in Aman season that was 14.54% followed by Aus 5.95% and Boro 2.93%, respectively (Fig 2). In Aman season, the highest coverage was 6.24% for Binadhan-17 and the lowest was 0.002% for Binadhan-23 as a newly developed variety. In Boro season, the highest coverage was 1.89% for Binadhan-10 and the lowest was 0.06% for Binadhan-24 as a newly developed variety. In Aus season, the highest coverage was 5.25% for Binadhan-19 and the lowest was 0.02% for Iratom-24 (Fig 3).

Table 1. Variety wise area coverage of BINA developed rice varieties in 2022-23

(In ha)				
Rice	Varieties	Cultivated Area	Varietal Adoption (%)	Area Coverage (%)
Boro	Binadhan-5	440	0.04	0.01
	Binadhan-6	2020	0.19	0.04
	Binadhan-8	15236	1.46	0.32
	Binadhan-10	90789	8.72	1.89
	Binadhan-14	25445	2.44	0.53
	Binadhan-18	3508	0.34	0.07
	Binadhan-24	2740	0.34	0.07
	Subtotal	141006	13.54	2.93
Aus	Iratom-24	200	0.02	0.02
	Binadhan-14	2840	0.27	0.25
	Binadhan-19	60848	5.84	5.25
	Binadhan-21	5042	0.48	0.43
	Subtotal	68930	6.62	5.95
Aman	Binashail	5144	0.49	0.09
	Binadhan-7	347352	33.35	6.07
	Binadhan-11	52642	5.05	0.92
	Binadhan-12	7980	0.77	0.14
	Binadhan-13	238	0.02	0.00
	Binadhan-15	120	0.01	0.00
	Binadhan-16	17417	1.67	0.30
	Binadhan-17	357177	34.29	6.24
	Binadhan-20	23496	2.26	0.41
	Binadhan-22	18688	1.79	0.33
Binadhan-23	1394	0.13	0.02	
	Subtotal	831649	79.84	14.54
	Total	1041585	100.00	8.91

Source: DAE data, 2022-23 and BBS, 2022

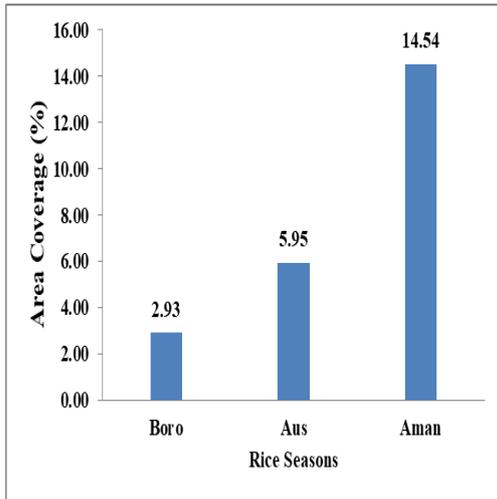


Fig 2. Cultivated areas of BINA developed rice varieties (%)

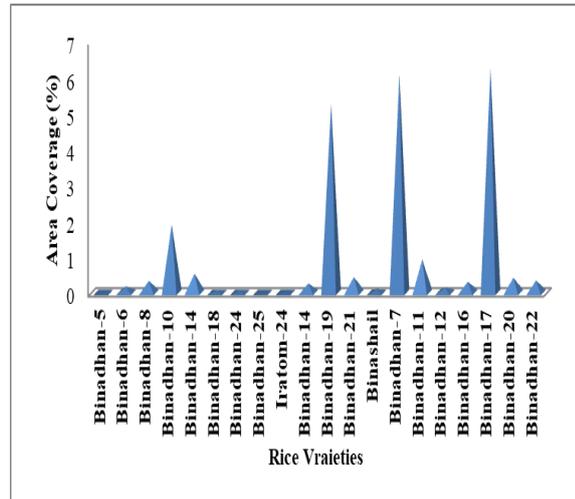


Fig 3. Area coverage of BINA developed rice varieties (%)

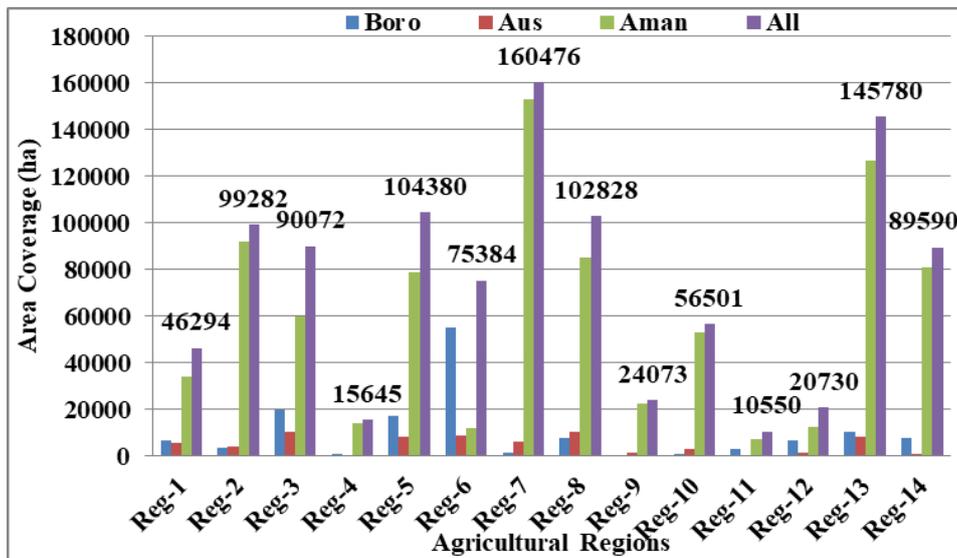


Fig 4. Regional coverage of BINA developed rice varieties during 2022-23

The results presented in Table 2 depicted that among three seasons, area coverage was the highest for Aman that was 79.84% followed by Boro 13.54% and it was the lowest for Aus i.e. 6.62%. Among the 14 agricultural regions the highest area coverage was found 15.41% in Rajshahi region (Reg-7) and the lowest found 1.01% in Dhaka region (Reg-11) (Fig 4.). In Rajshahi region, the highest area was found for Aman season 153128 ha and the lowest was found for Boro season 1320 ha. In Dhaka region, total Aman area was 7242 ha and Aus area was 300 ha.

Table 2. Region-wise area coverage of BINA developed rice varieties during 2022-23

Region	Boro		Aus		Aman		Total	
	Area (ha)	(%)						
Reg-1	6670	4.73	5488	7.96	34136	4.10	46294	4.44
Reg-2	3524	2.50	4000	5.80	91758	11.03	99282	9.53
Reg-3	19900	14.11	10524	15.27	59648	7.17	90072	8.65
Reg-4	846	0.60	587	0.85	14212	1.71	15645	1.50
Reg-5	17380	12.33	8274	12.00	78726	9.47	104380	10.02
Reg-6	55044	39.04	8664	12.57	11676	1.40	75384	7.24
Reg-7	1320	0.94	6028	8.75	153128	18.41	160476	15.41
Reg-8	7456	5.29	10492	15.22	84880	10.21	102828	9.87
Reg-9	388	0.28	1176	1.71	22509	2.71	24073	2.31
Reg-10	609	0.43	2796	4.06	53096	6.38	56501	5.42
Reg-11	3008	2.13	300	0.44	7242	0.87	10550	1.01
Reg-12	6626	4.70	1492	2.16	12612	1.52	20730	1.99
Reg-13	10552	7.48	8276	12.01	126952	15.27	145780	14.00
Reg-14	7683	5.45	833	1.21	81074	9.75	89590	8.60
All	141006	13.54	68930	6.62	831649	79.84	1041585	100

Source: DAE data, 2022-23

Note: **Reg-1:** Cumilla region, **Reg-2:** Mymensingh region, **Reg-3:** Sylhet region, **Reg-4:** Rangamati region, **Reg-5:** Khulna region, **Reg-6:** Barishal region, **Reg-7:** Rajshahi region, **Reg-8:** Rangpur region, **Reg-9:** Dinajpur region, **Reg-10:** Bogura region, **Reg-11:** Dhaka region, **Reg-12:** Chattogram region, **Reg-13:** Jashore region, and **Reg-14:** Faridpur region.

The results presented in Table 3 showed that the overall area coverage of BINA developed pulse varieties were 16.32%. The highest area coverage was found 16.54% for Mungbean and lowest was seen 1.07% in case of Chikpea (Fig 5). Among the pulses the highest area coverage was found in Binamoog-8 that was 11.03% followed by Binamasur-8 (4.13%) and Binamoog-6 (2.76%), respectively (Fig 6).

Table 3. Variety-wise area coverage of BINA developed pulse varieties in 2022-23

	Varieties	Cultivated Area	Varietal Adoption (%)	Area Coverage (%)
Lentil	Binamasur-1	50	0.08	0.03
	Binamasur-2	8	0.01	0.01
	Binamasur-3	63	0.10	0.04
	Binamasur-4	270	0.44	0.19
	Binamasur-5	2844	4.65	1.97
	Binamasur-6	240	0.39	0.17
	Binamasur-7	460	0.75	0.32
	Binamasur-8	5969	9.76	4.13
	Binamasur-9	164	0.27	0.11
	Binamasur-10	19	0.03	0.01
	Binamasur-12	48	0.08	0.03
		Sub-total	10133	16.56
Mungbean	Binamoog-2	25	0.04	0.01
	Binamoog-3	25	0.04	0.01
	Binamoog-4	625	1.02	0.25
	Binamoog-5	2178	3.56	0.88
	Binamoog-6	6795	11.11	2.76
	Binamoog-7	3758	6.14	1.53
	Binamoog-8	27153	44.38	11.03
	Binamoog-9	163	0.27	0.07
		Sub-total	40720	66.55
Chickpea	Binasola-4	65	0.11	0.09
	Binasola-6	11	0.02	0.01
	Binasola-7	300	0.49	0.41
	Binasola-8	428	0.70	0.58
	Binasola-9	8	0.01	0.01
	Sub-total	793	1.32	1.07
Grass pea	Binakhesari-1	8871	14.50	7.53
	Sub-total	8871	14.50	7.53
Black gram	Binamas-1	608	0.99	1.47
	Binamas-2	43	0.07	0.10
	Sub-total	651	1.06	1.57
	Total	61185	100.00	16.32

Source: DAE data, 2022-23 and BBS, 2022

It was observed from Table 4 and Fig 7, among the 14 regions the highest area coverage for pulses was found in the Barishal region 58.51% (Reg-6) and the lowest was found in the Rangamati region 0.001% (Reg-4), respectively.

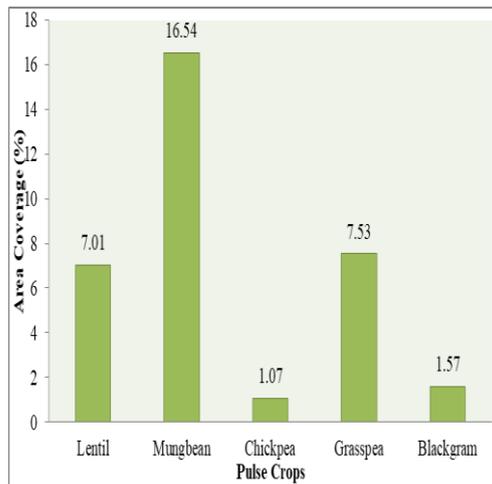


Fig 5. Cultivated areas of BINA developed pulse (%)

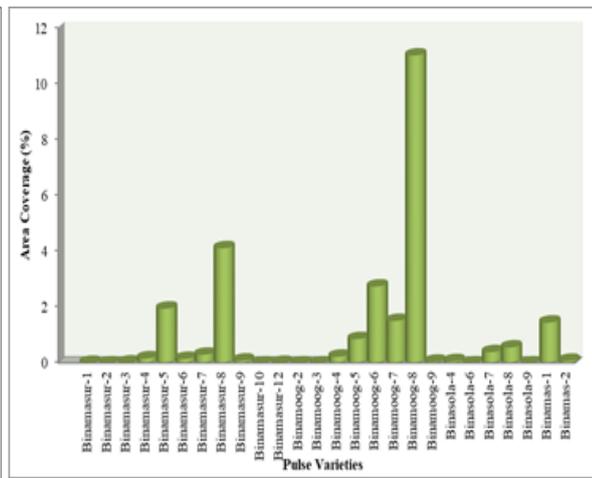


Fig 6. Area coverage of BINA developed pulse (%)

Table 4. Region-wise adoption of BINA developed Pulse varieties during 2022-23

(In ha)

Pulse	Lentil varieties		Mungbean varieties		Chickpea varieties		Grasspea varieties		Blackgram varieties		Total	
	area	%	area	%	area	%	area	%	area	%	area	%
Reg-1	5	0.05	0	0	0	0	105	1.18	0	0	110	0.18
Reg-2	53	0.52	65	0.16	0	0	80	0.9	0	0	198	0.32
Reg-3	0	0	148	0.36	0	0	10	0.11	0	0	158	0.26
Reg-4	0	0	0	0	0	0	0	0	0	0	0	0
Reg-5	748	7.38	786	1.93	0	0	13	0.14	0	0	1546	2.53
Reg-6	90	0.89	34253	84.12	0	0	1455	16.4	0	0	35798	58.51
Reg-7	950	9.38	2108	5.18	790	97.38	133	1.49	250	38.46	4230	6.91
Reg-8	220	2.17	288	0.71	8	0.92	285	3.21	210	32.31	1010	1.65
Reg-9	0	0	777	1.91	0	0	0	0	0	0	777	1.27
Reg-10	0	0	35	0.09	0	0	0	0	0	0	35	0.06
Reg-11	0	0	0	0	0	0	23	0.25	0	0	23	0.04
Reg-12	25	0.25	49	0.12	0	0	5991	67.53	0	0	6065	9.91
Reg-13	3728	36.79	1595	3.92	14	1.69	25	0.28	0	0	5361	8.76
Reg-14	4316	42.59	618	1.52	0	0	753	8.49	190	29.23	5876	9.6
All	10133	16.56	40720	66.55	811	1.33	8871	14.5	650	10.06	61185	100

Source: DAE data, 2022-23

Note: **Reg-1:** Cumilla region, **Reg-2:** Mymensingh region, **Reg-3:** Sylhet region, **Reg-4:** Rangamati region, **Reg-5:** Khulna region, **Reg-6:** Barishal region, **Reg-7:** Rajshahi region, **Reg-8:** Rangpur region, **Reg-9:** Dinajpur region, **Reg-10:** Bogura region, **Reg-11:** Dhaka region, **Reg-12:** Chattogram region, **Reg-13:** Jashore region, and **Reg-14:** Faridpur region.

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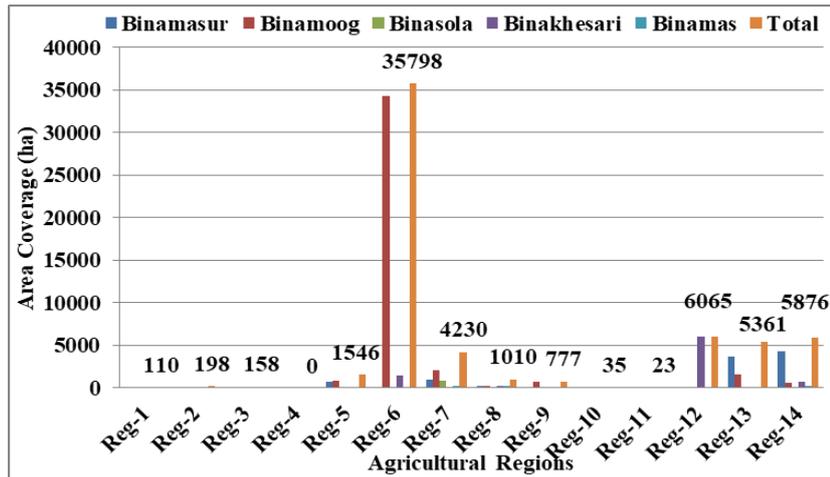


Fig 7: Region-wise adoption of BINA developed Pulse varieties during 2022-23

From Table 5, it was found that, the overall area coverage of BINA developed oilseed varieties were 23.46%. The highest area coverage was found 60.60% for sesame followed by groundnut (55.33%), mustard (21.92%) and soybean (4.22%) (Fig 8). The highest area coverage was found 35.97% for Binachinabadam-4 and the lowest 0.002% was seen in case of Binasoyabean-3 (Fig 9).

Table 5. Variety-wise area coverage of BINA developed oilseed varieties during 2022-23
(In ha)

	Varieties	Cultivated Area	Varietal Adoption (%)	Area Coverage (%)
Mustard varieties	Binasarisha-5	1835	1.56	0.55
	Binasarisha-6	1510	1.29	0.46
	Binasarisha-7	865	0.74	0.26
	Binasarisha-8	798	0.68	0.24
	Binasarisha-9	39201	33.41	11.85
	Binasarisha-10	1815	1.55	0.55
	Binasarisha-11	384	0.33	0.12
	Sub-total	72545	61.82	21.92
Soybean varieties	Binasoybean-2	13	0.01	0.02
	Binasoybean-3	11	0.01	0.02
	Binasoybean-4	48	0.04	0.08
	Binasoybean-5	116	0.10	0.20
	Binasoybean-6	2285	1.95	3.90
		Sub-total	2472	2.11
Groundnut varieties	Binachinabadam-1	75	0.06	0.19
	Binachinabadam-2	230	0.20	0.57
	Binachinabadam-3	150	0.13	0.37
	Binachinabadam-4	14490	12.35	35.97
	Binachinabadam-5	163	0.14	0.40
	Binachinabadam-6	445	0.38	1.10
	Binachinabadam-7	130	0.11	0.32
	Binachinabadam-8	4293	3.66	10.66
	Binachinabadam-9	796	0.68	1.98
	Binachinabadam-10	1518	1.29	3.77
	Sub-total	22289	18.99	55.33
Sesame varieties	Binatil-1	7747	6.60	23.43
	Binatil-2	2288	1.95	6.92
	Binatil-3	2145	1.83	6.49
	Binatil-4	7855	6.69	23.76
	Sub-total	20035	17.07	60.60
	Total	117341	100.00	23.46

Source: DAE data, 2022-23 and BBS, 2022.

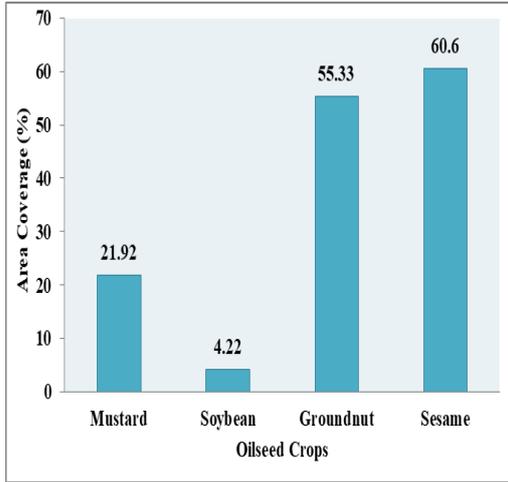


Fig 8. Cultivated areas of BINA developed oilseed varieties (%).

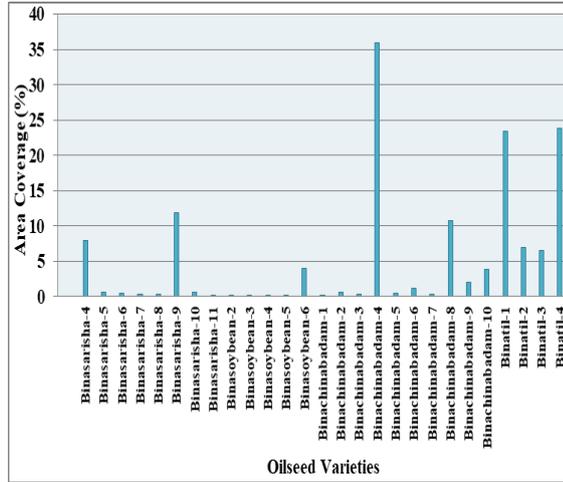


Fig 9. Area coverage of BINA developed oilseed varieties (%).

From Table 6, it was found that among the 14 regions the highest area coverage for oilseed was found in Jashore region 16.32% (Reg-13) and the lowest was found in Rangamati region 1.21% (Reg-10) (Fig 10).

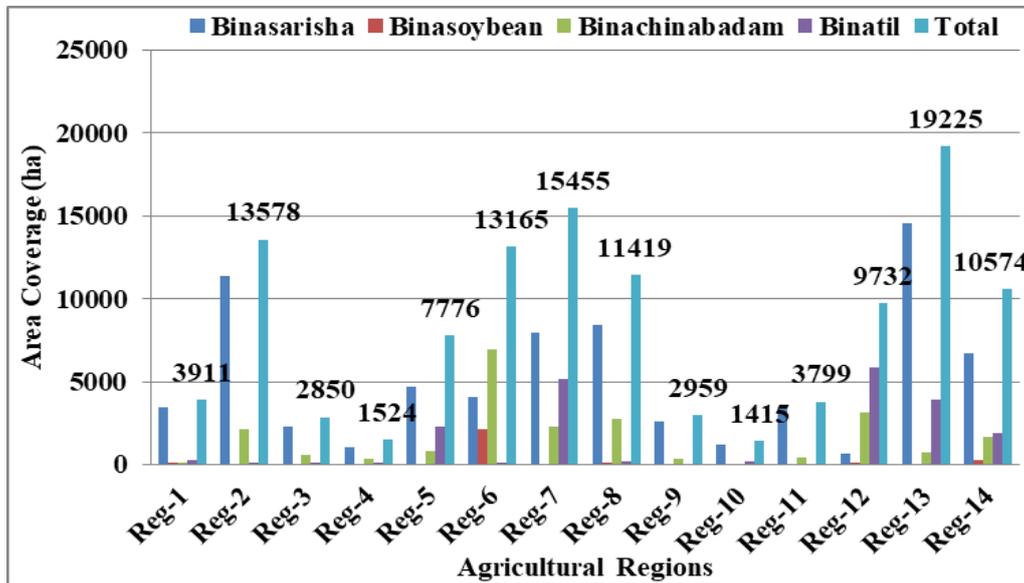


Fig 10. Cultivated areas of BINA developed oilseed varieties in 14 Ag. regions of Bangladesh.

Table-6. Region-wise adoption of BINA developed Oilseed varieties during 2022-23

Region	(In ha)									
	Mustard varieties		Soybean varieties		Groundnut varieties		Sesame varieties		Total	
	Area	%	Area	%	Area	%	Area	%	Area	%
Reg-1	3458	4.77	53	2.12	108	0.49	293	1.46	3911	3.33
Reg-2	11383	15.69	0	0	2148	9.63	48	0.24	13578	11.57
Reg-3	2253	3.1	0	0	575	2.58	23	0.11	2850	2.43
Reg-4	1070	1.47	0	0	378	1.69	76	0.38	1524	1.3
Reg-5	4718	6.5	0	0	783	3.51	2275	11.35	7776	6.63
Reg-6	4093	5.64	2130	86.17	6925	31.07	18	0.09	13165	11.22
Reg-7	7998	11.02	0	0	2303	10.33	5155	25.73	15455	13.17
Reg-8	8450	11.65	13	0.51	2756	12.37	200	1	11419	9.73
Reg-9	2609	3.6	0	0	350	1.57	0	0	2959	2.52
Reg-10	1240	1.71	0	0	0	0	175	0.87	1415	1.21
Reg-11	3343	4.61	0	0	418	1.87	0	0	3799	3.2
Reg-12	695	0.96	39	1.59	3114	13.97	5883	29.36	9732	8.29
Reg-13	14530	20.03	0	0	743	3.33	3953	19.73	19225	16.38
Reg-14	6707	9.25	238	9.61	1690	7.58	1938	9.67	10574	9.01
Total	72545	61.82	2472	2.11	22289	18.99	20035	17.07	117341	100

Source: DAE data, 2022-23

Note: **Reg-1:** Cumilla region, **Reg-2:** Mymensingh region, **Reg-3:** Sylhet region, **Reg-4:** Rangamati region, **Reg-5:** Khulna region, **Reg-6:** Barishal region, **Reg-7:** Rajshahi region, **Reg-8:** Rangpur region, **Reg-9:** Dinajpur region, **Reg-10:** Bogura region, **Reg-11:** Dhaka region, **Reg-12:** Chattogram region, **Reg-13:** Jashore region, and **Reg-14:** Faridpur region.

From Table 7, it was found that, the overall area coverage of BINA developed horticultural crop varieties were 0.26%. The highest cultivated area was found 546 ha for Binalebu-1 followed by Binaholud-1 (246 ha), Binarosun-1 (228 ha) and Binatomato-10 (105 ha) (Fig 11). The highest area coverage was found 4.52% for lemon and among the lemon varieties the highest area coverage was found 4.46% for Binalebu-1 followed by Binahalud-1 (0.93%), Binatomato-10 (0.36%) and Binarosun-1 (0.32%) (Fig 12).

Table 7. Variety-wise area coverage of BINA developed horticultural crop varieties during 2022-23

Varieties	Cultivated Area	Varietal Adoption (%)	Area Coverage (%)
Binalebu-1	546.00	46.73	4.46
Binalebu-2	7.50	0.64	0.06
Binarosun-1	228.00	19.51	0.32
Binatomato-7	36.00	3.08	0.12
Binatomato-10	105.00	8.99	0.36
Binahalud-1	246.00	21.05	0.93
Total	1168.50	100.00	0.26

Source: DAE data, 2022-23 and BBS, 2022

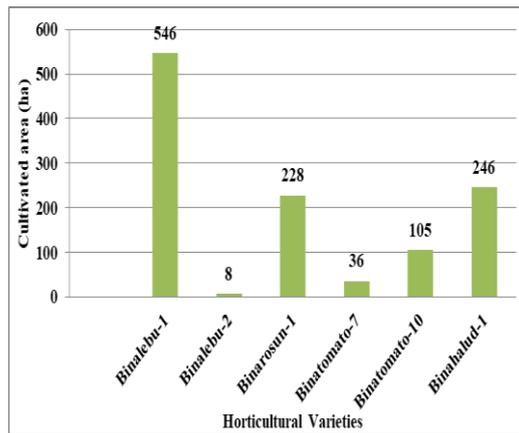


Fig 11. Cultivated areas of BINA developed horticultural crop varieties (%)

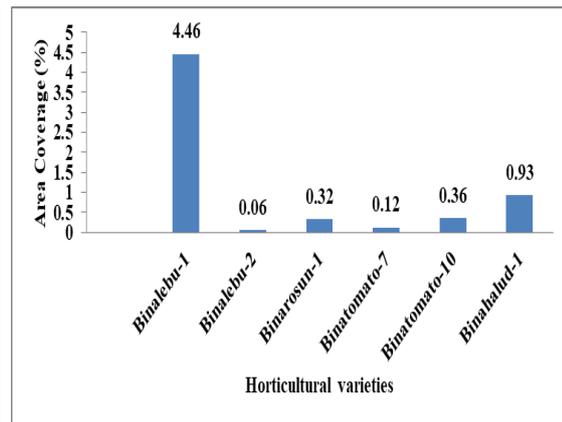


Fig 12. Area coverage of BINA developed horticultural crop varieties (%)

From Table 8, it was revealed that, among the 14 regions the highest area coverage for horticultural crop varieties was found 528 ha (45.19%) in Rangamati region (region-4). It was also observed that, among the 14 agricultural regions the highest area coverage for Binalebu-1 and Binahalud-1 were found in Rangamati region (region-4) about 483 ha (87.26%) and 45 ha (18.29%), respectively. But the highest area coverage for Binarosun-1 was found 195 ha (85.53%) in Rajshahi region (region-7).

Table 8. Region-wise adoption of BINA developed horticultural crop varieties during 2022-23
(In ha)

Region	Lemon varieties		Garlic varieties		Tomato varieties		Turmeric varieties		Total	
	Area	%	Area	%	Area	%	Area	%	Area	%
Reg-1	15	2.71	0	0	0	0	0	0	15	1.28
Reg-2	39	7.05	0	0	36	25.53	93	37.8	168	14.38
Reg-3	0	0	12	5.26	0	0	0	0	12	1.03
Reg-4	483	87.26	0	0	0	0	45	18.29	528	45.19
Reg-5	0	0	0	0	0	0	0	0	0	0
Reg-6	0	0	0	0	0	0	0	0	0	0
Reg-7	0	0	21	9.21	0	0	0	0	21	1.8
Reg-8	0	0	195	85.53	105	74.47	131.71	43.9	408	34.92
Reg-9	4.5	0.81	0	0	0	0	0	0	4.5	0.39
Reg-10	0	0	0	0	0	0	0	0	0	0
Reg-11	6	1.08	0	0	0	0	0	0	6	0.51
Reg-12	1.5	0.27	0	0	0	0	0	0	1.5	0.13
Reg-13	4.5	0.81	0	0	0	0	0	0	4.5	0.39
Reg-14	0	0	0	0	0	0	0	0	0	0
Total	553.5	60	228	24.72	141	15.28	246	26.67	1168.5	100

Source: DAE data, 2022-23

Note: **Reg-1:** Cumilla region, **Reg-2:** Mymensingh region, **Reg-3:** Sylhet region, **Reg-4:** Rangamati region, **Reg-5:** Khulna region, **Reg-6:** Barishal region, **Reg-7:** Rajshahi region, **Reg-8:** Rangpur region, **Reg-9:** Dinajpur region, **Reg-10:** Bogura region, **Reg-11:** Dhaka region, **Reg-12:** Chattogram region, **Reg-13:** Jashore region, and **Reg-14:** Faridpur region.

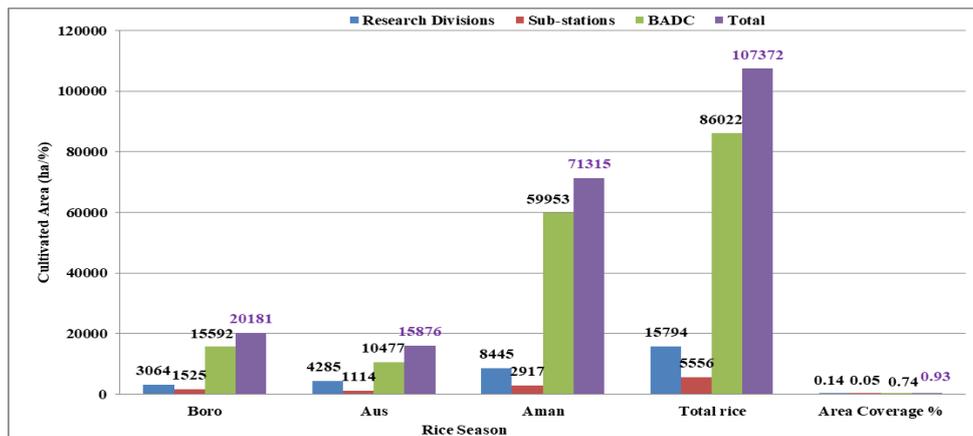


Fig 13. Cultivated areas of BINA developed rice varieties through own seed distribution, 2023.

From Fig 13, it was found that, the overall area coverage of BINA developed rice varieties with own seed distribution were 107372 ha (0.93%). The highest cultivated area was found 86022 ha (0.74%) for BADC followed by research divisions 15794 ha (0.14%), and BINA sub-stations 5556 ha (0.05%). Among the rice seasons the highest area coverage was found for Aman (71315 ha), followed by Boro (20181 ha) and Aus (15876 ha).

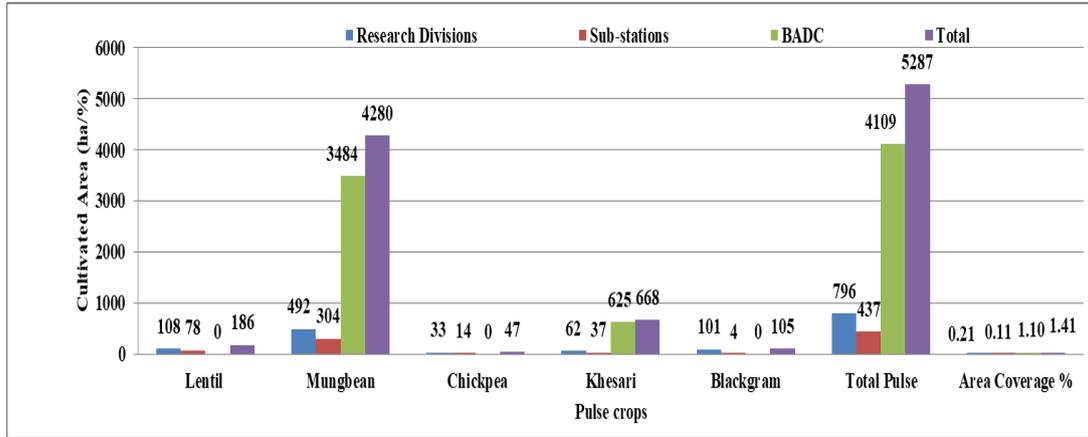


Fig 14. Cultivated areas of BINA developed pulse crops through seed distribution, 2023

It was observed from the Fig 14 that, the overall area coverage of BINA developed pulse crop varieties with own seed distribution were 5278 ha (1.41%). The highest cultivated area was found 4109 ha (1.10%) for BADC followed by research divisions 796 ha (0.21%), and BINA sub-stations 437 ha (0.11%). Among the pulse crop varieties the highest area coverage was found for mungbean varieties (4280 ha), followed by khesari varieties (668 ha), lentil varieties (186 ha), blackgram varieties (105 ha) and chickpea (47 ha).

From Fig-15, it was seen that the overall area coverage of BINA developed oilseed crop varieties with own seed distribution were 17250 ha (3.45%). The highest cultivated area was found 9619 ha (1.92%) for BADC followed by research divisions 5666 ha (1.13%), and BINA sub-stations 1965 ha (0.39%). Among the oilseed crop varieties the highest area coverage was found for sesame (8525 ha) followed by mustard (7205 ha) groundnut (1441 ha), and soybean (79 ha). It was also observed that the area coverage for research divisions distributed mustard seed was the highest (4583 ha).

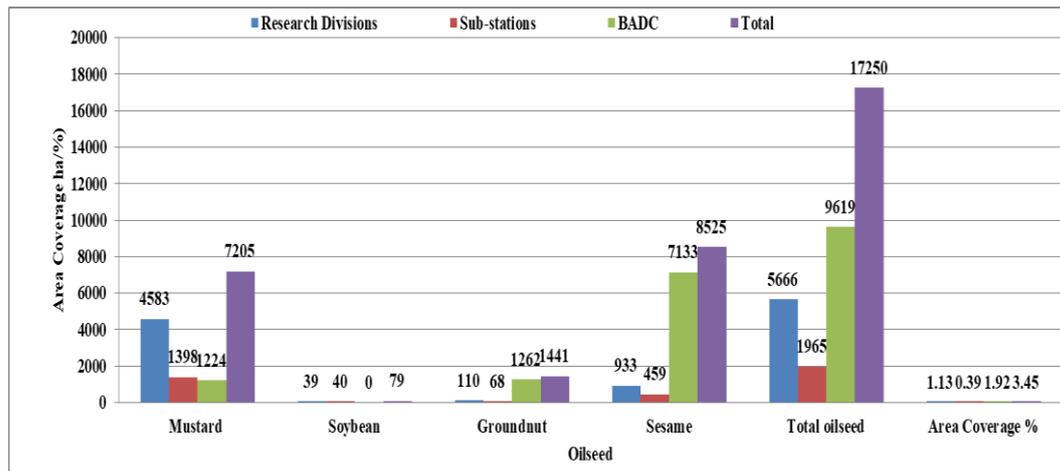


Fig 15. Cultivated areas of BINA developed oilseed crop varieties through own seed distribution, 2023

The study identifies some constraints of increasing area coverage of BINA developed varieties such as-Non availability of seeds which was ranked I; followed by lack of training, workshop and Field Day for DAE personnel, extension workers and farmers (rank II); lack of demonstrations of BINA developed different varieties to the farmers (rank III); lack of proper coordination with DAE, BADC and BINA (rank IV); lack of proper knowledge about BINA developed varieties of the farmers (rank V); lack of publicity of BINA developed varieties (rank VI); weak marketing linkage (rank VII); and lack of monitoring activities of BINA for the extension of BINA developed varieties (rank VIII). For increasing BINA variety cultivation, the highest suggestion was ensuring adequate seeds in every season at appropriate time which was ranked I; Arranging proper training to build proper conception about BINA developed varieties and technologies for the DAE personnel, extension workers and farmers (rank II); demonstrations and its fund should be increased (rank III); publicity is needed and distribute leaflets and booklets to popularize BINA developed varieties among the farmers through DAE and BADC (rank IV); more Field Day, farmers discussion meeting and Uthanbaitak should be arranged (rank V); BINA developed varieties should be included in different govt. incentive projects (rank VI); ensure proper coordination and strong linkage among DAE, BADC, BINA and farmers (rank VII) and ensuring proper monitoring activities by BINA for the extension of BINA developed varieties (rank VIII).

Table 9. Constraints and suggestions by DAE personnel

Item	No. of respondent	Percentage	Rank
Constraints			
Non availability of seeds	35	30	I
Lack of publicity of BINA developed varieties	11	9	VI
Lack of proper knowledge about BINA developed varieties of the farmers	13	11	V
Lack of training, workshop and Field Day for DAE personnel, extension workers and farmers	18	15	II
Lack of demonstrations of BINA developed different varieties to the farmers	17	14	III
Lack of proper coordination with DAE, BADC and BINA	15	13	IV
Lack of monitoring activities of BINA for the extension of BINA developed varieties	3	3	VIII
Weak marketing linkage	6	5	VII
Item	No. of respondent	Percentage	Rank
Suggestions			
Ensure adequate seeds in every season at appropriate time and supply seed to the market, seed dealer and selling center	36	21	I
Publicity is needed and distribute leaflets and booklets to popularize BINA developed varieties among the farmers through DAE & BADC	25	15	IV
Arrange proper training to build proper conception about BINA developed varieties and technologies for the DAE personnel, extension workers and farmers	35	21	II
Ensure proper coordination and strong linkage among DAE, BADC, BINA and farmers	12	7	VII
Ensure proper monitoring activities by BINA for the extension of BINA developed varieties	4	2	VIII
Demonstrations and its fund should be increased	31	18	III
BINA developed varieties should be included in different govt. incentive projects	11	6	VI
More Field Day, farmers discussion meeting and Uthanbaithak should be arranged	16	9	V

Source: DAE data, 2022-23

Conclusion

Area coverage BINA developed variety is increasing day by day and replacing traditional varieties. For continuation of variety expansion, the institute should ensure quality seed supply in proper time through strengthening strong collaboration among other research institutes, BADC, DAE and NGO's. Finally, sufficient number of training for extension workers and farmers, workshop/seminar, demonstration, field day and its budget should be increased which will support in extension/dissemination as well as minimize the future hazard of climate change for ensuring food and nutritional security.

Acknowledgements

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PROFITABILITY AND MARKETING SYSTEM OF Binachinabadam-8 IN SOME SELECTED NORTHERN CHAR AREAS OF BANGLADESH

M.M.A. Sarkar*, M.H. Rahman and S. Islam

Abstract

The present study was undertaken to assess the profitability, marketing system, production and marketing problems of a groundnut variety, Binachinabadam-8 in the river bed areas of Rangpur, Gaibandha and Kurigram districts during 2022–23. The study analyzed the efficiency of different marketing chains by ranking performance indicators using a composite index formula. The average per hectare production cost of Binachinabadam-8 was Tk. 90,470 and the net return was Tk. 68,064. The benefit-cost ratio was estimated at 1.75 and 2.89 on a full cost and variable cost basis. The average estimated marketing cost was highest (Tk. 925/quintal) for Stockist and lowest (Tk. 87/quintal) for Arathdar. Net marketing margin was also highest (Tk. 1787/quintal) for Stockist and lowest (Tk. 115/quintal) for Arathdar. Farmer's share in consumer prices of Binachinabadam-8 in different marketing chains was the highest in Chain-IV (65.46%) and the lowest was in Chain-I (63.98%). The performance indicators revealed that the Chain-IV was the most efficient of the chains. Farmers reported major problems including lack of irrigation facilities, high seed value, flood incidence, high fertilizer prices, lack of capital, lack of training and insect and pest. The farmers faced significant marketing challenges, including unstable prices, high transportation charges, lack of storage facilities and lack of cash capital. Therefore, the study will help to over undertake appropriate measures to overcome the production and marketing problems and thereby will increase the profitability and production of Binachinabadam-8 in the river bed areas of Bangladesh.

Key words: Profitability, Marketing chain, Marketing efficiency, Production and marketing problems, Binachinabadam-8, Char areas

Introduction

Groundnut, or peanut, is commonly called the poor man's nut. Today it is an important oilseed and food crop in Bangladesh. Bangladesh is the biggest delta in the world, as it is within overflow plains of three large rivers like Padma, Brahmaputra-Jamuna, and Meghna (Sarker, *et al.*, 2003). The provisional sandbars that emerged in the Bengal Delta, which are >100 m across with approximately ten years, are known as char (Rogers *et al.*, 2013). The overall land area was increased in Bangladesh by 4% from 1948 to 2006, typically due to the retrieval of char lands (Rahman, 2010). This trend is increasing gradually. About 10 million people live in these areas and survive mainly on agriculture including some people who are most defenseless and the poorest. They carry on their livelihood with hardship (Ashley *et al.*, 2000). The majority of the plant nutrients in these soils are scarce, having very low organic matter contents and low moisture-holding capacity, particularly in the northern char region of Bangladesh (SRDI, 2012).

Agricultural Economics Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

*Corresponding author: mohsinsarkar.bina@gmail.com

The cropping intensity of char land is increasing gradually. Now a days several crops such as maize, wheat, sesame, potato, groundnut etc. are produced in different parts of char lands in Bangladesh. It has substantially changed the socioeconomic life of the char lands people. The total groundnut production was 74,749 MT from 40,285 hectares of cultivated land in Bangladesh (BBS, 2022). Groundnut occupies only about 6.97% of the total area under all oil crops and contributes about 6.73% to the total oilseed production in Bangladesh (BBS, 2021). The soil and climate of Bangladesh are quite suitable for groundnut production. It is cultivated mostly in sandy soils and riverbeds (Nath and Alam, 2002). Apart from the fact that the groundnut is an excellent oil crop, it fixes nitrogen in its root nodule through symbiosis with bacteria and thus require less amount of chemical nitrogenous fertilizer. It is a good source of protein, minerals and vitamins like Vitamin D and Vitamin E. It is also a nutritious fodder for the cattle and profitable cash crop to the farmers. Because of lower farming cost and excellent market price with huge demand, char farmers have been expanding groundnut cultivation every year in the char lands.

Few years back the char people of northern part mostly affected by river erosion had compelled the people living over there to rush to towns for earnings either as day labor or rickshaw and van puller. Now they found a hope of survival by growing groundnut without much investment and hassle. Another advantage of groundnut cultivation is that no natural calamities except flood can damage this crop.

On the other hand Binachinabadam-8 cultivation has no adverse impact on environment rather its green plants help maintained ecological balance in the area. The sustainability of increased trend of production of groundnut in these river based greatly depends on its efficient marketing system. Different constraints from production to consumer's level have been emerged including transportation, price fluctuation, traders etc. may hamper in decision-making of the farmers to cultivate groundnut. Therefore, the study may show us an efficient marketing system of Binachinabadam-8. Recognizing the above importance and suitability of Binachinabadam-8 production and marketing in char areas the study was undertaken with the specific objectives (i) to estimate the costs and return of Binachinabadam-8 in the study areas (ii) to find out the key players involved in the marketing system of Binachinabadam-8 (iii) to determine the marketing cost, margin and marketing efficiency at different levels and (iv) to identify the major production and marketing problems of Binachinabadam-8.

Materials and Methods

Selection of the study area, sample size and sampling technique

This study was conducted in three Binachinabadam-8 growing northern districts having river beds, namely Rangpur, Gaibandha and Kurigram in Bangladesh. A total of 180 samples taking 60 from each district and among the 60 samples 40 farmers and 20

traders/intermediaries were selected following stratified random sampling with the help of Department of Agriculture Extension (DAE) personnel for interview. Data enumerator under the direct supervision of the researchers collected field level cross sectional data using pre-tested interview schedule for this study.

Method of data collection and period of study

Data for the present study were collected from Binachinabadam-8 farmers through a face-to-face interview method using a pre-tested interview schedule. Data were collected by researchers with the help of trained enumerators for the period of May–June 2023.

Analytical techniques

Cost and return analysis

Collected data were edited, summarized, tabulated and analyzed to fulfill the objectives of the study. The data were analyzed with the help of appropriate statistical measures as frequencies, percentages, mean and standard deviation. The following conventional profit equation was applied to examine farmer's profitability level of Binachinabadam-8 in the study areas.

$$\pi = \sum P_m Q_m + \sum P_f Q_f - \sum (P_x i X_i) - TFC$$

Where,

π = Net profit/Net returns from Binachinabadam-8 farming (Tk./ha);

P_m = Per unit price of Binachinabadam-8 (Tk./kg);

Q_m = Total quantity of the Binachinabadam-8 production (kg/ha);

Q_f = Per unit price of other relevant Binachinabadam-8 (Tk./kg);

P_f = Total quantity of other relevant Binachinabadam-8 (kg/ha);

$P_x i$ = Per unit price of i-th inputs (Tk.);

TFC = Total fixed cost (Tk.); and

X_i = Quantity of the i-th inputs (kg/ha); $i = 1, 2, 3, \dots, n$ (number of inputs).

Marketing cost: The total marketing cost was determined by the following formula

$$T_c = C_p + \sum M_{ci}$$

Where, T_c = Total cost of marketing, C_p = Producer cost of marketing and M_{ci} = Marketing cost by the i th trader

Marketing margin: The absolute margin of the middleman, wholesaler, trader and retailers were determined by the following formula

$$MM = SP - PP$$

Where, MM = Marketing margin, SP = Selling price, PP = Purchase price

The cost of marketing was calculated and the low cost marketing chain was ranked I and that which was the highest cost as the last. The same approach was followed in ranking the margin of middlemen in each chain.

Producer's share: The producer's share was calculated by the following formula and the chain which had the highest producer's share was ranked as 'I' (first) and vice-versa.

$$\text{Percentage of producers' share} = (P_{pi}/P_{ri}) \times 100$$

Where, P_{pi} = Producer's share in the i th chain, P_{ri} = Average price at the retail level in each chain, i = Number of chains ($i=1, 2, \dots, n$)

Marketing efficiency: Marketing efficiency is a complicated topic to be defined. It carries different meanings to different persons. Four methods like i) Shephred Method ii) Acharya and Agarwal Method iii) Composite Index Method and iv) Marketing Efficiency Index Method are usually used to calculate the marketing efficiency. However, Composite Index method was followed to estimate marketing efficiency for the present study. As per this method, the percentage of producer's price, marketing cost, marketing margin and marketing profit/gross margin per 100 kg of Binachinabadam-8 was calculated and were assigned ranks. Total scores were found by adding the respective ranks in each chain. The mean scores were calculated for each chain. Where the mean score was less, it was efficient chain.

$$R = (R_i/N_i)$$

Where, R_i = Total value of ranks of all indicators (I_1, I_2 and I_3), N_i = Number of indicators

Results and Discussion

Profitability of Binachinabadam-8 production

The cost of Binachinabadam-8 production, gross return, gross margin, net return and the benefit cost ratio (BCR) for Binachinabadam-8 cultivation are being discussed in the following sections.

Cost of Binachinabadam-8 production

The cost of human labour, power tiller, seed, fertilizers, pesticides and irrigation were taken into consideration, while calculating cost of Binachinabadam-8 production. Beside this, interest on operating capital was also considered as the cost of Binachinabadam-8 production. Total cost consists of variable cost and fixed cost that covered 60.73% and 39.27%, respectively, of total cost for Binachinabadam-8 production (Table 1).

The average costs of Binachinabadam-8 cultivation were Tk. 90,470 and Tk. 54,938 per hectare on full cost and cash cost basis, respectively. The highest production cost was for human labour (51.56%) followed by seed (17%), land use cost (9.54%), land preparation/power tiller (7.68%) and irrigation (5.66%). The cost of Binachinabadam-8

cultivation was found the highest at Rangpur (Tk. 94,215/ha) followed by Kurigram (Tk. 89,397/ha) and Gaibandha (Tk. 87,797/ha), respectively (Table1).

Table 1. Per hectare cost of Binachinabadam-8 production in different locations

Cost Component	Cost of production (Tk./hectare)			All area	% of total cost
	Rangpur	Gaibandha	Kurigram		
(A) Total Variable Cost	57,339	54,330	53,145	54,938	60.73
Hired labour (Man days)	21,721	19,452	18,065	19,746	21.83
Land preparation/Power tiller	7,480	6,854	6,523	6,952	7.68
Seed	14,960	15,201	15,984	15,382	17.00
Fertilizers:					
Urea	960	1050	925	978	1.08
TSP	2,157	2,078	2,216	2,150	2.38
MP	1,870	1,218	1,455	1,514	1.67
Gypsum	910	835	781	842	0.93
DAP	1,120	720	959	933	1.03
Pesticides	748	574	428	583	0.64
Irrigation	4,701	5,469	5,198	5,123	5.66
Int. on operating capital	712	878	611	733	0.81
(B) Total Fixed Cost	36,876	33,468	36,252	35,532	39.27
Family labour	27,259	25,112	28,323	26,898	29.73
Land use cost	9,617	8,356	7,928	8,634	9.54
Total Cost (A+B)	94,215	87,797	89,397	90,470	100.00

Source: Field survey, 2023

Return from Binachinabadam-8 production

The average return from Binachinabadam-8 production at different locations is shown in Table 2. The average yield of Binachinabadam-8 was 1761 kg/ha. The yield was highest at Rangpur (1875 kg/ha) followed by Gaibandha (1715 kg/ha) and Kurigram (1694 kg/ha). The average gross margin was found Tk. 1,03,596/ha on variable cost basis. Gross margin was the highest at Rangpur (Tk. 1,11,411/ha) followed by Gaibandha (Tk. 1,03,450/ha) and Kurigram (Tk. 95,927/ha). The average net return per hectare was Tk. 68,064. The net return was the highest at Rangpur (Tk. 74,535/ha) followed by Gaibandha (Tk. 69,983/ha) and Kurigram (Tk. 59,675/ha). Benefit cost ratio was estimated to be 1.75 and 2.89 on full cost and variable cost basis, respectively, implying that the Binachinabadam-8 cultivation at farm level was profitable.

Table 2. Profitability of Binachinabadam-8 cultivation at different locations

Type	Study areas			All area
	Rangpur	Gaibandha	Kurigram	
Yield from Binachinabadam-8 (Kg/ha.)	1,875	1,715	1,694	1,761
Average sale price (Tk./kg)	90	92	88	90
Total return (Tk./ha)	1,68,750	1,57,780	1,49,072	1,58,534
Total variable cost (Tk./ha)	57,339	54,330	53,145	54,938
Total Cost (Tk./ha)	94,215	87,797	89,397	90,470
Gross margin (Tk./ha)	1,11,411	1,03,450	95,927	1,03,596
Net return (Tk./ha)	74,535	69,983	59,675	68,064
Rate of return (BCR)				
BCR on full cost	1.79	1.80	1.67	1.75
BCR on variable cost	2.94	2.90	2.80	2.89

Source: Field survey, 2023

Groundnut marketing system

Marketing chain

Marketing chain is the alternative root of products flow from producers to consumers. Market chain analysis aims to provide information on profitability for the various agents along the market chain (Ferris *et al.*, 2001).

The following major marketing chains were found in the study areas:

- Chain-I: Farmer > Faria > Arathdar > Stockist > Paiker > Retailer > Consumer
- Chain-II: Farmer > Faria > Stockist > Bepari > Retailer > Consumer
- Chain-III: Farmer > Bepari > Arathdar > Paiker > Retailer > Consumer
- Chain-IV: Farmer > Paiker > Retailer > Consumer

Characteristics of actors involved in the groundnut marketing chain

Faria: Faria is a petty trader or small scale businessman who purchases groundnut from the producer from the village or from the local market and offer the same to the arathdar or bepari. Sometimes he sells his produce directly to the rural retailers or consumers.

Bepari: Bepari is a professional wholesale traders who make his purchase from producer or faria from the local market, bring their consignment to the urban wholesale market and sell them to the paiker and retailer through arathdar (commission agent).

Arathdar: Arathdar is a commission agent who has a fixed establishment and operates between bepari and retailers, or between farmer and paiker, or between bepari and paiker, or between faria and bepari. They take commission from both of the parties but generally they do not follow any standard rule to take commission.

Paiker: Wholesaler in consuming area is known as paiker, who purchases from bepari through arathdar and sell those to the retailer or consumer.

Retailer: The retailer, the last link in the marketing channel, buys groundnut from arathdar or wholesaler/paiker and sells these to the consumer.

Stockist: Stockist are working in the producing area who purchase wet groundnut from the farmers or faria and dry it in their chatal and store for some period for higher prices.

Marketing cost of different actors involved in groundnut marketing

The cost of marketing represents the cost of performing various marketing functions and operations by various agencies involved in the marketing process (Kohls and Uhl, 2005). In other words, the costs items, which are needed to move the product from producers to consumers, are ordinarily known as marketing cost. The per quintal marketing cost of Binachinabadam-8 of different actors like Faria Tk. 121, Bepari Tk. 278, Arathdar Tk. 87, Stockist Tk. 925, Paiker Tk. 141 and Retailer Tk. 145 in all areas. Marketing cost of stockist was the highest among the intermediaries. Weight loss and damage was the highest cost item for stockist. Because they purchase wet groundnut from farmers and dry it in their chatal. The other cost items of the actors were loading, unloading, packaging, Arathdar commission, khajna etc. The cost varied from area to area depending on distance (Table 3).

Table 3. Marketing cost of different actors involved in the chain (Tk./quintal)

Cost component	Faria	Bepari	Arathdar	Stockist	Paiker	Retailer
Transportation	32	61	-	38	38	21
Loading	9	22	-	14	18	14
Unloading	8	12	-	10	9	8
Wages and salaries	-	-	25	-	-	-
Packaging	15	22	-	-	17	-
Commission	20	55	-	-	22	67
Accountant cost	-	-	12	-	-	-
Drying & Grading	-	-	-	68	-	-
Khajna	19	19	-	-	20	-
Wastage and damage/Weight loss	-	65	-	562	-	-
Shop rent	-	-	8	-	-	-
Chatal cost	-	-	-	120	-	-
Storage cost	-	-	-	62	-	-
Market toll	-	-	-	-	-	16
sweeper	-	-	2	2	-	-
Electricity cost	-	-	8	10	-	-
Telephone/Mobile	8	10	15	20	9	9
Entertainment	10	12	17	19	8	10
Total	121	278	87	925	141	145

Source: Field survey, 2023

Marketing cost at different levels of marketing chain

Chain-wise marketing cost is shown in Table 4. It was observed that Chain-i incurred the highest marketing cost (Tk.1,697/quintal) followed by Chain-ii (Tk.1,440/quintal) and Chain-iii (Tk.1,286/quintal). Lowest marketing cost was found in Chain-iv and it was Tk. 863/quintal (Table 4). Highest numbers of intermediaries were involved in Chain-i which was the main reasons for the highest marketing cost.

Table 4. Marketing cost of Binachinabadam-8 for different marketing chain (Tk./quintal)

Cost component	Chain-I	Chain-II	Chain-III	Chain-IV
Transportation	190	184	181	174
Loading	77	75	74	65
Unloading	47	42	40	36
Wages and salaries	25	22	24	20
Packaging	54	51	50	42
Commission	164	162	160	115
Accountant cost	12	10	12	6
Drying & Grading	68	62	65	58
Khajna	58	55	58	52
Wastage and damage/Weight loss	627	426	288	110
Shop rent	8	8	7	6
Chatal cost	120	112	110	15
Storage cost	62	61	60	26
Market toll	16	14	14	10
sweeper	4	4	3	2
Electricity cost	18	14	18	16
Telephone/Mobile	71	66	70	60
Entertainment	76	72	52	50
Total	1,697	1,440	1,286	863

Source: Field survey, 2023

Marketing margin of different actors involved in the groundnut marketing

Marketing margin is the difference between the price paid by the consumer and price received by the producers. Marketing margin has two components marketing cost and net margin or profit. In broad sense, marketing margin is the difference between what is paid by the consumer and what is received by the producer. It is the price of all utility adding activities and functions that are performed by the intermediaries (Kohls and Uhl, 2005). It was revealed from the study that the net margin of the actors like Faria was Tk. 184, Bepari Tk. 297, Arathdar Tk. 115, Stockist Tk. 1787, Paiker Tk. 222 and Retailer Tk. 298 per quintal (Table 5). Among the intermediaries the stockiest added the highest margin followed by Retailer, Bepari, Paiker, Faria and Arathdar (Table 5). Because the stockist had done some marketing functions such as drying the groundnut in their chatal and stocked the quantity for higher price. On the other hand retailer has to sell small amount of groundnut for long period due to higher profit.

Table 5. Marketing margin of different stages of marketing chain (Tk./quintal)

Cost component	Faria	Bepari	Arathdar	Stockist	Paiker	etailer
A. Average sales price	9,155	9,730		8,962	9,541	9,984
B. Average purchase price	8,850	9,155		6,250	9,178	9,541
C. Gross margin (A-B)	305	575	202	2,712	363	443
D. Marketing cost	121	278	87	925	141	145
E. Marketing Margin (C-D)	184	297	115	1,787	222	298

Marketing Efficiency

Marketing efficiency is directly related to the cost involved in moving goods from the producer to the consumer and the quantity of services offered. If the cost incurred when compared with the service involved, is low, it will be efficient marketing. The improvement of marketing efficiency means the reduction of marketing cost without reducing the quantum of services to the consumer. Marketing efficiency is a complicated topic to be defined. It carries different meaning to different persons. The term marketing efficiency is seen in different perspectives by the marketing personnel and economist. Kohls *et al.* (2005) defined marketing efficiency as the maximization of input output ratio.

Farmer's share under different marketing chain of Binachinabadam-8

Farmer's share in consumer prices of Binachinabadam-8 in different marketing chains was the highest in Chain-IV followed by Chain-III and Chain-II and was the lowest in Chain-I (Table 6). It indicated that if farmer would sell their product through Farmer>Paiker>Retailer>Consumer, they would be most benefited. Unnecessary marketing tiers develops when there is market imperfection or producer-seller are inorganized and while there is lack of market information or the cost of gathering information is high.

Table 6. Farmer's share under different major marketing chain of Binachinabadam-8 (%)

Particulars	Chain-I	Chain-II	Chain-III	Chain-IV
Farmers price (Tk./100 kg)	8,800	8,914	8,967	9,105
Consumer/retail price (Tk./100 kg)	13,755	13,888	13,855	13,910
Percentage of farmers share	63.98	64.18	64.72	65.46
Rank(I ₁)	IV	III	II	I

Source: Field survey, 2023

Marketing cost and margin of different chains of Binachinabadam-8

The Chain-I of Binachinabadam-8 marketing has incurred the highest marketing cost whereas the lowest in case of Chain-IV (Table 7). It reveals if farmer sell their Binachinabadam-8 through Farmer > Faria > Arathdar > Stockist> Paiker > Retailer > Consumer, the marketing cost becomes the highest (Chain-I). On the other hand, if farmer

sell their product through Chain-IV (Farmer > Paiker > Retailer > Consumer) then the marketing cost is the lowest. The data reveals that the highest margin in Chain-I and the lowest in Chain-IV.

Table 7. Marketing cost and margins of different marketing chain for Binachinabadam-8 (Tk./ quintal)

Particulars	Chain-I	Chain-II	Chain-III	Chain-IV
Farmers price (Tk./100 kg)	8,800	8,914	8,967	9,105
Consumer/retail price (Tk./100 kg)	13,755	13,888	13,855	13,910
Marketing margin	4,955	4,974	4,888	4,805
Rank (I ₂)	III	IV	II	I
Marketing cost	1,697	1,440	1,286	863
Rank (I ₃)	IV	III	II	I

Source: Field survey, 2023

Efficiency of different marketing chains of Binachinabadam-8

The efficiency of different marketing chains was drawn as the basis of ranks of different performance indicators in different chains using composite index formula. The performance indicators revealed that the chain-IV is more efficient than that of other chains (Table 8).

Table 8. Marketing efficiency of Binachinabadam-8 under composite index method

Marketing channel	Source of performance indicator			Total score	Rank
	Farmer's share (%) (I ₁)	Marketing margin (Tk./quintal) (I ₂)	Marketing cost (Tk./quintal) (I ₃)		
Chain-i	63.98 (4)	4955 (3)	1697 (4)	11	IV
Chain-ii	64.18 (3)	4974 (4)	1440 (3)	10	III
Chain-iii	64.72 (2)	4888 (2)	1286 (2)	6	II
Chain-iv	65.46 (1)	4805 (1)	863 (1)	3	I

Source: Field survey, 2023

Major problems to Binachinabadam-8 production

Binachinabadam-8 is a profitable oilseed variety in the study areas. The farmers in the study areas faced some problems for Binachinabadam-8 production. The first ranked problem was lack of irrigation facilities (92%) in all the areas. Other problems were high value of seed (65%), incidence of flood (40%), high price of fertilizer (36%), lack of capital (26%), lack of training (14%) and insect and pest (12%) (Table 9).

Table 9. Major problems to Binachinabadam-8 production in the study areas

Type of problems	Percent of farmers responded				Rank
	Rangpur	Gaibandha	Kurigram	All area	
Lack of irrigation facilities	88	94	95	92	1
High value of seed	60	65	70	65	2
Incident of flood	30	82	8	40	3
High price of fertilizer	30	25	53	36	4
Lack of capital	17	50	12	26	5
Lack of training	10	15	18	14	6
Insect and pest	12	10	15	12	7

Source: Field survey, 2023

Marketing problems identified by the traders

The traders or intermediaries faced different marketing problems during their business. Eighty six percent farmers suffered unstable price during their business. Seventy six percent farmers had to pay high charge for transportation followed by lack of storage facilities (73%) and lack of cash capital (54%) (Table 10).

Table 10. Major marketing problems identified by the traders

Marketing problems	percent of traders responded				Rank
	Rangpur	Gaibandha	Kurigram	All areas	
Unstable price	90	82	85	86	1
High transportation cost	75	80	74	76	2
Lack of storage facilities	60	78	81	73	3
Lack of cash capital	38	55	68	54	4

Source: Field survey, 2023

Conclusion

Binachinabadam-8 production in the study areas is profitable. The study identified four major marketing chains for Binachinabadam-8 marketing. The actors in the Binachinabadam-8 marketing chain were Faria, Bepari, Arathdar, Stockiest, Paiker and Retailer. A good amount of marketing margin was received by the actors at different stages of the marketing chain. Out of four marketing chains, chain-IV was more efficient than those of the other chains. The study also identified seven production and four marketing problems of Binachinabadam-8 production in the study areas.

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CLAY MINERALS IDENTIFICATION BY X-RAY POWDER DIFFRACTION NEAR A MINING SITE, MALAYSIA

K.S. Arefin* and M.M.H. Sohag

Abstract

The soil samples collected from an agricultural region near the Sungai Chalit mine site in Raub, Pahang, Malaysia were subjected to X-ray powder diffraction (XRPD) analysis for the first time. The PanAnalytikX'pert Pro XRD equipment at the XRD laboratory, Material Technology, Nuclear Malaysia, was used to identify the clay minerals present in the samples. After thorough analysis of all samples, it has been determined that microcline is found in every sample, whereas bytownite has been detected in just three unique samples (DS2, DS15, and DS17). Two discrete variants of zeolite were discerned in two specimens, denoted as DS2 and DS17. Sample DS6 contains kaolinite. Sample C13 comprises four clay minerals: microcline, anorthite, birnessite, and tremolite. The occurrence of microcline in soil signifies the erosion of rocks abundant in feldspar, such as granite, resulting in a reduction of essential minerals like potassium and calcium. This depletion has the potential to affect plant nutrition and crop yield.

Key words: X-ray powder diffraction, Clay minerals, Agricultural soil.

Introduction

Clay minerals play a crucial role in the formation of soil formations. For instance, Garrels and Mackenzie's research in 1971 found that the percentage of clay minerals in sedimentary rocks is around 35% by weight. The soil composition consists of around 20-30% air in the pore space, 20-30% water, and 45-50% mineral soil solids, with 1-5% organic matter (Weaver & Pollard, 1975). The unique structures and surface properties of clay minerals, such as swelling, cation exchange capacity, and solid acidity, have been found to affect the characteristics of soils and rocks. This has been demonstrated in various studies (Moore and Reynolds, 1997; Okada *et al.*, 2006; Yuan *et al.*, 2008, 2009; Środoń and McCarty, 2008; Fan *et al.*, 2009; He *et al.*, 2010; Liu *et al.*, 2011, 2013, Zhou *et al.*, 2018; Ali *et al.*, 2022; Keya *et al.*, 2023).

X-ray diffraction (XRD) has become increasingly important as an analytical method for studying natural clay materials over the past century. It is used to qualitatively and quantitatively examine these materials. Various researchers have contributed to the development of XRD, including Taylor in 1978, Bish and Howard in 1988, Bish and

Electronics and Health Physics, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202

*Corresponding author's e-mail: jewel.ape@gmail.com

Chipera in 1994, Środoń in 2002, and Chipera and Bish in 2013. A crucial concern in the domain of mineral analysis, especially when dealing with clay minerals, is the precise evaluation of quantity.

The many characteristics of clay minerals, such as their broad variety of chemical compositions, preferred orientation, structural disorder, and major structural changes, are the main reasons for this (Hillier, 2000; Środoń *et al.*, 2001; Środoń, 2002, 2013; Bergaya *et al.*, 2006). Quantifying clay minerals is more challenging compared to quantifying other minerals due to the distinctive structures they possess, including different polytypes and types of flaws. Additionally, clay minerals may vary in terms of chemical composition and the influence of preferred orientation (Środoń, 2002). The alignment and orientation of clay mineral particles influences the X-ray diffraction (Brindley, 1980; Moore *et al.*, 1997) intensity of powdered samples. Nevertheless, the majority of clay mineral particles have a flat, plate-like structure. Commonly used preparation techniques, such as front-loading, tend to result in the alignment of clay minerals in a desired orientation (Hillier, 1999). The XRD reflection intensities of clay minerals are often amplified by the preferred orientation, as observed by Lippmann in 1970. Consequently, the preferred orientation is considered a significant source of mistake in the quantitative analysis of clay minerals, as stated by Środoń *et al.* in 2001.

In the past, qualitative phase analysis, quantitative phase analysis using calibration techniques, and crystal structure determinations were separate fields of study. The Rietveld approach emerged as the primary technique for quantitative phase analysis (QPDA) in the late 20th century (Rietveld, 1967, 1969), replacing older methods. Subsequently, quantitative phase analysis has necessitated the use of crystal structures and fitting instead of calibrations. Simultaneously, the number of reference patterns for phase identification grew as crystal structure data was included, and databases were quickly populated with reference powder patterns derived from single crystal structure data. The software's primary objective is to perform phase identification, which serves as the first stage in X-ray diffraction (XRD) powder analysis. The user has the ability to do identification by analyzing the peak locations (d spacing and relative intensities) and/or by examining the whole net profile of the experimental pattern.

However, often using both strategies yields optimal outcomes, including significant and minor stages. For a search-match job, it is advantageous to merge many commercial reference databases (Allen *et al.*, 1987; Belsky *et al.*, 2002; Villars *et al.*, 2002, 2004; Downs and Hall-Wallace, 2003; Gražulis *et al.*, 2009, 2012; ICDD) together with user databases. Consequently, contemporary powder pattern analysis software must not only manage peaks, background, profile, and reference data, but also accommodate crystal structures, enable space group transformations, and facilitate various fits, as well as support related methods like microstructure analysis. Emerging one and two dimensional detectors provide expedited and enhanced data acquisition, necessitating the use of statistical

techniques and a preliminary identification of "noteworthy" data for further analysis. Automation and data throughput have grown in significance at the same time (Degen *et al.*, 2014). The objective of the study was to identify the clay minerals present in the samples by utilizing X-ray powder diffraction. The minerals were identified by employing the diffraction pattern peaks and peak positions obtained from the inorganic crystal structures database (ICSD) of FIZ Karlsruhe, followed by analysis using the Rietveld refinement in HighScore software of PANalytical. The quality of analysis were ensured by Material Technology Division, Malaysia Nuclear Agency.

Method and Material

i) Principle of X-ray Diffraction

X-ray diffraction is the phenomenon where X-rays are dispersed by atoms that are organized in a regular pattern inside crystals. The atoms act as scattering centers (Moore and Reynolds, 1997), producing X-rays in all directions at the same wavelength as the incoming radiation (coherent scattering). The atoms' organized configuration causes the X-rays to disperse in a pattern where they are synchronized in some directions determined by the crystal's symmetry and atomic distances, while they are unsynchronized in all other directions (Figure-1).

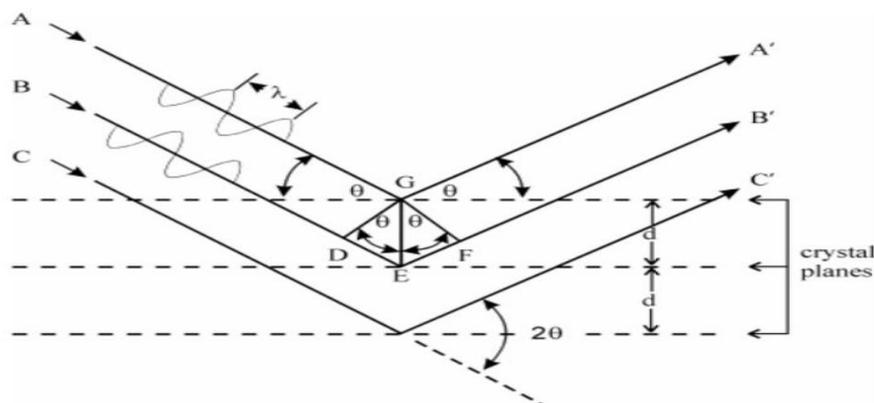


Fig. 1. Diagram illustrating X-ray diffraction (XRD) using evenly spaced atomic planes inside a crystal.

The distance between a given set of planes is termed d-spacing. The d-spacing, although on a scale of Angstroms, can be determined quite accurately using XRD. The principles underlying this determination are elegantly expressed by the Bragg equation: $n\lambda = 2d \sin \theta$;

where n is an integer, λ is wavelength of the radiation, d is d-spacing, and θ is the angle between the planes and the incident X-ray beam.

The factor in the Bragg equation of interest to mineralogists is the d-spacing, which can be determined in XRD analysis by fixing λ and measuring the θ angle where a peak in X-ray intensity occurs.

ii) Sample Collection and preparation

Five soil samples were collected from Sungai Chalit, Raub, Pahang, Malaysia, for the purpose of identifying and analyzing the structure of clay minerals. The samples were prepared for X-ray diffraction (XRD) examination after conducting element analysis using X-ray fluorescence (XRF). X-ray diffraction (XRD) is mostly used for the identification of various compounds based on their diffraction patterns or diffractograms, making it the most often employed application of XRD (Zwell *et al.*, 1975; Chipera *et al.*, 2013; Salma *et al.*, 2023 Wasel *et al.*, 2023).



Fig. 2. Soil samples for clay mineral analysis.

Before doing XRD measurements, it is crucial to adequately prepare the sample. The XRD sample preparations were carried out using standard techniques. The five samples were oven dried and subsequently crushed using a mortar and pestle. After the grinding operation, dry sieving was conducted. Consequently, a filter size of 500 microns was employed. Only samples less than 500 microns were collected and appropriately labeled. The sample preparation method involved using many pieces of equipment, including the cover glass, PW 1172/01 sample holder with a back plate, and slide glasses. The powder sample was placed into the holder, which was connected to the back plate. A shard of glass was employed to compact the powder. The surplus was removed by employing a glass plate, thereby prepared the sample for measurement (Figure 3).

iii) X-ray powder diffraction of samples

The PanAnalytikX'pert Pro XRD equipment (Figure 4) at the XRD laboratory, Material Technology, Nuclear Malaysia, was used to examine all samples. The inputted scan settings were as follows: The scan axis is set to Gonio. The start position is $10.0297^{\circ}2\theta$, the end position is $79.9567^{\circ}2\theta$, and the step size was $0.0330^{\circ}2\theta$. The scan step duration was 19.4436 seconds, and the scan type was PSD (2.12). The divergence slit was set at a fixed value of 0.50 for all specimens with a length of 10 mm. The anode material used for the measurement in this X'pert Pro XRD instrument was copper (Cu), with specific wavelengths of K-Alpha1 (1.54060 \AA), K-Alpha2 (1.54443 \AA), K-Beta (1.39225 \AA), and a ratio of K-A2 to K-A1 of 0.50000. The generator was tuned to a current of 30 milliamperes and a voltage of 40 kilovolts for all diffraction investigation.

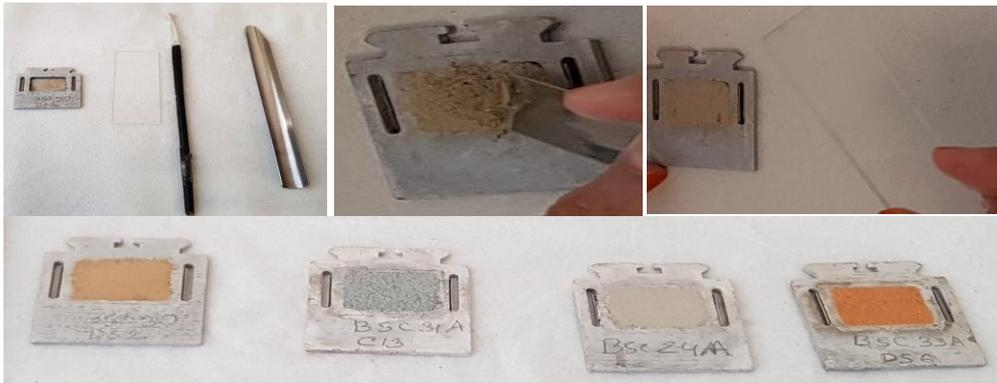


Fig. 3. Sample preparation for XRD measurement.



Fig. 4. PANalytical X'Pert PRO XRD system at MTEG.

iv) Data Processing

Subsequently, all diffractograms underwent processing, refinement, and comparison with the powder diffraction file (PDF) using High Score suite, PANalytical's own program for analyzing powder diffraction. This tool enables users to conduct a wide range of crystallographic experiments, including the capability to determine crystal structures using powder diffraction data. The program employed qualitative phase identification, profile fits, and phase rietveld fits to perform quantitative determinations (Zhou et al., 2018). In addition, it was utilized for graphical comparisons, resolving inorganic crystal structures, identifying unit cells, and conducting statistical analyses to verify the presence of certain clay minerals (Degen *et al.*, 2014).

Result and discussion

The diffraction pattern of sample C13 in Figure 5 shows the existence of Anorthite, Birnessite, Microcline, and Tremolite. Anorthite is a very prevalent mineral that has a distinct, pointed appearance. It consists of a crystalline material. The sample's quantitative composition was determined using the rietveld refinement process, which identified the presence of Anorthite (56.2%), Birnessite (1.9%), Microcline (7.2%), and Tremolite (34.7%). Hence, Anorthite and Tremolite have greater importance in comparison to other minerals, accounting for 56.2% and 34.7% respectively.

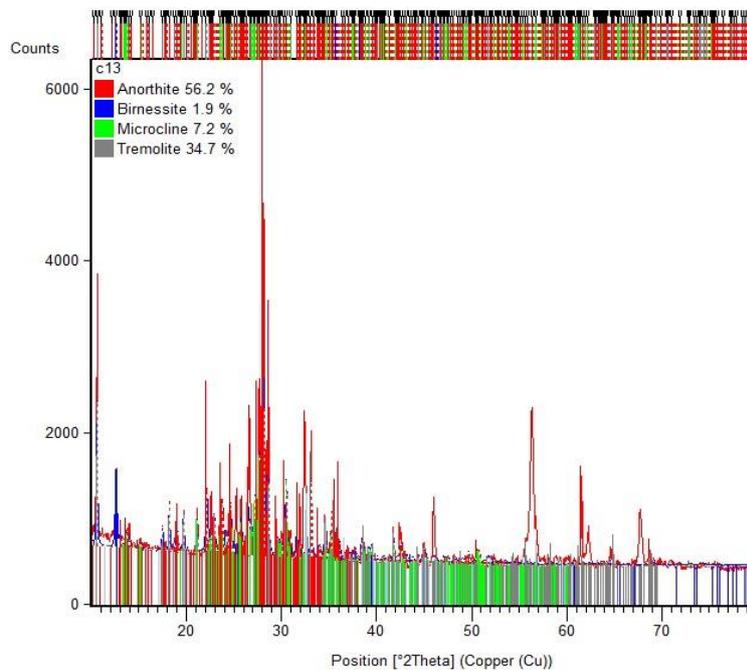


Fig. 5. XRD diffractogram of sample C13. (2θ vs Counts).

The diffraction pattern of sample DS2 in Figure 6 indicates the existence of Microcline, Bytownite, and Zeolite SIZ-7. Microcline is more prevalent than other minerals and has a distinct cleavage. The sample contains Microcline (56.1%), Bytownite (24.7%), and Zeolite SIZ-7 (19.2%). Hence, Microcline and Bytownite exhibit notable prominence in comparison to other minerals, accounting for 56.1% and 24.7% respectively.

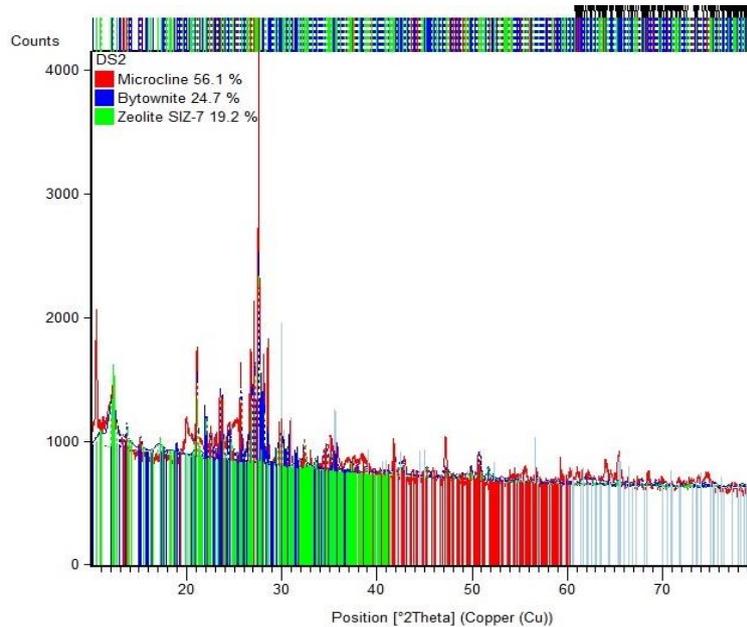


Fig. 6. XRD diffractogram of sample DS2. (2θ vs Counts).

The diffraction pattern of sample DS6 in Figure 7 indicates the existence of Microcline and Kaolinite 1A. Kaolinite 1A is the most prevalent mineral and has a distinct fracture. It consists of a crystalline material. The composition of the sample consists of Microcline (25.4%) and Kaolinite 1A (74.6%). Thus, Kaolinite 1A has more importance in comparison to Microcline.

The diffractogram of sample DS15 in Figure 8 indicates the existence of Microcline and Bytownite. Both Microcline and Bytownite possess a distinct cleavage and were made of crystalline matter. Microcline (41.1%) and Bytownite (58.9%) are the constituents of the sample.

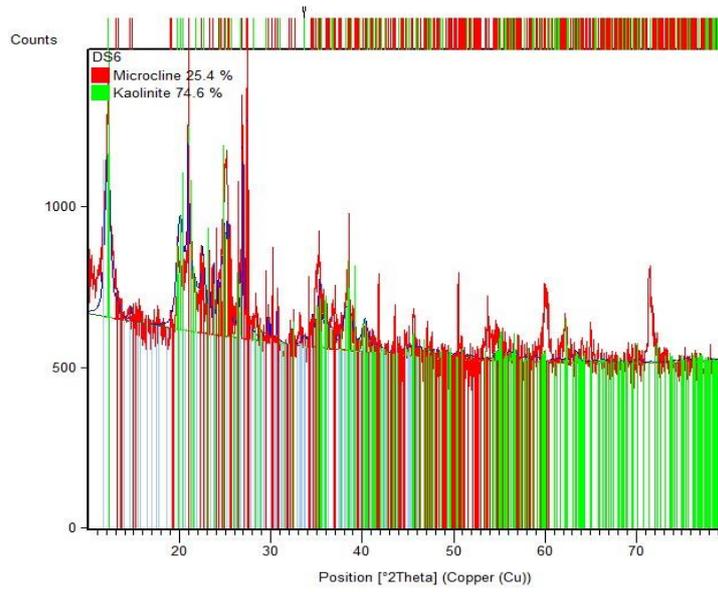


Fig. 7. XRD diffractogram of sample DS6. (2θ vs Counts).

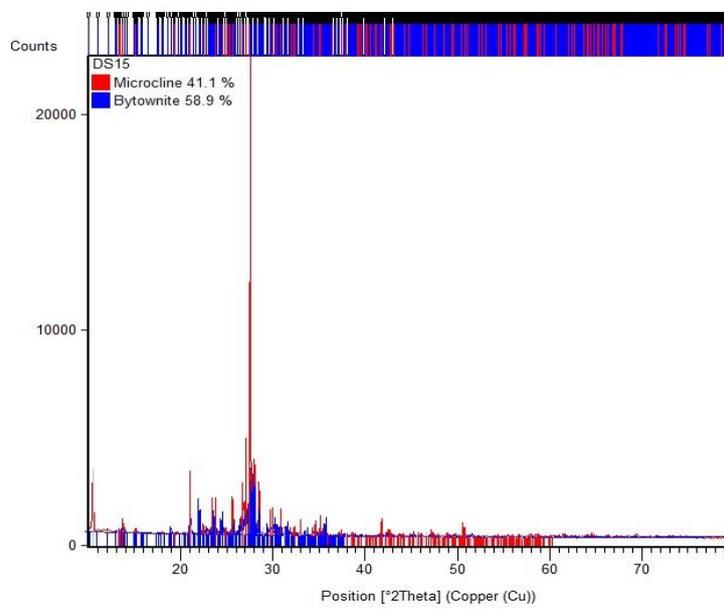


Fig. 8. XRD diffractogram of sample DS15. (2θ vs Counts).

Figure 9 displays the diffractogram of sample DS17, indicating the existence of Microcline, Bytownite, and Zeolite. Minerals has a distinct and pointed beak. It consists of a crystalline material. Microcline (35.5%), Bytownite (27.2%), and Zeolite A (39.3 %) are the minerals found in the sample. Hence, Microcline and Zeolite A exhibit greater importance in comparison to other minerals, accounting for 35.5% and 39.3% respectively.

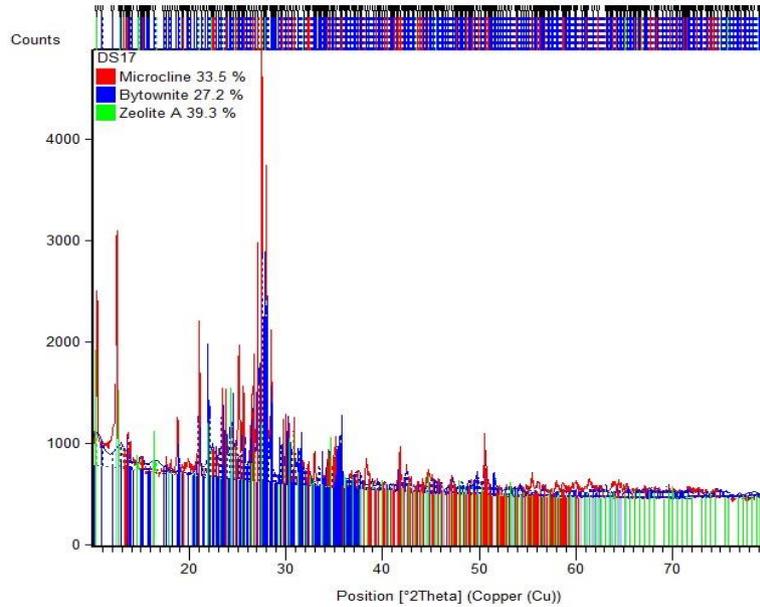


Figure 9: XRD diffractogram of sample DS17. (2θ vs Counts).

Minerals found in samples named C13, DS2, DS6, DS15 and DS17 with their ICSD reference and chemical formula are stated in table 1. Microcline has been identified in all samples, whereas Bytownite has only been recognized in three distinct samples (DS2, DS15, and DS17). It may be deduced from the presence of microcline in soil that feldspar-rich rocks such as granite have been worn. Minerals composed of feldspar are sensitive to the chemical weathering process, which is initiated when water and carbon dioxide combine to generate carbonic acid. Potassium, sodium, and calcium all dissolve as a result of the reaction between carbonic acid and feldspar. This causes the components in the soil to become more mobile, where they may be carried away by runoff, rivers, and the ocean. Two distinct forms of zeolite were identified in two samples, referred to as DS2 and DS17.

Table 1. Minerals found in samples with ICSD reference.

Sample Name	ICSD Ref. Code	Compound Name	Chemical Formula
C13	98-001-7945	Anorthite	Al ₂ Ca ₁ O ₈ Si ₂
	98-002-8787	Birnessite	H ₃ Mg _{0.29} Mn ₂ O _{5.5}
	98-001-7678	Microcline	Al _{0.99} K _{0.94} Na _{0.06} O ₈ Si _{3.01}
	98-001-2569	Tremolite	H ₂ Al _{0.29} Ca _{1.69} Fe _{0.49} Mg _{4.6} Na _{0.11} O ₂₄ Si _{7.82}
DS2	98-001-7357	Microcline	Al ₁ K ₁ O ₈ Si ₃
	98-001-7029	Bytownite	Al _{1.94} Ca _{0.85} Na _{0.14} O ₈ Si _{2.06}
	98-008-5669	Zeolite SIZ-7	Al _{19.2} Co _{12.8} O ₁₂₈ P ₃₂
DS6	98-004-5844	Microcline	Al ₁ K _{0.95} Na _{0.05} O ₈ Si ₃
	98-002-1819	Kaolinite 1A	H ₄ Al ₂ O ₉ Si ₂
DS15	98-001-7357	Microcline	Al ₁ K ₁ O ₈ Si ₃
	98-001-7029	Bytownite	Al _{1.94} Ca _{0.85} Na _{0.14} O ₈ Si _{2.06}
DS17	98-001-7357	Microcline	Al ₁ K ₁ O ₈ Si ₃
	98-001-7029	Bytownite	Al _{1.94} Ca _{0.85} Na _{0.14} O ₈ Si _{2.06}
	98-004-0992	Zeolite A	H ₂₂ Al ₁₂ Co ₄ Na ₄ O ₄₈ S ₁₁ Si ₁₂

Only the DS6 sample has kaolinite in its composition. Kaolinite, are formed when the residual components of feldspar, such as aluminum and silica, are exposed to heat and pressure. According to Bell (n.d.), clay minerals are essential to the structure and fertility of the soil because of their ability to store water and nutrients and to offer a home for soil bacteria. The C13 sample consists of four clay minerals: microcline, anorthite, birnessite, and tremolite. Mineral identification relies on the measurement of d-spacings and the comparison of relative peak intensities. Every material produces many diffraction peaks. Identification becomes significantly easier when the sample contains only a single mineral. The presence of many minerals in a mixture can result in intricate XRD patterns, making it difficult to identify the individual minerals (Ruan & Ward, 2002). Nevertheless, there are various aspect that partially alleviate the difficulty for soils. The majority of soils consist of a limited number of minerals, which tend to divide into different particle size groups. These groups are often examined individually using X-ray fluorescence (XRF) analysis in order to simplify the analysis process (Arefin et al., 2018). In addition, the minerals that are found in soil on a regular basis make up only a small portion of the more than 40,000 different minerals that have been discovered.

Conclusion

The presence of microcline in soil is evidence of the weathering of rocks rich in feldspar, such as granite, which has led to a depletion of important minerals like potassium and calcium. The depletion of this resource has the potential to have an impact not just on the yield of crops but also on the nutrients that plants get.

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A REVIEW OF NATURALLY OCCURRING RADIOACTIVE ELEMENTS IN COASTAL REGIONS OF BANGLADESH

K.S. Arefin*, M.S.H. Suny, M. Hassan and M.M.H. Sohag

Abstract

Extensive and systematic study has been carried out globally to get a thorough understanding of the underlying dynamics of radionuclides in the environment. This study was aimed to examine the natural radioactivity level in coastal areas of Bangladesh by combining the main research carried out on environmental radioactivity, predominantly employing established nuclear methods and methodologies during the past. The concentrations of important radionuclides, including ^{226}Ra , ^{232}Th , ^{40}K , ^{238}U , and ^{235}U , were measured in those investigations. Soil samples showed the highest concentration of ^{226}Ra at Inani Beach, Cox's Bazar ($21^{\circ}8' \text{ N}$ latitude, $92^{\circ}4' \text{ E}$ longitude), with a value of 61.66 Bqkg^{-1} . On the other hand, Cox's Bazar Sea Beach has the lowest ^{226}Ra concentration at 10.8 Bqkg^{-1} . In contrast, Cox's Bazar had the highest ^{226}Ra activity in the sand and silt samples (147.93 Bqkg^{-1}), whereas Kuakata Sea Beach ($21^{\circ}48' \text{ N}$ latitude and $90^{\circ}10' \text{ E}$ longitude) had the lowest (2.82 Bqkg^{-1}). The research found that ^{226}Th activity varied from $1085.99 \text{ Bqkg}^{-1}$ to 27.4 Bqkg^{-1} in soil samples from Cox's Bazar Sea Beach. In Cox's Bazar, the sand and silt samples showed the highest ^{226}Th activity at 1085.9 Bqkg^{-1} . In contrast, Kuakata Sea Beach has the lowest radioactivity at 9.22 Bqkg^{-1} . Soil samples showed that ^{40}K content was highest at Inani Beach ($21^{\circ}13' \text{ N}$ latitude and $92^{\circ}3' \text{ E}$ longitude), Cox's Bazar ($1304.11 \text{ Bqkg}^{-1}$) and lowest at Cox's Bazar Sea Beach (25.16 Bqkg^{-1}). The sand and silt sample from Kuakata Sea Beach had the greatest ^{40}K activity, measuring 852 Bqkg^{-1} . However, the sample from Cox's Bazar Sea Beach had the lowest amount (21.9 Bqkg^{-1}). Soil samples from Cox's Bazar Sea Beach showed ^{238}U activity of 455.99 Bqkg^{-1} and 110.84 Bqkg^{-1} , respectively. The highest ^{238}U activity sand sample was found in Cox's Bazar, measuring 460.5 Bqkg^{-1} . The Patenga region ($22^{\circ}14' \text{ N}$ latitude and $91^{\circ}47' \text{ E}$ longitude) in Chattogram has the lowest radioactive level, 45.2 Bqkg^{-1} . The study's findings have been meticulously recorded as crucial foundational knowledge for forthcoming investigations on radionuclides in this coastal area. A comprehensive evaluation of prior published analyses of naturally occurring radioactive elements in the coastal regions of Bangladesh has shown that the level of activity is completely non-hazardous. The experimental areas' soils are ideal for a variety of agricultural and other purposes.

Key words: Natural Radioactivity, Radionuclide, Gamma spectrometry, Activity level.

Electronics and Health Physics, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202.

*Corresponding author's e-mail: jewel.ape@gmail.com

Introduction

Radiation is present in every ecosystem, whether it is on the surface, underneath the Earth, or in the atmosphere. According to UNSCEAR (1993), about 87% of the radiation dosage that people get, originates from natural radiation sources, while the remaining 13% is attributed to anthropogenic radiation (Kannan *et al.*, 2002). The Earth's environment contains natural radioactivity that is extensively spread and may be detected in many geological formations, such as soils, rocks, plants, sand, water, and air (Rozanski & Froehlich, 1996). Therefore, it is essential to get information about their local natural environment in terms of the possible ramifications of radiation arising from both naturally occurring and anthropogenic radioactive elements. (US EPA, 2023).

According to Shahbazi-Gahrouei *et al.* (2013), the main radioactive elements discovered in soil include primordial radionuclides from the uranium series, thorium series, and ^{40}K . "Fallout" is the term used to describe the radioactive materials that are released into the atmosphere and deposited on the Earth's surface as a result of nuclear weapons testing. The dispersion of this fallout in the environment varies depending on the nuclear device's yield, burst mode, and ambient conditions. Due to nuclear weapon testing and accidents, the environment contains notable amounts of artificial radionuclides such as ^{137}Cs and ^{90}Sr (IAEA, 1989). Naturally occurring radioactive nuclides are present in the soil, water, food, air, and even inside human bodies (Aswood *et al.*, 2013). These originate from the primary decay series of ^{238}U , ^{235}U , and ^{232}Th and their respective offspring. Long-lived radioactive nuclides such as ^{40}K may also be found (IAEA, 2007; UNSCEAR, 2008).

The weathering process that takes place inside the earth's crust is vital in releasing radioactive nuclides into the soil, making it the main source of natural background radiation. Animals ingest plants and plants may absorb these nuclides via their roots or leaves. The transfer of radioactive substances to the human body happens when we eat plants or drink water that contains these substances. It may also occur indirectly when we consume animal products like meat or milk that have been exposed to these substances (Alharbi, 2013). An accurate evaluation of the released radioactivity into the environment is crucial for protecting the health of the general population, especially when the released radioactivity becomes part of the food chain (El-Taher and Al-Turki, 2014).

Multiple inquiries have been carried out in these areas for the last thirty years to determine the concentrations of ^{226}Ra , ^{232}Th , ^{40}K , ^{238}U , and ^{235}U in samples of beach sand and soil. Considering the information provided from the earlier research, a comprehensive review has been conducted on the movement and behavior of natural radioactive elements in the coastal regions of Bangladesh.

Methodology

Sampling Technique

Upon reviewing the extensive research conducted on the south coast over the past thirty years, it was found that the majority researchers utilize a high-purity germanium (HPGe) detector for a minimum duration of 10,000 seconds with appropriate shielding to count the activity of the radionuclides (Islam *et al.*, 2012). Conversely, only a small number of researchers employ a survey or radiation monitoring system. When employing HPGe for detection, the process involved configuring characteristics such as resolution and peak-to-compton ratio, as well as ensuring that the detectors maintained a minimal level of activity, among other considerations. All prior studies collected soil and sediment samples from the research locations and environs to estimate natural radionuclides. Each sample was collected one kilometer apart. The sample weighed (0.75-1) kilogram. Before being taken to the lab, each sample was bagged in polythene. Before being collected, each sample was weighed, dried in an oven at 110°C for 24 hours, roughly pulverized using a grinder, and filtered through a 10-mesh screen. A homogenized sample was placed in a sealed plastic container for measurements. All sample containers were air-tightened with polythene packs and sealed with caps before being wrapped with Teflon and thick vinyl tape around their screw necks and stored for minimum four weeks prior to counting allowing establishment of secular equilibrium between long-lived ^{238}U and ^{232}Th and their decay products (Islam *et al.*, 2012).

Study Area

The majority of the studies on natural radioactivity in the coastal region of Bangladesh were conducted at specific locations: Cox's Bazar (21° 8' N latitude and 92° 4' E longitude), Kuakata Sea Beach (21° 48' N latitude and 90°10' E longitude), Patenga, Chattogram (22°14' N latitude and 91°47' E longitude), and Moheshkhali, Teknaf (20° 51' N latitude and 92°16' E longitude). Figure 1 illustrates the geographical locations where the study was carried out.

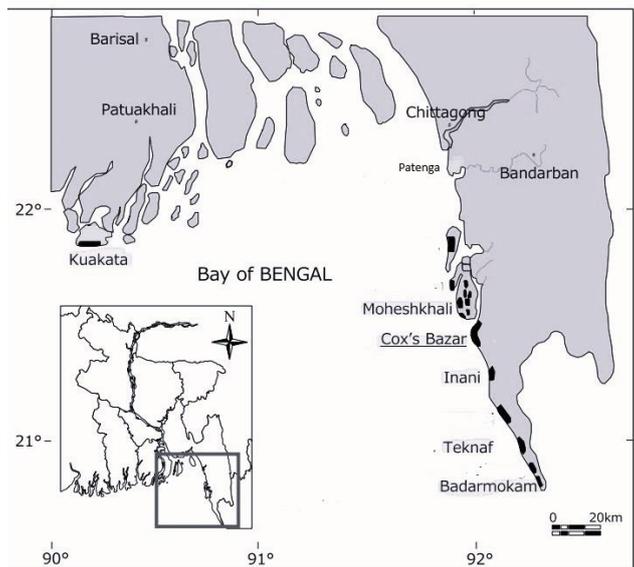


Fig. 1. Map of Coastal Bangladesh indicating environmental radioactivity study sites.

Radionuclide Analysis

Gamma-ray spectrometry enables the identification of gamma-ray energies and, by extension, the radioactive species responsible for their emission. The region enclosed by the peak in a gamma-ray spectrum corresponds to the number of counts obtained only for that specific gamma-ray energy. The peak regions were used to determine the concentration of radioactivity for the radionuclides found in the sample. By taking the peak energy region away from a linear background distribution of the pulse height spectra, the net count of the sample was found. The activity concentration of the radionuclides was determined by calculating it from the net counts of the samples using the given formula.

$$A = \frac{CPS \times 1000}{\epsilon(abs) \times I_r(abs) \times W}$$

where, *A* is the activity concentration in Bqkg⁻¹, CPS is the net peak counts per second of the samples, *W* is the weight of the sample in gm, $\epsilon(abs)$ is the absolute gamma peak detection efficiency, $I_r(abs)$ is the absolute gamma intensity of the corresponding gamma ray energy. Gamma ray's intensities were taken from the literature (IAEA, 1989).

Findings of earlier studies and discussion

Since environmental radioactivity directly affects how much ionizing radiation the public is exposed to, it is essential to consider it while evaluating the radiological hazard associated with seashore. Numerous research have been carried out over time to look into the radioactive levels of various sea beaches in Bangladesh. The results of these investigations are reviewed and summarized in this part, which also offers information on the radiological danger connected to these regions.

In 1999, Chakraborty undertook a study to assess the levels of radiation and radioactivity in Bangladesh. While the research does not primarily focus on seashore, its findings are crucial for understanding the country's fundamental radiation levels. The study utilized a survey meter, specifically the PDR ISV provided by NE Technology Limited in England, to conduct a comprehensive field survey. Additionally, an HPGe detector, specifically the Intrinsic Germanium p-Type Coaxial supplied by SILENA Detektor Systeme GmbH in Germany, was used to measure the radioactivity levels in soil and water samples collected from various locations in Bangladesh. The mean activity concentrations of ^{232}Th , ^{238}U and ^{40}K in the Kuakata Sea beach were determined to be $269.04 \pm 11.62 \text{ Bqkg}^{-1}$, $110.84 \pm 10.22 \text{ Bqkg}^{-1}$ and $266.0 \pm 24.8 \text{ Bqkg}^{-1}$, respectively. In the Cox's Bazar Sea beach, the mean activity concentrations were $1085.99 \pm 20.01 \text{ Bqkg}^{-1}$, $455.99 \pm 16.35 \text{ Bqkg}^{-1}$ and $25.16 \pm 5.39 \text{ Bqkg}^{-1}$ for ^{232}Th , ^{238}U and ^{40}K , respectively. The investigation revealed that the level of radiation exposure in sandy areas with a brown hue and glossy appearance was measured at $4.20 \pm 0.88 \text{ mSvy}^{-1}$ for Kuakata Sea Beach and $8.94 \pm 3.15 \text{ mSvy}^{-1}$ for Cox's Bazar Sea Beach. Subsequent investigations can utilize the data obtained from this study as a benchmark.

After that a study conducted by Alam *et al.* (1999) aimed to evaluate the levels of activity of naturally occurring radionuclides, specifically ^{226}Ra , ^{232}Th and ^{40}K , in beach sand minerals and beach soils. This was achieved by utilizing a γ -ray spectrometry technique with a p-type coaxial high-purity Ge (HPGe) detector. The HPGe detector had a relative efficiency of 35%, an active volume of 132 cm^3 , and a Full Width at Half Maximum (FWHM) of 1.8 keV for the 1332.5 keV γ -ray of ^{60}Co . The study was conducted in Cox's Bazar, Bangladesh. The occurrence of these radioactive isotopes in coastal ecosystems was worrisome since it poses possible radiological hazards to both local inhabitants and visitors. Significant fluctuations in the activity concentrations of the three examined radionuclides were seen in the Cox's Bazar region, as reported by the authors. The measured concentration of ^{226}Ra in the soil samples taken from the tourist zone's sea beach varied from 10.8 ± 1.6 to $27.3 \pm 2.6 \text{ Bqkg}^{-1}$, with an average concentration of $19.0 \pm 4.8 \text{ Bqkg}^{-1}$. The measured range for the isotope ^{40}K was from 117 ± 25 to $688 \pm 33 \text{ Bqkg}^{-1}$, with an average value of $458 \pm 160 \text{ Bqkg}^{-1}$. On the other hand, the range for the isotope ^{232}Th was found to be from 27.4 ± 3.7 to $49.4 \pm 5.0 \text{ Bqkg}^{-1}$, with an average value of $36.7 \pm 6.5 \text{ Bqkg}^{-1}$. Furthermore, it was calculated the absorbed dose rates caused by different radionuclides in

sand minerals located more than one meter away from the plant's stacks. The average dosage rate was found to be $4.30 \mu\text{Gy h}^{-1}$, ranging from 0.05 to $13.0 \mu\text{Gy h}^{-1}$.

Kuakata, a coastal area, is situated in the Patuakhali district. In a study conducted by Chowdhury *et al.* (2006), the levels of primordial radionuclides were examined in this region. The specific activities of ^{226}Ra in three soil samples were found to be 36 ± 2 , 39 ± 3 , and $37 \pm 2 \text{ Bqkg}^{-1}$. For ^{232}Th , the specific activities were measured to be 52 ± 5 , 99 ± 8 , and $93 \pm 8 \text{ Bqkg}^{-1}$ in the same soil samples. Additionally, the specific activities of ^{40}K were discovered to be 549 ± 24 , 631 ± 25 , and $874 \pm 28 \text{ Bqkg}^{-1}$. The measurements were conducted using a p-type coaxial lead shielded intrinsic high-purity germanium detector. The detector had a volume of 132 cm^3 and a relative efficiency of 35%. It had a resolution (FWHM) of 1.83 keV for the gamma-ray energy of 1332 keV emitted by ^{60}Co . The representative level index was determined to be 1.45, while the radium equivalent activity was measured at 195 Bqkg^{-1} .

In 2012, a research undertaken by Islam *et al.* at Kuakata Sea coastal is among the first to directly examine the levels of environmental radiation in a coastal region in Bangladesh. The study utilized a HPGe detector with a relative efficiency of 40% to measure radioactivity levels in sand and soil samples from Kuakata. This investigation provided valuable insights into the radioactive hazards linked to this well-liked tourist spot. The activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K in the sand samples from Kuakata seabeach varied from 2.82 ± 4.89 to $87.96 \pm 4.45 \text{ Bqkg}^{-1}$, 21.72 ± 16.27 to $290.93 \pm 18.15 \text{ Bqkg}^{-1}$, and 26.24 ± 0.35 to $852.05 \pm 142.15 \text{ Bqkg}^{-1}$, respectively. The average values for ^{226}Ra , ^{232}Th , and ^{40}K were $29.48 \pm 3.85 \text{ Bqkg}^{-1}$, $93.72 \pm 15.62 \text{ Bqkg}^{-1}$, and $551.24 \pm 109.95 \text{ Bqkg}^{-1}$, respectively. The activity concentrations of the corresponding radioactive isotopes in soil samples ranged from 20.98 ± 3.96 to $42.92 \pm 4.76 \text{ Bqkg}^{-1}$, 59.25 ± 15.62 to $144.34 \pm 18.52 \text{ Bqkg}^{-1}$, and 570.43 ± 100.3 to $1165 \pm 166.27 \text{ Bqkg}^{-1}$, with an average value of $29.19 \pm 4.88 \text{ Bqkg}^{-1}$, $90.56 \pm 17.94 \text{ Bqkg}^{-1}$, and $874.89 \pm 119.96 \text{ Bqkg}^{-1}$, respectively. The sand was estimated to contain radionuclides with an average absorbed dose rate of 98.33 nGyh^{-1} , ranging from 51.84 to 246.55 nGyh^{-1} . The mean absorbed dose rate resulting from radionuclides in soil was calculated to be 110.04 nGyh^{-1} , with a variation ranging from 76.63 to 142.36 nGyh^{-1} .

In another investigation, Zaman *et al.* at 2012 discovered considerably higher levels of naturally occurring radionuclides in beach sands that were rich in heavy minerals. The research was crucial for comprehending the increased levels of radionuclides found in beach sands abundant in heavy minerals in the Cox's Bazar area of Bangladesh. This study investigates the levels of activity in five specific mineral fractions extracted from beach sands, as well as in the overall bulk beach sands. The measurements were conducted using two coaxial low-energy high-purity germanium detectors, specifically an n-type detector (ORTEC) with a 39 cm^3 active volume and a 0.5 mm Be window. The purpose of this study was to assess the potential environmental impact of radioactivity and the feasibility of utilizing the mineral deposits as a source of uranium and thorium. The study revealed that

the levels of ^{232}Th , ^{40}K , ^{235}U , and ^{238}U in the beach sand samples were notably elevated and had a positive correlation with the sand's heavy mineral content. The zircon component of the mineral fractions exhibited the highest levels of activity concentrations, with garnet, rutile, ilmenite, and magnetite following in descending order. At the foredune and backdune sites that were examined, the beach sands had Raef activity levels that were 33, 18, and 15 times higher than the worldwide standard value of 370 Bqkg^{-1} (IAEA, 1996). Similarly, the beach sands had activity levels that were 10, 8, and 6 times higher than the global standard value.

The 2014 study conducted by Chakraborty and Alam provides a comprehensive assessment of the natural radioactivity levels throughout the beaches of Bangladesh. It helps to place the radiation dangers in these areas within a broader and more complete framework. The measurements were conducted using a high-resolution HPGe gamma-ray spectrometer equipment, which included a vertically mounted p-type intrinsic germanium coaxial detector (model: GX3018) linked to an 8 K multichannel analyzer (Canberra). The mean activity concentration of ^{232}Th was $461 \pm 16 \text{ Bqkg}^{-1}$, with a minimum value of 55.4 Bqkg^{-1} and a maximum value of 1085.9 Bqkg^{-1} . The ^{238}U exhibited a range of 23.3 to 460.5 Bqkg^{-1} , with an average of $208 \pm 11 \text{ Bqkg}^{-1}$. The sand collected from Cox's Bazar Sea beach exhibited a range of activity concentrations of ^{40}K , ranging from 21.9 to 41.6 Bqkg^{-1} , with an average of $27 \pm 6 \text{ Bqkg}^{-1}$. The average activity concentration of ^{232}Th in Kuakata was $158 \pm 9 \text{ Bqkg}^{-1}$, ranging from 105.4 to 269.0 Bqkg^{-1} . The average concentration of ^{238}U was $72 \pm 11 \text{ Bqkg}^{-1}$, ranging from 51.9 to 110.8 Bqkg^{-1} . The average activity concentration of ^{40}K was $237 \pm 24 \text{ Bqkg}^{-1}$, ranging from 192.9 to 292.8 Bqkg^{-1} . The average dose level at one meter above ground was 395 nGyh^{-1} for Cox's Bazaar sea beach and 146 nGyh^{-1} for Kuakata sea beach, both of which had brilliant brown sandy parts.

In 2014, Ahmed *et al.*, conducted a study to investigate the levels of natural radioactivity and the resulting radiological dangers in environmental samples obtained from Inani Beach in Cox's Bazar, Bangladesh. The study specifically investigates the radioactivity levels of sand, silt, and soil samples, analyzing their composition of naturally occurring radioactive elements such as uranium, thorium, and potassium. Their research unveiled disparities in radioactive levels across the various sample types, with sand displaying greater quantities in comparison to silt and soil. The gamma spectrometry system, equipped with a p-type co-axial HPGe detector of 93 cm^3 active volume and 20% relative efficiency (CANBERRA Model GC-2018), was used to detect and measure radionuclides in the samples. The sand samples exhibited varying activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K , ranging from 15.14 ± 2.62 to $25.16 \pm 3.05 \text{ Bqkg}^{-1}$, 24.39 ± 2.50 to $49.46 \pm 3.58 \text{ Bqkg}^{-1}$, and 362.00 ± 79.61 to $560.87 \pm 81.40 \text{ Bqkg}^{-1}$, respectively. The mean values of these three variables were $21.26 \pm 2.87 \text{ Bqkg}^{-1}$, $36.64 \pm 3.20 \text{ Bqkg}^{-1}$ and $477.57 \pm 79.80 \text{ Bqkg}^{-1}$, respectively. The average values closely resemble those recorded at Kuakata Sea-Beach, Bangladesh, by K.M.N. Islam and colleagues (2012). The sediment samples exhibited varying levels of radioactivity for ^{226}Ra , ^{232}Th , and ^{40}K , ranging from 18.09 ± 2.66

to $53.32 \pm 4.01 \text{ Bqkg}^{-1}$, 31.01 ± 2.73 to $78.37 \pm 4.35 \text{ Bqkg}^{-1}$, and 390.26 ± 76.37 to $733.61 \pm 85.80 \text{ Bqkg}^{-1}$, respectively. The respective average activity concentrations were $28.17 \pm 3.12 \text{ Bqkg}^{-1}$, $48.57 \pm 3.43 \text{ Bqkg}^{-1}$, and $490.59 \pm 81.04 \text{ Bqkg}^{-1}$. The mean values obtained in this study closely resemble those found in a separate investigation undertaken by Miah *et al.* (2012) using sand samples collected from a popular tourist spot in Bangladesh. The mean activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K in soil samples were $44.39 \pm 4.91 \text{ Bqkg}^{-1}$, $69.79 \pm 5.52 \text{ Bqkg}^{-1}$, and $1007.25 \pm 130.85 \text{ Bqkg}^{-1}$, respectively. The activity concentrations for ^{226}Ra , ^{232}Th , and ^{40}K ranged from 26.11 ± 2.99 to $61.66 \pm 5.88 \text{ Bqkg}^{-1}$, 41.93 ± 4.18 to $89.39 \pm 6.15 \text{ Bqkg}^{-1}$, and 467.16 ± 77.62 to $1304.11 \pm 147.07 \text{ Bqkg}^{-1}$, respectively. Chowdhury *et al.* (2006) reported that the activity levels in sea-beach soil are practically the same as those in mainland soil, as indicated by the findings of the present study.

Das *et al.* (2015) conducted a thorough examination of the levels of natural radioactivity and the resulting radioactive dangers in soil, sand and sediment samples gathered from the coastal area of Cox's Bazar, Bangladesh. The results suggest that the research region displays different amounts of inherent radioactivity, with the sand samples usually demonstrating elevated values. The samples were analyzed for radionuclides using a gamma spectrometry system equipped with a p-type co-axial HPGe detector. The detector had an active volume of 93 cm^3 and a relative efficiency of 20%. The soil samples exhibited a range of values for ^{226}Ra , ^{232}Th , and ^{40}K , spanning from 15.34 ± 1.66 to $33.70 \pm 2.08 \text{ Bqkg}^{-1}$, 30.09 ± 3.62 to $58.06 \pm 4.23 \text{ Bqkg}^{-1}$, and 379.99 ± 33.43 to $755.26 \pm 38.03 \text{ Bqkg}^{-1}$, respectively, among others. The sand samples exhibited activity values ranging from 8.98 ± 1.49 to $31.33 \pm 2.04 \text{ Bqkg}^{-1}$, 16.82 ± 3.24 to $63.28 \pm 4.37 \text{ Bqkg}^{-1}$, and 252.42 ± 31.72 to $565.72 \pm 35.78 \text{ Bqkg}^{-1}$, respectively. The sediment sample yielded findings of 13.90 ± 1.64 to $38.41 \pm 2.19 \text{ Bqkg}^{-1}$, 37.94 ± 3.82 to $51.92 \pm 4.11 \text{ Bqkg}^{-1}$, and 367.70 ± 33.28 to $665.05 \pm 36.97 \text{ Bqkg}^{-1}$, respectively. As stated by Ahmed *et al.* (2014), the mean value is similar. The measured absorbed dose rates for soil, sand, and sediment samples were determined to be 79.98, 77.17, and 57.86 nGyh^{-1} , respectively. The yearly effective doses received outdoors were 0.49, 0.47, and 0.36 mSvh^{-1} .

The researchers Yeasmin *et al.* (2015) examined the levels of radioactivity in the sand of a recently formed beach area in Cox's Bazar, a coastal region located in the southern part of Bangladesh. The examination identified varying levels of inherent radioactivity, with particular focus on the presence of radioactive elements such as potassium, thorium, and uranium. The sand samples included radionuclides ^{226}Ra , ^{238}U , ^{232}Th , ^{40}K , and ^{137}Cs with average activity values of 147.93 ± 3.13 , 164.27 ± 4.82 , 254.41 ± 5.43 , 321.54 ± 17.32 , and $7.21 \pm 0.80 \text{ Bqkg}^{-1}$, respectively. The average values for radium equivalent activity, external and internal risks, and outdoor and indoor absorbed dose rates were 536.50 Bqkg^{-1} , 1.45 and 1.85, 245.48 nGyh^{-1} and 294.58 nGyh^{-1} , respectively.

A study by Ahmed *et al.* (2016) investigated the levels of natural radioactivity in sand and soil samples collected from Kuakata Beach, Bangladesh. The samples were

analyzed for radionuclides using a gamma spectrometry system equipped with a p-type coaxial HPGe detector. The detector had an active volume of 93 cm³ and a relative efficiency of 20%. The researchers discovered that the levels of ²²⁶Ra, ²³²Th, and ⁴⁰K in sand samples from the Kuakata sea beach varied from 22.83±4.11 to 100.21±2.39 Bqkg⁻¹, 68.76±2.86 to 297.37±4.32 Bqkg⁻¹ and 75.87±15.75 to 161.81±19.90 Bqkg⁻¹, respectively. The average values for these elements were 48.76, 126.11, and 292.38 Bqkg⁻¹, respectively. The mean values of the linked radionuclides in the sediment samples were 19.67, 32.13 and 243.38 Bqkg⁻¹, respectively. The values in the range for the other radionuclides were 12.11±1.99 to 31.64±1.64 Bqkg⁻¹, 18.94±1.90 to 71.11±2.88 Bqkg⁻¹, 182.73±19.81 Bqkg⁻¹ and 345.77±21.07 Bqkg⁻¹. The computed range of the absorbed dose rate was between 30.50 and 242.93 nGyh⁻¹, with an average value of 74.87 nGyh⁻¹.

Zaman *et al.* (2016) investigates the levels of uranium and thorium in zircon assemblages extracted from beach sands in Cox's Bazar, Bangladesh. The results indicate that zircon rocks, commonly present in the coastal sands of the region, may contain radioactive elements. Neutron Activation Analysis (NAA) determined that the mean concentrations of uranium and thorium were 122 and 220 ppm, respectively. The analysis revealed that the most elevated levels of thorium and uranium were found to be 506 and 141 ppm, respectively. A limited number of supplementary investigations (Alam *et al.*, 1999; Chowdhury, 2003; Kabir *et al.*, 2010) have utilized gamma spectrometry to quantify the levels of ²³⁸U and ²³²Th, together with other radioactive elements like as K, in certain soils and dense minerals found in the Cox's Bazar region.

In their study, Begum *et al.* (2018) conducted a thorough investigation to evaluate the levels of background radiation in different places around Bangladesh. It has been discovered that the coastal regions in the Cox's Bazar region have significantly increased levels of background radiation, with the highest recorded level being 38.89 mSvy⁻¹. However, outside of Cox's Bazar seashore, the amount of background radiation is significantly lower in and around Cox's Bazar town, with a minimum level of 1.40 mSvy⁻¹. The background radiation level in Cox's Bazar district ranges from 1.40 to 38.89 mSvy⁻¹. The researchers utilized Geiger-Muller based micro-Roentgen survey meter and digital survey meter (GAMMA SCOUT) to measure the radiation levels in their investigation. The elevated background radiation level in the coastal regions of Cox's Bazar region is attributed to the presence of monazite and zircon in the sand.

In this study, Uddin *et al.* (2019) examined and analyzed the extent to which the population in Bangladesh is exposed to elevated amounts of natural background radiation. Although the literature was a conference proceeding and offers limited information, its primary focus was on comprehending the radiological situation in Bangladesh. This study aims to investigate the levels of natural background radiation in Bangladesh and evaluate its possible effects on the population. The study was anticipated to address the presence of certain locations or places in Bangladesh that have higher amounts of natural background radiation. The provided data includes the radioactivity levels of ²²⁶Ra, ²³²Th, and ⁴⁰K

isotopes in soil samples from Kuakata Sea Beach. The activity levels were found to be 23 Bqkg^{-1} , 80 Bqkg^{-1} , and 700 Bqkg^{-1} for ^{226}Ra , ^{232}Th , and ^{40}K , respectively.

The 2020 study conducted by Deeba, Rahman and Kabir specifically examined the levels of radon in both soil and groundwater in the western coastal region of Bangladesh. The study also assessed the overall yearly effective dosage resulting from the intake and inhalation of radon. Radon plays a substantial role in the overall amount of radiation that occurs naturally. It is vital to comprehend the levels of radon in these regions. The research area was assessed for radon levels using the AlphaGUARD PQ2000 PRO radon detector. The radon levels in soil samples collected from Kuakata were evaluated at depths of 0, 20 and 40 cm below the surface. The radon concentration at the surface ranges from 10 ± 04 to $122 \pm 28 \text{ Bqm}^{-3}$. At depths of 20 cm to 40 cm, the concentration ranges from 12 ± 05 to $2800 \pm 44 \text{ Bqm}^{-3}$. At depths of 40 cm to 4790 $\pm 51 \text{ Bqm}^{-3}$, the concentration is $4790 \pm 51 \text{ Bqm}^{-3}$. The radon concentrations in a given area are influenced by the depth of the ground surface and the placements of the sample sites in that region, as indicated by the findings of the soil radon concentration.

In 2021, Sarker and his colleagues utilized a gamma-ray spectroscopy device with an HPGe detector to quantify the radiation levels in sand samples collected from the Kuakata and Cox's Bazar Sea beaches. Their discoveries provided valuable information on the radioactive hazards present in these highly sought-after coastal areas, which attract both domestic and foreign visitors. The sand samples from Kuakata Sea Beach have activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K ranging from 24.48 ± 2.17 to $76.14 \pm 3.25 \text{ Bqkg}^{-1}$, 9.22 ± 2.15 to $46.80 \pm 2.17 \text{ Bqkg}^{-1}$, and 76.23 ± 12.34 to $458.65 \pm 17.04 \text{ Bqkg}^{-1}$, respectively. Sand samples from Cox's Bazar Sea Beach show activity concentrations of ^{226}Ra , ^{232}Th , and ^{40}K ranging from 14.12 ± 2.75 to $75.54 \pm 3.15 \text{ Bqkg}^{-1}$, 10.28 ± 1.95 to $53.37 \pm 2.75 \text{ Bqkg}^{-1}$ and 56.42 ± 10.16 to $341.22 \pm 14.64 \text{ Bqkg}^{-1}$. The mean activity concentration of the natural radionuclides ^{226}Ra , ^{232}Th , and ^{40}K in sand samples collected at Kuakata beach is 41.22 ± 2.56 , 21.78 ± 2.27 and $218.41 \pm 13.63 \text{ Bqkg}^{-1}$, respectively. In Cox's Bazar, the corresponding values are 54.01 ± 2.65 , 36.16 ± 2.42 and $200.51 \pm 12.74 \text{ Bqkg}^{-1}$. The radium equivalent activity of sand samples collected from Cox's Bazar Sea beach ranges from 49.17 to 153.1 Bqkg^{-1} , whereas samples from Kuakata sea beach show a radium equivalent activity ranging from 61.02 to 163.14 Bqkg^{-1} . The sand samples from Cox's Bazar show a radium equivalent activity of $121.15 \pm 39.13 \text{ Bqkg}^{-1}$, whereas the samples from the Kuakata beach have an average of $83.94 \pm 35.99 \text{ Bqkg}^{-1}$. The mean internal and external hazard indices in Cox's Bazar Sea beach are 0.48 ± 0.16 and 0.33 ± 0.11 , respectively. In Kuakata seashore, the corresponding values are 0.35 ± 0.13 and 0.23 ± 0.09 . The average annual effective dose in Kuakata is $0.29 \pm 0.11 \text{ mSvy}^{-1}$, whereas at Cox's Bazar Sea beach it is $0.39 \pm 0.13 \text{ mSvy}^{-1}$.

The study clearly indicates that the majority of the research focused on measuring the levels of natural radioactivity in the soil, sand and beach sediments in the coastal areas of Kuakata and Cox's Bazar. Typically, the HPGe detector was utilized for estimating the

activity. The exterior gamma absorbed dose measurement was conducted using survey meters, portable plastic scintillometers, and thermoluminescent dosimeters (TLDs). Tables 1 to 9 provide clear and well-documented evidence of regional differences in the levels of radionuclides, including ^{226}Ra , ^{232}Th , ^{40}K , ^{238}U and ^{235}U .

The examination of the soil samples indicated that the concentration of ^{226}Ra was greatest at Inani Beach, Cox's Bazar ($21^{\circ} 8' \text{ N}$ latitude and $92^{\circ} 4' \text{ E}$ longitude) with a value of 61.66 Bqkg^{-1} (Ahmed *et al.*, 2014). On the other hand, the lowest concentration of ^{226}Ra was seen at Cox's Bazar Sea Beach, measuring 10.8 Bqkg^{-1} (Alam *et al.*, 1999).

In contrast, the sand and silt sample exhibited the maximum level of ^{226}Ra activity in Cox's Bazar (147.93 Bqkg^{-1}) (Yeasmin *et al.*, 2015), whilst the lowest activity was recorded at Kuakata Sea Beach (2.82 Bqkg^{-1}) (Islam *et al.*, 2012).

The study of the soil sample indicated that the ^{226}Th activity ranged from its highest value of $1085.99 \text{ Bqkg}^{-1}$ (Chakraborty, 1999) to its lowest value of 27.4 Bqkg^{-1} (Alam *et al.*, 1999) at Cox's Bazar Sea Beach.

The sand and silt samples had the most pronounced level of ^{226}Th activity in the Cox's Bazar area, at 1085.9 Bqkg^{-1} (Chakraborty *et al.*, 2014). Conversely, the Kuakata Sea Beach had the lowest amount of radioactivity, measuring at 9.22 Bqkg^{-1} (Sarker *et al.*, 2021).

The study of the soil samples indicated that the concentration of ^{40}K was greatest at Inani Beach, Cox's Bazar, with a measurement of $1304.11 \text{ Bqkg}^{-1}$ (Ahmed *et al.*, 2014), while it was lowest at Cox's Bazar Sea beach, with a measurement of 25.16 Bqkg^{-1} (Chakraborty, S. R., 1999).

The sand and silt sample collected from Kuakata Sea Beach exhibited the highest amount of ^{40}K activity, measuring 852 Bqkg^{-1} (Islam *et al.*, 2012). In contrast, the sample obtained from Cox's Bazar Sea Beach had the lowest level, measuring 21.9 Bqkg^{-1} (Chakraborty *et al.*, 2014).

The soil samples collected from Cox's Bazar Sea Beach exhibited an activity of ^{238}U at a level of 455.99 Bqkg^{-1} and 110.84 Bqkg^{-1} (Chakraborty, S. R., 1999), respectively.

The sand sample with the highest level of ^{238}U activity was discovered at Cox's Bazar, measuring 460.5 Bqkg^{-1} (Chakraborty *et al.*, 2014). The Patenga area in Chattogram had the lowest radioactive level, measuring 45.2 Bqkg^{-1} (Chakraborty *et al.*, 2014).

Table-9 displays the presence of ^{235}U in the minerals extracted from sand. The maximum concentration of ^{235}U was found in Bodarmukam, Cox's Bazar (specifically in the Fore dune region) at a level of 145 Bqkg^{-1} (Zaman *et al.*, 2012). Conversely, the lowest concentration was observed in Fakirahata, Cox's Bazar (specifically in the Backdune area) at a level of 30 Bqkg^{-1} (Zaman *et al.*, 2012).

Table 1. Comparison of activity concentration of ^{226}Ra in the soil

^{226}Ra (Bqkg ⁻¹)	Location	Reference
36±2 to 39±2	Patuakhali District	Chowdhury <i>et al.</i> , 2006
20.98 ± 3.96 to 42.92 ± 4.76	Kuakata Sea Beach	Islam <i>et al.</i> , 2012
23	Kuakata Sea Beach	Uddin <i>et al.</i> , 2019
10.8 ± 1.6 to 27.3 ± 2.6	Cox's Bazar Sea Beach	Alam <i>et al.</i> , 1999
26.11 ± 2.99 to 61.66 ± 5.88	Inani Beach, Cox's Bazar	Ahmed <i>et al.</i> , 2014
15.34 ± 1.66 to 33.70 ± 2.08	Coastal Area, Cox s Bazar	Das <i>et al.</i> , 2015

Table 2. Comparison of activity concentration of ^{226}Ra in the sand and sediment

^{226}Ra (Bqkg ⁻¹)	Location	Sample type	Reference
2.82 ± 4.89 to 87.96 ± 4.45	Kuakata Sea Beach	Sand	Islam <i>et al.</i> , 2012
22.83 ± 4.11 to 100.21 ± 2.39	Kuakata beach	Sand	Ahmed <i>et al.</i> , 2016
12.11 ± 1.99 to 31.64 ± 1.64	Kuakata beach	Sediment	Ahmed <i>et al.</i> , 2016
24.48 ± 2.17 to 76.14 ± 3.25	Kuakata Sea Beach	Sand	Sarker <i>et al.</i> , 2021
14.12 ± 2.75 to 75.54 ± 3.15	Cox's Bazar	Sand	Sarker <i>et al.</i> , 2021
15.14 ± 2.62 to 25.16 ± 3.05	Inani Beach, Cox's Bazar	Sand	Ahmed <i>et al.</i> , 2014
18.09 ± 2.66 to 53.32 ± 4.01	Inani Beach, Cox's Bazar	Sediment	Ahmed <i>et al.</i> , 2014
8.98 ± 1.49 to 31.33 ± 2.04	Coastal Area, Cox s Bazar	Sand	Das <i>et al.</i> , 2015
13.90 ± 1.64 to 38.41 ± 2.19	Coastal Area, Cox s Bazar	Sediment	Das <i>et al.</i> , 2015
147.93 ± 3.13 (mean activity concentration)	Cox's Bazar	Sand	Yeasmin <i>et al.</i> , 2015

Table 3. Comparison of activity concentration of ^{232}Th in the soil

^{232}Th (Bqkg ⁻¹)	Location	Reference
269.04 ± 11.62 (average)	Kuakata Sea Beach	Chakraborty, 1999
1085.99 ± 20.01	Cox's Bazar Sea Beach	Chakraborty, 1999
52 ± 5 to 99±8	Patuakhali District	Chowdhury <i>et al.</i> , 2006
59.25 ± 15.62 to 144.34 ± 18.52	Kuakata Sea Beach	Islam <i>et al.</i> , 2012
80	Kuakata Sea Beach	Uddin <i>et al.</i> , 2019
27.4 ± 3.7 to 49.4 ± 5.0	Cox's Bazar Sea Beach	Alam <i>et al.</i> , 1999
41.93 ± 4.18 to 89.39 ± 6.15	Inani Beach, Cox's Bazar	Ahmed <i>et al.</i> , 2014
30.09 ± 3.62 to 58.06 ± 4.23	Coastal Area, Cox s Bazar	Das <i>et al.</i> , 2015

Table 4. Comparison of activity concentration of ²³²Th in the sand and sediment

²³² Th (Bqkg ⁻¹)	Location	Sample type	Reference
21.72 ± 16.27 to 290.93 ± 18.15	Kuakata Sea Beach	Sand	Islam <i>et al.</i> , 2012
55.4±7.9–1085.9±20.0	Cox's Bazar	Sand	Chakraborty <i>et al.</i> , 2014
105.4±9.2–269.0±11.6	Kuakata	Sand	Chakraborty <i>et al.</i> , 2014
96.8±9.1–121.7±9.7	Potenga	Sand	Chakraborty <i>et al.</i> , 2014
68.76 ± 2.86 to 297.37 ± 4.32	Kuakata beach	Sand	Ahmed <i>et al.</i> , 2016
18.94 ± 1.90 to 71.11 ± 2.88	Kuakata beach	Sediment	Ahmed <i>et al.</i> , 2016
9.22 ± 2.15 to 46.80 ± 2.17	Kuakata Sea Beach	Sand	Sarker <i>et al.</i> , 2021
10.28 ± 1.95 to 53.37 ± 2.75	Cox's Bazar	Sand	Sarker <i>et al.</i> , 2021
24.39 ± 2.50 to 49.46 ± 3.58	Inani Beach, Cox's Bazar	Sand	Ahmed <i>et al.</i> , 2014
31.01 ± 2.73 to 78.37 ± 4.35	Inani Beach, Cox's Bazar	Sediment	Ahmed <i>et al.</i> , 2014
16.82 ± 3.24 to 63.28 ± 4.37	Coastal Area, Cox s Bazar	Sand	Das <i>et al.</i> , 2015
37.94 ± 3.82 to 51.92 ± 4.11	Coastal Area, Cox s Bazar	Sediment	Das <i>et al.</i> , 2015
254.41 ± 5.43		Sand	Yeasmin <i>et al.</i> , 2015
(mean activity concentration)	Cox's Bazar		

Table 5. Comparison of activity concentration of ⁴⁰K in the soil

⁴⁰ K (Bqkg ⁻¹)	Location	Reference
266.00 ± 24.80	Kuakata Sea Beach	Chakraborty, S. R., 1999
25.16 ± 5.39	Cox's Bazar Sea Beach	Chakraborty, S. R., 1999
549±24 - 874±28	Patuakhali District	Chowdhury <i>et al.</i> , 2006
570.43 ± 100.3 to 1165 ±166.27	Kuakata Sea Beach	Islam <i>et al.</i> , 2012
700	Kuakata Sea Beach	Uddin <i>et al.</i> , 2019
117 ± 25 to 688 ± 33	Cox's Bazar Sea Beach	Alam <i>et al.</i> , 1999
467.16±77.62 to 1304.11±147.07	Inani Beach, Cox's Bazar	Ahmed <i>et al.</i> , 2014
379.99 ± 33.43 to 755.26 ± 38.03	Coastal Area, Cox s Bazar	Das <i>et al.</i> , 2015

Table 6. Comparison of activity concentration of ^{40}K in the Sand and Sediment

^{40}K (Bqkg $^{-1}$)	Location	Sample type	Reference
26.24± 0.35 to 852.05 ± 142.15	Kuakata Sea Beach	Sand	Islam <i>et al.</i> , 2012
21.9±5.1–41.6±6.6	Cox's Bazar	Sand	Chakraborty <i>et al.</i> , 2014
192.9 ± 20.4–292.8 ± 25.4	Kuakata	Sand	Chakraborty <i>et al.</i> , 2014
236.5±24.4–293.8±25.6	Potenga	Sand	Chakraborty <i>et al.</i> , 2014
75.87 ± 15.75 to 161.81± 19.90	Kuakata beach	Sand	Ahmed <i>et al.</i> , 2016
182.73 ± 19.81 to 345.77 ± 21.07	Kuakata beach	Sediment	Ahmed <i>et al.</i> , 2016
76.23 ± 12.34 to 458.65 ± 17.04	Kuakata Sea Beach	Sand	Sarker <i>et al.</i> , 2021
56.42 ± 10.16 to 341.22 ± 14.64	Cox's Bazar	Sand	Sarker <i>et al.</i> , 2021
362.00 ± 79.61 to 560.87 ± 81.40	Inani Beach, Cox's Bazar	Sand	Ahmed <i>et al.</i> , 2014
390.26 ± 76.37 to 733.61 ± 85.80	Inani Beach, Cox's Bazar	Sediment	Ahmed <i>et al.</i> , 2014
252.42 ± 31.72 to 565.72 ± 35.78	Coastal Area, Cox s Bazar	Sand	Das <i>et al.</i> , 2015
367.70 ± 33.28 to 665.05 ± 36.97	Coastal Area, Cox s Bazar	Sediment	Das <i>et al.</i> , 2015
321.54±17.32 (mean activity concentration)	Shaplapur, Inani and Himchori villages of Cox's Bazar	Sand	Yeasmin <i>et al.</i> , 2015

Table 7. Comparison of activity concentration of ^{238}U in the soil

^{238}U (Bqkg $^{-1}$)	Location	Reference
110.84 ±10.22	Kuakata Sea Beach	Chakraborty, S. R., 1999
455.99 ± 16.35	Cox's Bazar Sea Beach	

Table 8. Comparison of activity concentration of ^{238}U in the sand

^{238}U (Bqkg $^{-1}$)	Location	Sample type	Reference
23.3±7.8–460.5±16.4	Cox's Bazar	Sand	Chakraborty <i>et al.</i> , 2014
51.9±8.6–110.8±10.2	Kuakata	Sand	Chakraborty <i>et al.</i> , 2014
45.2±8.2–59.4±8.9	Potenga	Sand	Chakraborty <i>et al.</i> , 2014
164.27±4.82 (mean activity concentration)	Shaplapur, Inani and Himchori villages of Cox's Bazar	Sand	Yeasmin <i>et al.</i> , 2015

Table 9. Comparison of activity concentration of ^{235}U in the separated minerals from sand

^{235}U (Bqkg ⁻¹)	Location	Reference
145 ± 18	Bodarmukam, Cox's Bazar (Foredune area)	Zaman <i>et al.</i> , 2012
87 ± 14	Monkhali, Cox's Bazar (Foredune area)	
65 ± 13	Kalatoli, Cox's Bazar (Foredune area)	
43 ± 12	Lomburi, Cox's Bazar (Backdune area)	
39 ± 10	Shaplapur, Cox's Bazar (Backdune area)	
30 ± 8	Fakirahata, Cox's Bazar (Backdune area)	

Conclusion

The coastal region of Bangladesh has great prospective not only in tourism but also in agriculture. Based on the available information, a thorough review has been carried out on the naturally occurring radioactive elements in the coastal areas of Bangladesh and found that the level of activity is not harmful at all (Islam *et al.*, 2012; Ahmed *et al.*, 2014). The soil of the study areas could be used for the agricultural and other purpose. The study's findings are extensively recorded as foundational knowledge for future investigations on radionuclides, particularly the transmission of radionuclides from soil to different crops and ecosystems as well.

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YIELD PERFORMANCE OF MUTANT BLACKGRAM VARIETY Binamash-2 OVER LOCATIONS IN BANGLADESH

M.M. Islam^{1*}, M.M. Hoque¹, M.E. Hossain¹, S. Begum¹, M.R.I. Akondo², S.M.A. Alim², M.S. Rahman³ and M.A.A. Topu³

Abstract

Blackgram is one of the most important pulse crops extensively grown in Bangladesh with multiple uses. An experiment was conducted to examine the suitability and productivity of a gamma irradiated pulse variety Binamash-2 in Kharif-II season for two consecutive years 2021 and 2022 over four locations, Mymensingh, Chapainawabganj, Gopalganj and Magura districts. The experiment was consisted of two varieties viz. Binamash-2 and BARI Mash-3. Binamash-2 produced higher seed yield than BARI mash3 at all four locations. Binamash-2 produced bolder seeds with early maturity than BARI mash3. The seed coat color and size of Binamash-2 was more attractive than BARI mash3 and farmers of four studied areas are very much interested to cultivate Binamash-2 in the upcoming years.

Key words: Binamash-2, blackgram, mutant, pod, yield

Blackgram (*Vigna mungo* L.) is one of the most important food legumes in Bangladesh. It is a healthy legume that is typically designed to withstand stress and is an affordable supply of vegetable protein, amino acids, and so on for the underprivileged. Due to capacity of fixing atmospheric nitrogen, the crop is very effective in increasing soil fertility. It is also well adapted for many cropping systems, including dry farming and intercropping. In Bangladesh during 2019-2020, blackgram was grown over an area of 0.467 lakh hectares with the production of 0.537 lakh metric tons where the average yield of 1150 kg ha⁻¹ was low as compared to the cereal crops (BAS, 2021). In order to break the yield bottle neck in black gram, efforts are needed to develop high yielding varieties with better growth habit. Research on this species has lagged behind that on cereals and other legumes because genetic improvement is severely hampered by the crop's limited genetic diversity. Therefore, it is necessary to develop this crop by making use of the genetic diversity that is already there. A crop's genetic improvement for sustainable food supply and other features may be aided by increasing genetic diversity. Induced mutagenesis produces fresh variations in the qualities important for genetic improvement, such as increased yield and other polygenic traits, without altering the plant's fundamental chromosome structure. In order to improve cultivars in certain specific features and complement existing germplasm, mutation

¹Adaptive Research and Extension Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

²Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

³Entomology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

*Correspondence: mohimenu008ag@gmail.com

induction has established itself as a strategy in plant breeding. (Kurobane *et al.*, 1979). Mutagenesis has been widely employed as a potential and advance technique to increase diversity for crop development. (Singh and Singh, 2001). Genetic variation can be created through induced mutation utilizing physical and chemical mutagens, leading to the development of new variety with improved characteristics (Wongpiyasatid, 2000). In self-pollinated crops like blackgram, mutation breeding is an appropriate method for generating variety. Therefore, the current mutation breeding effort was started to find mutants with high yield potential, earliness, erect, and determinate type plant growth habits of blackgram. Many research institutions in Bangladesh are currently working to develop high-yielding blackgram varieties. Farmers still grow native varieties, which yield modest yields, however ignorance prevents them from doing so. As a result, utilizing the right production techniques on superior cultivars will assist farmers in Bangladesh in increasing yield and promoting blackgram production. Therefore, the aim of this study was to investigate the yield potential of gamma irradiated blackgram varieties developed by BINA under different agro-ecological conditions of Bangladesh.

The field experiments were performed at four agro-ecological zones of Bangladesh such as Mymensingh, Magura, Gopalganj and Chapainawabganj districts in Kharif-II (August-November) season of two consecutive years of 2021 and 2022. Seeds of Binamash-2 and BARI mash3 were used as planting material. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Seeds were sown between 25 to 27 August in both years. Seeds were sown at a depth of 2-3 cm by line sowing. Unit plot size was 30 m². Distance between plant to plant was 5 cm in a row while line to line distance was maintained 40 cm. The trials were conducted in the farmers' field following farmers' management practices. A fertilizer dose of 15, 35 and 20 kg ha⁻¹ of Urea, DAP and MoP, respectively were applied at the time of sowing as basal dose. Intercultural practices like weeding and thinning were done for its maximum growth and pesticide was applied to control diseases as and when necessary. Reaction to major diseases like *Cercospora* leaf spot, Powdery mildew and Yellow Mosaic Virus and insect-pests infestation under field condition were recorded in both years. At harvest, data on days to maturity, plant height, primary branches plant⁻¹, pods plant⁻¹, seeds pod⁻¹, 1000-seed weight and seed yield were recorded. Plot yield was converted to ton per ha and farmers' reactions were recorded on the basis of studied parameters. The collected data were analyzed statistically by using computer package program, MSTAT-C.

Considering mean over locations showed that varietal variation on days to maturity, number of primary branches and pods plant⁻¹, number of seeds pod⁻¹, 1000-seed weight and seed yield was significant at 1st year (Table 1). But plant height variation was non-significant. Results indicated that Binamash-2 matured almost 3 days earlier than BARI mash3. Binamash-2 produced higher number of primary branches and pods plant⁻¹ and also produced bolder seeds than BARI mash3. There was significant difference regarding seed yield between the two tested varieties (Table 1). However, seed yield was apparently higher

in Binamash-2 (1.45 tha⁻¹) than BARI mash3 (1.39 tha⁻¹). The seed yield was higher in Binamash-2 than BARI mash3 might be due to greater number of pods plant⁻¹, higher number of seeds pod⁻¹ and bolder seeds. On the other hand, pod number per plant was higher in Binamash-2 than BARI mash3 for greater number of branches plant⁻¹. Relevant reports (Nag *et al.*, 2000 and Amanullah *et al.*, 2016) have found genotypic heterogeneity with respect to yield attributes and seed yield which has corroborated currently conducted experiments. Farmers' also very much interested to cultivate Binamash-2 in those districts due to early maturity and bushy plant type.

Table 1. Yield and yield contributing characters of Binamash-2 over four locations during 2021

Location(s)	Variety(s)	Days to maturity	Plant height (cm)	Primary branches plant ⁻¹ (no.)	Pods plant ⁻¹ (no.)	Seeds pod ⁻¹ (no.)	1000-seed weight. (g)	Seed yield (tha ⁻¹)
Mymensingh	Binamash-2	75.4b	52.3b	3.72a	32.13a	6.50a	38.25a	1.40a
	BARI mash3	78.2a	54.0a	2.53b	25.50b	6.30b	35.20b	1.31b
Chapainawabganj	Binamash-2	74.80	35.3a	3.15a	41.76a	6.42a	42.30a	1.52a
	BARI mash3	76.5a	32.7b	2.35b	36.54b	6.15b	39.00b	1.45b
Gopalganj	Binamash-2	72.2b	50.5b	3.70a	38.62a	6.43a	41.76a	1.45ns
	BARI mash3	75.6a	54.4a	3.23b	32.58b	5.92b	38.42b	1.42
Magura	Binamash-2	73.4b	48.2a	3.52a	38.67a	6.25a	38.50a	1.41ns
	BARI mash3	75.3a	46.3b	3.15b	32.55b	5.85b	35.26b	1.38
Combined	Binamash-2	73.7b	46.6 ns	3.52a	37.80a	6.40a	40.20a	1.45a
	BARI mash3	76.4a	46.8	2.82b	31.79b	6.06b	36.97b	1.39b
	CV%	4.84	6.52	5.20	8.74	1.75	4.26	6.23

*Figures followed by same letter in a column did not differ significantly at 5% level

Regarding mean over locations showed that varietal variation on days to maturity, plant height, number of primary branches and pods plant⁻¹, number of seedspod⁻¹ and 1000-seed weight was significant at 2nd year (Table 2). But seed yield variation was non-significant. Results indicated that Binamash-2 matured 2 days earlier than BARI mash3. BARI mash3 (47.58 cm) was taller than Binamash-2 (47.35 cm). Binamash-2 produced higher number of primary branches (3.71) and pods plant⁻¹ (41.21) and also produced bolder seeds than BARI mash3 (38.03 and 3.08 respectively). There was no significant difference regarding seed yield between the two tested varieties (Table 2). However, seed yield was apparently higher in Binamash-2 (1.45 tha⁻¹) than BARI mash3 (1.41 tha⁻¹). The seed yield was higher in Binamash-2 than BARI mash3 might be due to greater number of pods plant⁻¹, higher number of seeds pod⁻¹ and bolder seeds. On the other hand, pod number per plant was higher in Binamash-2 than BARI mash3 for greater number of branches plant⁻¹. The current experimental results were corroborated by previous numerous reports (Ghafoor *et al.*, 2005) that noted genotypic diversity with relation to yield attributes and seed yield. Farmers' were keen to cultivate Binamash-2 due to earliness and good pod numbers.

Table 2. Yield and yield contributing characters of Binamash-2 over four locations during 2022

Location(s)	Variety(s)	Days to maturity	Plant height (cm)	Primary branches plant ⁻¹ (no.)	Pods plant ⁻¹ (no.)	Seeds pod ⁻¹ (no.)	1000-seed weight (g)	Seed yield (tha ⁻¹)
Mymensingh	Binamash-2	74.20b	50.23b	3.51a	37.56a	6.72a	41.25a	1.42ns
	BARI mash3	76.43a	51.76a	3.13b	29.63b	6.15b	37.45b	1.38
Chapainawabganj	Binamash-2	76.34b	41.12b	4.17a	43.35a	6.51a	43.23a	1.56a
	BARI mash3	79.40a	37.32a	3.81b	39.35b	6.23b	38.67b	1.46b
Gopalganj	Binamash-2	71.27b	52.33b	3.82a	41.34b	6.32a	41.67a	1.41ns
	BARI mash3	74.15a	53.70a	3.32b	43.62a	5.91b	39.78b	1.39
Magura	Binamash-2	70.41b	45.73b	3.35a	42.57a	6.45a	39.80a	1.42ns
	BARI mash3	73.47a	47.32a	3.10b	39.52b	6.12b	37.42b	1.40
Combined	Binamash-2	73.06b	47.35ns	3.71a	41.21a	6.48a	41.49a	1.45ns
	BARI mash3	75.86a	47.58	3.38b	38.03b	6.11b	38.33b	1.41
	CV%	3.92	5.45	4.85	7.26	2.10	5.27	4.14

*Figures followed by same letter in a column did not differ significantly at 5% level

Considering mean over locations and years showed that varietal variation on days to maturity, plant height, number of primary branches and pods plant⁻¹, number of seeds pod⁻¹ and 1000 seed weight was significant (Table 3). But seed yield variation was non-significant. Results indicated that Binamash-2 matured 3 days earlier than BARI mash3. BARI mash3 (47.0 cm) was taller than Binamash-2 (48.8 cm). Binamash-2 produced higher number of primary branches and pods plant⁻¹ and also produced bolder seeds than BARI mash3. There was no significant difference regarding seed yield between the two tested varieties (Table 3). However, seed yield was apparently higher in Binamash-2 (1.45 tha⁻¹) than BARI mash3 (1.40 tha⁻¹). The seed yield was higher in Binamash-2 than BARI mash3 might be due to greater number of pods plant⁻¹, higher number of seeds pod⁻¹ and bolder seeds. On the other hand, pod number per plant was higher in Binamash-2 than BARI mash3 for greater number of branches plant⁻¹. Genotypic variation regarding yield attributes and seed yield was observed by earlier many reports (Sharma *et al.*, 2000; Sing and Sing, 2000 and Gupta *et al.*, 2007) that supported present experimental results. Interesting thing is that two varieties performance regarding seed yield of each location was almost similar. It means these two blackgram varieties are highly stable over locations. Farmers' preferred Binamash-2 due to earliness and good pod numbers and pod sizes in those areas.

Table 3. Mean varietal variation on days to maturity, morphological, yield attributes and seed yield over locations and years

Location(s)	Variety(s)	Days to maturity	Plant height (cm)	Primary branches plant ⁻¹ (no.)	Pods plant ⁻¹ (no.)	Seeds pod ⁻¹ (no.)	1000-seed weight. (g)	Seed yield (tha ⁻¹)
Mymensingh	Binamash-2	74.8b	51.3ns	3.62a	34.85a	6.61a	39.75a	1.41a
	BARI mash3	77.3a	52.9	2.88b	27.57b	6.23b	36.33b	1.15b
Chapainawabganj	Binamash-2	75.6b	38.2a	3.66a	42.56a	6.47a	42.77a	1.54ns
	BARI mash3	78.0a	35.0b	3.08b	37.95b	6.19b	38.84b	1.46
Gopalganj	Binamash-2	71.8b	51.4b	3.76a	39.98a	6.38a	41.72a	1.43 ns
	BARI mash3	74.9a	54.0a	3.28b	38.10b	5.92b	39.10b	1.41
Magura	Binamash-2	71.4b	47.0ns	3.44b	40.62a	6.30a	39.15a	1.42ns
	BARI mash3	74.4a	46.8	3.16a	36.04b	6.00b	36.34b	1.39
Combined	Binamash-2	73.4b	47.0b	3.62a	39.50a	6.44a	40.85a	1.45ns
	BARI mash3	76.1a	48.8a	3.10b	34.91b	6.08b	37.65b	1.40
	CV%	3.51	5.72	4.45	6.92	2.13	4.89	7.72

*Figures followed by same letter in a column did not differ significantly at 5% level

Disease reaction against *Cercospora* leaf spot, Powdery mildew and Yellow Mosaic were examined under field condition during 2021 and 2022 at over four locations. Results were presented in table 4. There was not much disease incidence in Binamash-2 and check variety for *Cercospora* leaf spot and Powdery mildew in field. Binamash-2 along with check variety, BARI Mash-3 showed moderately susceptible to the diseases. All the tested entries along with a check variety, BARI Mash-3 showed moderately susceptible to the Yellow Mosaic Virus disease.

Table 4. Incidence of *Cercospora* leaf spot, powdery mildew and yellow mosaic disease in Binamash-2 over four locations for two consecutive years (mean: 2021, 2022)

Variety(s)	Location(s)	<i>Cercospora</i> leaf spot			Powdery mildew			Yellow Mosaic		
		PDI	DS	Reaction	PDI	DS	Reaction	PDI	DS	Reaction
Binamash-2	Mymensingh	54.23	32.35	MS	61.15	42.31	MS	6.41	17.23	MS
	Chapainawabganj	45.12	26.13	MS	49.42	35.17	MS	4.13	14.85	MS
	Gopalganj	51.57	28.92	MS	54.83	37.92	MS	5.65	15.48	MS
	Magura	53.78	30.75	MS	58.52	40.62	MS	5.96	16.38	MS
BARI mash3	Mymensingh	61.52	36.42	MS	62.43	45.28	MS	28.65	42.74	S
	Chapainawabganj	55.78	32.19	MS	55.76	38.67	MS	23.42	32.17	MS
	Gopalganj	57.42	35.15	MS	56.35	41.72	MS	25.31	35.85	MS
	Magura	60.20	35.85	MS	59.28	43.75	MS	30.83	45.21	S

MS = Moderately Susceptible, S = Susceptible; DS = Diseases severity; PDI = Percent Disease Index

Table 5. Infestation of hairy caterpillar in Binamash-2 over four locations for two consecutive years (mean: 2021, 2022)

Variety(s)	Location(s)	Hairy caterpillar	
		0-6 Scale	Reaction
Binamash-2	Mymensingh	03	MR
	Chapainawabganj	03	MR
	Gopalganj	03	MR
	Magura	03	MR
BARI mash3	Mymensingh	04	MS
	Chapainawabganj	03	MR
	Gopalganj	03	MR
	Magura	04	MS

MS = Moderately Susceptible, MR = Moderately Resistant.

Naturally low insect pest infestation is occurred in black gram and pod borer infestation was not found in black gram. The hairy caterpillar infestation was occurred in black gram during experimentation (Table 5). Tested varieties were moderately resistant and moderately susceptible to this insect pest. According to Sudhir *et al.* (2018) hairy caterpillars, galeruid beetle, stem fly and white fly, pod borer complex insects and yellow mosaic virus (YMV), *crcospora* leaf spots (*C. cruenta*) and powdery mildew (*Erysiphe polygoni*) disease are considered of major importance for black gram.

It may be concluded that high yielding cultivar, Binamash-2 has higher number of branches and pods plant⁻¹, higher number of seeds pod⁻¹ with bolder seed size and also matured almost 3 days earlier than the low yielder, BARI mash3. Farmers of studied areas choice Binamash-2 in future cultivation for higher seed yield with early maturity that might be fit the existing cropping pattern in Bangladesh.

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