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EVALUATION OF INDUCED GENETIC VARIABILITY IN GAMMA RAY IRRADIATED RAPESEED MUTANTS

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Abstract

Bangladesh is suffering from a severe lack of edible oil. As a result, we import oil and oilseeds to overcome the shortage. To select the superior rapeseed mutants an experiment was conducted with thirteen mutants following randomized complete block design. The mutants showed significant genetic variation for most of the characters. Phenotypic variations were higher than genotypic variances for the majority of the traits, the high genotypic coefficient of variation value was also observed for seed yield, number of seeds siliqua^{-1} and number of siliqua plant^{-1}. Number of seeds siliqua^{-1} exhibited the highest value of heritability followed by plant height while siliqua length exhibited the lowest value of heritability. Path co-efficient analysis revealed that number of branch plant^{-1}, siliqua length, number of siliqua plant^{-1}, and number of seed siliqua^{-1} had a direct positive effect on yield. Considering the yield performance genotypes RNM-8, RNM-18, RNM-20 and RNM-33 might be recommended for future varietal improvement program of rapeseeds.

Key words: Rapeseed, Mutants, Genetic Variability, Correlation coefficient, Path coefficient analysis and Yield performance

Introduction

Mustard (*Brassica napus*) a member of the Brassicaceae family, is the most important edible oil crop in Bangladesh. We need to spend a significant amount of foreign currency to import edible oil. After soybeans and groundnuts, mustard is one of the most important oil crops in the world (www.fao.org). Mustard oil meets the country's one-third edible oil demand as noted by Ahmed (2008). It is used as a condiment, salad, green manure, fodder crop, and the leaf and stem are used as a vegetable in the many mustard cultivating regions. Its oil is also utilized as a green biofuel, an ingredient in animal feed and a key component in a variety of chemical and pharmaceutical applications (Huang *et al.*, 2016). After oil extraction, the by product from rapeseeds provides a protein-rich (38-44%) animal feed. Oilseed rape may be regarded as an alternate source of protein instead of soya in subtropical monsoon region such as Bangladesh, due to its nutritional benefits. This meal has a good amino acid profile, with a high concentration of sulfur containing amino acids, methionine and cysteine. Furthermore, the meal is high in minerals including calcium, magnesium, and phosphorus, as well as vitamins B4 and E (Bocianowski *et al.*, 2011).

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Meeting the ever-increasing demand for high-quality human food and animal feed is a challenge for academics, farmers and food producers. Variety plays an important role for the yield of mustard, because different varieties perform differently for their genotypic characters. Improved variety is the first and foremost requirement for initiation and acceleration crop production program. High yielding varieties (HYVs) can contribute to getting optimum yield as well as the highest percentage of oil. The presence of genetic variability in the gene pool for high yielding diverse economic features is required for the development of a successful breeding program. Induced mutation is a common source of creating genetic variation. The most common method of mutation induction is gamma rays, which account for 64% of all radiation-induced mutant types. Induced mutation has been employed to improve a variety of crops, including oilseeds, with great success. More variability of rapeseed germplasms can be created via mutagenesis (Amosova *et al.*, 2019). Understanding the level of variability existing in a population is critical to determine the best breeding approach for improving trait (s). Traits connected with yield component, can be employed as the greatest guidance for successful indirect selection for yield improvement. With genetic variability, there must be a thorough knowledge of inheritance, kind of gene action and the relative magnitude for most of yield contributing characters. So in the context of the above mentioned situation, the present work was undertaken to select elite mutant genotypes to utilize in future breeding programs.

Material and Methods

Plant Materials and Experimental Design

The studied mutants were derived from a gamma ray irradiated local rapeseed germplasm collected from Nachol (Rajshahi). The list of mutants derived from collected germplasm is given in Table 1. The experiment was carried out at BINA Headquarters farm, Mymensingh following a randomized complete bock design (RCBD) with 3 replications in 2019.

Table 1. List of rapeseed genotypes used in this study

Genotype	Doses of gamma rays	Source	Genotype	Doses of gamma rays	Source
RNM-3	500 Gy	BINA	RNM-22	700Gy	BINA
RNM-5	500 Gy	BINA	RNM-27	700Gy	BINA
RNM-7	600 Gy	BINA	RNM-33	700Gy	BINA
RNM-8	600 Gy	BINA	RNM-35	700Gy	BINA
RNM-10	600 Gy	BINA	RNM-66	700Gy	BINA
RNM-18	800Gy	BINA	BARI Sarisha-14	Check	BINA
RNM-20	800Gy	BINA	Nachoal (Local)	Parent	BINA
RNM-21	700Gy	BINA			

The unit plot size was 10 m with 10 rows and line to line and plant to plant distances were 30 cm and 5 to 7cm, respectively. The recommended fertilizers and cultural practices

were done to maintain a healthy crop growth. The harvesting was done separately for each genotype when 80% of plants were matured.

Data Collection

The data were recorded from 10 randomly selected plants for days to maturity, plant height (cm), number of branches plant^{-1}, number of siliquae plant^{-1}, siliqua length (cm), number of seeds siliqua^{-1}, and seed yield.

Statistical Analysis

The data were analyzed for different components. Phenotypic and genotypic variance was estimated by the formula used by Johnson *et al.,* (1955). Heritability and genetic advance were measured using the formula given by Singh and Chaudhury (1985). Genotypic and phenotypic co-efficient of variation were calculated by the formula of Burton (1952). Correlation coefficient analysis was done by the formula proposed by Miller *et al.,* (1958). Path coefficient analysis was performed according to the method described by Dewey and Lu (1959).

Result and Discussion

The analysis of variance (ANOVA) revealed the existence of significant variations for days to maturity, plant height, number of siliquae plant^{-1}, number of seeds silique^{-1} and seed yield among 15 rapeseed genotypes (Table 2). This suggests that there is an opportunity for the genetic improvement of rapeseed for these traits by using mutation techniques.

Table 2. Estimation of genetic parameters for 11 characters among 15 genotypes in rapeseed

Traits	GMS	σ^2g	σ^2p	GCV	PCV	h^2b	GA	GA(%)
Maturity	24.85**	3.5	17.7	2.24	4.99	20.18	1.79	2.08
Plant height (cm)	196.38*	34.09	128.19	6.12	11.87	26.60	6.2	6.5
Number of branch plant^{-1}	0.89	0.11	1.12	7.26	22.93	10.03	0.22	4.74
Number of siliqua plant^{-1}	605.26**	66.88	471.49	11.57	30.73	14.19	6.3	8.9
Siliqua length	0.55	0.04	0.56	1.18	14.36	9.66	0.105	2.14
Number of seed siliqua^{-1}	4.89**	8.14	14.42	13.42	17.85	56.49	4.42	20.78
Yield (kg/ha)	163724*	26024.2	111674.28	13.13	27.21	23.3	60.42	13.06

** = Significant at 1%; * = Significant at 5%.

GMS = Genotypic mean sum of Square, σ^2g = Genotypic variance, σ^2p = Phenotypic variance, GCV = Genotypic coefficient of variation and PCV = Phenotypic coefficient of variation h^2b = heritability and GA= Genetic advance.

The higher phenotypic variance (σ^2p) compared to the genotypic variance (σ^2g) for the studied traits indicates the prevalence of environmental effect on the phenotypic expression of these traits (Table 2). The higher σ^2g was found for seed yield (kg ha^{-1}), number of siliquae plant^{-1} and plant height followed by number of seeds siliquae^{-1} and maturity period, where as lower σ^2g was found for siliqua length followed by number of

branch plant^{-1}. The results indicate the presence of high genetic variability for these traits. There were differences between PCV and GCV for almost all characters. Highest GCV (13.13) and PCV (27.21) were shown for seed yield followed by number of siliqua plant^{-1} and number of seed siliqua^{-1}. It's indicating that a greater amount of genetic variability is present for this characters which provide greater scope for selection followed by number of branch plant^{-1} and plant height. Lowest values GCV (1.18) and PCV (14.36) were obtained from siliqua length followed by maturity values indicating the limited scope for improvement by selection. For maturity period lowest GCV and PCV were also observed by Aradhana *et al.,* (2003) and Bhuiyan *et al.,* (2019). Broad sense heritability estimates ranged from 9.66 to 56.49% for all characters. Highest values were obtained from number of seed siliqua^{-1}, plant height and maturity and lowest value was found for the siliqua length. In order to estimate the selection effects, heritability accompanied with genetic advance is rather useful than heritability alone. Heritability and genetic advance were maximum for seed yield (kg ha^{-1}), number of seed siliqua^{-1}and plant height. Thus suggests that these traits under additive genetic control and selection might be effective for these traits. Like this study Zare and Sharafzadeh (2012) also found low broad sense heritability for siliqua length in rapeseed. High heritability with high genetic advance for number of siliquae plant^{-1} reported by Sadat *et al.,* (2010) also support our result. High heritability for number of seeds per siliqua was observed by Yadava *et al.,* (1993). Aytac and Kinaci (2009) mentioned the high heritability and genetic advance for seed yield selection for this character would be effective. Yield is a complex trait and controlled by polygene's and very often influenced by environment. Therefore, phenotypic selection based only on yield is not effective. Correlation and path co-efficient pave the way to select plant for breeding purpose by the plant breeders. Genotypic and phenotypic correlation coefficients among 6 characters are presented in Table 3.

Table 3. Genotypic (r_g) and phenotype (r_p) correlation coefficients among different pairs of yield and yield contributing characters for different genotype of mustard

Traits		PH	NBP	NSP	SL	NSS	Yield
DM	r_g	0.5896**	-0.1948	-0.3974	-0.087**	0.4938**	0.4544**
	r_p	0.5485**	-0.2798	-0.03	-0.5596**	0.6391**	0.3947**
PH	r_g		0.2145	0.5389*	-0.5674	0.6765	0.1978
	r_p		0.1776	0.3702 *	-0.1656	0.5772 **	0.1883
NBP	r_g			0.0684**	0.766**	0.6668**	0.134**
	r_p			0.4843**	0.6901**	0.4823 **	0.5456 **
NSP	r_g				0.3333	0.1064	0.463**
	r_p				0.1961	0.0741	0.806**
SL	r_g					0.8552 **	0.0528 **
	r_p					0.2624 **	0.8111 **
NSS	r_g						0.994 **
	r_p						0.604 **

** = Significant at 1%; * = Significant at 5%.

DM = Days to maturity, PH = Plant height (cm), NBP = Number of branches per plant, NSP = Number of siliqua per plant, NSS = Number of seed per siliqua, SL = Siliqua length

In most instances, there was a close agreement between genetic correlations and phenotypic correlations. The genotypic correlations were higher than phenotypic correlations for all the studied traits that indicate the minimum influence of environments for the expression of those characters. Seed yield had a highly significant positive correlation with days to maturity, number of branches plant^{-1}, number of siliqua plant^{-1}, siliqua length and number of seeds silique^{-1}.

Among these, number of seeds siliqua^{-1} and number of siliqua plant^{-1} have highly significant and strong positive correlation with seed yield at both levels suggests the high degree of association between these traits. Malik *et al.,* (200) and Jeromela *et al.,* (2007) reported positive correlation between siliqua plant^{-1} and seed yield. Rameeh (2015) reported that number of seeds siliqua^{-1} was positively correlated with seed yield. As genetic correlation coefficient was higher than phenotypic correlation coefficient, it reveals that the apparent association of two characters is mainly due to genetic effects. The direct and indirect effects of yield contributing traits on seed yield were analyzed by path analysis. Seed yield per plant was considered as effect (dependent variable) while remaining traits were treated as causes (independent variables) and shown in Table 4.

Table 4. Path coefficient analysis showing indirect effects of different characters on yield of mustard

Traits	DM	PH	NBP	NSP	SL	NSS	r_g with Yield
DM		-0.288	0.025	0.510	0.698	0.997	-0.17
PH	-0.323		0.007	0.408	0.282	0.913	0.01*
NBP	0.294	0.075		-0.620	0.212	-0.927	-0.06
NSP	0.744	0.531	0.079		0.651	1.681	0.03**
SL	0.849	0.389	0.102	-0.465		-0.360	-0.21**
NSS	0.486	0.394	0.039	0.557	0.469		0.014**
Residual Effect = 0.211							

**, * indicates significant at the 0.01 and 0.05 level, respectively.
DM = Days to Maturity, PH= Plant height (cm), NBP= Number of primary branches plant^{-1}
NSP= Number of siliqua plant^{-1}, SL= Siliqua length, NSS= Number of seed siliqua^{-1}

The results showed that number of siliqua plant^{-1} had maximum direct effect (Fig. 1) followed by number of seeds siliqua^{-1} on seed yield. Number of siliqua plant^{-1} had a positive indirect effect to all the traits and finally this trait had a highly significant positive correlation with number of seeds siliqua^{-1}.

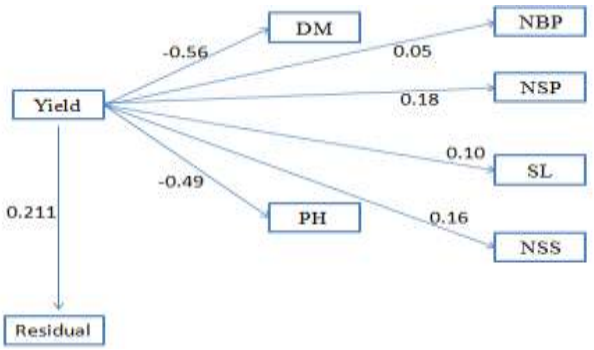

Fig. 1. Path coefficient analysis showing direct and effects of different characters on yield of mustard

Similarly, number of seeds siliqua^{-1} had a positive indirect effect via all the traits and have strong positive correlation with number of siliqua plant^{-1}. These results indicate that the correlation is mainly due to the direct effect of a character and it was realized via indirect positive and negative effects. It reveals the true relationship between them and direct selection for this trait will be rewarding for the genetic improvement of yield. Sharafi *et al.*, (2015) found the number of siliqua plant^{-1} had the highest direct effect on seed yield. They also reported that number of seeds siliqua^{-1} had direct positive effect on yield per plant. On the contrary, a negative direct effect for seeds siliqua^{-1} on seed yield per plant has been reported by Basalma (2008). The value of residual effect was 0.211. It indicated that beside the component characters, there was an influence of some other attributes (approx. 21.1%) on seed yield.

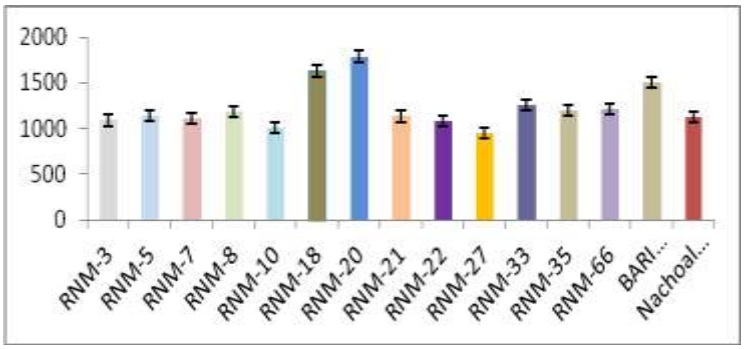

Fig. 2. Yield performance of rapeseed mutants and varieties

The mutants RNM-20 produced the highest seed yield of 1789 kgha^{-1} followed by RNM-18 (1630 kgha^{-1}) and BARI Sarisha-14 (1505) (Fig. 2). Lowest seed yield was reported from RNM-10 and RNM-27. Mother variety Nachoal (Local) produced the yield of 1127 kg ha^{-1}. In rapeseed-mustard and other oilseed mutants having higher seed yield over mother varieties also reported by Akter *et al.*, 2020 and Mondal *et al.*, 2020.

Conclusion

By selecting high yielding genotypes, there is a lot of room to increase production. We know that yield attribute characteristics like siliqua plant^{-1} and seeds siliqua^{-1} might improve seed yield. Various genotypes differ in their yield regulating characteristics which play an essential role in yields of rapeseed and mustard. It was observed that among the mutants and mother variety mutants RNM-8, RNM-18, RNM-20 and RNM-33 performed better for seed yield and yield contributing characters which can be selected for further multi locational yield trials. Moreover, this study suggests that for rapeseed or mustard improvement breeding program, researcher needs to consider the characters like seed siliqua^{-1} and siliqua plant^{-1}. It also concludes that gamma rays irradiation can be fruitfully applied to induce mutations in rapeseed with higher seed yield and other improved agronomic traits.

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GENOTYPE-ENVIRONMENT (G×E) INTERACTION, STABILITY AND ADAPTABILITY STUDY ON GRAIN YIELD IN ADVANCED RICE LINES

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Abstract

Stability and adaptability of a rice genotype is crucial to release it as a variety for commercial cultivation in a wide range of growing conditions. The present study was conducted during 2020-21 to assess the Genotype×Environment (G×E) interaction and to identify the stable rice lines for varietal development. Nine rice genotypes consisting advanced lines and released variety were investigated for stability in grain yield across three environments by Additive Main effects and Multiplicative Interaction (AMMI) and the Genotype Main Effect and Genotype by Environment interaction effects (GGE) analyses. AMMI and GGE analyses revealed significant G×E interactions indicating the variability among the genotypes and environments. As per AMMI1 and AMMI2 biplot models the genotypes BN-P-317 and BN-P-318 were identified as the best performer and suited for the environment Jamalpur. GGE biplot analysis showed that the genotypes BN-P-114, BN-P-115 and BN-P-317 were adapted to the environment Jamalpur, whereas BN-P-318 was more suitable for Nalitabari. According to GGE biplot-genotype view graph, the genotype BN-P-317 was identified as the ideal genotype for grain yield followed by BN-P-318. The GGE biplot- polygon view graph showed that the genotype BN-P-317 performed better in both the environments Jamalpur and Nalitabari. The genotypes BN-P-317 and BN-P-318 could be selected for further evaluation to release as variety.

Key words: AMMI, GGE, G×E interaction, Rice

Introduction

Rice is a staple food crop for half of the world's population, and its cultivation is widely adapted in different agroecological zones. Although the wide adaptation, rice cultivation is becoming challenging due to the adverse effect of climate change. Climate change increases the variability in weather conditions and negatively impacts the genotype × environment (G×E) interactions which adversely affects the yield potential of rice varieties (Khumairoh *et al*., 2018).

There is a need to develop stable and widely adapted varieties that can ensure superior yield and quality performance across a wide range of environmental conditions for sustainable rice production. Thus, success in a breeding program lies in developing a variety that is widely adapted to diverse environments (Malosetti *et al.*, 2013, Andiku *et al.*, 2020).

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The stability of a quantitative trait like yield potential is a cumulative result of different interactions between the genetic make-up of the variety and the conditions where the variety is cultivated (Malosetti *et al.*, 2013). Moreover, the grain yield of rice is a complex polygenic trait (Renukadevi and Subbalakshmi 2006) and is largely affected by environmental conditions like precipitation, temperature, humidity and other biotic and abiotic factors.

Before releasing a crop variety for commercial cultivation, it is very important to understand the genotype by environment interaction (Sabaghpour *et al.*, 2012). The performance of the newly developed lines is compared across different environmental conditions for assessing the interaction. Multi-environment trial (MET) is decisive in identifying a stable genotype across the environments or detecting the most suitable environment for a specific genotype (Yan *et al.*, 2000). Cultivars grown in different environments react differently, and this reaction is the G×E interaction. The study of G×E interaction is important for the plant breeders to select the suitable varieties releasing for commercial cultivation. A number of studies have been conducted to evaluate the yield stability of rice in diverse environmental conditions (Cooper and Somrith, 1997; Flores *et al.*, 1998; Ouk *et al.*, 2007; Anandan *et al.*, 2009; Katsura *et al.*, 2016).

Combined analysis of variance can quantify the G×E interactions but cannot explain it (Asnake *et al.*, 2013). Several statistical models have been developed to explain the G×E interaction. But more than one model should be applied for getting the best comparison (Lubadde *et al.*, 2017). Additive Main effects and Multiplicative Interaction (AMMI) and the Genotype Main Effect and Genotype by Environment interaction effects (GGE) models are widely applied to analyze the G×E interaction in a MET (Nyaligwa *et al.*, 2018). AMMI determine the G×E interaction by analyzing the main effects (additive effect) and the non-additive residual effect through the analysis of variance (ANOVA) and principal component analysis (PCA) (Crossa and Cornelius, 1997). However, GGE biplots exhibit both genotype and genotype by environment difference for a trait (Crossa *et al.*, 2002). Stability and adaptability of a specific variety can be detected by studying the G×E interaction analyzed with AMMI and GGE biplot models (Jadhav *et al.*, 2019). Thus, the present study was conducted to evaluate the grain yield performance of advanced rice lines for their stability and adaptability across three environments.

Materials and Methods

Experimental design and plant materials

The experiment was conducted at three environments *viz.* Mymensingh, Jamalpur and Nalitabari of Bangladesh during dry season in 2020-21. Nine genotypes consisting of eight blast resistant advanced rice lines and one released variety were used in the study (Table 1). The experiment was carried out following the randomized complete block design (RCBD) with three replications. Thirty-five-day-old seedlings were transplanted to the field in a 3.0 m × 2.0 m size plot with 20 cm spacing between rows and 15 cm between plants within row. All the recommended management practices were adopted to raise a healthy crop under all

the tested environments. Each line was harvested separately and grain yield was assessed plot basis. Grain yield was recorded at 14% moisture level and finally converted to t ha^{-1}.

Table 1. List of genotypes used in the study

Code	Genotype	Origin	Variety status	Special attributes
G1	BN-P-102	IRRI	Pure line	Blast resistant & HYV
G2	BN-P-110	IRRI	Pure line	Blast resistant & HYV
G3	BN-P-114	IRRI	Pure line	Blast resistant & HYV
G4	BN-P-115	IRRI	Pure line	Blast resistant & HYV
G5	BN-P-120	IRRI	Pure line	Blast resistant & HYV
G6	BN-P-310	IRRI	Pure line	Blast resistant & HYV
G7	BN-P-317	IRRI	Pure line	Blast resistant & HYV
G8	BN-P-318	IRRI	Pure line	Blast resistant & HYV
G9	BRRI dhan58	Bangladesh	Released variety	HYV

Statistical analysis

Combined ANOVA was constructed based on the grain yield data using RStudio (RStudio Team, 2020). The homogeneity test of the variance was conducted by the Barlette methods (Bartlett, 1932). The combined ANOVA was proceeded further to assess the G×E interaction and stability of the genotypes across the three locations. AMMI and GGE biplot multivariate models were used to determine the genotype and environment interaction and its relationship with stability parameters. AMMI and GGE biplot were constructed using PB Tools ver. 5 statistical software (http://bbi.irri.org/products) and RStudio (RStudio Team, 2020). The AMMI and GGE model are a combination of ANOVA and PCA. The analytical models are given as:

$$Y_{ij} = \mu + \delta_i + \beta_j + \sum_{k=1}^{K} \lambda_k \, \delta_{ik} \beta_{jk} + \epsilon_{ij} \text{ (AMMI model)}$$

$$Y_{ij} = \mu + \beta_j + \sum_{k=1}^{K} \lambda_k \, \delta_{ik} \beta_{jk} + \epsilon_{ij} \text{ (GGE model)}$$

Where, Y_{ij} is the mean yield of i^{th} genotype in j^{th} environment, μ is the overall mean, δ_i is the genotypic effect, β_j is the environment effect, λ_k is the singular value for PC axis k: δ_{ik} is the genotype eigenvector value for PC axis n, βjk is the environment eigenvector value for PC axis k and ε_{ij} is the residual error assumed to be normally and independently distributed (0, σ^2 /r), σ^2 is the pooled error variance, and r is the number of replicates (Crossa *et al.*, 2002; Gauch *et al.*, 2008).

Results and discussions

Combined analysis of variance

A wide variation was observed among the tested genotypes for grain yield across the environments (Table 2). In the environment Mymensingh, grain yield ranged from 4.34 to 5.75 t ha^{-1} with 5.42 tha^{-1} as median, whereas it ranged from 5.00 to 6.20 t ha^{-1} and 4.90 to 6.20 t ha^{-1} in Jamalpur and Nalitabari, respectively (Fig. 1). After confirming the homogeneity of the variance across the environments through the Bartlett test, a combined analysis of variance was performed. It revealed highly significant differences among the

genotypes for grain yield (Table 3). Homogeneity of variance indicates that the genetic variances among the genotypes are inherent. According to the combined ANOVA, the grain yield was significantly affected by genotype (36.45%), environment (16.08%) and G×E interaction (38.88%) (Table 3). The significant mean sum of squares for genotypes indicates the divergence of the tested lines/varieties for the mean yield potential (Xu *et al.,* 2014). The significant G×E interaction effect confirms the diverse response of the genotypes to the differences in the environmental conditions (Jadhav *et al.,* 2019).

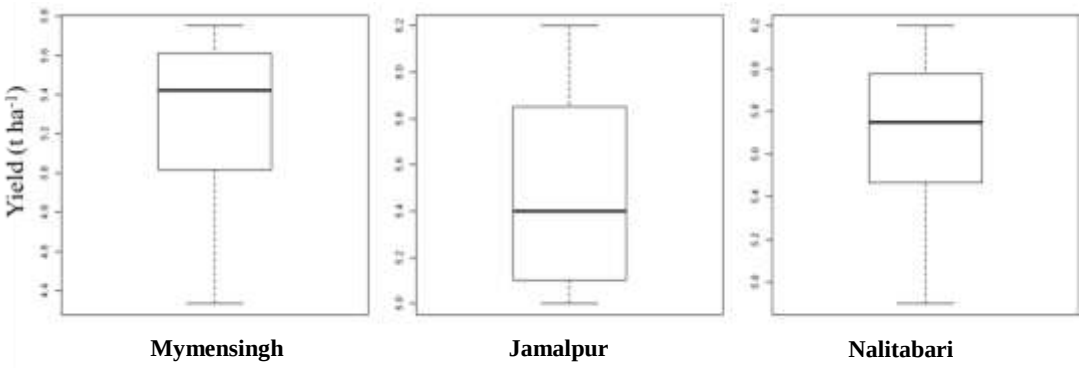

Fig. 1. Box plot of genotype performance for grain yield across the three environments

Table 2. Phenotypic variability and descriptive statistics of grain yield of nine genotypes for three environments

Code	Environment	Parameters								
		Min	Max	Mean	Var	SE	CV	Skewness	Kurtosis	IQR
E1	Mymensingh	4.34	5.75	5.28	0.15	0.07	7.37	-0.61	-0.36	0.59
E2	Jamalpur	5.00	6.20	5.49	0.17	0.08	7.57	0.41	-1.34	0.75
E3	Nalitabari	4.90	6.20	5.69	0.14	0.07	6.58	-0.73	-0.34	0.51

Min = Minimum, Max = Maximum, Var = variance, SE = Standard Error CV = Coefficient of Variation, IQR = Interquartile Range

Table 3. Combined analysis of variance of grain yield for rice genotypes evaluated at three environments

Source	df	SS	MS	Explained SS (%)
Genotype (G)	8	5.26	0.66***	36.45
Repeat (E)	6	0.06	0.01	0.42
Environment (E)	2	2.32	1.16***	16.08
G×E	16	5.61	0.35***	38.88
Residuals	48	1.19	0.02	8.25
Total	80	14.43	0.18	
Chi square Value (Bartlett's Test)	2		0.29	

NB: ***indicates significance at $p < 0.001$ probability level
df = degree of freedom; SS = Sum of squares; MS = Mean of squares

AMMI and GGE analysis of variance

The AMMI analysis of variance indicates that the genotypes and environments contributed 26.26% and 11.58% of the total sum of squares, respectively, whereas 28.00% of the total sum of squares was attributed by G×E interaction effects (Table 4). A larger genotype sum of square than the environment, indicates the substantial differences among the genotypes (Bose *et al.*, 2014). A considerable portion of variation explained by environments indicating that the environments were diverse. It might be due to the divergence in precipitation pattern, temperature regime and other biotic and abiotic factors (Zewdu *et al.*, 2020). The AMMI model demonstrates the presence of significant genotype by environment interaction. This interaction was portioned in the first two interaction principal component axis (IPCA) and these two components were significant at $p < 0.001$ in a postdictive assessment (Table 4). The first two principal components explain the contribution of genotype and their environment. The PC1 (71.1) and PC2 (26.9) values together explained 100% of the G×E interaction which means the interaction of the studied 9 genotypes can easily be predicted by using this two IPCAs (Gauch and Zobel, 1996). Previously it was reported that the first two IPCAs contribute more to explain the G×E interactions (Susanto *et al.*, 2015; Jadhav *et al.*, 2019; Senguttuvel *et al.*, 2021).

The principal components from the GGE analysis explain the contribution of genotype, environment and the interaction of G×E (Malosetti *et al.*, 2013). From the GGE analysis it was observed that the PC1 value (50.3) was higher than PC2 (37.3) and PC2 (12.4) (Table 5). Higher PC1 implies the higher contribution of genotype in the total sum of squares (Malosetti *et al.*, 2013; Jadhav *et al.*, 2019).

Table 4. Additive main effects and multiplicative interaction (AMMI) analysis of variance for grain yield (t ha^{-1}) of rice genotypes across three environments

Source	Df	SS	MS	Explained SS (%)	Proportion	Accumulated
Genotype (G)	8	5.26	0.66***	26.26		
Repeat (E)	6	0.06	0.01	0.30		
Environment (E)	2	2.32	1.16***	11.58		
G×E	16	5.61	0.35***	28.00		
IPCA1	9	4.10	0.46***	20.47	73.1	73.1
IPCA2	7	1.51	0.22***	7.54	26.9	100
Residuals	48	1.19	0.02			
Total	96	20.03	0.21			

NB: ***indicates significance at P<0.001 probability level; IPCA=Interaction Principal Component Axis

Table 5. ANOVA and Sum of Squares percentage on G, E and G x E derived from analysis of variance for GGE stability model

Source of variation	df	Sum of Squares percentage
PC1	9	50.3
PC2	7	37.3
PC3	5	12.4

Stability analysis of genotypes for grain yield across the environments

In AMMI1 biplot, PC1 is plotted on vertical axis and mean yield on the horizontal axis. The genotypes that are perpendicular to each other have same mean yield performance and the genotypes which are on a horizontal line have the same interaction pattern (Ebdon and Gauch, 2002). As per AMMI1 biplot model the genotypes BN-P-110, BN-P-115, BN-P-120, BN-P-317 and BN-P-318 exhibited the high yield with high main (additive) effects as they are situated at the right side of the mean yield performance (Fig. 2). Among these 5 genotypes BN-P-115 and BN-P-317 had the positive IPCA1 score and the genotype BN-P-317 being the highest yield producing genotype. The genotype BN-P-317 was specially adapted to the environment Jamalpur. The genotypes or environments that have high PC1 score has large interaction effect irrespective of their positive or negative sign, whereas the genotypes having the low or near to zero PC1 value have less interaction and is considered as a stable genotype (Crossa *et al.*, 1990). The genotype BN-P-114 positioned the farthest from the origin indicates that it contributed the largest towards the interaction. The genotypes BN-P-102 and BN-P-310 produced grain yield lower than the average and with negative IPCA1 value. The genotype BRRI dhan58 also produced grain yield lower than the average but had IPCA1 value near to zero indicating its stability across the environments. The genotypes BN-P-115 and BN-P-318 also had IPCA1 value near to zero means these were also stable across the environments.

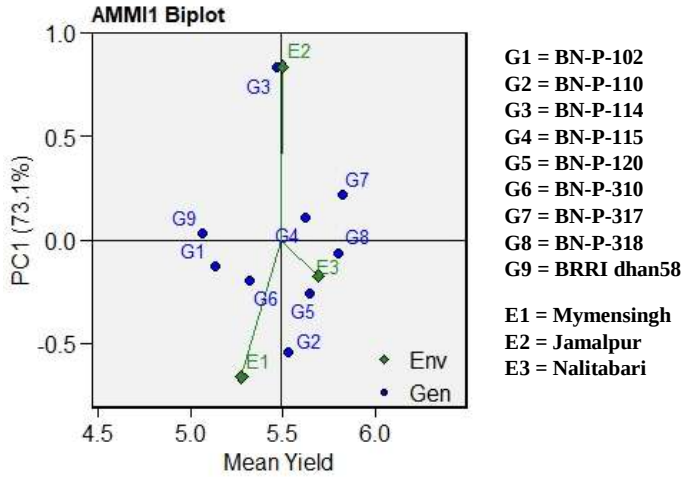

Fig. 2. AMMI1 biplot for grain yield (t ha^{-1}) of nine rice genotypes and three environments using genotypic and environmental IPCA scores

According to the AMMI2 biplot, the environments fall into three sections (Fig. 3). The genotypes BN-P-110, BN-P-114, BN-P-115 and BRRI dhan58 were more responsive as they are more distant from the origin. BN-P-114 and BRRI dhan58 were the best genotypes with respect to the best enhancing environment Jamalpur. The other genotypes BN-P-102, BN-P-120, BN-P-310, BN-P-317 and BN-P-318 were less sensitive to the environmental interactive forces as these are close to zero.

G×E interaction is a complex phenomenon and it can be simplified by GGE biplot analysis by dissecting this complex phenomenon into various PC (Yan and Tinker, 2006). GGE biplot-environment view can assess the relationship among the test environments. The relationship is visualized by the line connecting to the biplot origin and calculated by the cosine of the angle of two environments (Yan and Tinker, 2006). According to GGE biplot-environment view, environments Mymensingh & Nalitabari and Jamalpur & Nalitabari had positive relationship as they have acute angle (Fig. 4). But Mymensingh and Jamalpur had no relationship as they have a right angle. According to the GGE biplot, genotype BN-P-120 was more suitable for the environment Mymensingh. In Jamalpur, the genotypes BN-P-114, BN-P-115 and BN-P-317 were more adaptable and BN-P-318 was more suitable in Nalitabari.

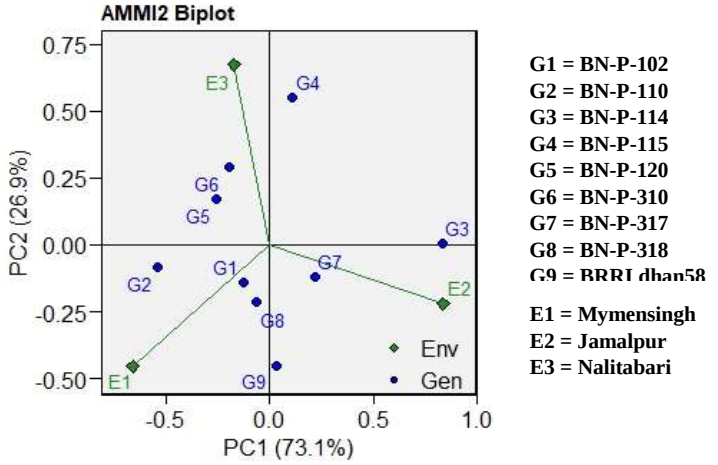

Fig. 3. AMMI2 biplot for grain yield (tha^{-1}) showing the interaction of IPCA2 against IPCA1 scores of nine rice genotypes in three environments

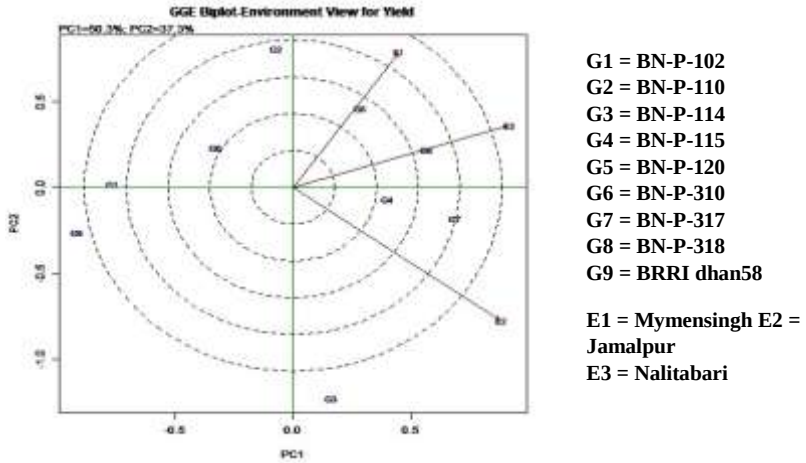

Fig. 4. GGE biplot for grain yield (t ha^{-1}) of nine genotypes tested in three environments

Adaptability of genotypes across the environments

Based on the GGE biplot-genotype and GGE biplot polygon view graph, adaptability of the genotypes was assessed across the environments. The ideal genotypes for grain yield were selected from the GGE biplot-genotype view graph and the best suited genotype for a specific environment was identified by the GGE biplot-polygon view graph. In GGE biplot-genotype graph, the genotype BN-P-317 was identified as the ideal for grain yield (Fig. 5). BN-P-318 which is next to the BN-P-317 in the ideal genotype graph was more stable than BN-P-317 as this genotype is closer to the average environment axis (AEA). Genotypes BN-P-115, BN-P-120, BN-P-317 and BN-P-318 were selected as more stable with high performance. The genotype BRRI dhan58 was also more stable as closer to AEA but with below average performance. The genotypes BN-P-110 and BN-P-114 were highly environment specific as they located in distant position from the AEA.

In GGE biplot polygon view graph, different polygons comprise one or several environments and one or several genotypes are used to detect that which genotype is performing best in which environments (Jadhav *et al.*, 2019). According to the the GGE biplot-polygon view graph BN-P-110 and BN-P-120 were the most suitable genotypes in the environment Mymensingh. BN-P-317 and BN-P-318 were suitable genotypes for the environment Jamalpur and Nalitabari, respectively. Moreover, genotype BN-P-317 performed better in both the environments Jamalpur and Nalitabari (Fig. 6).

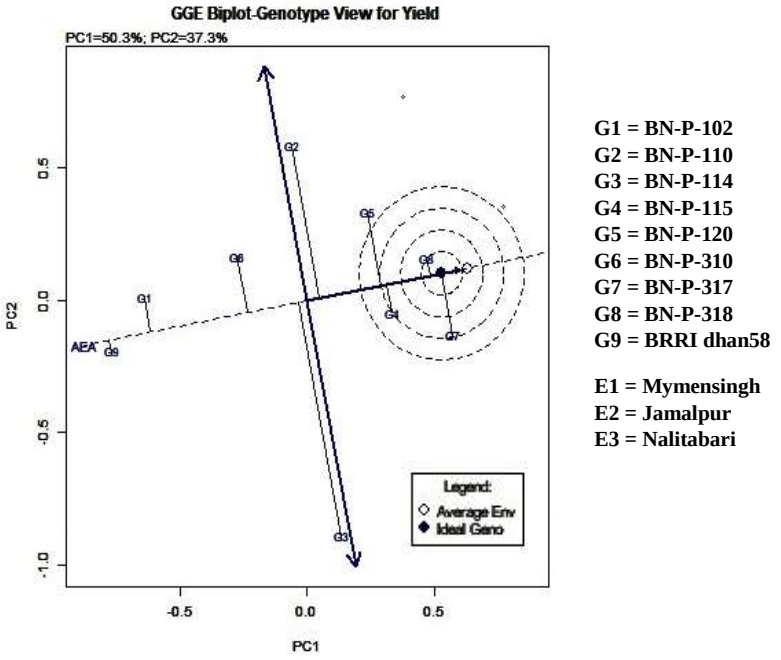

Fig. 5. GGE biplot-Genotype view, including performance of test genotypes in comparison to an estimated average environment and ideal genotype

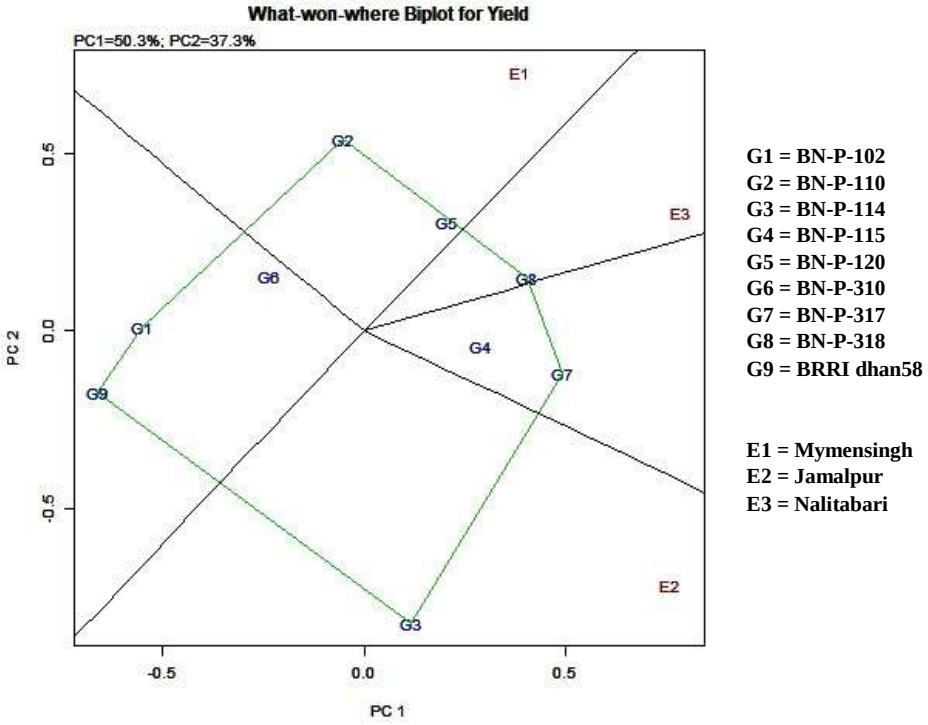

Fig. 6. Polygon views of the GGE biplot based on symmetrical scaling for 'which-won-where' pattern of rice genotypes in three environments showing genotype performed the best in particular environment.

Conclusion

The present research work reveals that the genotype BN-P-317 was the highest yield producer across the environments and the best suitable environment was Jamalpur. Moreover, BN-P-318 also can produce a high yield next to BN-P-317 and was more stable across the environments. These two advanced lines can be further evaluated and released as new varieties.

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EVALUATION OF IRON AND ZINC ENRICHED RICE (*Oryza sativa* L.) GENOTYPES IN DIFFERENT LOCATIONS OF BANGLADESH

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Abstract

Malnutrition among women and children are extremely prevalent in Bangladesh. Bangladeshi children become stunted and underweight due to micronutrients deficiencies particularly iron (Fe) and zinc (Zn). Anemia is also highly prevalent among children and women in the country due to Fe deficiency. Biofortification of rice (*Oryza sativa* L.) with micronutrients is widely recognized as a sustainable strategy to alleviate human Fe and Zn deficiencies in Bangladesh where rice is the staple food. With this view, four advanced brown rice genotypes: IZSD-10, IZSD-26, IZSD-44 and IZSD-45 along with Binadhan-20 as check variety were analyzed for grain Fe and Zn concentration using energy Dispersive X-ray Fluorescence Spectrophotometer (ED-XRF). Advanced yield trial was conducted in three different locations of Bangladesh during Aman season of 2020 in a randomized complete block design (RCBD) with three replications in each location. The Fe concentration varied from 9 to 15 mg kg^{-1} and 1 to 4 mg kg^{-1} whereas Zn concentration ranged from 45 to 59 mg kg^{-1} and 29 to 40 mg kg^{-1} in unpolished and polished rice, respectively. Almost higher Fe loss (~60 to 94 %) was observed compared to Zn (~18 to 42%) at 10% polishing throughout the grain shape that was responsible due to loss of embryo, pericarp and aleurone layer. Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations. The genotype IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days with yield 5.36 t ha^{-1}). Considering earliness, Fe and Zn content and higher yield, the genotypes IZSD-26 and IZSD-10 might be recommended for further regional yield trial to develop Fe and Zn enriched varieties. Moreover, other genotypes IZSD-44 and IZSD-45 with high Zn concentration were identified, which have the potential to be used in rice improvement for bio fortification.

Key words: Advanced yield trial, Bangladesh, Biofortification, Iron (Fe), Zinc (Zn) Rice (*Oryza sativa* L.)

Introduction

Rice is the staple food for about 164.6 million people in Bangladesh (BBS, 2019) which inevitably meets most of the nutritional demand of the majority of its people (Shelley *et al.*, 2016). But rice can't provide all the nutrition a human being requires and many poor

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people throughout the world particularly in Asia face acute nutritional deficits. Millions of individuals have a danger of sickness, disease, growth failure and more simply, deficiencies in Fe, Zn and the vitamins needed. It is currently a significant problem in Bangladesh also (FAO, IFAD, UNICEF, WFP and WHO 2021). It is estimated that micronutrient deficiencies affect approximately 1.6 billion people globally, particularly children, pregnant and lactating women, in low and middle-income countries. (Chandu *et al.,* 2020). Biofortification can be used to improve nutritional quality by increasing the content of important micronutrients in rice (Hasanzadeh and Hazrati, 2020). Since rice fortifies a large part of the diet, it can be a revolutionary remedy for micronutrients needed. Afterwards, rice yield differs due to growth conditions, e.g., various locations, seasonal fluctuations, diverse planting dates and so on (Sarker, 2002). With this view, Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA) has developed some Fe and Zn enriched genotypes. Therefore, multi-locations trial is very important to evaluate the performance of rice varieties through appropriate cultural practices to get maximum yield and quality before releasing the rice promising genotypes as national varieties and passed on to the farmers as end users. The objectives of the present study were to identify brown rice genotypes with enriched Fe and Zn concentration using ED-XRF method and to evaluate the yield performance of those genotypes in different locations of Bangladesh.

Materials and Methods

Plant materials

Four advanced brown rice genotypes: IZSD-10, IZSD-26, IZSD-44, IZSD-45 were gained from crosses between Binadhan-7 and IR66946-3R-178-1-1 in the year of 2012 to develop iron and zinc enriched variety in Bangladesh Institute of Nuclear Agriculture (BINA). Binadhan-20 was used as check that was released as Fe and Zn enriched rice variety in Bangladesh.

Estimation of Fe and Zn concentration

Well dried 10g of paddy sample from each genotype was de-husked using non-metallic de-husker (Krishi international 810 de-husker) having a roller made of polymer to avoid Fe and Zn contamination. De-husked rice was cleaned by removing broken grains and debris and 5 g of each sample was weighed and transferred to sample cups. The sample cups were gently shaken for uniform distribution of samples and kept for analysis. Fe and Zn concentration in brown rice samples was estimated using non-destructive, energy-dispersive X-ray fluorescence spectrometry (ED-XRF) instruments (model X-Supreme 8000; Oxford Instruments plc, Abingdon, UK) from IRRI, Bangladesh Office. Concentration of Fe and Zn was expressed in micrograms per gram ($\mu g\ g^{-1}$) or parts per million (ppm) and converted into milligrams per kilogram ($mg\ kg^{-1}$).

Advanced yield trial

The genotypes were evaluated in three locations such as BINA HQ Mymensingh, BINA sub-stations Jamalpur and Nalitabari as advanced yield trial during Aman season of 2020. The trial was replicated three times in each location. The unit plot size for each entry was 20 m^2 (5 m × 4 m). Seedling ages varied from 25 to 30 days at the locations due to different unavoidable circumstances during transplanting time. Seedlings were transplanted at 25 cm × 15 cm spacing on 5th August with a single number. At the rate of 120, 20, 60, 20 and 4 kg by N, P, K, S and Zn containing fertilizers per hectare were applied, respectively. All fertilizers except urea were applied as a basal dose and whereas urea was applied in 3 equal splits at 15, 30 and 45 days after transplanting. Other standard management practices were followed as and when necessary. Appropriate measures were taken to control pests and diseases. Data on days to flowering, days to maturity, plant height, total tillers $hill^{-1}$, effective tillers $hill^{-1}$, panicle length, grains $panicle^{-1}$, sterile spikelets $panicle^{-1}$, 1000-grain weight and grain yield were recorded. For yield estimation, 10 m^2 sample area from each plot was harvested at maturity and grain yield was adjusted to 14% moisture content. The yield, yield contributing traits and other parameters were statistically analyzed using the software Statistix 10.

Results and Discussion

The mean value of grain Fe concentration of rice genotypes ranged from 9 to 15 mg kg^{-1} and 1 to 4 mg kg^{-1} in unpolished and polished rice, respectively (Fig. 1). Besides, the mean value of Zn concentration of rice genotypes ranged from 45 to 59 mg kg^{-1} and 29 to 40 mg kg^{-1} in unpolished and polished rice, respectively (Fig. 2). The lowest concentration of Fe was observed in IZSD-10 and Binadhan-20 with 9 mg kg^{-1} and that of Zn in IZSD-44 with 45 mg kg^{-1} while the highest grain Fe and Zn concentration was observed in IZSD-26 with 15 mg kg^{-1} and 59 mg kg^{-1}, respectively in unpolished rice. In polished rice, the lowest concentration of Fe was observed in IZSD-44 with 0.8 mg $kg^{-1}$and that of Zn in Binadhan-20 with 29 mg kg^{-1} while the highest grain Fe and Zn concentration was observed in IZSD-10 with 4 mg kg^{-1} and 40 mg kg^{-1}, respectively. Higher heterogeneity in Fe and Zn levels among the genotypes were observed following polishing where Fe loss (~60 to 94 %) was almost higher at 10% polishing throughout the grain shapes compared to Zn (~18 to 42%). More loss of Fe than Zn was caused during polishing that might be owing to a loss of embryo, pericarp and aleurone layer, partly or total, in the polishing process; because of embryo has more Fe followed by pericarp and aleurone layer with endosperm, variation in pericarp or aleurone layer thickness or both (Matres *et al.,* 2021; Cakmak and Kutman, 2018; Díaz-Benito *et al.,* 2018; Kawakami and Bhullar, 2018; Trijatmiko *et al.,* 2016; Gregorio, 2002). It happened due to mechanical damage and removing of outermost layer to make thin rice grain (Majumder *et al.,* 2019). Almost all the genotypes had higher Fe and Zn concentration than the check variety. Similar findings were found by Anuradha *et al.* (2012) from analyzed 126 accessions of brown rice genotypes for Fe (6.2-71.6 ppm) and Zn (26.2-67.3 ppm) concentration using Atomic Absorption Spectrophotometer (AAS). The

analysis of 100 genotypes of rice for Fe and Zn content using ED-XRF method also performed by Chandu *et al.* (2020). The authors found Fe concentration varied from 1.6 to 15.2 ppm whereas Zn concentration ranged from 6.2 to 33.2 ppm of the tested germplasms. Banerjee *et al. (*2010) screened 46 rice lines including cultivated and wild accessions and showed that wild rice accessions have higher grain Fe and Zn concentration.

Significant differences were observed among the genotypes and the check variety for most of the yield and yield attributing characters for three individual locations and mean over locations from advanced yield trial presented in Table 1. The IZSD-26 performed better among the genotypes and check variety Binadhan-20 in terms of yield at all the locations. The highest yield was found on IZSD-26 at Nalitabari (5.71 t ha^{-1}) and the lowest was in IZSD-44 (4.27 t ha^{-1}) at Jamalpur. All the tested genotypes matured earlier than the check variety. Days to maturity ranged from 107-130 days for the three locations. From mean over locations, it was appeared that the IZSD-26 had significantly shorter duration (111 days) and higher number of filled grains (171.29) at all locations than the check variety followed by IZSD-45. Regarding plant height, significant differences among the genotypes were found ranging from 95-128 cm at all the locations. From mean over locations, check variety Binadhan-20 was the tallest (124.42 cm), while the genotype IZSD-45 was the shortest (102.40 cm) among the genotypes. There was no significant difference between the tested genotypes and check variety for the number of total tillers, number of effective tillers and panicle length. The lowest (22.06 g) 1000-grain weight was found in IZSD-44 and the highest was found in Binadhan-20 (24.03 g). Grain size was medium fine with red pericarp (Fig. 3). Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations than the check variety. But the genotypes IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days) (Table1). Kader *et al.* (2020) reported that a promising line which was later released as a Zn enriched variety BRRI dhan84 could produce 6.0-6.5 t ha^{-1} grain yield and mature 140 days in the dry (Boro) season. Besides, early maturing and high yielding varieties with good quality grain are chosen by farmers to cultivate (Mamin *et al.,* 2015). So, further yield trials in several years at different locations are needed to confirm the genotypes' stability (Inabangan-Asilo *et al.,* 2019) that was found in this experiment to release as a variety.

Conclusions

Based on the analysis, micronutrient enriched IZSD-26 and IZSD-10 genotypes contained higher Fe and Zn than the check variety Binadhan-20 both in unpolished and polished rice. Grain yield of IZSD-26 was considerably higher (5.37 t ha^{-1}) but not significantly different at mean over locations, also the genotypes IZSD-26 and IZSD-10 were matured (116 and 114 days) earlier than the check variety (129 days). Considering earliness, Fe and Zn content with higher yield, the genotypes IZSD-26 and IZSD-10 might be recommended for regional yield trial in next Aman season. Furthermore, other genotypes having higher Fe and Zn content can be used as plant materials for biofortification breeding programs in future.

Table 1. Agronomic performance of Fe and Zn enriched rice genotypes along with check variety at different locations of Bangladesh during Aman season 2020.

Locations	Genotypes	Days to flowering	Days to maturity	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Grains panicle^{-1} (no.)	Sterile spikelets panicle^{-1} (no.)	1000-grain weight (g)	Grain yield (t ha^{-1})
BINA HQ, Mymensingh	IZSD-10	88 b	119 b	100.13 b	11.33 a	10.0 a	24.87 a	160.00 bc	44.00 a	21.87 a	5.32 a
	IZSD-26	88 b	118 b	100.67 b	11.6 a	10.67 a	25.20 a	176.33 a	56.33 a	22.21 a	5.24 a
	IZSD-44	84 c	114 b	97.43 b	10.00 a	9.33 a	23.53 a	143.00 c	46.67 a	22.05 a	4.60 c
	IZSD-45	85 bc	117 b	96.40 b	11.40 a	10.40 a	23.00 a	175.00 ab	42.66 a	22.69 a	4.87 b
	Binadhan-20	97 a	129 a	123.67 a	11.67 a	10.00 a	23.67 a	165.33 bc	47.33 a	23.33 a	5.30 a
BINA Sub-station Jamalpur	IZSD-10	86 bc	108 b	112.67 b	10.67 a	10.33 a	26.00 a	155.67 ab	44.67 a	22.32 a	5.23 a
	IZSD-26	88 b	114 a	114.0 b	12.33 a	11.33 a	25.67 a	167.00 a	39.33 a	22.57 a	5.17 b
	IZSD-44	83 c	107 b	115.13 b	10.67 a	9.67 a	25.67 a	124.33 b	41.00 a	21.67 a	4.27 c
	IZSD-45	83 c	107 b	114.34 b	11.67 a	11.00 a	26.00 a	150.67 ab	40.67 a	22.23 a	5.12 b
	Binadhan-20	94 a	116 a	127.33 a	11.67 a	10.67 a	25.00 a	167.00 a	44.00 a	23.34 a	5.29 a
BINA Sub-station Nalitabari	IZSD-10	86 b	116 bc	100.00 b	11.13 a	10.33 ab	25.80 a	153.20 bc	31.00 a	24.36 ab	5.19 c
	IZSD-26	86 b	117 b	98.93 b	11.00 a	10.4 ab	26.73 a	170.53 a	44.73 a	23.33 bc	5.71 a
	IZSD-44	84 b	113 c	95.40 b	10.20 a	9.53 b	25.13 a	132.60 c	45.73 a	22.46 c	5.16 c
	IZSD-45	84 b	115 bc	96.47 b	11.13 a	10.7 a	27.00 a	158.73 bc	33.80 a	24.36 ab	5.59 ab
	Binadhan-20	98 a	129 a	128.27 a	11.60 a	106 ab	27.06 a	161.33 ab	35.80 a	25.43 a	5.50 b
Mean over locations	IZSD-10	87 bc	114 bc	104.27 b	11.04 a	10.33 a	25.56 a	159.62 ab	39.89 a	22.86 ab	5.22 a
	IZSD-26	87 b	116 b	104.53 b	11.93 a	10.8 a	25.87 a	171.29 a	46.80 a	22.74 ab	5.37 a
	IZSD-44	84 c	111 c	102.72 b	10.29 a	9.51 a	24.78 a	133.31 c	44.47 a	22.06 b	4.67 b
	IZSD-45	84 bc	113 bc	102.40 b	11.40 a	10.7 a	25.33 a	161.47 ab	39.04 a	23.13 ab	5.19 a
	Binadhan-20	96 a	129 a	124.42 a	11.64 a	10.42 a	25.24 a	164.56 a	42.30 a	24.03 a	5.36 a

In a column, values with the same letter(s) for individual location/combined means do not differ significantly at 5% level.

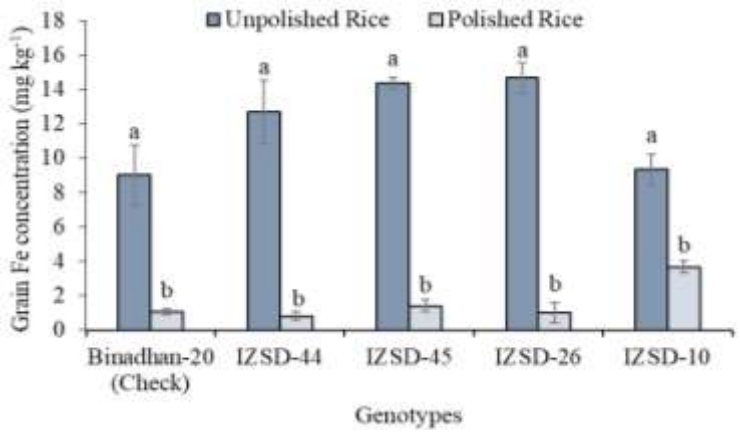

Fig. 1. Grain Fe concentration in unpolished and polished rice of the four genotypes along with check. Standard error indicated by error bars and lettering was done at 5 % level of Tukey's honest significant difference test.

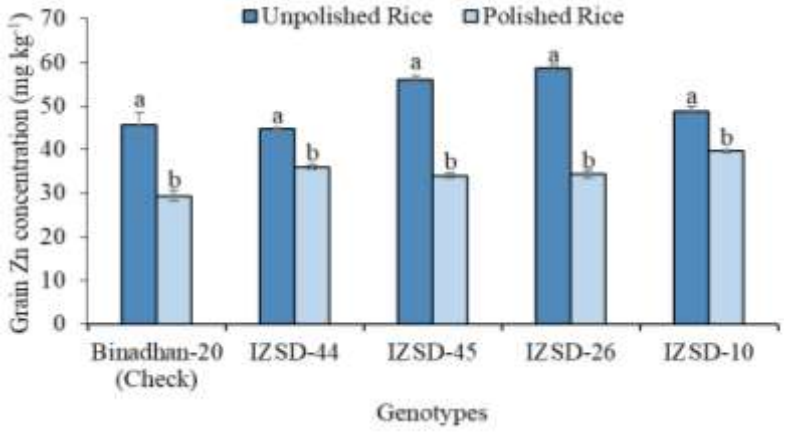

Fig. 2. Grain Zn concentration in unpolished and polished rice of the four genotypes along with check. Standard error indicated by error bars and lettering was done at 5 % level of Tukey's honest significant difference test.

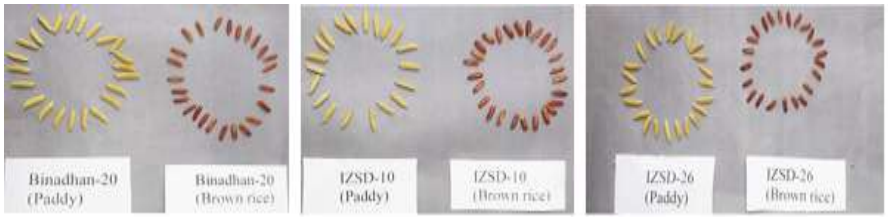

Fig. 3. Pictorial view of paddy with brown rice of Fe and Zn enriched genotypes (IZSD-10, IZSD-26) along with check variety Binadhan-20.

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COMPARATIVE ASSESSMENT OF TOMATO MUTANTS BASED ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL AND REPRODUCTIVE CHARACTERS, YIELD AND QUALITY

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Abstract

A field experiment was conducted at the experimental farm of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during the period from October 2012 to March 2013 to investigate some morphological, growth, reproductive characters, yield attributes and fruit yield in ten tomato mutants/varieties. The tested mutants/varieties were Binatomato-4, Binatomato-5, TM-113, TM-127, TM-128, TM-131, TM-132, TM-133, TM-134 and TM-160. Results revealed that in general, high yielding genotypes showed superior performance in leaf area, total dry mass production, absolute growth rate, nitrate reductase activity and total sugar content in leaves, number of flower cluster and flowers plant^{-1} which resulted higher number of fruits plant^{-1} compared to low yielding ones. Chlorophyll and reproductive efficiency had no contribution to fruit yield. Fruit yield had highly significant positive correlation with leaf area, total dry mass, absolute growth rate, number of flower clusters and fruits plant^{-1}. On the other hand, fruit size had significant negative association with fruit number. This result indicates that the improvement of fruit number plant^{-1} could be achieved by selecting increased number of effective flower cluster plant^{-1}. The mutant, TM-133 maintained superiority in most of the yield related traits and produced the highest fruit yield (100.1 t ha^{-1}).

Key words: Growth, biochemical parameters, reproductive characters, yield, tomato.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important popular and nutritious vegetable crops in the world. It ranks next to potato and sweet potato in respect of production in the world (FAO, 2015). But in South Asia, it ranks 2nd which is next to potato (SAARC, 2015) and top the list of canned vegetables. Its food value is very rich because of higher contents of vitamin A, B and C including calcium, minerals, carotene and iron (Mondal *et al*., 2016). It is extensively used in the canning industry.

The yield of tomato in this country is not satisfactory in comparison with other advanced tomato growing countries (FAO, 2015). The average yield of tomato is 26.72 t ha^{-1}

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(BBS, 2015) that is quite low than the tomato growing countries (54.55 t ha^{-1}) according to FAO (2015). To meet nutritional demand of population, it is highly important to increase the yield of tomato per unit area of land. Increase of production depends upon many factors such as use of improved varieties and proper management practices. So, using different types of techniques such as conventional breeding methods, nuclear technique and genetic engineering may improve production level and quality under the existing environmental conditions.

Varietal improvement of tomato is essentially needed to increase fruit yield by creating variability in the available germplasm followed by appropriate selection procedures. The induced mutation breeding is an effective technique for creating substantial genetic variability in plant species. Many workers have attempted to exploit somaclonal variation for crop improvement through physical mutagens particularly gamma radiation (Begum, 2005). This technique has been successfully utilized by BINA and many other research institutes in the world. The mutation breeding can play an efficient role in developing an ideal plant type having superior physiological performance as well as high yield (Malek *et al*., 2014). To increase productivity in tomato, it is therefore necessary to create variability and select desirable type with stable yield. However, biochemical properties are related to yield of tomato plant (Dutta, 2004). The higher chlorophyll, nitrate reductase activity and total sugar are helpful in increasing fruit yield in tomato (Dutta, 2001). On the other hand, component characters for yield are interdependent to each other while one character may express at the expense of other (Sharma *et al*., 2006). The importance of correlation in any breeding programme is well documented for various crop species as if provides a basis for effective selection. Correlation index acts as a guide to the reliability of phenotypic and genotype values and determines success in crop improvement (Mondal *et al*., 2011).

Under these circumstances, the scientists of BINA have developed several promising mutants of tomato with high yield potentials. These mutants need to be assessed for their morphological and physiological manoeuvring that takes place compared to the existing tomato cultivars. The present research work was therefore designed to assess the performances of eight tomato mutants along with two local improved varieties on the basis of morpho-physiological features and yield attributes.

Materials and Methods

The field experiments were conducted at the experimental field of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during winter season (November-April) of 2012-13. Eight promising mutants and two released varieties were undertaken in this study. The name of the genotypes/varieties is presented in Table 1. The soil of the experimental area is silty loam having 0.06% nitrogen, 1.15% organic matter, 18.5 ppm available phosphorus, 0.28 meq/100g exchangeable potassium, 18 ppm sulphur and 6.8 pH. The experiments were laid out in a randomized complete block design with three replicates.

The unit plot size was 4.0 m × 3.5 m with plant spacing of 50 cm × 50 cm. All agricultural practices of cultivation were performed as recommended by the Hand Book of Agricultural Technology Published by BARI (2014), Bangladesh.

Seeds were sown in seed bed and the seedlings were transplanted in the field at 25 days after sowing. Gap filling was made at seven days after transplanting (DAT) to keep same plant population density for every plot. After 30 DAT, each plant was staked with bamboo sticks to keep them erect. Recommended intercultural practices such as weeding, irrigation, application of pesticides were followed for proper growth and development of the plants. Urea, triple super phosphate (TSP), muriate of potash (MP), gypsum and cowdung were applied at the rate of 280, 250, 180, 80 and 10000 kg ha^{-1}, respectively (BARC, 2012). Total amount of TSP, MP, gypsum and cowdung were applied as basal doses during final land preparation. Half of urea was applied as top dress at 21 days after transplanting and rest half was applied at 45 days after transplanting.

To study growth characteristics, a total of two harvests were made. The second rows of each plot were used for sampling. The first and second crop sampling was done at 45 and 65 DAT. From each sampling, five plants were randomly selected from each plot and uprooted for collecting data on necessary parameters. The plants were separated into roots, stems, leaves and fruits, and the corresponding dry weights were recorded after oven drying at 80 ± 2 ^{0}C for 72 hours. The leaf area was measured by automatic leaf area meter (Model: LICOR 3000, USA) at 80 DAT, just before starting of harvesting fruits. The growth analysis like absolute growth rate (AGR) and relative growth rate was carried out following the formula of Hunt (1978). All biochemical parameters were recorded at 50-60 DAT, the fruiting stage. Nitrate reductase (NR) activity was determined by following the method of Steward and Orebanjo (1979). Total sugar, reducing sugar and chlorophyll were determined following the method of Yoshida *et al*. (1976). Photosynthesis was measured at flowering and fruit development stage using portable photosynthesis meter (LI- 6400XT, USA).

At harvest, ten plants from each plot were selected randomly for data recording on morpho-physiological, reproductive, yield and yield related traits. Per cent fruit set to opened flowers i.e. reproductive efficiency (RE) was estimated as: % fruit set = (Number of fruits $plant^{-1}$ ÷ Number of flowers $plant^{-1}$) × 100. The number of effective and non-effective flower clusters $plant^{-1}$ was counted of the sampled plant at 80 DAT. The effective flower cluster denotes as when it bears at least one fruit. The non-effective effective flower cluster denotes as when it bears no fruits. Fruit yield was collected from each plot excluding border line and converted into tonnes per hectare. Harvesting was done at different dates depending on fruit ripening.

The collected data were analyzed statistically following the analysis of variance (ANOVA) technique and the mean differences were adjudged by Duncan's Multiple Range Test (DMRT) using the statistical computer package program, MSTAT-C.

Results and Discussion

Morphological parameters

There were significant genotypic variations on morphological characters such as plant height, branch and leaf number and leaf area (Table 1). Results showed that high yielding genotypes maintained moderate plant height, medium branch number, increased leaf number and leaf area. In contrast, low yielding genotypes showed shorter plant, fewer branches (except Binatomato-5) and leaves. However, among the genotypes, the longest plant was recorded in TM-113 (110.2 cm) and the shortest was in TM-160 (68.7 cm). Results indicated that the plants those produced higher number of leaves, also showed higher leaf area. TM-128 having second highest leaves number with the highest leaf area might be due to broader leaves. In contrast, Binatomato-5 produced the highest number of leaves with lower leaf area might be due to shorter/narrower leaf. Genotypic variability in plant height, branch and leaf production was also observed by many workers in tomato (Asati *et al*., 2008; Prashanth *et al*., 2008) that also supported the present experimental results. The differential response of branching and leaves in the genotypes could be attributed to its genetic potentiality. The variation in leaf area might occur due to the variation in number of leaves and the expansion of leaf. The result obtained from the present study is consistent with result of Heuvelink (1999) in tomato who stated that variation in LA could be attributed to the changes in number of leaves. The results are also supported by the result of Andriolo *et al*. (1998) in tomato.

Table 1. Genotypic effect on morphological characters of tomato

Mutants/varieties	Plant height (cm)	Branches/plant (no.)	Leaves/plant† (no.)	Leaf area/plant† (cm^2)
Binatomato-4	70.5 e	4.89 ef	45.1 bc	1867 g
Binatomato-5	99.0 bc	6.89 a	53.3 a	3320 ef
TM-113	110.2 a	6.11 bc	42.4 c	5565 cd
TM-127	86.6 d	5.29 de	43.3 c	5230 d
TM-128	104.0 b	6.44 ab	49.6 ab	7937 a
TM-131	83.1 d	4.67 f	36.1 d	6065 bc
TM-132	68.3 e	4.78 ef	33.8 de	2091 g
TM-133	93.9 c	5.78 cd	49.7 ab	6206 b
TM-134	83.1 d	5.00 ef	36.0 d	3721 e
TM-160	68.7 e	4.00 g	30.2 e	2783 f
F-test	**	**	**	**
CV (%)	3.78	5.97	6.70	7.91

In a column figures having same letter (s) do not differ significantly at $P \leq 0.05$;

** indicates significant at 1% level of probability; †: Data was collected at 80 DAT.

Growth parameters

The effect of genotypes on root and total dry mass (TDM), absolute growth rate (AGR) and relative growth rate (RGR) was significant (Table 2). Results showed that high yielding genotypes maintained increased leaf number and leaf area, root weight and TDM plant^{-1} and AGR. RGR had no relation with yield. In contrast, low yielding genotypes showed shorter plant, fewer branches (except Binatomato-5) and leaves, lower AGR and also produced lower TDM plant^{-1}. However, among the genotypes, TM-128 showed higher/highest branch number, leaf number, leaf area, root weight and TDM plant^{-1} and showed higher fruit yield. In contrast, two low yielding mutants, TM-132 and TM-160 produced lower branches, leaves and leaf area, root weight, TDM, AGR and RGR. Increased TDM in TM-128 and TM-133 was possibly might be due to greater LA. Many researcher reported that TDM was positively correlated with leaf area in tomato (Andriolo *et al*., 1998; Mehta and Asati, 2008) that supported present experimental results.

Table 2. Effect of genotype on growth characters of tomato

Mutants/varieties	Root weight/ plant† (g)	Total dry mass/ plant† (g)	Absolute growth rate at 45-65 DAT (g/plant/day)	Relative growth rate at 45-65 DAT (mg/g/day)
Binatomato-4	4.90 f	40.0 f	1.52 ef	71.1 a
Binatomato-5	6.18 e	84.9 c	3.11 bc	66.0 ab
TM-113	6.40 e	57.7 e	2.19 d	71.1 a
TM-127	8.35 c	93.8 b	3.40 ab	64.4 ab
TM-128	11.2 a	102.5 a	3.23 bc	49.0 cd
TM-131	9.37 b	87.9 bc	2.87 c	52.9 c
TM-132	4.31 f	41.0 f	1.21 f	44.5 d
TM-133	8.82 bc	107.3 a	3.75 a	60.1 b
TM-134	7.30 d	71.5 d	2.19 d	47.3 cd
TM-160	4.49 f	54.2 e	1.78 de	53.4 c
F-test	**	**	**	**
CV (%)	5.91	5.28	9.05	6.30

In a column figures having same letter (s) do not differ significantly at $P \leq 0.05$;
** indicates significant at 1% level of probability; †: Data was collected at 80 DAT.

Biochemical parameters

The effect of genotypes on chlorophyll, nitrate reductase (NR) and total sugar in leaves and Vit-C in ripen fruits was significant (Table 3). High yielding genotypes, in general, showed higher NR and total sugar than low yielding ones. Vit-C was greater in small size fruits than bolder ones. Chlorophyll had no relation with yield. Mondal *et al.* (2016) reported that fruit yield was positively correlated with chlorophyll content in leaf. In the present investigation, the mutant TM-133 was high yielder with medium chlorophyll content in leaves. This result indicates that chlorophyll content in leaves is not obligatory for

getting higher fruit yield in tomato. However, the highest NR and total sugar was recorded in TM-133, a high yielding genotype. The lowest NR and total sugar was recorded in TM-132, a low yielding genotype. Vit-C was higher in Binatomato-4 and TM-160 whilst TM-127 showed the lowest. Result indicated that in general, small size fruit had higher Vit-C than large size ones. Genotypic variation in chlorophyll content in leaves of tomato was also observed by BINA (2007) that supported the present experimental results.

Table 3. Genotypic effect on biochemical characters of tomato†

Mutants/varieties	Chlorophyll (mg/gfw) †	Nitrate reductase (µmol NO_2^- /gfw) †	Total sugar (mg/gfw) †	Vit-C in ripen tomato (mg/100 gfw)
Binatomato-4	2.65 ab	6.40 c	72.4 cde	20.6 a
Binatomato-5	2.36 b	7.88 a	75.6 a-d	19.1 abc
TM-113	2.68 ab	7.86 a	80.2 ab	18.4 a-d
TM-127	2.60 ab	7.22 b	74.3 bcd	14.8 e
TM-128	2.46 ab	7.90 a	80.6 ab	16.4 cde
TM-131	2.36 b	6.30 c	78.3 abc	15.5 de
TM-132	2.64 ab	5.41 d	66.3 e	20.1 ab
TM-133	2.42 ab	8.11 a	82.5 a	17.3 b-e
TM-134	2.75 a	7.62 ab	73.2 cd	20.0 ab
TM-160	2.64 ab	5.63 d	68.7 de	21.0 a
F-test	*	**	**	**
CV (%)	7.38	4.28	4.89	8.73

In a column figures having same letter (s) do not differ significantly at $P \leq 0.05$;
* and ** indicate significant at 5% and 1% level of probability, respectively;
†: Data was collected at 65 DAT i.e. flowering and fruiting stage.

Reproductive characters

The effect of genotypes on reproductive characters such as number of effective flower clusters plant^{-1}, non-effective flower clusters plant^{-1}, flowers plant^{-1} and reproductive efficiency was significant (Table 4). High yielding genotypes in general produced higher number of effective flower clusters and flowers plant^{-1} than low yielding ones. The highest number of effective flower clusters plant^{-1} as well as reproductive efficiency was recorded in TM-133. The lower number of effective flower clusters, flowers plant^{-1} and reproductive efficiency was observed in TM-113 and TM-132, the low yielding mutants. Result revealed that fruit yield had no relation with non-effective flower cluster plant^{-1}. For example, TM-128, the high yielding mutant, produced the highest number of non-effective flower clusters plant^{-1} (10.0) where as Binatomato-5, the low yielding variety, produced higher number of non-effective flower clusters plant^{-1} (9.89). The lowest number of non-effective flower clusters plant^{-1} was recorded in TM-160 (5.78). Genotypic variation in flower number was also observed by BINA (2008) in tomato that supported the present experimental result.

Table 4. Reproductive characters of ten tomato mutants/varieties

Mutants/varieties	Effective flower clusters/plant (no.)	Non-effective flower clusters/plant (no.)	Flowers/ Plant (no.)	Reproductive efficiency (%)
Binatomato-4	14.9 cd	9.11 cd	69.2 cd	73.0 a
Binatomato-5	18.4 b	9.89 ab	81.7 a	52.8 cd
TM-113	10.1 g	6.22 fg	52.3 f	56.5 cd
TM-127	13.5 ef	9.33 bc	62.0 e	66.4 b
TM-128	16.0 c	10.0 a	80.2 a	58.7 c
TM-131	14.6 de	6.66 f	67.7 cd	54.9 cd
TM-132	13.2 f	9.11 cd	74.6 b	51.9 d
TM-133	19.7 a	7.33 e	70.9 bc	78.2 a
TM-134	18.4 b	8.62 d	65.5 de	75.7 a
TM-160	13.8 def	5.78 g	43.1 g	78.9 a
F-test	**	**	**	**
CV (%)	4.28	4.41	4.21	5.11

In a column figures having same letter (s) do not differ significantly at $P \leq 0.05$;
** indicates significant at 1% level of probability.

Yield contributing characters and fruit yield

The effect of genotypes on yield contributing characters and yield was significant (Table 5). Result revealed that five mutants out of eight showed higher fruit yield than the two check cultivars. The highest fruit yield both per plant and hectare was recorded in TM-133 (2.86 kg plant^{-1} and 100.1 t ha^{-1}) due to production of highest number of fruits plant^{-1}. The lower fruit yield was recorded in TM-113 (1.48 kg plant^{-1} and 51.8 t ha^{-1}) and TM-132 (1.35 kg plant^{-1} and 47.3 t ha^{-1}) due to production of fewer fruits plant^{-1}. Most of the researchers reported that fruit yield in tomato mostly depend on fruit number and fruit size (Mondal *et al*., 2004; Sharma *et al*., 2006; Mondal *et al*., 2016) that supported the present experimental results.

Table 5. Genotypic effect on yield and yield attributes of tomato

Mutants/varieties	Fruits/plant (no.)	Single fruit weight (g)	Fruit weight/plant (kg)	Fruit yield (t/ha)
Binatomato-4	44.0 d	43.6 e	1.92 d	67.2 e
Binatomato-5	48.3 cd	38.0 f	1.84 d	64.4 e
TM-113	19.5 g	75.7 a	1.48 e	51.8 f
TM-127	47.2 cd	48.3 cd	2.28 c	79.8 d
TM-128	49.8 bc	60.4 b	2.67 ab	93.4 b
TM-131	54.0 ab	53.0 c	2.54 ab	88.9 bc
TM-132	28.9 f	46.7 de	1.35 e	47.3 f
TM-133	58.1 a	49.2 cd	2.86 a	100.1 a
TM-134	51.2 bc	48.9 cd	2.40 bc	84.0 cd
TM-160	35.4 e	51.4 cd	1.82 d	63.7 e
F-test	**	**	**	**
CV (%)	6.88	6.03	9.38	5.48

In a column figures having same letter (s) do not differ significantly at $P \leq 0.05$;
** indicates significant at 1% level of probability.

Correlation

Phenotypic correlation coefficients among different quantitative characters are presented in the Table 6. Fruit yield plant^{-1} was positively and significantly correlated with leaf area (r = 0.62**), total dry mass (r = 0.78**), nitrate reductase (r = 0.42*), total sugar (r = 0.52**), absolute growth rate (r = 0.69**), the number of flower cluster (r = 0.58**) and fruits plant^{-1} (r = 0.81**) but showed negative association with chlorophyll (r = -0.34) and relative growth rate (r = -0.15). Therefore, fruit yield could be improved by selecting increased leaf area, TDM and increased number of flower cluster in tomato.

However, number of fruits plant^{-1} was highly significant and positively correlated with TDM plant^{-1} (r = 0.70**), AGR (r = 0.63**) and number of flower cluster plant^{-1} (r = 0.80**) but significantly negative associated with fruit size (r = -0.46**). It means fruit size and number is negatively associated with each other. Interestingly, chlorophyll and RGR had no significant positive association in most of the plant characters indicating chlorophyll content in leaves and RGR should not be considered for tomato improvement but nitrate reductase and AGR should be considered. The above results are supported by many workers in tomato (Singh and Raj, 2004; Sharma *et al*., 2006).

Table 6. Simple correlation coefficient among different characters of tomato mutants/variety†

Characters	Leaf area plant^{-1} (cm^2)	Total dry mass plant^{-1} (g)	Chlorophyll (mg g^{-1} fw)	Nitrate reductase (µmol NO_2^- /gfw)	Total sugar (mg g^{-1} fw)	Absolute growth rate (g p^{-1} d^{-1})	Relative growth rate (mg g^{-1} d^{-1})	Flower clusters plant^{-1} (no.)	Flowers plant^{-1} (no.)	Reproductive efficiency (%)	Fruits plant^{-1} (no.)	Single fruit weight (g)
Fruit yield	0.62**	0.78**	- 0.34	0.42*	0.52**	0.69**	- 0.15	0.58**	0.26	0.30	0.81**	0.09
Fruit number	0.44*	0.70**	- 0.45*	0.45*	0.34	0.63**	- 0.18	0.80**	0.45*	0.32	---	- 0.46**
Single fruit weight	0.52**	0.003	0.12	0.26	0.38*	0.01	0.07	- 0.55**	- 0.42*	- 0.15	- 0.46*	---
Plant height	0.71**	0.57**	- 0.22	0.84**	0.67**	0.62**	0.30	0.06	0.21	- 0.30	0.04	0.54**
Branch number	0.44*	0.47**	- 0.23	0.77**	0.51**	0.52**	0.36*	0.25	0.53**	- 0.41*	0.08	0.14
Leaf area	---	0.80**	- 0.31	0.60**	0.72**	0.74**	- 0.09	0.08	0.16	- 0.15	0.44*	0.52**
Total dry mass		---	- 0.51**	0.64**	0.61**	0.95**	- 0.08	0.51**	0.35*	0.002	0.70**	0.003
Chlorophyll			---	- 0.22	- 0.25	- 0.42*	- 0.04	- 0.28	- 0.31	0.23	- 0.45*	0.12
Nitrate reductase				---	0.68**	0.66**	0.31	0.42*	0.29	0.05	0.45*	0.26
Total sugar					---	0.64**	0.29	0.22	0.16	- 0.10	0.34	0.38*
Absolute growth rate						---	0.13	0.44*	0.30	0.004	0.63**	0.008
Rrelative growth rate							---	- 0.19	- 0.14	0.03	- 0.18	0.07
Flower cluster number								---	0.54**	0.34	0.80**	- 0.55**
Flower number									---	- 0.42*	0.45*	- 0.42*

*, ** significant at 5 and 1% level of probability; †: Fruit yield and other yield attributes had no significant positive correlation with plant height and branch number.

Conclusion

From the results above, it may be concluded that (i) Leaf area was the most important sources that determined TDM yield, (ii) Number of flower cluster was the main sink determining organ of flowers and fruits production and (iii) Correlation analysis indicated that number of flower clusters plant^{-1}, fruits plant^{-1} and fruit size contributed maximum to fruit yield in tomato, and the mutants TM-133 and TM-128 maintained superiority in the above characters, resulting higher fruit yield. This information may be implemented in the future plant breeding programme.

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COMPARATIVE STUDY OF HYBRID MAIZE VARIETIES IN RELATION TO YIELD BASED ON MORPHOLOGY AND GENETIC PARAMETERS

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Abstract

An experiment was conducted to study on morphological characters for yield and its contributing characters among 15 genotypes of hybrid maize. The study was carried out at the experimental field of the Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of November, 2016 to May, 2017. The results indicated that the genotypes differed regarding all the characters studied. The phenotypic variance and coefficients of variation were higher than the genotypic variance and coefficients of variation in all the characters studied. Moderate to high heritability was observed for all characters except cob per plot and row per cob. High heritability coupled with high genetic advance in percent mean were observed for days to tasseling, days to pollen shedding, days to silking, plant height, ear height, cob length, cob breadth, kernel per row, thousands seed weight and seed yield. The characters days to silking, plant height, ear height, field weight, moisture percentage, kernel per row, row per kernel and thousand seed weight showed positive direct effect on yield. All the genotypes were grouped into five clusters having 5, 4, 3, 2 and 1 genotypes, respectively. Cluster V comprised the maximum number (5) of genotypes followed by cluster III (4) and II (3). The highest inter-cluster distance (33.06) was observed between the cluster IV and V and the highest distant genotypes were G6 (PAC-999 supper) and G15 (PAC-339). Among the characters studied days to tasseling, days to pollen shedding, days to silking, plant height, number of leaf, cob per plot, cob length, cob breadth, row per cob, thousands seed weight were the important component characters having higher contribution to the genetic divergence.

Key words: Evaluation, Hybrid, Phenotypic variance, Genotypic variance, Heritability

Introduction

Maize (*Zea mays* L.) is an annual plant belonging to the grass family (*Graminae* or *Poaceae*) (Sprague and Dudley, 1988). Though it has wider environmental plasticity in general, it is a warm season crop and most production takes place between temperatures of 21-27^{0}C and in regions receiving rainfall of 500-700 mm per annum (Lafitte, 1994). Maize is believed to have originated in Mexico (Mangelsdorf *et al.*, 1964) about 6,000-7,000 years ago (Goodman 1988, Hallauer, 1994) and it does not survive in its wild form probably

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because of the highly cross pollinated nature. It is currently the third most traded cereal, after wheat and rice with a total production of 822 million tons in over 160 million hectares (FAOSTAT, 2010). Maize grain today is recognized worldwide as a strategic food and feed crop that provides an enormous amount of protein and energy for humans and livestock. Among all the crop plants, maize is the most versatile one as it has high nutritive value containing 66.2% starch 11.1% protein, 7.12% oil and 1.5% minerals. Moreover, 100 g maize grains contain 90 mg carotene, 1.8 mg niacin 0.8 mg thiamin and 0.1 mg riboflavin (Chowdhury and Islam, 1993). Crop improvement depends upon the magnitude of genetic variability and extent to which the desirable character are heritable. High heritability alone is not enough to make efficient selection in segregating generation, unless the information is accompanied for substantial amount of genetic advance.

Genetic improvement of a crop is pivoted on the strength of genetic diversity within the crop species. Adequate variability provides options from which selections are made for improvement and possible hybridization. Genotypic correlations had been used as an effective tool to determine the relationships among agronomic traits in genetically diverse population for enhanced progress in crop improvement (Bello *et al*., 2006). Heritability and genetic advance help in determining the influence of environment expression of the characters and the extent to which improvement is possible after selection (Robinson *et al*., 1949). Considering the above mention facts the study was undertaken with screen out the superior hybrid maize for yield and yield contributing characters comparing the morphophysiological and genetic parameters for knowing the nature of association of traits and identifying the genetically divergent parents for future breeding programme.

Materials and Methods

An experiment was conducted at the experimental field of Sher-e-Bangla Agricultural University, Dhaka during the period from November 2016 to June 2017 to study on the genetic diversity, correlation and path coefficient analysis in Maize (*Zea mays* L). Fifteen (15) genotypes were used in the study. These are G1 = BHM-5, G2 = BHM-6, G3 = BHM-7, G4 = BHM-9, G5 = Prince, G6 = PAC-999, G7 = Kaveri 25 k 60, G8 = 9120 (Palomoni), G9 = Elit, G10 = Pioneer, P-3396, G11 = Sun shine, G12 = CP- 808, G13 = HP- 701, G14 = 981 and G15 = PAC-339. The experiment was laid in Randomized Complete Block Design (RCBD) with three replications. Each entry was sown in single row of 4 m length with a spacing of 20 cm between rows and 60 cm between plants. The experimental plots were ploughed and raised the bed, applied the recommended dose of fertilizers and farm yard manures (FYM). Five plants in each entry were selected randomly and were tagged. These tagged plants were used for recording observations for the characters. Mean data of the characters was subjected to multivariate analysis. Univariate analyses of the individual character were done for all characters under study using the mean values (Singh and Chaudhury, 1985) and were estimated using MSTAT-C computer programme. Duncan's Multiple Range Test (DMRT) was performed for all the characters to test the differences between the means of the genotypes. Mean, range and co-efficient of variation (CV%) were estimated using MSTAT-C.

Results and Discussion

The highest moisture percentage was found 22.64 in BARI hybrid maize-6 and lowest moisture percentage was found 17.27 in PAC-3339 genotype. The maximum cob length was found 20.67 cm in HP-701 whereas minimum cob length was found 15.17 cm in CP-808. The lowest cob breadth was recorded 15.00 cm in BARI hybrid maize-5 whereas the highest cob breadth was recorded 18.50 cm in CP-808. The maximum kernel per row was found 46.33 in elit whereas prince showed minimum kernel per row 34.00. The lowest row per cob was found 13.00 in prince whereas maximum row per cob was found 16.00 in elit. The maximum thousand seed weight was recorded 370.00g in PAC-999 super whereas the minimum thousands seed weight was 320.00g in 981 genotype. The maximum seed yield was recorded 11.1 ton/ha Palmoni-9120 whereas minimum seed yield was 6.46 ton/ha in BARI hybrid maize-5. (Table 1)

The phenotypic coefficients of variation were higher than genotypic coefficients of variation in all the characters under study showed in table-1. Phenotypic coefficients of variation were also near to genotypic coefficients of variation for all the characters under study. High heritability (>50%) was observed for all the characters under study except cob per plot, moisture percentage, and row per cob. The highest heritability was found for thousand seed weight (99.25%). The high heritability coupled with high genetic advance in percent of mean observed in days to tasseling, days to pollen shedding, days to silking, plant height, ear height, cob length, kernel per row, 1000 seed weight and seed yield which would be selected for future breeding program. High heritability coupled with low genetic advance in percent of mean was observed in number of leaf, cob per plot, field weight, moisture percentage, cob breadth, row per cob.

The characters which had positive direct effects are days to silking (0.612), plant height (0.155), ear hight (0.735), field weight (2.048), moisture percentage (0.168), kernel per row (0.940), row per cob (0.114) and thousand seed weight (0.812) . However characters viz., days to tasseling (-0.265), days to pollen shedding (-0.775) and number of leaf (-0.374), cob per plot (-0.359), cob length (-1.771), cob breadth (-o.375) had negative direct effect on seed yield per plant (Table 5). Path coefficient analyses revealed that seed yield per plant was directly influenced by days to silking, plant height, ear hight, field weight, moisture percentage, kernel per row, row per kernel, and thousand seed weight. Hence selection of any of these traits can be done for the improvement of genotypes. Wright (1921) and Dewey and Lu (1959) observed direct and indirect contribution of various characters on yield by path coefficient analysis.

Days to tasseling showed a indirect positive effect on seed yield through days to silking, plant height, ear hight, cob per plot, moisture percentage, kernel per row, row per cob, thousand seed weight . However it had indirect negative effect on seed yield through days to pollen shedding, leaf number, field weight, cob length, cob breadth.

Days to pollen shedding showed a positive indirect effect on seed yield through days to silking, plant height, cob per plot, moisture percentage, cob length, kernel per row, row per cob, and thousand seed weight. Though it showed a negative indirect effect on seed yield through days to tasseling, ear height, number of leaf, field weight, cob breadth, day to silking showed a positive indirect effect on seed yield through plant height, ear height, cob per plot, moisture percentage, cob breadth, kernel per row, row per cob, thousand seed weight. Though it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, number of leaf, field weight, and cob length.

Plant height showed a positive indirect effect on seed yield through days to silking, ear height, number of leaf, cob per plot, field weight, moisture percentage, kernel per row. However it showed negative indirect effect days to tasseling, days to pollen shedding, cob length, cob breadth, row per cob, thousand seed weight. Ear height showed a positive indirect effect on seed yield through days to pollen shedding, days to silking, plant height, cob per plot, moisture percentage. Saidaiah *et al.* (2008) and Shinde *et al.* (2009) reported almost similar result. Though it showed negative indirect effect days to tasseling, number of leaf, field weight, cob length, cob breadth, kernel per row, row per cob, thousand seed weight. Number of leaf showed a positive indirect effect on seed yield through days to silking, ear height, moisture percentage, cob length whereas it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, plant height, cob per plot, field weight, cob breadth, kernel per row, row per cob, thousand seed weight. Cob per plot showed a positive indirect effect on seed yield through days to tasseling, days to pollen shedding, field weight, and cob length. Though it showed a negative indirect effect on seed yield through days to silking, plant height, ear height, number of leaf, moisture percentage, cob breadth, kernel per row, row per cob, and thousand seed weight (-0.148).

Field weight showed a positive indirect effect on seed yield through days to tasseling, days to pollen shedding, plant height, number of leaf, moisture percentage, kernel per row, row per kernel**.** Though it showed a negative indirect effect on seed yield through days to silking, ear height, cob per plot, cob length, cob breadth and thousand seed weight. Moisture percentage showed a positive indirect effect on seed yield through days to silking, plant height, ear height, cob per plot, field weight, kernel per row, and row per cob. Though it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, number of leaf, cob length, cob breadth, thousand seed weight. Cob length showed a positive indirect effect on seed yield through days to pollen shedding, days to silking, plant height, ear height, number of leaf, cob per plot, field weight, moisture percentage, cob breadth, kernel per row, row per cob, thousand seed weight. Though it showed a negative indirect effect on seed yield through days to tasseling. Cob breadth showed a positive indirect effect on seed yield through plant height, ear height, field weight, moisture percentage, cob length and row per cob. Though it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, days to silking, number of leaf, cob per plot, kernel per row, and thousand seed weight.

Kernel per row showed a positive indirect effect on seed yield through days to silking, plant height, number of leaf, cob per plot, field weight, moisture percentage, cob breadth, row per cob. Though it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, ear height, cob length and thousand seed weight. Row per cob showed a positive indirect effect on seed yield through days to silking, number of leaf, cob per plot, field weight, moisture percentage, kernel per row, Though it showed a negative indirect effect on seed yield through days to tasseling, days to pollen shedding, plant height, ear height, cob length, cob breadth, thousand seed weight. Thousand seed weight showed a positive indirect effect on seed yield through showed a positive indirect effect on seed yield through days to silking, number of leaf, cob per plot, cob breadth. Though it showed a negative indirect effect on seed yield days to tasseling, days to pollen shedding, plant height, ear hight, field weight, moisture percentage, cob length, kernel per row, row per cob. Similar result was observed by Kumar *et al.* (2015) and Huda (2015).

Genetic diversity of fifteen maize hybrid genotypes based on fourteen characters was measured through multivariate analysis. The 15 genotypes clustered into five distant clusters. The cluster V comprised the maximum number 5 of genotypes followed by cluster III 4. The cluster III, II, I AND IV comprised 4, 3, 2 and 1 genotypes, respectively. The highest inter-cluster distance (33.06) was observed between the cluster I and V and the highest distant genotypes were G6 (PAC-999 supper) and G15 (PAC-339). The lowest inter-cluster distance (5.11) was observed between the cluster V and II and the lowest distance genotypes were G8 (Palmoni-9120) and G2 (BARI hybrid maize-6).

The inter-cluster distances were larger than the intra-cluster distances. The intra cluster distances in the entire five clusters were more or less low indicating that the genotypes within the same cluster were closely related. Days to tasseling, days to pollen shedding, days to silking, plant height, number of leaf, cob per plot, cob length, cob breadth, row per cob, thousands seed weight were the important component characters having higher contribution to the genetic divergence. Beyene *et al.* (2005) also reported that the first principal component cluster analysis revealed that 40.4% of the total variation was due to days to tasseling and silking, plant and ear height, leaf length and days to maturity.

The result of the present study exposed that a wide variability exists among the studied and collected hybrid maize genotypes. In addition, there was also genotype of different yield contributing characters with yield of maize.

Table 1. Mean performance of different morphological characters of 15 hybrid Maize genotypes

Genotypes	DT	DPS	DS	PH	EH	LN	Cob	FW
BHM-5	86.00F	88.00G	90.00DE	200A	106AB	14.67AB	24AB	3.12D
BHM-6	88.00D	90.00E	92.00BC	192AB	121A	14.67AB	27AB	4.30C
BHM-7	90.00B	92.00C	93.00B	180A-D	114A	14.67AB	25AB	4.47BC
BHM-9	85.00G	89.00F	92.33B	180A-D	91BC	14.00A-C	27AB	4.50BC
Prince	82.33I	85.33I	89.00EF	155E	83CD	15.00A	30A	5.28A-C
PAC-999	93.00A	95.00A	97.00A	172B-E	79CD	14.00A-C	29A	4.98A-C
Kaveri 25 k 60	88.00D	92.00C	92.00BC	180A-D	81CD	12.67BC	25AB	5.40AB
9120 (Palomoni)	87.00E	91.00D	90.00DE	166C-E	71D	12.00C	26AB	5.75A
Elit	86.00F	89.00F	91.00CD	175B-E	86CD	14.00A-C	24AB	5.15A-C
Pioneer,P-3396	83.00I	86.00H	88.00F	158DE	84CD	12.00C	30A	5.19A-C
Sun shine	84.00H	90.00E	91.00CD	172BC-E	91BC	14.00A-C	30A	5.33AB
CP- 808	90.00B	93.00B	92.00BC	169B-E	88B-D	15.00A	29A	5.22A-C
HP- 701	89.00C	91.00D	92.00BC	188A-C	85CD	13.00A-C	25AB	5.07A-C
981	83.00I	85.00I	89.00EF	162DE	89B-D	13.67A-C	28AB	4.95A-C
PAC- 339	89.00C	92.00C	92.67B	1250F	78CD	14.33AB	22B	3.05D

DT= days to tasseling, DPS= days to pollen shedding, DS= days to silking, PH= plant height (cm), EH= Ear height (cm), LN= Leaf no., Cob= Cob per plot, FW= Field weight (g).

Table 1. Continued.

Genotypes	M (%)	CL	CB	KPR	RPC	TSW	YTH
BHM-5	19.90A-D	17.67CD	15.00F	39.00C-E	14.00AB	344.0G	6.46F
BHM-6	22.64A	20.00AB	16.83BC	41.00A-D	15.00AB	341.3H	8.22DE
BHM-7	20.57A-C	18.33B-D	16.17CDE	38.67C-E	15.00AB	365.7B	9.01CD
BHM-9	20.33A-D	18.67B-D	15.50EF	43.00A-C	15.33AB	324.7J	9.26CD
Prince	18.13CD	18.00CD	16.17C-E	34.00E	13.00B	354.7E	9.50A-D
PAC-999	18.87B-D	18.50B-D	15.50EF	41.67A-C	15.00AB	370.0A	9.35B-D
Kaveri 25 k 60	19.58A-D	19.33A-C	16.00CD-F	40.67A-D	15.00AB	344.3G	11.0AB
9120 (Palomoni)	19.63A-D	20.00AB	16.17CDE	45.00AB	15.00AB	360.3D	11.1A
Elit	21.13A-C	20.00AB	17.33B	46.33A	16.00A	346.0G	10.7A-C
Pioneer,P-3396	18.70B-D	18.33B-D	15.67D-F	39.67B-E	13.67AB	352.0F	9.63A-D
Sun shine	18.75B-D	17.67CD	15.83C-F	34.67E	15.00AB	353.3EF	9.65A-D
CP- 808	19.13B-D	15.17E	18.50A	35.33DE	16.00A	330.3I	9.45A-D
HP- 701	21.47AB	20.67A	16.83BC	41.33A-C	16.00A	353.3EF	9.90A-D
981	19.20B-D	17.67CD	16.67B-D	41.00A-D	15.00AB	320.7K	9.02CD
PAC- 339	17.27D	17.00D	15.67D-F	37.67C-E	16.00A	363.0C	6.90EF

M (%) = Moisture, CL = Cob length (cm), CB = Cob breadth (cm), KPR = Kernel per row, RPC = Row per cob, TSW = 1000 Seed weight (g), YTH = Yield (t/ha).

Table 2. Estimation of genetic parameters in fifteen characters of 15 hybrid maize genotypes

Parameters	σ^2p	σ^2g	$\sigma^2 e$	PCV	GCV	ECV	Heritability	Genetic advance (5%)	Genetic advance (% mean)
Days to tasseling	9.57	9.42	0.16	3.56	3.53	0.45	98.38	6.27	7.22
Days to Pollen Shedding	8.39	8.30	0.09	3.22	3.20	0.33	98.94	5.90	6.57
Days to Silking	4.96	4.50	0.46	2.44	2.32	0.74	90.78	4.16	4.56
Plant height (cm)	431.49	265.77	165.72	12.11	9.50	7.50	61.59	26.36	15.37
Ear height (cm)	248.07	155.10	92.97	17.52	13.85	10.73	62.52	20.29	22.57
Leaf no.	1.73	0.63	1.10	9.50	5.74	7.57	36.51	0.99	7.15
Cob per plot	12.70	2.31	10.38	13.31	5.68	12.03	18.20	1.34	4.99
Field weight (g)	0.80	0.53	0.27	18.64	15.20	10.77	66.57	1.22	25.55
Moisture (%)	3.65	1.02	2.63	9.70	5.14	8.23	28.05	1.10	5.61
Cob length (cm)	2.65	1.66	0.99	8.82	6.98	5.39	62.70	2.10	11.39
Cob breadth (cm)	0.98	0.66	0.33	6.10	4.98	3.52	66.65	1.36	8.38
Kernel per row	18.66	9.48	9.18	10.82	7.71	7.59	50.81	4.52	11.32
Row per cob	2.02	0.16	1.86	9.47	2.64	9.10	7.78	0.23	1.52
1000 Seed weight (g)	213.29	211.70	1.59	4.19	4.18	0.36	99.25	29.86	8.57
Yield (t/ha)	2.25	1.46	0.79	16.15	12.99	9.59	64.75	2.00	21.54

σ^2p = Phenotypic variance, σ^2g = Genotypic variance and $\sigma^2 e$ = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation.

Table 3. Genotypic correlation coefficients among different pairs of yield and yield contributing characters for different genotype of maize

	DT	DPS	DS	PH	EH	LN	Cob	FW	M (%)	CL	CB	KPR	RPC	TSW
DPS	0.931**													
DS	0.878**	0.869**												
PH	0.148	0.083	0.118											
EH	0.051	-0.101	0.030	0.549**										
LN	0.206	0.090	0.340*	-0.045	0.608**									
Cob	-0.50**	-0.39**	-0.242	-0.134	-0.253	0.173								
FW	-0.185	-0.031	-0.180	0.048	-0.553**	-0.594**	0.697**							
M (%)	0.254	0.091	0.163	1.385**	1.064**	0.042	-0.199	0.096						
CL	0.012	-0.020	0.030	0.526**	0.012	-0.757**	-0.395**	0.276	0.908**					
CB	0.168	0.127	-0.068	0.027	0.009	0.179	0.346*	0.458**	0.404**	-0.197				
KPR	0.155	0.094	0.149	0.348*	-0.213	-0.817**	-0.765**	0.080	0.791**	0.757**	-0.068			
RPC	1.201**	1.302**	1.09**	-0.106	-0.295*	-0.285	-1.050**	0.155	0.418**	0.065	0.869**	1.026**		
TSW	0.419**	0.437**	0.366*	-0.260	-0.206	-0.147	-0.183	-0.028	-0.308*	0.239	-0.386**	-0.084	-0.238	
YTH	-0.080	0.087	-0.098	0.086	-0.613**	-0.768**	0.632**	1.009**	0.162	0.482**	0.337*	0.428**	0.428**	0.021

Table 4. Phenotypic correlation coefficients among different pairs of yield and yield contributing characters for different genotype of maize

	DT	DPS	DS	PH	EH	LN	Cob	FW	M (%)	CL	CB	KPR	RPC	TSW
DPS	0.928**													
DS	0.850**	0.844**												
PH	0.130	0.073	0.118											
EH	0.059	-0.061	0.052	0.611**										
LN	0.108	0.046	0.205	0.170	0.463**									
Cob	-0.216	-0.182	-0.103	0.011	-0.015	-0.113								
FW	-0.133	-0.017	-0.118	0.026	-0.253	-0.364*	0.532**							
M (%)	0.118	0.050	0.068	0.311*	0.250	0.006	-0.263	0.006						
CL	0.022	-0.009	0.030	0.258	0.035	-0.250	-0.172	0.239	0.456**					
CB	0.168	0.128	0.000	0.037	0.097	0.223	-0.115	0.300*	0.240	-0.027				
KPR	0.127	0.064	0.091	0.200	-0.034	-0.216	-0.173	0.194	0.330*	0.644**	0.064			
RPC	0.359*	0.388**	0.304*	0.019	-0.014	0.134	-0.366*	-0.174	0.246	0.059	0.407**	0.119		
TSW	0.413**	0.431**	0.347*	-0.200	-0.164	-0.099	-0.084	-0.027	-0.169	0.175	-0.312*	-0.069	-0.093	
YTH	-0.048	0.087	-0.019	0.027	-0.300*	-0.382**	0.044	0.830**	0.132	0.373*	0.354*	0.290	-0.013	0.021

** = Significant at 1%; * = Significant at 5%.

DT= days to tasseling, DPS= days to pollen shedding, D = days to silking, PH= plant height (cm), EH = Ear height (cm), LN = Leaf no., Cob = cob per plot, FW = Field weight (g). M (%)= Moisture, C= Cob length (cm), C= Cob breadth (cm), KPR= Kernel per row, RPC= Row per cob, TSW= 1000 Seed weight (g), YTH= Yield (t ha^{-1}).

Table 5. Partitioning of genotypic correlations into direct (bold) and indirect effects of ten important characters by path analysis of Maize

	DT	DPS	DS	PH	EH	LN	Cob	FW	M (%)	CL	CB	KPR	RPC	TSW	Genotypic correlation with yield
DT	**-0.265**	-0.721	0.537	0.023	0.037	-0.077	0.181	-0.378	0.043	-0.021	-0.063	0.145	0.137	0.340	-0.08
DPS	-0.247	**-0.775**	0.532	0.013	-0.074	-0.034	0.140	-0.064	0.015	0.035	-0.048	0.089	0.149	0.355	0.087
DS	-0.233	-0.673	**0.612**	0.018	0.022	-0.127	0.087	-0.369	0.027	-0.052	0.026	0.140	0.126	0.297	-0.098
PH	-0.039	-0.064	0.072	**0.155**	0.403	0.017	0.048	0.098	0.233	-0.932	-0.010	0.328	-0.012	-0.211	0.086
EH	-0.013	0.078	0.018	0.085	**0.735**	-0.227	0.091	-1.133	0.179	-0.022	-0.003	-0.201	-0.034	-0.167	-0.613**
LN	-0.055	-0.070	0.208	-0.007	0.447	**-0.374**	-0.062	-1.216	0.007	1.341	-0.067	-0.768	-0.033	-0.120	-0.768**
Cob	0.134	0.302	-0.148	-0.021	-0.186	-0.065	**-0.359**	1.426	-0.034	0.700	-0.130	-0.720	-0.120	-0.148	0.632**
FW	0.049	0.024	-0.110	0.007	-0.407	0.222	-0.250	**2.048**	0.016	-0.489	-0.172	0.075	0.018	-0.023	1.009**
M (%)	-0.067	-0.071	0.100	0.215	0.782	-0.016	0.071	0.197	**0.168**	-1.608	-0.151	0.744	0.048	-0.250	0.162
CL	-0.003	0.015	0.018	0.082	0.009	0.283	0.142	0.566	0.153	**-1.771**	0.074	0.712	0.007	0.194	0.482**
CB	-0.045	-0.099	-0.042	0.004	0.006	-0.067	-0.124	0.939	0.068	0.349	**-0.375**	-0.064	0.099	-0.314	0.337*
KPR	-0.041	-0.073	0.091	0.054	-0.157	0.306	0.275	0.164	0.133	-1.340	0.026	**0.940**	0.117	-0.068	0.428**
RPC	-0.318	-1.009	0.672	-0.017	-0.217	0.106	0.377	0.318	0.070	-0.115	-0.326	0.965	**0.114**	-0.193	0.428**
TSW	-0.111	-0.338	0.224	-0.040	-0.152	0.055	0.066	-0.057	-0.052	-0.424	0.145	-0.079	-0.027	**0.812**	0.021

Residual effect: 0.131 ** = Significant at 1%; * = Significant at 5%.

DT = days to tasseling, DPS = days to pollen shedding, DS = days to silking, PH = plant height (cm), EH = Ear height (cm), LN = Leaf no., Cob = cob per plot, FW = Field weight (g). M (%) = Moisture, CL = Cob length (cm), CB = Cob breadth (cm), KPR = Kernel per row, RPC = Row per cob, TSW = 1000 Seed weight (g), YTH = Yield (t/ha).

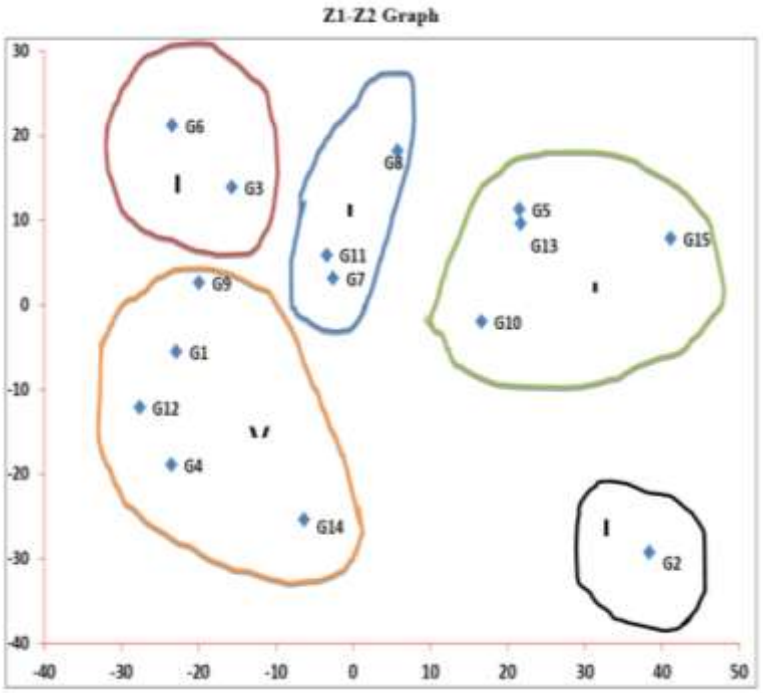

Fig. 1. Scatter diagram of 15 maize genotypes based on their principal component scores.

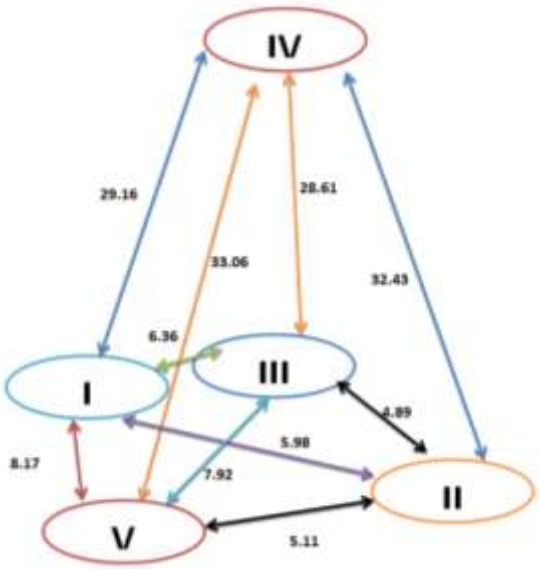

Fig. 2. Intra and inter cluster distances of 15 genotypes in maize

Conclusions

The genotype of clusters I was more diverse from the genotypes of cluster V. Wide range of genetic diversity present among the maize genotypes. Wide genetic diversity was found in 15 genotypes of maize, which were grouped into five clusters and most diverse genotypes were G6 (PAC-999 supper) and G15 (PAC-339). That variability could be used for future breeding program of maize in Bangladesh. Field weight, cob length, cob breadth, kernel per row, row per cob showed highly significant and positive correlation with seed yield at both genotypic and phenotypic levels. This results suggested that seed yield per plant can be increased by improving these characters. High heritability coupled with high genetic advance in percent of mean was observed in days to tasseling, days to pollen shedding, days to silking, plant height, ear height, cob length, cob breadth, kernel per row, thousands seed weight and seed yield. Hence, yield improvement in maize would be achieved through selection of these characters. Days to silking, plant height, ear height, field weight, moisture percentage, kernel per row, row per kernel, thousand seed weight showed positive direct effect on seed yield. So yield improvement was associated with these characters. Days to pollen shedding, days to silking, plant height, ear height, cob length, cob breadth, kernel per row, thousands seed weight were found responsible for the maximum diversity. On the other hand, days to tasselling, moisture percentage, and cob per plot have the least responsibility of both the primary and secondary differentiation of genotypes. Further collection of maize hybrid genotypes would be continued for getting more variability and desired qualities and higher yield in Maize in Bangladesh.

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DEVELOPING HIGH YIELDING, LODGING TOLERANT BIROI TYPE RICE LINES THROUGH MUTATION BREEDING TECHNIQUE

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Abstract

Red rice is increasing its popularity for its functionality and healthy food value hence raised the market demand now-a-days. But the yield of this group of rice is very poor. Local rice Biroi having red pericarp, low yielding and lodging susceptible variety was irradiated with five (100, 150, 200, 250 & 300 Gy) doses of gamma ray. A total of 6 M_3 plants were first selected from irradiated 1805 M_2 plants. Two years replicated yield trial experiments were conducted in different locations of Bangladesh. Among them two mutants were selected for higher yield and moderate lodging tolerance. The selected mutants Biroi-250-2-2 and Biroi-250-2-3 showed 11% to 13% higher yield than their original parent which gave red pericarp. These two mutants might be a good breeding material for red rice, lodging resistance variety development program.

Key words: Red pericarp, lodging tolerance, high yield, biroi

Introduction

Rice is the main food for more than half of the world's population and is the staple food for the people of Bangladesh, constituting over 91% of the food grain production, and providing 62% of the calorie with 46% of the protein intake in the average daily diet (HIES, 2010). The rice production area in Bangladesh is approximately 15.4 million hectares (ha) producing 63.64 million tons of rice annually (BBS, 2019).

There are around 40,000 variants of rice in the world. Red colored rice is a variety of rice that contains anthocyanin. During the milling process, only the husk is removed from the rice grains but retain all nutrients, vitamins, and minerals intact in the bran layer and in the germ. Red rice is enriched with antioxidants and magnesium compared to polished rice. It is used in breads, nutty, colored pasta, vinegar, alcoholic beverage, drugs, and cosmetics (Patindol *et al*., 2006). It has antioxidant activity with procyanidins (Oko *et al*., 2012) and exists great gene diversity which make red rice important to cultivate as resistance to drought, flood, submergence, alkalinity, salinity, and resistance to pests and diseases (Chaudhary and Tran, 2001). But traditional red rice varieties also typically have weak stems, low tillering ability, and long droopy leaves, turn yellow during grain development, and become logged at maturity. However, farmers still plant them widely because they can

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be grown under low inputs and produce a reasonable yield under the seasonal environmental conditions to which modern varieties are not adapted. Nonetheless, this limited yield makes farmers less interested in cultivating red rice despite its high medicinal value and use as a functional food (BRRI, 2016).

Biroi is a traditional local rice variety of Bangladesh containing red pericarp in grain and considered as red rice. Higher plant height, longer duration and lodging susceptibility are the key characteristics of Biroi cultivar (BINA, 2017). Stem lodging hindrances the photosynthetic effectiveness of the canopy that affects the grain filling (Weng *et al*., 2017). Hence the rice grain yield and quality reduced by 60-80% as photosynthesis is directly associated with lodging (Jency *et al*., 2020).

Modern breeding techniques including mutation could improve the lodging (Jency *et al*., 2020) and yield of red rice to overcome this problem. Successful breeding for crop improvement, however, depends on genetic variation in the parents (BRRI, 2016) which limit breeding progress and/or yield and quality crop improvements (Corneous and Sneller, 2012).

Mutation can play a vital role in improving desired characters of red rice. The technique has been successfully utilized by Bangladesh Institute of Nuclear Agriculture (BINA) and many other research institutes on different crops (Miah and Bhatti, 1968; Azad *et al*., 2012).

As red rice is a very good source of human health concern, we designed an experiment to improve this rice through mutation breeding. We had developed some lines of Biroi those possess earliness, high yielding and moderate lodging tolerant comparing to parent. The objective of this research is to develop a lodging tolerant premium quality red pericarp rice variety that will maintain the nutritional balance as well as the food demand in the world prospective.

Material & Methods

The local popular germplasm Biroi was used as experimental material and collected from different area of Gafargaon and Fulpur Upazila, Mymensingh were irradiated through physical mutagen (gamma rays) from Electronics Section, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh. About 500 mature and viable seeds (moisture 12%) of the Biroi variety were packed in butter paper covers and supplied for each dose of irradiation at 150, 200, 250, 300 and 350Gy with 60_{Co} (Cobalt-60) irradiator (Model: IAEA-TECDOC-539, IAEA, 1990) at BINA irradiation chamber, Mymensingh. Germination and survival test were done from irradiated seeds. Seeds were grown dose wise with dense spacing in the raised nursery beds established at the plant breeding division, BINA. After a month, the seedlings were transplanted in a non-replicated design with 10 m^2 area with the spacing of 15 cm in plant and 20 cm in row (Kato *et al.*, 2020). Recommended doses of nitrogen, phosphorus, potassium, sulphur and zinc were applied in the form of Urea, TSP,

MoP, Gypsum and Zinc Sulphate @ 195, 50, 70, 55, and 5.6 kg ha^{-1} respectively. Cultural and intercultural practices were followed as and when necessitated. Data on plant height (cm), effective tillers $hill^{-1}$, panicle length (cm), filled and unfilled grains $panicle^{-1}$ were recorded from five randomly selected competitive hills at maturity. Maturity time was assessed plot basis. Grain yield was recorded from an area of 1.0 m^2 which later converted to t ha^{-1}.

Results & Discussions

Determination of Lethal Dose (LD_{50}) of Mutagens

As the relative effectiveness of the mutagens is essential to determine the correct dose/concentration of the mutagens (Zhao *et al*., 2016) LD_{50} was calculated (Fig. 1). Probit analysis (Finney, 1978) was carried out using seed germination values (Poornima *et al.*, 2017) for gamma rays to determine the LD_{50}. The expected LD_{50} value for the seeds was 250 Gy (Fig. 1) and LD_{50} was found 300 for the hard coated seeds of rice (Huang *et al.*, 2009). To comprehend, higher doses caused injury to the cell, which may be vital, and inhibited many cellular activities, eventually causing death of the cells. It had been noticed that, due to these chaos of the mutagens, seeds treated at high doses 350 Gy most of the seeds did not germinate, or their seedlings could not survive beyond a few days (Liu *et al*., 2012). The social acceptance of gamma-ray irradiation is quite high and numerous useful practical mutant varieties have been developed in the past 60 years (Nakagawa and Kato, 2017). This method has been proven to be useful in generating higher yield mutants, which hence contribute to the discovery of novel genes for higher yield.

Growing of M_1 generation of Biroi rice in T. Aman season

Germination% was decreased with the increase of the irradiation doses (Fig. 1). The result was agreed with Liu *et al*., 2012. The plant height was also decreased gradually with the increase of gamma rays doses. Zanzibar and Sudrajat (2016) was reported the similar result. Finally, the M_1 seeds from the survived plants were bulked dose wise and kept for growing as M_2 generation in the next growing season.

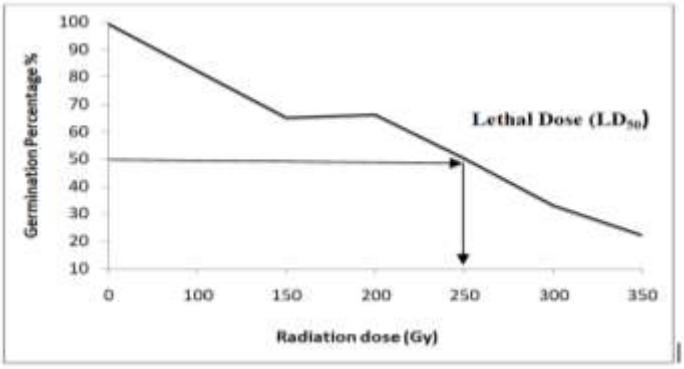

Fig. 1. Determination of LD_{50} based on germination percentage of irradiated seed

Screening of the candidate mutant of M_2 plants and M_3 lines

A wide range of variations was noticed among the M_2 generations for novel altered phenotypes in the traits *viz*., plant height, effective tiller hill^{-1}, panicle length, filled grain panicle^{-1}, unfilled grain panicle^{-1}, yield plant^{-1}. Five seeds of each panicle were bulked together from each dose (150, 200, 250, 300 and 350 Gy) of irradiation. The total number of 1805 M_2 plants was collected from irradiated Biroi variety according to the homogeneity of the panicles. Then M_3 population was grown in the field for selection. In our selection, we considered their positional advantage over other plants and their position in the field so that the effect of non-uniform soil fertility could be eliminated. In M_3 generations several mutant has been selected from different doses. Two mutants 2-1 & 2-2 were selected from 300 Gy and eight mutants were selected from 200 Gy of irradiation. The progeny were recorded as Biroi-300-2-1, Biroi-300-2-2, Biroi-200-2-1, Biroi-200-2-2, Biroi-200-2-3, Biroi-200-2-4, Biroi-200-2-5, Biroi-200-2-6, Biroi-200-2-7 and Biroi-200-2-8 respectively (Table 1). Likewise, six M_3 mutants were selected from 250 Gy of irradiation and named as Biroi-250-2-1 to Biroi-250-2-6. Total 16 mutants from M_3 generation were planted along with biroi variety as a check. Five plants from each line excluding border plants were harvested for yield evaluation. All the panicles of each plant were cut at the neck and collected into individual paper bags. The panicle weight of each plant was measured as the individual plant yield. To fulfill the research objectives the mutants having white pericarp and lodging susceptible was discarded even they are short duration and moderately high yielding mutant (Table 1). Only six mutants from 250 Gy dose having red pericarp and moderately tolerant to lodging were selected and planted for M_4 generation.

Table 1. Grain color, lodging status and yield of some M_3 mutants of Biroi during 2016-17

Variety/mutant	Duration (days)	Pericarp color	Lodging condition	Yield (tha^{-1})
Biroi-300-2-1	136	White	Susceptible	4.30
Biroi-300-2-2	138	White	Susceptible	4.46
Biroi-200-2-1	141	White	Susceptible	5.20
Biroi-200-2-2	133	White	Susceptible	5.33
Biroi-200-2-3	145	White	Susceptible	5.80
Biroi-200-2-4	146	White	Susceptible	5.65
Biroi-200-2-5	142	White	Susceptible	4.98
Biroi-200-2-6	137	White	Susceptible	4.50
Biroi-200-2-7	128	White	Susceptible	4.25
Biroi-200-2-8	135	White	Susceptible	4.32
Biroi-250-2-1	140	Red	Moderately tolerant	5.30
Biroi-250-2-2	139	Red	Moderately tolerant	6.50
Biroi-250-2-3	145	Red	Moderately tolerant	6.00
Biroi-250-2-4	143	Red	Moderately tolerant	6.66
Biroi-250-2-5	137	Red	Moderately tolerant	5.10

Variety/mutant	Duration (days)	Pericarp color	Lodging condition	Yield (tha^{-1})
Biroi-250-2-6	142	Red	Moderately tolerant	5.56
Biroi (Parent)	148	Red	Susceptible	4.30

Performance of selected mutants in M_4 and M_5 generation

In M_4 generation, maturity date among the mutants ranged from 123-141 days (Table 2) and was shorter than parent (145 days). Among the mutants three had relatively longer days (133-141) to mature and produced higher yield. The long maturity duration allows the plants to attain higher yield and biomass (Dixit *et al*., 2014). Plant height ranged between 85.60 to 136.8 cm with Biroi-250-2-3 being the shortest and Biroi-250-2-1 the tallest. Number of effective tillers ranged between 7.0 to 10.6 with the parent being the highest and the mutants Biroi-250-2-2 and Biroi-250-2-6 the lowest. Panicle length ranged between 14.87 to 26.8 cm with the mutant Biroi-250-2-6 being the longest and Biroi-250-2-5 the shortest (Table 2). Filled grains $panicle^{-1}$ ranged between 122.0 to 233.6 with the parent being the lowest and the mutant Biroi-250-2-6 the highest. Unfilled grains $panicle^{-1}$ ranged 23 to 57.6. Grain yield ranged between 3.9 to 6.86 t ha^{-1} with the mutant Biroi-250-2-5 being the lowest and Biroi-250-2-3 the highest (Table 2). Three mutants (2-2, 2-3 & 2-4) were red colored similar maturity period (133-141 days), intermediate plant height (85-96 cm), 7-8 no. effective tiller $hill^{-1}$, average no. of filled grain $panicle^{-1}$ was 166-194 and thus the yield (>6 ton/ha) which was higher than the parent and within the mutants as well. The mutants having red grain color with medium plant height possess moderate lodging resistance (Mackill *et al*., 1996). In addition, lower positioning of panicles in the plant's canopy is known to be associated with increased tolerance of lodging (Setter *et al*., 1997). Two dominant genes with complementary gene action (ISASaT, 2017), one increases the pigment content and other accumulates the pigments (Nagao *et al*., 1957) might be responsible for red pericarp. Therefore, the mutants with red decorated grain are needed to further confirmation by molecular analysis.

Table 2. Grain yield and yield components of some M_4 mutants of Biroi during 2018-19

Variety/ mutant	Days to maturity	Plant height (cm)	Effective tillers $hill^{-1}$ (no.)	Panicle length (cm)	Filled grains $panicle^{-1}$ (no.)	Unfilled grains $panicle^{-1}$	Grain yield (t ha^{-1})
Biroi-250-2-1	127	136.8±2.63	8.0±0.55	25.0±0.95	205.8±6.26	44.0±9.33	4.0±0.02
Biroi-250-2-2	141	92.8±1.94	7.0±0.45	24.8±0.49	166.6±9.93	57.6±7.11	6.18±0.08
Biroi-250-2-3	133	85.60±1.70	7.4±0.81	24.8±0.49	194.4±14.96	55.6±9.37	6.86±0.05
Biroi-250-2-4	133	96.2±1.98	8.0±0.71	23.8±1.02	166.2±18.79	23.00±6.24	6.43±0.04
Biroi-250-2-5	127	134.2±2.03	7.6±0.60	14.87±0.37	180.0±6.61	24.6±1.63	3.9±0.03
Biroi-250-2-6	123	130.4±1.21	7.0±0.63	26.8±1.30	233.6±16.63	35.2±6.98	5.34±0.09
Biroi (Parent)	143	125.8±1.85	10.6±0.68	24.2±0.58	122.0±5.15	24.2±1.91	4.05±0.02

In Table 3, results showed significant variation among the mutants and check for most of the characters in combined over locations and individual locations from combined analysis, it

was observed that plant height of all the genotypes ranged from 101.29-146.74 cm., effective tillers was found lowest (8.93) in mutant Biroi-250-2-3 and highest (19.08) in Biroi-250-2-6. Significant variation was found in panicle length, filled grain panicle^{-1} and unfilled grain panicle^{-1}. Some mutants showed lower effective tillers hill^{-1} than the parent but produced longer panicle length, higher filled grains panicle^{-1} and lower unfilled grains panicle^{-1} which contributed to the higher yield. In Rice, higher Leaf Area Index (LAI), more filled grains panicle^{-1} and significant panicle length are the pre-requisite for maximizing yield (Wu *et al*., 2005).

Table 3. Grain yield and yield components of some M_5 mutants of Biroi at different locations during 2019-20

Location	Mutants/Variety	Plant height (cm)	Effective tillers hill^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Yield (t ha^{-1})
Mymensingh	Biroi	141.73 b	10.93 b	21.86 c	126.93 d	44.33 ab	3.91 d
	Biroi-250-2-1	140.47 b	9.66 bc	25.20 a	207.33 a	39.06 ab	4.01 d
	Biroi-250-2-2	92.73 d	10.20 bc	24.73 ab	177.07 abc	39.06 ab	6.10 a
	Biroi-250-2-3	87.67 d	9.20 cd	25.13 a	200.73 a	39.80 ab	6.23 a
	Biroi-250-2-4	98.33 c	8.20 d	23.53 b	161.93 bc	30.60 b	5.56 b
	Biroi-250-2-5	154.00 a	14.40 a	26.06 a	191.00 ab	47.26 a	3.95 d
	Biroi-250-2-6	142.59 b	13.39 a	24.86 ab	156.95 cd	41.95 ab	4.59 c
Nalitabari	Biroi	120.93 a	10.40 b	24.36 a	91.60 a	32.73 ab	3.96 c
	Biroi-250-2-1	153.00 a	9.93 b	24.80 a	106.73 a	34.00 ab	4.13 bc
	Biroi-250-2-2	130.70 a	10.46 b	25.43 a	96.67 a	16.66 b	6.03 a
	Biroi-250-2-3	114.67 a	8.86 b	22.93 a	89.93 a	23.40 ab	6.06 a
	Biroi-250-2-4	116.47 a	10.60 b	23.00 a	98.60 a	19.26 ab	5.66 a
	Biroi-250-2-5	133.73 a	9.93 b	24.30 a	105.73 a	29.20 ab	4.10 bc
	Biroi-250-2-6	137.40 a	25.03 a	21.26 b	109.47 a	37.06 a	4.56 b
Combined mean over location	Biroi	131.33abc	10.66 ab	23.12 b	109.27 b	38.53 a	3.94 d
	Biroi-250-2-1	146.74 a	9.33 b	25.00 a	157.03 a	36.53 ab	4.07 d
	Biroi-250-2-2	109.49 abc	10.15 b	25.36 a	138.63 ab	26.13 ab	6.02 a
	Biroi-250-2-3	101.29 c	8.93 b	23.96 b	145.47 ab	31.70 ab	6.03 a
	Biroi-250-2-4	107.40 bc	9.40 b	23.26 b	130.26 ab	24.93 b	5.62 b
	Biroi-250-2-5	143.87 ab	12.16 ab	25.18 a	148.37 ab	38.23 a	4.02 d
	Biroi-250-2-6	139.57 ab	19.08 a	17.06 c	135.40 ab	39.03 a	4.54 c

Table 4. Grain yield and yield components of some M_6 mutants of Biroi during 2020-21

Mutants/Variety	Plant height (cm)	Effective tillers hill^{-1}	Panicle length (cm)	Filled grains panicle^{-1}	Unfilled grains panicle^{-1}	Yield (t ha^{-1})	BLB severity (%)
Biroi	141.73 b	10.93 a	21.86 c	126.93 d	44.33 d	3.91 d	-
Biroi-250-2-1	143.73 b	7.13 d	25.27 b	159.80 a	45.67 cd	4.00 bc	30
Biroi-250-2-2	92.93 d	8.32 cd	22.04 d	146.33 bc	79.20 ab	6.00a	-

Biroi-250-2-3	87.93 d	9.87 b	24.80 bc	141.00 bc	85.80 a	5.97 a	-
Biroi-250-2-4	98.53 c	8.50 cd	23.87 cd	130.87 cd	60.00 cd	5.41 bc	25
Biroi-250-2-5	151.80 a	9.20 bc	26.60 a	153.47 b	82.93 ab	4.03 ab	20
Biroi-250-2-6	142.27 b	9.73 bc	23.53 cd	131.93 cd	58.67 bc	3.95c	15

During aman season 2020-21, considering the yield performance and lodging tolerant ability of the mutant further trial was done for M_6 generation and bacterial leaf blight (BLB) severity test also done (Table 4). Among six mutants, Biroi-250-2-1, Biroi-250-2-4, Biroi-250-2-5 and Biroi-20-2-6 were BLB susceptible. Mutant Biroi-250-2-2 & Biroi-250-2-3 was BLB tolerant and produced higher yield than parent and other mutants. These two mutants also showed higher yield in Nalitabri substation, and Mymensingh Headquaters of BINA in M_5 generation (Table 3) and M_4 generation (Table 2). Although mutant Biroi-250-2-4 had good performance regarding yield and lodging tolerant ability in different generations we discarded this mutant because of BLB susceptibility (Table 4). Finally two mutants were selected based on moderate plant height, lodging tolerance, red colored pericarp and BLB tolerance which will need further trail in next season.

Conclusions

The higher yields of the selected mutants were likely to have been caused by the increase of positive allele frequency in mutants through mutagenesis. Biroi-250-2-2 and Biroi-250-2-3 mutants can be evaluated at the next trail to develop a high yielding moderate lodging tolerant reddish grain with having BLB tolerant and semi dwarf biroi type rice variety.

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BINAMASH-2, A GAMMA RAY INDUCED HIGH YIELDING BLACKGRAM MUTANT VARIETY

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Abstract

Blackgram is one of the most important pulse crops extensively grown in Bangladesh with multiple uses. Genetic improvement of blackgram is highly impeded due to narrow genetic base whilst hybridization and recombination is rather difficult because of its auto-gamous nature. Viewing these constraints, an induced mutation, using gamma irradiation, creating genetic variation, resulting in the creation of new varieties with better characteristic was undertaken. The mutants were derived from three popular varieties, BARI Mash-1, BARI Mash-2 and BARI Mash-3 which were treated with gamma irradiation (400, 500 and 600 Gy doses) subsequent selection and field trials were conducted with a view to select mutant with desired traits during 2011 to 2020. The mutant BM-404 out yielded the checks and other mutants with other desired characters like earliness, bolder seed and erect plant type. This mutant also showed moderately resistant to Yellow Mosaic Virus, cercospora leaf spot and Hairy caterpillar. Therefore, we applied to the National Seed Board (NSB) of Bangladesh for registration and the mutant line BM-404 was registered namely Binamash-2 as a national modern variety in 2021.

Key words: Black gram, high yielding, mutant, variety

Introduction

Blackgram or urdbean (*Vigna mungo*) is widely cultivated in the Indian subcontinent including Bangladesh and to a lesser extent in Thailand, Australia, and other Asian and South Pacific countries. It is a nutritious and most commonly tailored stress-tolerant legume with a cheap source of vegetable protein, amino acids and so for poor people. The crop plays a major role in improving soil fertility owing to their ability to fix atmospheric nitrogen and it is also well suited for various cropping systems like dry farming and intercropping. In Bangladesh during 2018-2019, urdbean was grown over an area of 0.618 lakh hectares with the production of 0.690 lakh metric tonnes whilst the average yield of 1100 kg ha^{-1} was low as compared to the cereal crops. In order to break the yield bottleneck in black gram, efforts are needed to develop high yielding varieties with better growth habit.

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Genetic improvement is highly impeded due to narrow genetic base of the crop as a result research on this species has lagged behind that of cereals and other legumes. Therefore, improvement of this crop is needed through utilization of available genetic diversity. Expanding genetic variation may offer better traits for the genetic improvement of the crop for sustainable food production and other qualities. Induced mutagenesis creates a new variation in the traits of interest for genetic enhancement like improved yield and other polygenic characters with no disruption in the plant's basic chromosome structure. Mutation induction has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain specific traits (Kurobane *et al.,* 1979). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid, 2000). Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs *et al.,* 2002). Mutation breeding is suitable choice of creating variability in self-pollinated crops such as blackgram. Therefore, the present mutation breeding programme was initiated to identify mutants with high yield potential, earliness, erect, and determinate type plant growth habit of blackgram.

Materials and Methods

Dry, healthy and uniform sized seeds of black gram variety, BARI Mash-1, BARI Mash-2 and BARI Mash-3 were treated with gamma rays at 350, 400, 450 and 500Gy doses from BINA radiation source, [Cobalt sixty (Co^{60})]. The irradiated seeds were immediately sown at BINA headquarters farm, Mymensingh in 2011 and the M_1 population was grown at close spacing where the first two pods formed on each M_1 plants were harvested considering mutation experiments as and kept separately dose and variety wise to grow M_2 generation in the next season. A total of 192 plant-progeny-rows were grown from four doses in M_2 generation and 28 M_2 plants were selected to grow M_3 generation and subsequent M_4 generation was grown and selection were made with desirable mutants. Through preliminary yield trial seven mutants of good performed lines were selected considering their field performance as compared to the checks (Binamash-1, BARI Mash-2 and BARI Mash-3) during 2016 and they were put into Advanced Yield Trial (AYT) along with a popular check variety, BARI Mash-3 during Kharif 2017. Considering the superior performance of the selected mutant line BM-404 of BARI Mash-3 derived from 400Gy, multi-location yield trials were conducted with this line in various agro-ecological zones of the country during 2018 and 2019. Unit plot size was 4m x 3m. Row to row and plant to plant distances were 40 cm and 8-10 cm, respectively. Data on days to maturity, plant height (cm), primary branches plant^{-1}, pods plant^{-1}, seeds pod^{-1}, 100-seed weight and yield plot^{-1} were recorded. Plot yield was converted to kg ha^{-1}. The trials were conducted simultaneously both in the experimental farms and in the farmers' field following two management practices (research management and farmers' management). The most popular variety and mother BARI Mash-3 was used in all the multilocation trials as check. In the research management practice trials

were replicated and farmers' practices were non-replicated. 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 during 2020.

Results and discussion

The mutants BM-211, BM-401, BM-102, BM-404, BM-108, BM-401 and BM-409 were put under preliminary yield trials with three checks at two locations, Magura and Chapainwabganj during 2016. From the Table 1, it was observed that significant differences were found for all the characters among the mutants grown except number of seeds pod^{-1} at both the locations. The mutant BM-409 was the tallest whilst BM-211, BM-102 and BM-404 were the shortest among the mutants and checks in combined mean of two locations. In case of primary branches plant^{-1} BM-401 and BM-404 had the highest number of branches per plant. The mutants BM-201 and BM-404 produced the highest number of pods plant$^{-1.}$ The higher 100-seed weight were found in the mutants BM-404 and BM-409 as well as higher seed yield at both the locations. Singh (1996) characterized mutations obtained in *Vigna mungo* as gene mutations and found long pod and bold seeded mutants with better yield which is in support of the bold seeded mutants BM-409, BM-404 and BM-211. Combining mean of two locations showed that mutant BM-409 produced the highest seed yield followed by the mutants BM-108 and BM-404.

The advanced yield trials were conducted with three promising blackgram mutants along with a check varietiy BARI Mash-3 at Magura and Chapainawabganj during 2017. Results revealed that there was significant difference for most of the characters except seeds pod^{-1} (Table 2). BM-404 was the shortest among the mutants and checks at Magura and Chapainawabganj. In case of primary branches and pods plant^{-1}, BM-404 had the highest number of branches and pods plant^{-1}. Higher 100-seed weight (g) was found in the BM-409 and BM-404. Seed yield was the highest for BM-404 because of its higher 100-seed weight and higher number of pods plant^{-1}. Jain (1975) suggested that improvement in grain yield in legume crops could be achieved through restructuring of plant types to determinate, erect and compact growth habits which totally in support of the mutant BM-404 with erect, short, determinate type plant growth habit and higher yield.

The zonal yield trials were conducted with two promising blackgram mutants along with a check varietiy BARI Mash-3 at Magura and Chapainawabganj during 2018. Results revealed that there was significant difference for most of the characters except seeds per pod (Table 3). The mutant line BM-404 matured earlier than the other mutant and the check variety, BARI Mash-3 in both locations Magura and Chapainawabganj. Sonu *et al.* (2019) found mutants with different gamma rays produced early mutants which was similar to this result. The check variety, BARI Mash-3 was the tallest among the mutant and check. In case of number of pods per plant, BM-404 had the higher number of pods than the other mutant and the check variety, BARI Mash-3. Seed yield was the highest for the BM-404 because of its higher number of pods per plant and also higher 100-seed weight (g). Combining mean

of two locations, it was observed that BM-404 produced the highest seed yield (1.45 t ha^{-1}). This result was the similar with the findings that the early mutants with altered agronomic characteristics like yield and growth habit were isolated in *Vigna mungo* (Kumar *et al.* 2009).

Table 1. Performance of seven promising mutants along with three check varieties, BARI Mash-1, BARI Mash-2 and BARI Mash-3 grown at Magura and Chapainawabganj during 2016

Mutants/Variety	Plant height (cm)	Primary branches $plant^{-1}$ (no.)	Pods $plant^{-1}$	Seeds pod^{-1} (no.)	100 seeds weight (g)	Yield (kg ha^{-1})
Magura						
BM-211	40.8 abc	1.4 b	35.6 cdc	5.8	4.1 bc	1235ab
BM-401	41.5ab	1.7 b	39.6 abc	5.5	3.6 bc	1196ab
BM-102	40.2 bc	1.7 b	37.5 bcd	5.8	3.5 bc	1166ab
BM-404	37.8 c	3.8 a	42.2 ab	5.9	5.2a	1350a
BM-108	38.2 c	1.7 b	32.0 de	5.8	3.4c	1186ab
BM-409	43.7 a	1.6b	40.0 abc	6.0	5.5a	1283a
BM-201	39.5 bc	1.8 b	44.5 a	5.9	3.8bc	1183ab
BARI Mash-2	41.6 ab	1.4 b	31.3 e	5.9	4.2 b	1243ab
BARI Mash-3	38.9bc	1.9 b	31.7 de	5.7	3.9 bc	1166ab
BARI Mash-1	40.4 bc	1.8 b	40.7 abc	5.4	3.8 bc	1231a
CV(%)	4.36	30	12.5	3.2	17	4.7
Chapainawabganj						
BM-211	40.4 c	1.3 bc	26.2 ab	6.2	6.6a	1266bc
BM-401	43.2 abc	1.2 bc	24.8 abc	6.1	5.3ab	1300ab
BM-102	41.2 bc	1.0 c	23.5 bc	6.1	4.0c	1266bc
BM-404	44.4 a	1.9 a	27.0 ab	6.1	7.0a	1200c
BM-108	46.4 a	1.2 a	28.2 a	6.3	4.1c	1350a
BM-409	45.5 ab	1.5 ab	21.2 c	6.2	6.3a	1293abc
BM-201	43.2 abc	1.1bc	27.5 ab	5.5	6.3a	1283abc
BARI Mash-2	43.9 abc	1.2bc	25.7 ab	6.0	7.0a	1266bc
BARI Mash-3	45.6 ab6	1.2 bc	26.5 ab	6.3	6.6a	1233bc
BARI Mash-1	45.8ab	1.2 bc	26.9 ab	5.9	7.0	1187bc
CV(%)	4.55	19.68	8.12	3.87	19.16	3.81
Combined over locations						
BM-211	40.6	1.3 b	30.9	6.0	5.3a	1250ab
BM-401	42.3	2.9 a	32.0	5.8	4.4b	1248ab
BM-102	40.7	1.3 b	30.5	5.9	3.7c	1216bc
BM-404	41.1	2.8 a	34.6	6.0	6.1a	1275a
BM-108	42.3	1.4 b	30.1	6.0	3.7c	1268a
BM-409	44.6	1.5 b	30.6	6.1	5.9a	1288a
BM-201	41.3	1.5 b	36.0	5.7	4.5b	1233bc
BARI Mash-2	42.7	1.3 b	28.5	5.9	5.6a	1254d
BARI Mash-3	42.2	1.5 b	29.1	6.1	5.2a	1199c
BARI Mash-1	43.1	1.5 b	33.8	5.6	4.9b	1209bc
CV(%)	4.425	27.84	10.31	3.535	18.08	4.255

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

Table 2. Mean of yield and yield contributing characters of three selected mutants of blackgram grown at two locations, Magura and Chapainawabganj during 2017

Mutants/Variety	Plant height (cm)	Primary branches plant^{-1} (no.)	Pods plant^{-1}	Seeds pod^{-1} (no.)	100-seeds weight (g)	Yield (kg ha^{-1})
Magura						
BM-404	38.2 bc	3.8 a	42.2 ab	5.9	3.7 bc	1538a
BM-108	38.6 bc	1.7 b	32.0 de	5.8	3.1 c	1514a
BM-409	43.7 a	1.6 b	40.0 abc	6.0	5.5 a	1352b
BARI Mash-3	40.9ab	1.9 b	31.7 de	5.7	3.9 bc	1508a
CV%	6.27	26.25	14.85	2.20	25.30	5.75
Chapainawabganj						
BM-404	42.1b	2.9 a	37.0 a	6.1	6.3a	1838a
BM-108	46.4 a	1.2 bc	28.2 ab	6.3	4.6b	1722ab
BM-409	45.5 ab	1.5 ab	21.2 c	6.2	6.3a	1714ab
BARI Mash-3	46.6 a	1.2 bc	26.5 ab	6.3	6.0a	1590b
CV%	4.84	27.78	23.26	1.53	14.00	5.90
Combined						
BM-404	44.1a	3.3a	39.6a	6.0	5.0a	1688a
BM-108	40.5b	1.4b	30.1	6.0	3.85c	1618a
BM-409	44.6a	1.5b	30.6	6.1	5.90a	1533ab
BARI Mash-3	42.2ab	1.5b	29.1	6.1	4.95b	1549ab
CV%	4.38	27.68	15.06	0.95	17.55	4.44

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

Table 3. Mean of yield and yield contributing characters of two promising mutants of blackgram grown at two locations, Magura and Chapainawabganj during 2018

Variety/mutants	Days to maturity	Plant height (cm)	Primary branches plant^{-1} (no.)	Pods plant^{-1}	Seeds pod^{-1} (no.)	100-seeds weight (g)	Yield (kg ha^{-1})
Magura							
BM-404	74b	51.9 ab	3.8	42.2 a	6.6	4.37 a	1502a
BM-108	75b	48.2 c	2.7	35.0 a	6.7	3.80b	1341b
BARI Mash-3	79a	56.0 ab	2.2	27.1b	6.6	4.52a	1400b
CV%	3.48	7.49		21.72	0.87	8.875	5.75
Chapainwabganj							
BM-404	74c	32.10	1.8	40.0 a	6.1	4.49a	1412 a
BM-108	76ab	32.7	1.2	31.5 a	6.3	3.60b	1320 b
BARI Mash-3	78a	35.0	2.2	29.2 b	6.3	4.56a	1300 b
CV(%)	2.63	4.60	24.74	16.95	1.85	12.69	4.44
Combined							
BM-404	74c	42.5	2.8	41.1a	6.35	4.43a	1456a
BM-108	76b	40.4	1.9	33.2b	6.50	3.70b	1330b
BARI Mash-3	79a	45.0	2.2	28.1b	6.45	4.54a	1350b
CV(%)	3.297	5.40	40.78	19.18	1.187	10.81	4.91

On-station and farmers' field trials were carried out with two promising lines and a most popular check variety with BARI Mash-3 at Mymensingh, Magura, Chapainawabganj and Gopalgonaj during 2019 and 2020. The mutant line BM-404 produced the highest seed yield followed by BM-108 and check variety, BARI Mash-3 in the research management practice at all the locations (Table 4). Similar trend of seed yield was found by the line in farmers' management practices (Table 5). Average seed yield of BM-404, BM-108 and BARI Mash-3 were 1644, 1485, and 1452 kg per hectare, respectively, (Table 6).

It was observed from five years average mean yield (Table 7) that the mutant BM-404 produced the highest yield (1480 Kg/ha) followed by the mutant BM-108 and the check BARI Mash-3 (1394 and 1359 Kg/ha) respectively. Mutagens have been successfully utilized to generate promising traits particularly for isolating mutants with desirable characters of economic importance (Shah *et al*. 2008; Usharani and Kumar 2015) which is in support of the high yielding mutant BM-404 of blackgram with determinate plant type and higher number of pods with bolder seeds.

Table 4. Seed yield of mutant lines along with the check variety BARI Mash-3 grown at four research stations during 2019 and 2020

Mutants/ Variety	Seed yield (kg ha^{-1})							
	2019			2020				
	Mymensingh	Magura	Average	Mymen.	Magura	Chapai	Gopalganj	Average
BM-404	1421a	1408a	1415a	1732a	1531a	2074a	1721a	1719a
BM-108	1331ab	1325ab	1328ab	1503b	1389b	1895b	1473b	1595b
BARI Mash-3	1208b	1315ab	1261b	1479c	1412b	1836b	1461b	1547b
CV (%)	8.1	3.78	5.78	8.89	5.27	6.41	9.46	5.47

Table 5. Seed yield of selected mutant lines grown at farmer's field at Mymensingh, Magura, Faridpur and Gopalganj during 2019 and 2020

Mutants/ Variety	Seed yield (kg ha^{-1})						
	2019			2020			
	Mymensingh	Magura	Average	Mymensingh	Faridpur	Gopalganj	Average
BM-404	1311a	1304a	1307a	1623a	1669a	1416a	1569a
BM-108	1205b	1225b	1215b	1560b	1338b	1223b	1374b
BARI Mash-3	1246b	1216b	1231b	1550b	1318b	1201b	1356b
CV (%)	4.26	3.88	3.93	2.51	13.67	9.24	8.24

Table 6. Comparative seed yield (kg/ha) of the selected mutants/variety grown at research station and farmer's field during 2019 and 2020

Mutants/Variety	Seed yield (kg ha^{-1})					
	Research management (kg ha^{-1})		Farmer's management (kg ha^{-1})		Average	
	2019	2020	2019	2020	2019	2020
BM-404	1415a	1719a	1307a	1569a	1362a	1644a
BM-108	1328b	1595b	1215b	1374b	1272b	1485b
BARI Mash-3	1261bc	1547b	1231b	1356b	1246b	1452b
CV (%)	5.78	5.47	3.92	8.24	4.71	6.72

Table 7. Combined means over five years for seed yield (kg ha^{-1}) of the two mutants along withe the check variety, BARI Mash-3

Mutants/Variety	2016	2017	2018	2019	2020	Mean over five year
BM-404	1251a	1688a	1456a	1361a	1644a	1480a
BM-108	1268a	1618a	1330b	1272b	1485b	1394b
BARI Mash-3	1199b	1549b	1350b	1246b	1452b	1359b
CV (%)	2.9	4.29	1.0	4.66	6.72	4.41

Remarks about the susceptibility to diseases and insect-pests

A. Disease reaction

Disease reaction against Cercospora leaf spot and Powdery mildew and Yellow Mosaic were examined under field condition during 2020 at Mymensingh. Results were presented in table 8, 9 and 10. There was not much disease incidence in test mutants and check variety for Cercospora leaf spot and Powdery mildew in field. All the tested mutants along with a check variety, BARI Mash-3 showed moderately susceptible to the diseases.

Table 8. Incidence of Cercospora leaf spot disease in some selected mutants/variety of blackgram at Mymensingh during 2020

Genotypes/Variety	Cercospora leaf spot		
	PDI	DS	Reaction
BM-404	55.00	23.28	MS
BM-108	58.33	20.72	MS
BARI Mash-3	66.67	25.04	MS

Table 9. Incidence of Powdery mildew disease in mutants/variety of blackgram at Mymensingh during 2020

Genotypes/Variety	Powdery mildew		
	PDI	DS	Reaction
BM-404	60.00	44.67	MS
BM-108	63.33	32.09	MS
BARI Mash-3	60.00	39.33	MS

All the tested entries along with a check variety, BARI Mash-3 showed moderately susceptible to susceptible to the Yellow Mosaic Virus disease.

Table 10. Incidence of Yellow Mosaic in selected mutants/variety of Blackgram at Mymensingh during 2020

Genotypes/ Variety	Yellow Mosaic		
	PDI	DS	Reaction
BM-404	4.76	14.07	MS
BM-108	6.14	18.09	MS
BARI Mash-3	20.68	36.42	S

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

B. Insect-pests infestation

Naturally low insect pest infestation is occurred in black gram and there was no pod borer infestation in blackgram. The caterpillar infestation was occurred in black gram during experimentation (Table 11). Tested mutants and check were moderately tolerant and moderately susceptible to this insect pest.

Table 11. Infestation of Hairy caterpillar in selected mutants/variety of blackgram at Mymensingh during 2020

Genotypes/Variety	Hairy caterpillar	
	(0-9 scale)	Reaction
BM-404	3	MR
BM-108	5	MS
BARI Mash-3	7	MS

In respect of yield potential, earliness, erectness with determinate type plant habit and diseases reaction, BM-404 performed the best over year and location. Therefore, the mutant has been registered as a variety, Binamash-2 for cultivation all over the country.

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ASSESSMENT OF GENETIC DIVERSITY AMONG SIXTY FIVE BRINJAL GENOTYPES USING SSR MARKERS

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Abstract

The present study was conducted at the Biotechnology Laboratory and Horticulture Field Laboratory of the Bangladesh Institute of Nuclear Agriculture (BINA) to study the genetic diversity among 65 brinjal genotypes using single sequence repeats (SSR) markers. Molecular characterization of the collected genotypes was performed using six SSR markers. The selected primers, namely EM107, EM114, EM133, EM139, EM140 and EM145 showed polymorphism. The values of pair-wise comparison of Nei's genetic distance (D) between genotypes computed from combined data for the 6 primers ranged from 0.17 to 1.00. The highest genetic dissimilarity (100%) was observed among various combinations, such as Islampuri (S31), Thapara (S32), Mentar (S33), Iribegun (S35), Eye-red (S36), Deembegun (S37), Comilla-L (S38) and Chega (S39) with Dohazari G (S10), Borka (S11), Khatkhatia B (S12), Khatkhatia BAU (S13), ISD-006 (S2) and Laffa-M (S3). The UPGMA cluster based on Nei's genetic distance analysis led to the grouping of the 65 brinjal genotypes into three major clusters with nine sub-clusters.

Key words: Brinjal, SSR marker, molecular characterization, genetic diversity, dendogram

Introduction

Brinjal (*Solanum melongena* Linn.), also known as eggplant or aubergine, is an important solanaceous vegetable crop grown widely in the Central, South and South-East Asian countries, and in a number of African countries of the world (Kalloo, 1993; Kumar *et al.*, 2003; Shaukat *et al*., 2009). Brinjal is believed to have been originated in India, as the people of this subcontinent were reported to grow brinjal since last 4000 years (Dunlop, 2006). Brinjal occupies a distinct place in the realm of vegetable crops globally. The current global production of brinjal is estimated as 54.08 million tons of which 93% is contributed by the Asian countries (FAO, 2020). In terms of production, China ranked the top (45% of world output) followed by India (24% of world output) (FAO, 2020). Brinjal is one of the most nutritious and culturally important vegetables and is a good source of minerals and vitamins. It is also one of the most common, popular and principal vegetables grown both in summer and winter seasons in Bangladesh. To meet domestic demand, Bangladesh produces substantial amounts of brinjal every year. During 2018-19, 531 thousand metric tons of brinjal were produced from 53 thousand ha of land (BBS, 2019).

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The identification of crop plant has become increasingly important for the documentation of genetic resource and to protect breeders' interests. For recipes and food industries, this is especially important for different varieties of brinjal having wide range of morphological, eating and marketing qualities. Farmers need varietal identity for protection of their proprietary rights. Identification process must assure varietal identity and free from mixture. Examination of morphological characters was used to be the common method of identification, but not all of them can be distinguished on morphological basis only.

Molecular characterization of genotypes is considered a powerful and efficient technique for assessment of genetic variability required for successful breeding programme. Molecular markers are used now-a-days as recent innovation for characterization and evaluation of genetic diversity within and between species and population. These markers can provide significant information which may enhance the scope of diversified use of genotypes in crop improvement programme. Characterization at molecular level reveals the extent of relationship among the genotypes and in the estimation of genetic diversity or relatedness. SSR marker was first developed for brinjal by Nunome *et al.* (2003), where they confirmed the usefulness of these markers for genetic analysis and found useful for agronomically-important traits in brinjal that could facilitate marker assisted breeding. Despite widespread cultivation, and nutritional, cultural and economic importance, brinjal genome has not yet been extensively evaluated as compared to those of other solanaceous vegetables, namely tomato, potato, pepper and so on. Few studies have been conducted to determine genetic diversity of brinjal using RAPD (Nunome *et al*., 2001), AFLP (Mace *et al*., 1999), RAPD and AFLP (Nunome *et al*., 2001), SSR (Nunome *et al*., 2003; Stagel *et al*., 2008; Nunome *et al*., 2009) and ISSR (Isshiki *et al*., 2008) markers.

In Bangladesh, information on molecular characterization using SSR marker is scanty and for brinjal it is a new attempt. Very few studies with smaller number of genotypes were conducted and reported in Bangladesh. However, reports of in-depth investigation with most of the available genotypes in Bangladesh and potential exotic lines are hardly found available in the in Bangladesh. Therefore, the main objectives of the present investigation were to precisely describe the genetic variation, genetic distance and genetic dissimilarities among the 65 brinjal genotypes sourced from home and abroad using SSR markers in Bangladesh.

Materials and Methods

The experiment was conducted at the Field Laboratory of BINA with 65 brinjal genotypes primarily obtained from IPM Lab, BAU; BARI, Gazipur and BINA, Myemnsingh. The collected genotypes were assigned to accession numbers starting from S1-S65. The seedlings were grown in the pot up to 25-30 days. To carry out the analysis, a fresh young leaf from each of the brinjal genotypes was collected and held in zip-lock plastic bags and preserved in icebox. Then the samples in icebox were brought to the laboratory and cut into 2-3 cm pieces for extraction of genomic DNA. Then 800 µl DNA

extraction buffer was added and samples were grounded with mortar and pestle and was taken into eppendrof (2 ml) tube and vortex well to suspend the powder. Then the eppendrof tubes were placed in 65^{o}C water bath in a tube holder for 20 minutes (after 10 minutes solution were mixes by inverting and returned to the water bath). Then the tube were removed, mixed inverting and bring to a chemical fume hood. Later 800 µl chloroform mix (Phenol:Chloroform:Isomyl alcohol=24:25:1) was added. The tightly closed tubes were placed in a tube rack covered with paper towels and hold a second tube rack against the top of the tubes and invert repeatedly for three minutes. Then the tubes were centrifuge for eight minutes at 11,000 rpm in a micro centrifuge. Then 500 µl of upper aqueous layer was removed then to a new 1.5 ml tube being careful not to pipette near the dirty layer. 1000 µl of cold 100% ethanol was added and mixed by inverting. Then the samples were centrifuged for 12 minutes at maximum speed (13,200 rpm), a small pellet was visible. Later the supernatant was decanted carefully by pouring into a beaker. The DNA pellet was washed with cold 70% ethanol and allowed to air dry until they were completely dried using a concentrator for maximum 10 min. The DNA pellets were re-suspended into 100 µl of 10×TE buffer, and was dissolved the pellet. After the pellet was dissolved, it was stored at -20 ^{o}C. DNA quality were assessed on a 0.80% agarose gel at 120 volt for one and half an hour stained with ethium bromide andvisualization under UV light using Gel Doc system (Biometra, Japan). The qauntity of DNA concentration was measured by NanoDrop Spectrophotometer (Thermo Scientific NanoDrop^{TM} 1000) by taking UV absorbance at 260 nm.

SSR-PCR analysis

Twenty primers were used for survey with 27 randomly selected genotypes (Table 1). Among them, 5 polymorphic micro satellite markers were selected based on intensity of bands, PCR product size (bp), annealing temperature, presence of smearing and potential of population discrimination, to evaluate the collected 65 brinjal genotypes for molecular characterization. The polymerase Chain Reaction (PCR) mixture contained 2 µl genomic DNA, Primer forward 1.00 µl, Primer reverse 1.00 µl, 10x buffer 1.00 µl, dNTPs 1.00 µl, $MgCl_2$ 0.60 µl, Taq polymerase 0.20 µl, ddH_2O 3.20 µl in a total volume of 10 µl. PCR amplification was performed using Biometra T3 thermal cycler. The amplification conditions involved initial denaturation of 5 min at 94 ^{o}C, followed by 35 cycles of 30 sec at 94^{o}C, 1 min at 65^{o}C and 2 min at 72^{o}C, with a final extension at 72^{o}C for 7 min. The PCR product were separated on 8% polyacrylamide gel in 1x TBE buffer with 90 volts and 500 mA for 1.5-2 h and visualized under ultraviolet light (Whatman Biometra gel Documentation System, prod nr: 1603209) after staining in ethidium bromide and capture image of each gel and saved in a computer as a JPEG file. The 100 bp DNA ladder plus molecular weight was used to compare the molecular weight of amplified product (Fig. 1-4). The six SSR primers were previously selected for assortment of genetic diversity of brinjal genotypes.

Data analysis

The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to molecular weight size marker (100 bp DNA Ladder) with the help of Alpha VIEW (Version 3.2.8) software. Data of each SSR locus, the number of alleles, allele size, major frequency allele, genetic diversity and polymorphism information content (PIC) ware determined using the POWER MARKER version 3.23 (Liu and Muse, 2005). The score were obtained in the form of matrix with '1' and '0', which indicate presence and absence of bands in each variety, respectively. This observation was further analysed with NTSYS-pc version 2.2 (Rohlf, 2000). NTSYS-pc was used to construct a UPGMA (Unweighted Pair Group method with Arithmetic Mean Averages) dendogram showing the distance based on interrelationship among the genotypes.

Result and Discussion

Selection of primers

Primers were selected on the basis of band resolution, intensity, presence of smearing, consistency within individuals and potential for population discrimination. All the selected primers (EM107, EM114, EM133, EM139, EM140 and EM145) showed clear polymorphism which were used for further analysis. The properties of the selected primers are furnished in Table 2.

Overall allelic diversity

Among the six SSR motifs used in the present study five SSR motifs were clearly polymorphic, and produced varying number of alleles with different size ranges (Table 3). A total of 26 alleles were detected among the 65 brinjal genotypes. The number of allele locus^{-1} ranged from 3.0 to 6.0 with an average of 4.33 alleles locus^{-1}. The primers EM107 and EM114 had the highest number of alleles (6) and EM139 and EM145 had the lowest number of alleles (3). However EM140 had high genetic diversity (0.7730), while EM133 had lowest genetic diversity (0.5960) with a mean diversity of (0.6746) (Table 3). The overall size of amplified products ranged from 100 bp in locus EM107 to 340 bp in locus EM139. On an average, 41.03% of the 65 brinjal genotypes shared a common major allele ranging from 30.77% (EM107) to 46.15% (EM114) common allele at each locus (Table 3). These results revealed that markers EM140 (0.7730) would be the best in screening the collected brinjal genotypes followed by the markers EM107 (0.7115), EM139 (0.6627), EM114 (0.6551), EM145 (0.6495) and EM133 (0.5960).

PIC (Polymorphism Information Content)

The PIC value is a reflection of allele diversity and frequency among genotypes. PIC value of each marker can be evaluated on the basis of alleles. In the present investigation, PIC varied markedly for all the tested SSR loci. Polymorphism among the 65 studied genotypes was evaluated by calculating PIC values for each of the six SSR loci, which

ranged from 0.5120 (EM133) to 0.7387 (EM140) with an average of 0.6128 locus^{-1} (Table 3). Reports on molecular characterization of available brinjal genotypes using SSR markers are meager in Bangladesh. However, Demir *et al*. (2010) conducted molecular characterization of eggplant genotypes collected from different geographical regions in Turkey using both SSR and RAPD markers. They found that with amplification of five SSR loci, the number of alleles per microsatellite locus ranged from 2 to 10, with a total of 24 alleles. The greatest number of alleles was found at the emf21H22 locus (10 alleles) followed by emh11O01 (5 alleles) and emf21C11 (4 alleles). The average number of alleles locus^{-1} was 4.8. Adeniji *et al.* (2012) also reported that the proportion of polymorphic alleles for EM 114 and 145, and these SSR markers are adequate for detecting genetic diversity among seven *Solanum* species.

Pair-wise genetic dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the 65 genotypes studied. The pair-wise genetic dissimilarity co-efficient was measured among the test entries. The values of pair-wise comparison of Nei's (1973) genetic distance (D) between plants were computed from combine data for the 6 selected primers ranged from 0.17 to 1.00. The pair-wise genetic dissimilarity indices indicated that the highest genetic dissimilarity (100%) was observed among various combinations of the genotypes. For instance, the highest similarity was observed among Islampuri (S31), Eye-red (S36), Deembegun (S37), Comilla-L (S38) and Chega (S39) with Dohazari G (S10), Borka (S11), Khatkhatia BAU (S13), ISD-006 (S2) and Laffa-M (S3). The highest genetic dissimilarity also existed among Marich Begun-E (S58) with ISD-006 (S2), Shingnath-S (S29), Longla Talbegun (S30) and Islampuri (S31). The lowest genetic dissimilarity (17%) among the collected brinjal genotypes was observed as: Dohazari G (S10) and Borka (S11) with Zhumki (S1), Khatkhatia-B (S12) and Khatkhatia-BAU (S13). Similarly, Kaikka-G (S15) and Islampuri-BADC (S16) with Dohazari G(S10), Borka (S11), Khatkhatia B (S12), Khatkhatia BAU (S13) and Kaikka-N (S14) and Jessore L(S17). There were a number of combinations of genotypes where no genetic dissimilarity (0%) existed such as: Borka (S11) with Dohazari G (S10); Khatkhatia BAU(S13) with Khatkhatia B (S12); Islampuri, BADC (S16) with Kaikka-G (S15); Jessore L(S17) with Kaikka-N (S14); China oblong (S24) Ishurdi-WS (S25) and Putabegun (S27); Pahuza-2 (S50) and Magura Local (S51) and Long Lived High Plant (S52), Bholanath (S46), BAU Begun-1 (S47) and Pahuza-1 (S49); Purple Long (S53) with Laffa BAU (S55) and Katabegun WS (S56); Natore Local (Round) (S61) with Marich Begun-E (S58). Similar research was conducted by Khorsheduzzaman *et al.* (2008) with very small number of genotypes (5) using SSR markers. Behera *et. al*. (2006) found broader genetic diversity in 92 South Asian brinjal accessions (genetic similarity between 0.37 and 0.90) using microsatellite markers. The genotypes showed considerable variation in respect of morphological, anatomical and biochemical aspects. For study of relatedness, plant genomic DNA was extracted using 11 randomly selected primers which developed 22 bands through PCR amplification, and out of which 15 from 3 primers were

polymorphic. The similarity value ranged from 0.83 to 1.00 which indicated the presence of narrow range of genetic diversity at molecular level but have still a possibility of crossing among the genotypes of two clusters.

Construction of phylogenetic tree

A phylogenetic tree was constructed based on the Nei's genetic distance using 6 SSR markers among 65 brinjal genotypes (Nei, 1973). This is also called UPGMA cluster dendrogram. UPGMA based dendrogram was obtained from DNA profiles of the analyzed 65 brinjal genotypes (Fig 5). The UPGMA cluster based on the Nei'sgenetic distance led to the grouping of the collected 65 brinjal genotypes into three major clusters with nine sub-clusters. The Cluster-I led to three Sub-clusters. The Sub-cluster-I comprised 4 genotypes, namely S63, S65, S62 and S64. The Sub-cluster-II led to 13 genotypes, namely S48, S56, S53, S55, S51, S52, S46, S47, S49, S50, S60, S58 and S61, and the Sub-Cluster-III comprised 3 genotypes, namely S54, S57 and S59. Altogether, Cluster-I consisted of 20 out of 65 genotypes (Table 4). The Cluster-II led to three Sub-clusters. Sub-Cluster-I comprised 5 genotypes, namely S6, S8, S2, S12 and S13. The Sub-Cluster-II under Cluster-II consisted of 8 genotypes, namely S1, S9, S10, S11, S3, S4, S5 and S7. The Sub-Cluster-III accommodated 6 genotypes such as S14, S17, S15, S16, S18 and S19. Altogether, Cluster-II comprised 19 out of 65 genotypes (Table 4). The Cluster-III led to three Sub-clusters. The Sub-cluster-I comprised 10 genotypes (S43, S44, S45, S42, S40, S41, S36, S37, S38 and S39). The Sub-cluster II consisted of 11 genotypes, namely S28, S29, S30, S24, S25, S27, S21, S23, S20, S22 and S26. On the other hand, the Sub-cluster III consisted of 5 genotypes (S31, S32, S34, S33 and S35). Altogether, the Cluster-III consisted of 26 genotypes (Table 4). The tolerant and moderately tolerant genotypes were observed to be located across all three clusters. For example, the Cluster-I comprised 5 tolerant to moderately tolerant genotypes (S8, S9, S11, S13 and S17). Cluster-II consisted of 9 tolerant to moderately tolerant genotypes (S26, S43, S22, S23, S24, S30, S35, S38, S39) and the Cluster-III contained 8 tolerant and moderately tolerant genotypes (namely S57, S47, S55, S58, S61, S62, S63 and S65) (Table 4). In a similar study with 5 genotypes a dendrogram generated two clusters and they were clearly distinct and separated from each other (Khorsheduzzaman *et al.* 2008).

Table 1. List of SSR markers used in molecular characterization of 65 brinjal genotypes

Sl. No	Name of the primers	Primer sequence (5′-3′)	Repeat motif	Motif length	Annealing Temperature (^{0}C)	Product size (reference)	Expected product size
1	EM 107	F- GGC CCT AGA CTG AGC CTG AAATGT T	$(AC)_{13}$ $(AT)_{13}$	25	65	214	100-240
		R-TGG TAC AAC CAA CAC AAC CCT CAA		24			
2	EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC	$(AC)_{13}$	24	65	221	159-250
		R-GAA GCT TTA AGA GCC TTC TAT GCA G		25			
3	EM 116	F-TTA GAA ATT TGC GAA CAA AGA GA	$(AC)_{12}$ $(AT)_{8}$	23	65	246	150-230
		R-CCA CAT GAA ACT TGG ACC AAT GAG		24			
4	EM 117	F-GAT CAT CAC TGG TTT GGG CTA CAA	$(AC)_{19}$ $(AT)_{11}$	24	65	160	120-220
		R-AGG GGA GAG GAA ACT TGATTG GAC		24			
5	EM 119	F-CCC CAC CCC ATT TGT GTT ATG TT	$(GGAGG)_5(AT)_8$	23	65	210	100-210
		R-ACC CGA GAG CTA TGG AGT GTT CTG		24			
6	EM 120a	F-GGA TCA ACT GAA GAG CTG GTG GTT	$(AC)_{16}$	24	65	160	100-218
		R-CAG AGC TTC AAT GTT CCA TTT CAC A		25			
7	EM 120b	F-CAA AAG ATA AAA AGC TGC CGC ATG	$(AC)_{16}$	24	65	248	80-240
		R-CATGCG TGA GTT TTG GAG AGA GAG		24			
8	EM 127	F-CAG ACA CAA TGC TGA GCC AAA AT	$(AC)_{13}$ $(AT)_{13}$	23	65	200	150-230
		R-CGG TTT AAT CAT AGC GGT GAC CTT		24			
9	EM 128	F- TAG CGG TGC TAG GTC CAT CAT CTC A	$(CA)_{26}$ $(TA)_{19}$	25	60	295	100-230
		R-TTC TCA AGA AGT TGC TCC AAA GGA		24			
10	EM 131	F-TCT GCA ACA CCA AGT GAA AAA TCA	$(AT)_5(AC)_3A(AC)_{14}$	24	65	213	120-220
		R-TGC GTT TTT GGC TCC TCT ATG AAT	$(AT)_7GTA(TG)_5(TA)_3$	24			
11	EM 133	F-GCG GAT CAC CTG CAGTTA CAT TAC	$(AC)_{13}$ $(AT)_6$	24	65	177	120-220
		R-TCC TTT GAC CTA TAG TGG CAC GTA GT		26			
12	EM 134	F-AGT AAG GGA AAG TGC TGA CGA AGG	$(GT)_2GC(GT)_6$	24	65	168	120-300
		R-CAG AGT CAT CGT TAT GGG GAG GTT		24			
13	EM 135	F-ATC CTG TTG CTGCTC ATT TTC CTC	$(CA)_{11}(GA)_{20}$		65		256-260
		R-AGG AGG ATC CAAAGA GGT TTGTTGA					
14	EM 139	F-TGC TAA GTC GTC ATC CAACAA GAA	$(AC)_3AT(AC)_{11}(AT)_{10}$	24	65	258	130-340
		R-GAT TTT GGC TCC TTG ACC ATT TTG		24			
15	EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C	$(AC)_4GC$ $(AC)_5T(AC)_3$	25	65	268	150-300
		R-GAC CAG AAT GCC CCT CAA ATT AAA	$ATGC(AC)_4AT(AC)_6$ $(AT)_6G(TA)_{13}$	24			
16	EM 141	F-TCT GCA TCG AAT GTC TAC ACC AAA	$(AT)_{16}(GT)_{19}$	24	65	228	100-260
		R-AAA AGC GCT TGC ACT ACA CCT GAA T		25			
17	EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG	$(TG)_3TA(TG)_8(TA)_8$	26	65	165	145-220
		R-GAC TCC TCA AGC CTT TAC CTC CAA		24			
18	EM 146	F-GGA CCA AAG CGA AAT TTT CAC AAC	$(AC)_{19}(AT)_{11}AC(AT)_2$	24	63	288	120-350
		R-TTG CAC CAATTG GGA AGT AAC ACA		24			
19	EM 155	F-CAA AAG ATA AAA AGC TGC CGG ATG	$(CT)_{38}$		65	248	256-260
		R-CAT GCG TGA GTT TTG GAG AGA GAG					
20	EM 104a	F-TGG ATC GTC GTC ATC CAACAA GAA	$(TC)_9(AC)_{38}(AT)_{19}$	25	60	246	230-350
		R-GAT TTT GGC TCC TTG ACC ATT TTG		23			

Table 2. Properties of the six selected primers

Sl. No.	Name of primers	Primer sequence (5′-3′)	Repeat motif	Motif length	Annealing Temperature (^{o}C)	Product size (reference)	Expected product size
1	EM 107	F- GGC CCT AGA CTG AGC CTG AAATGT T R-TGG TAC AAC CAA CAC AAC CCT CAA	$(AC)_{13}$ $(AT)_{13}$	25 24	65	214	100-240
2	EM 114	F-AGC CTA AAC TTG GTT GGT TTT TGC R-GAA GCT TTA AGA GCC TTC TAT GCA G	$(AC)_{13}$	24 25	65	221	159-250
3	EM 133	F-GCG GAT CAC CTG CAGTTA CAT TAC R-TCC TTT GAC CTA TAG TGG CAC GTA GT	$(AC)_{13}$ $(AT)_{6}$	24 26	65	177	120-220
4	EM 139	F-TGC TAA GTC GTC ATC CAACAA GAA R-GAT TTT GGC TCC TTG ACC ATT TTG	$(AC)_3AT(AC)_{11}(AT)_{10}$	24 24	65	258	130-340
5	EM 140	F-CCA AAA CAA TTT CCA GTG ACT GTG C R-GAC CAG AAT GCC CCT CAA ATT AAA	$(AC)_4GC$ $(AC)_5T(AC)_3$ $ATGC(AC)_4AT(AC)_6$ $(AT)_6G(TA)_{13}$	25 24	65	268	150-300
6	EM 145	F-TGA TTT GGC CCT TAA GCC TAA GTA TG R-GAC TCC TCA AGC CTT TAC CTC CAA	$(TG)_3TA(TG)_8(TA)_8$	26 24	65	165	145-220

Table 3. Name of the marker, sample size, allele no., major allele frequency, genetic diversity and polymorphism information content (PIC) for six markers

Name of the marker	Sample size	Allele No.	Major allele frequency	Genetic diversity	PIC
EM107	65.0	6.0	0.4308	0.7115	0.6688
EM114	65.0	4.0	0.4615	0.6551	0.5931
EM133	65.0	4.0	0.4462	0.5960	0.5120
EM139	65.0	3.0	0.3846	0.6627	0.5888
EM140	65.0	6.0	0.3077	0.7730	0.7387
EM145	65.0	3.0	0.4308	0.6495	0.5756
Mean	65.0	4.33	0.4103	0.6746	0.6128

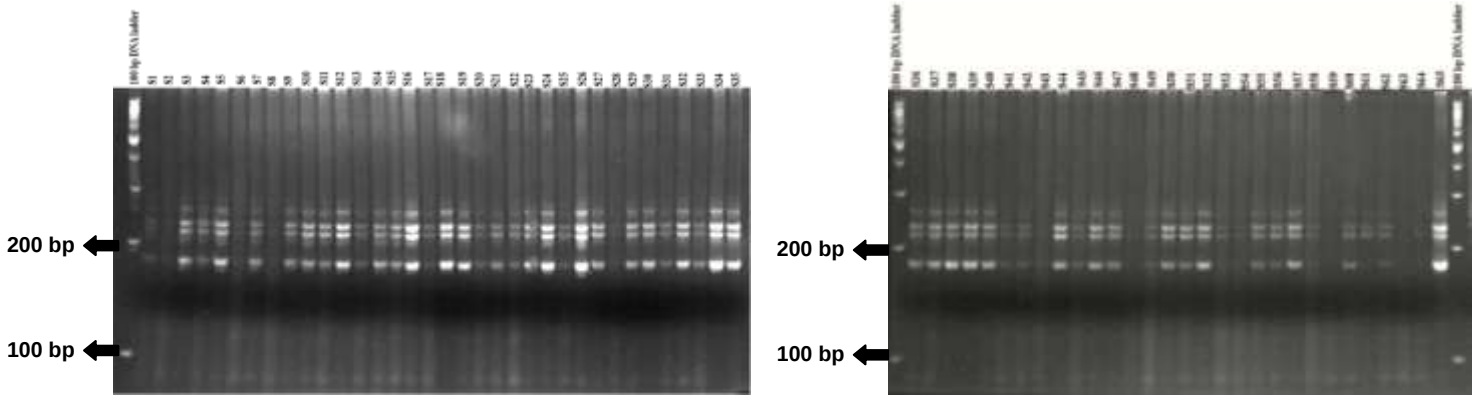

Fig. 1. DNA profile of the 65 brinjal genotypes (A-35 and B-30) using the primer EM107.

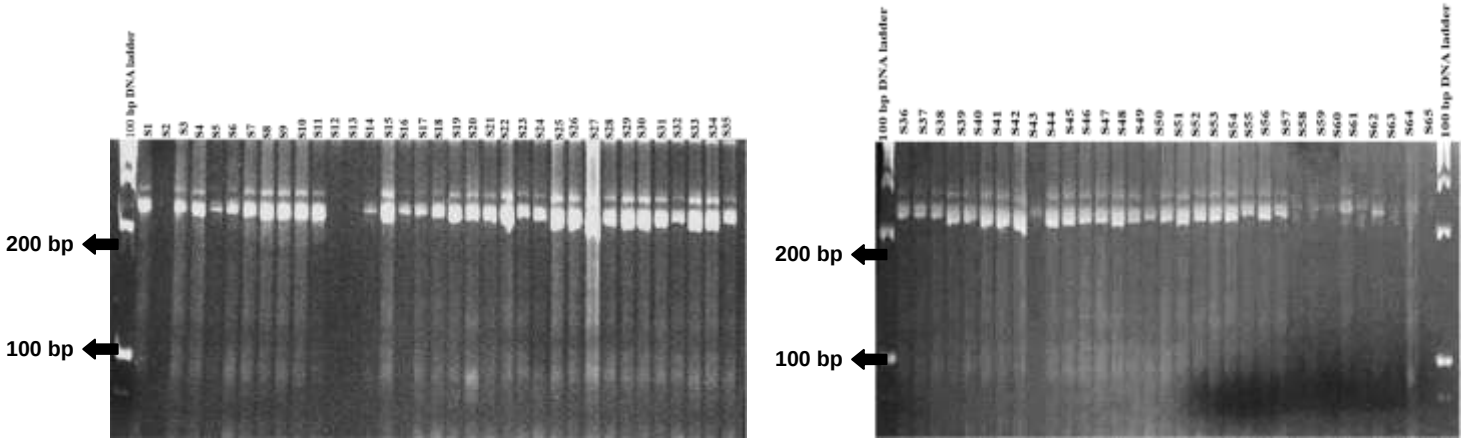

Fig. 2. DNA profile of the 65 brinjal genotypes (A- 35 and B-30) using the primer EM114.

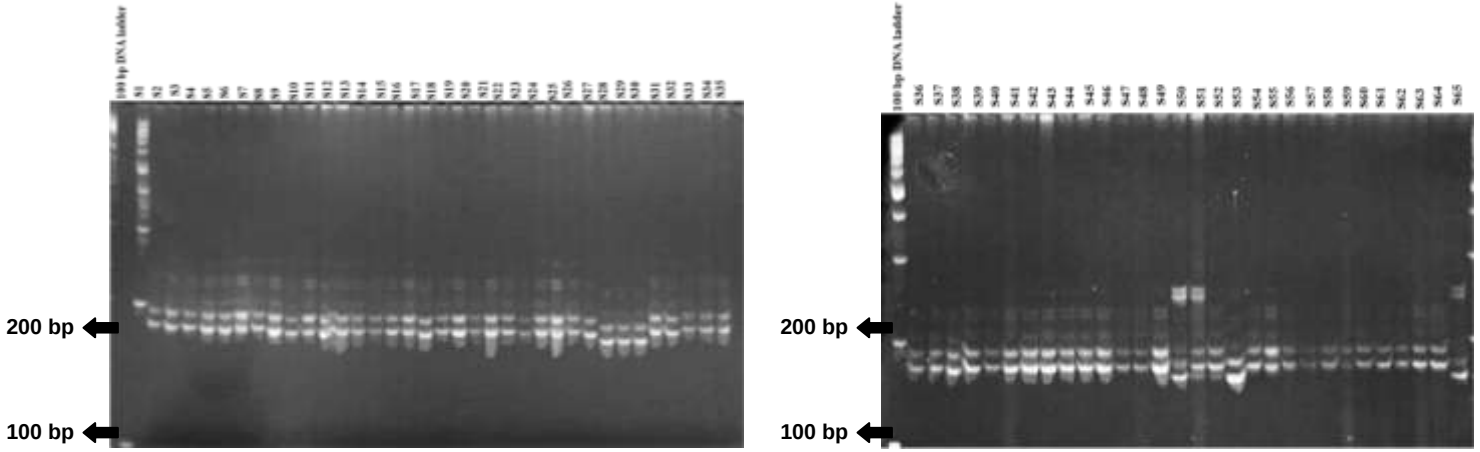

Fig. 3. DNA profile of the 65 brinjal genotypes (A- 35 and B-30) using the primer EM133.

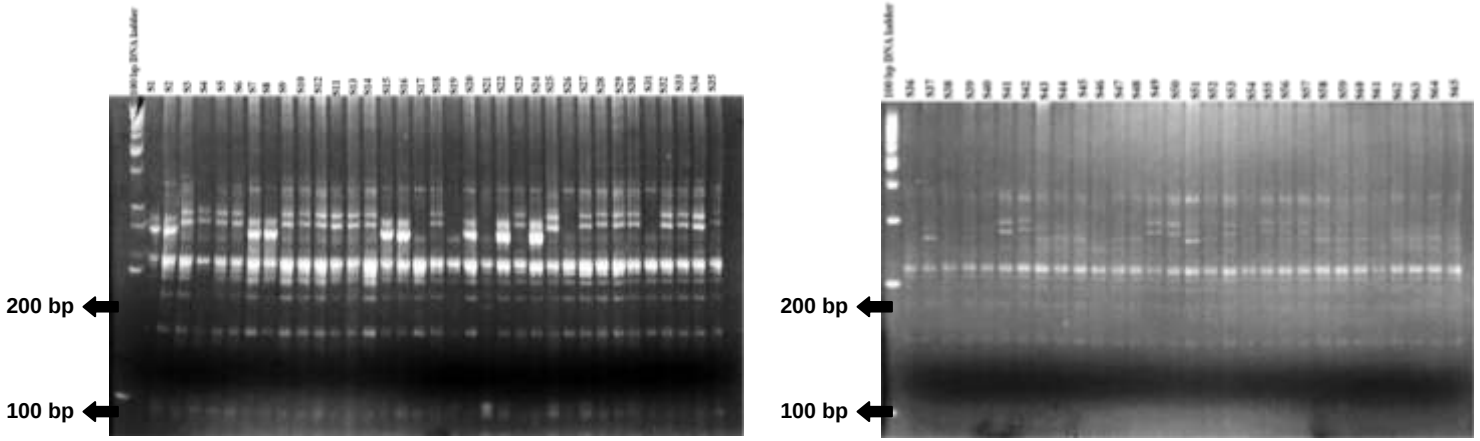

Fig. 4. DNA profile of the 65 brinjal genotypes (A- 35 and B- 30) using the primer EM140.

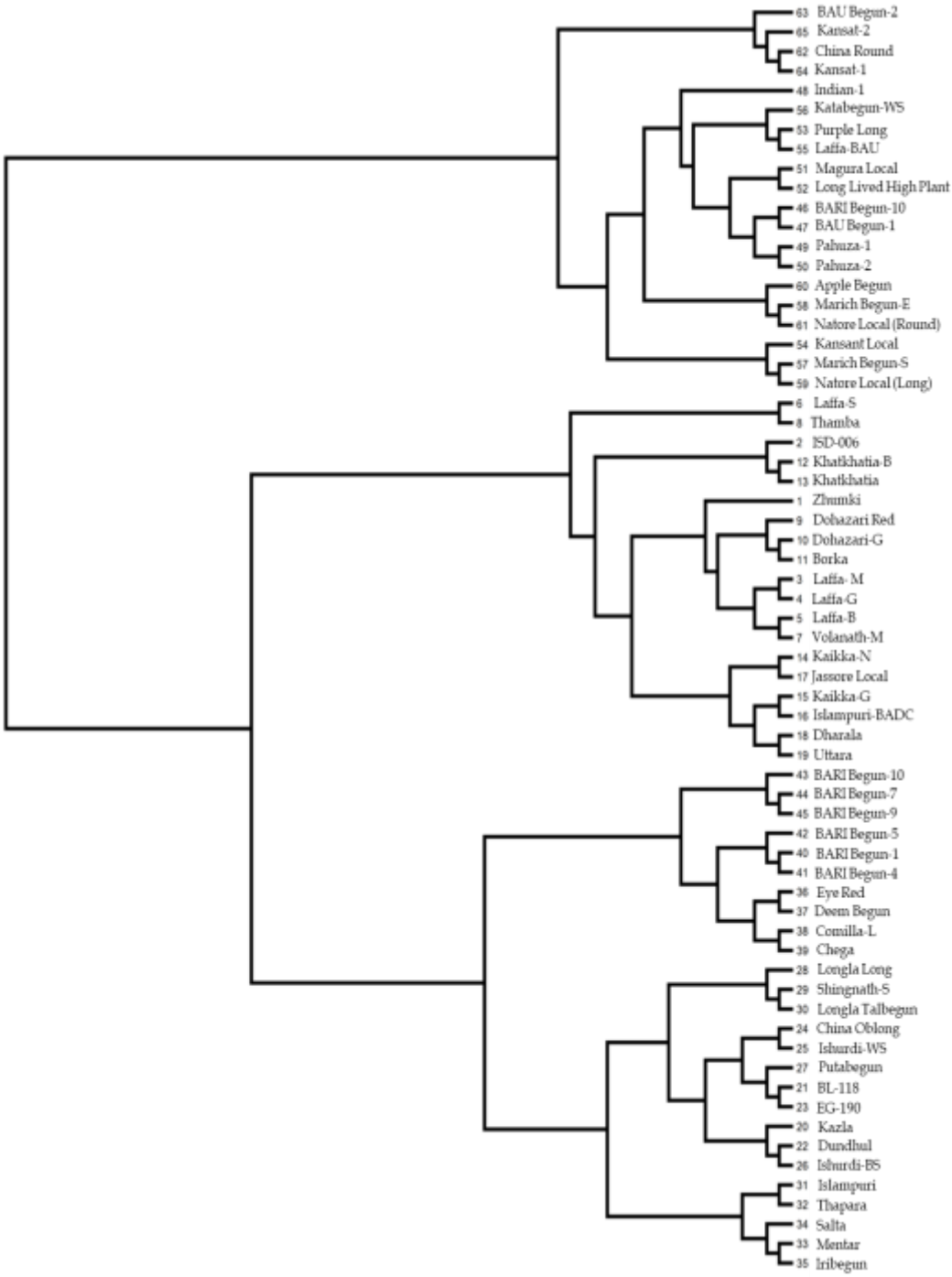

Fig. 5. UPGMA cluster dendrogram obtained from DNA profiles of 65 brinjal genotypes.

Table 4. Clusters and sub-clusters of the 65 brinjal genotypes based on the Nei's (1973) genetic distance using 6 SSR primers

Cluster	Sub-Cluster	Genotypes
I	I	S63, S65, S62, S64
	II	S48, S56, S53, S55, S51, S52, S46, S47, S49, S50, S60, S58, S61
	III	S54, S57, S59
II	I	S6, S8, S2, S12, S13
	II	S1, S9, S10, S11, S3, S4, S5, S7
	III	S14, S17, S15, S16, S18, S19
III	I	S43, S44, S45, S42, S40, S41, S36, S37, S38, S39
	II	S28, S29, S30, S24, S25, S27, S21, S23, S20, S22, S26
	III	S31, S32, S34, S33, S35

Conclusion

The present investigation provided new information about the genetic diversity and relationship among brinjal (*Solanum*) genotypes using microsatellite markers. To perform molecular characterization using SSR markers for identifying genetic diversity and relatedness among the genotypes at molecular level. Molecular analysis using SSR markers led to the grouping of the 65 genotypes into 3 major clusters with 9 sub-clusters. The relatedness among the 65 genotypes was also estimated.

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DEVELOPMENT OF HIGH YIELDING, YEAR-ROUND, SCENTED AND ALMOST SEEDLESS VARIETY OF LEMON

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Abstract

Three lemon germplasms selected from the preliminary evaluation of twenty local and exotic lemons were evaluated in two locations of Bangladesh. Through consequently three years trials, two germplasm viz. LVG-1 and LVG-2 were selected considering their agronomic performance at Mymensingh and Tangail. The LVG-1 and LVG-2 has been collected from Vietnam. It was observed that, LVG-1 was superior to other exotic genotypes and also to check variety BAU Lebu-3, reactions to major diseases and insect pest infestations were also found to be tolerant. During 2015-16, 2016-17 and 2017-18 at Mymensingh, the highest fruit yield per plant (18.1, 19.1, and 20.0 kg), vitamin C content (66.8, 65.1 and 65.9 mg 100 g^{-1}), fruit length (7.7, 8.4 and 8.6 cm), number of fruits $plant^{-1}$ (190.3, 210.3 and 254.6), juice content $fruit^{-1}$ (34.1%, 32.4% and 35.5%) and the lowest seed $fruit^{-1}$ (3 or 2 nos.) was recorded fromLVG-1 from all the three consecutive year and also at Tangail the highest fruit yield per plant (20.6, 19.5, and 20.1 kg), vitamin C content (67.7, 65.7 and 66.1 mg 100 g^{-1}), fruit length (8.2, 8.5 and 7.4 cm), number of fruits $plant^{-1}$ (198.6, 241.6 and 268.6), juice content $fruit^{-1}$ (34.6%, 37.6% and 38.2%) and lowest seed $fruit^{-1}$ (3, 4 and 4 nos.) was recorded from LVG-1compared to LVG-2 and BAU Lebu-3. Considering the better performance of fruit yield, vitamin C content, juice content and less number of seed, LVG-1 genotype was selected as a new variety (Binalebu-1) to cultivate commercially all over Bangladesh.

Key words: Lemon, almost seedless variety, high yield, scented, year round

Introduction

Citrus fruits are very important in respect of their food values, especially being very rich in vitamin C. It is a source of macro and micronutrients (Ting, 1980) and of dietary fiber (Marin *et al.,* 2007). They are also rich in antioxidants compounds (Liu *et al.,* 2012), reveal anticancer, and anti-inflammatory properties (Ma *et al.,* 2020), and are effective at reducing the risk of cardiovascular disease, osteoporosis and type-2 diabetes (Cirmi *et al.,* 2016). It also contains some organic compounds, which work against asthma, antidepressant, stress relief, aids digestion, colds, flu, fever, nosebleeds, mouth ulcers, throat infection and boils (Sfgate, 2017).

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Bangladesh is a country of 168.95 million people and approximately 70% people suffer from malnutrition problem, most importantly different types of vitamins, viz. A, C etc. (Rahman M. and Rahman J., 2014). Reports show that around 93% people of Bangladesh suffer from vitamin C deficiency and unlike other vitamins; it cannot be stored in the body (Mamun *et al.,* 2015). Vitamin C intake of is far below from the recommended dietary allowance (Nielsen, 2000; Hels *et al.,* 2003; Khan and Ahmed, 2005). Thus, intake of vitamin C fruits at regular basis is necessary to maintain the supply of vitamin C in the body.

Despite enormous health benefits of citrus, Bangladesh stands in a very low position in respect of the production of citrus fruits. According to the available statistics, the total area under lemon cultivation was 156440 acres while total production was 58552 M. tons in the year 2019-20 (BBS, 2020). Major lemon producing regions of Bangladesh are Sylhet, Chittagong, and the Chittagong Hill Tracts in the orchards as well as in the homestead areas of many households (Umar *et al.,* 2015). It is, therefore, necessary to give proper attention to increase the production of lemon and to improve their qualities to meet the increasing demand of the people of Bangladesh. The main reasons of low yield are lacking high yielding varieties as well as their traditional cultivation practices. The yield of lemon can be increased through modern high yielding varieties with adopting improved production technologies. Various strategies to increase genetic variability can be pursued by such as seed introduction, hybridization, and mutation (Soeranto, 2011). Introduction as well as selection is one of the main strategies used to improve agronomic traits. In many citrus species, several varieties have developed through selection (Caruso *et al.*, 2020). Hence the objectives of this study were to select a high quality lemon genotype (in terms of size, vitamin C and juice content and seedlessness), year round, tolerant to different abiotic and biotic threats, and with high productivity.

Materials and Methods

Twenty lemon germplasm were collected from home and abroad. Among them LVG-1 and LVG-2 has been collected from Vietnam and selected for their high yielding, year round and seedless characters. Collected genotypes (LVG-1 & LVG-2) along with check variety BAU Lebu-3 were evaluated during three consecutive years (2015-16, 2016-17 and 2017-18) at two different locations viz; Kashiarchar, Mymensingh and Delduwar, Tangail. All the trials were laid out in randomized complete block design with three replications. Pit size was 0.5 × 0.5 m^2 with plant-to-plant distance 2.5 m maintained for all the trials. 20 kg cowdung, 300 g mustard oilcake, 500 g bone meal and 2 kg ash were applied into the pit before planting and after every 3 months 200 g urea, 250 g TSP and 250 g MoP fertilizer was applied to every plant. Intercultural operations as irrigation, weeding, insecticide and fungicide were applied when necessary to ensure normal plant growth and development. Well drainage system was maintained to avoid water logging condition in the experimental site. Data on various characters, such as plant height, fruit length, fruit breath,

individual fruit weight, number of fruits plant^{-1}, juice content fruit^{-1}, rind thickness, number of seed fruits^{-1}, vitamin Ccontent and fruit yield were taken from each plant. The analysis of variance for yield and yield contributing characters of various fruits were done following the principle of F-statistics. Mean comparisons of the treatments were adjudged by the Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results

Results showed significant difference in lemon yield, yield contributing parameters and quality between the evaluated genotypes. Among the genotypes, LVG-1 showed better field performance considering fruit yield plant^{-1}, individual fruit weight, vitamin C content and other important agronomic characters. It was also observed that, LVG-1 was tolerant compare to another genotype and check variety BAU Lebu-3, in cases of reactions to major diseases and insect pest infestations.

Mymensingh

During 2015-16 two germplasm viz. LVG-1 and LVG-2 with check variety BAU Lebu-3 were evaluated at Kashiarchar, Mymensingh. The tallest plant (188.98 cm) was recorded in LVG-1 followed by LVG-2 (176.8 cm) and BAU Lebu-3 (154.6 cm). The fruit length ranged 8.33cm (LVG-1) to 5.32 cm (LVG-2) and fruit breadth was 6.7 cm (BAU Lebu-3) to 4.5 cm (LVG-2). The highest juice fruit^{-1} (34.1%) was recorded fromLVG-1and lowest (21.9%) from BAU Lebu-3. The highest rind thickness (6.63 mm) was obtained from LVG-2 and the lowest rind thickness (4.16 mm) from LVG-1. The highest number of seedfruit^{-1} (15) was found in LVG-2 and the lowest no of seed fruit^{-1} (3) was present in LVG-1. The highest vitamin C content (66.8 mg 100 g^{-1}) was found in LVG-1and the lowest vitamin C content (60.7 mg 100 g^{-1}) was found in LVG-2 followed by BAU Lebu-3 (63.5 mg 100 g^{-1}).Maximum number of fruits plant^{-1} (190.3) was obtained from LVG-1 followed by BAU Lebu-3 (155.7) and LVG-2 (174.7). In case of fruit yield, highest yield (18.1 kg tree^{-1}) was obtained from LVG-1 followed by BAU Lebu-3 (12.1 kg tree^{-1}).

In 2016-17, the tallest plant (197.6 cm) was recorded in LVG-1 genotype at Mymensingh while the shortest plant (172.6 cm) was recorded in BAU Lebu-3. The fruit length ranged 8.4 cm (LVG-1) to 5.6 cm (LVG-2) and fruit breadth was 6.5 cm (BAU Lebu-3) to 5.3 cm (LVG-2). The highest juice fruit^{-1} (32.4%) was recorded from LVG-1 and lowest (22.0%) from BAU Lebu-3. The highest rind thickness (7.34 mm) was obtained from LVG-2 and the lowest rind thickness (4.03 mm) was from LVG-1. The highest number of seeds fruit^{-1} (17) was present in LVG-2 and the lowest number of seeds fruit^{-1} (2) was present in LVG-1. The highest vitamin C content (65.1 mg 100 g^{-1}) was found in LVG-1and the lowest vitamin C content (59.6 mg 100 g^{-1}) was found in LVG-2 followed by BAU Lebu-3 (62.6 mg 100 g^{-1}). In case of fruit yield, highest fruit yield (19.1 kg tree^{-1}) was recorded from LVG-1 and the second highest fruit yield (13.5 kg tree^{-1}) from BAU Lebu-3. The lowest fruit yield (11.5 kg tree^{-1}) was recorded from LVG-2 germplasm.

In 2017-18, the longest plant (217.7 cm) was found in LVG-1 genotype at Mymensingh while the smallest plant (199.6 cm) was recorded in BAU Lebu-3. The fruit length range 8.6 cm (LVG-1) to 5.3 cm (LVG-2) and fruit breadth was 6.7cm (BAU Lebu-3) to 4.5 cm (LVG-2). Maximum no. of fruits plant^{-1} (254.6) was obtained from LVG-1 followed by BAU Lebu-3 (213.6) and LVG-2 (205.6). The highest juice fruit^{-1} (35.5%) was recorded from LVG-1and the lowest (18.8%) from BAU Lebu-3. The highest rind thickness (6.6 mm) was obtained from LVG-2 and the lowest rind thickness (4.1 mm) was LVG-1. The highest number of seeds fruit^{-1} (15) was present in LVG-2 and the lowest number of seed fruit^{-1} (3) was present in LVG-1. The highest vitamin C content (65.9 mg 100 g^{-1}) was found in LVG-1and the lowest (60.2 mg 100 g^{-1}) was found in LVG-2 followed by BAU Lebu-3 (62.9 mg 100 g^{-1}). In case of fruit yield, the highest yield (20.0 kg tree^{-1}) was obtained from LVG-1, the second highest yield (14.4 kg tree^{-1}) was recorded in BAU Lebu-3 and the lowest (12.5 kg tree^{-1}) LVG-2 (Table 1).

Tangail

During 2015-16, two germplasm viz. LVG-1 and LVG-2 with check variety BAU Lebu-3 were evaluated at Deldwar, Tangail. The longest plant (176.8 cm) was recorded in LVG-1 followed by LVG-2 (169.7 cm) and BAU Lebu-3 (154.5 cm). The fruit length ranged 8.8 cm (BAU Lebu-3) to 5.3 cm (LVG-2) and fruit breadth was 5.7 cm (BAU Lebu-3) to 4.8 cm (LVG-2). The highest juice fruit^{-1} (34.6%) was recorded from LVG-1 and lowest (21.2%) from BAU Lebu-3. The highest rind thickness was also obtained from LVG-2 (7.3 mm) and the lowest rind thickness was LVG-1 (4.7 mm). Maximum number of fruits plant^{-1} (198.6) was obtained from Binalebu-1 followed by BAU Lebu-3 (185.3) and LVG-2 (163.5). In case of fruit yield, the highest yield (20.6 kg tree^{-1}) was obtained from LVG-1 and the second highest yield (14.7 kg tree^{-1}) was recorded in BAU Lebu-3 and the lowest (11.8 kg tree^{-1}) from LVG-2.

In 2016-17, the tallest plant (201.4 cm) was recorded in LVG-1 genotype at Deldwar, Tangail followed by LVG-2 (170.7 cm).The highest juice fruit^{-1} (37.6%) was recorded from LVG-1 and lowest (22.1%) from BAU Lebu-3. In case of fruit yield highest yield (19.5 kg tree^{-1}) was found in Binalebu-1 (LVG-1) followed by BAU Lebu-3. The lowest fruit yield (14.7 kg tree^{-1}) was recorded in LVG-2 genotype.

In 2017-18, the significant tallest plant height (215 cm) was found in LVG-1genotype at Tangail, while the smallest plant (174.3 cm) was recorded in BAU Lebu-3. Maximum no. of fruits plant^{-1} (268.6) was obtained from LVG-1 followed by BAU Lebu-3 (246.3) and LVG-2 (215.3). The highest juice fruit^{-1} (38.2%) was recorded from LVG-1 and lowest (20.7%) from BAU Lebu-3. In case of fruit yield, the highest yield (20.1 kg tree^{-1}) was obtained from LVG-1 and the second highest yield (16.4 kg tree^{-1}) was recorded in BAU Lebu-3 (Table 2).

Table 1: Fruit yield, Physicochemical characteristics of three lemon genotypes at Mymensingh during 2015-18

Genotypes	Plant height (cm)	Fruit length (cm)	Fruit breadth (cm)	Individual fruit weight (g)	No. of fruits plant^{-1}	Juice fruit^{-1} (%)	Rind thickness (mm)	Number of seeds fruit^{-1}	Vitamin C (mg 100 g^{-1})	Fruit yield (kg tree^{-1})
2015-16										
LVG-1	188.9a	7.7b	5.1a	121b	190.3a	34.1a	4.16b	3b	66.8a	18.1a
LVG-2	176.8b	5.3c	4.5b	91.8c	174.7b	23.3b	6.63a	15a	60.7b	11.5b
BAU Lebu-3	154.6c	8.3a	6.7a	130a	155.7c	21.9b	5.07a	5b	63.5b	12.1b
2016-17										
LVGI	197.6a	8.4a	5.3b	123b	210.3a	32.4a	4.03b	2c	65.1a	19.1a
LVG-2	181.8b	5.6b	5.3b	111.8c	164.7b	22.9b	7.34a	17a	59.6b	11.5b
BAU Lebu-3	172.6b	9.0a	6.5a	138a	175.9b	22.0b	5.98a	6b	62.6b	13.5b
2017-18										
LVG-1	217.7a	8.6a	5.1b	127.9b	254.6a	35.5a	4.16c	3b	65.9a	20.0a
LVG-2	203.1b	5.3b	4.5b	120.5b	205.6b	23.7b	6.63a	15a	60.2b	12.5b
BAU Lebu-3	199.6b	9.4a	6.7a	143a	213.6b	18.8b	5.07b	5b	62.9b	14.4b

Table 2: Fruit yield, Physicochemical characteristics of three lemon genotypes at Tangail during 2015-18

Genotypes	Plant height (cm)	Fruit length (cm)	Fruit breadth (cm)	Individual fruit weight (g)	No. of fruits plant^{-1}	Juice fruit^{-1} (%)	Rind thickness (mm)	Number of seeds fruit^{-1}	Vitamin C (mg 100 g^{-1})	Fruit Yield (kg tree^{-1})
2015-16										
LVG-1	176.8a	8.2a	5.67a	111.7b	198.6a	34.6a	4.7b	3b	67.7a	20.6a
LVG-2	169.7a	5.3b	4.82b	97.3c	163.5b	24.2b	7.3a	13a	61.4b	11.8c
BAU Lebu-3	154.5b	8.8a	5.69a	127.6a	185.3a	21.2b	6.6a	5b	63.6b	14.7b
2016-17										
LVG-1	201.4a	8.5a	5.70a	109.9b	241.6a	37.6a	4.5b	5b	65.7a	19.5a
LVG-2	170.7b	5.5b	4.63b	119.3b	213.6c	23.9b	5.0a	14a	59.4b	14.7b
BAU Lebu-3	158.5b	8.90a	5.59a	138.9a	228.4b	22.1b	4.6b	6b	61.6b	15.8b
2017-18										
LVG-1	215a	7.4b	5.54a	118c	268.6a	38.2a	4.1a	5b	66.1a	20.1a
LVG-2	184.4b	5.2c	4.35b	129b	215.3c	20.6b	5.1b	11a	61.4b	14.3b
BAU Lebu-3	174.3b	9.1a	5.57a	139a	246.3b	20.7b	5.5b	7b	62.6b	16.4b

The present study revealed that LVG-1 genotype showed better fruit yield, vitamin C, juice content and less seed than LVG-2 and check variety BAU Lebu-3 which is the most important characters to select a suitable variety. LVG-1 performed 30.2%, 4.5% and 23.9% higher fruit yield, vitamin C, juice content and 48.9% lower seed at Mymensingh and 19.6%, 5.9% and 21.5% higher fruit yield, vitamin C, juice content and 28.5% lower seed at Tangail respectively than check variety. Similar variations in fruit characters in lemon cultivars was reported by Arora and Daulta (1991), Fallahi *et al.* (1990) and Prasad et al. (1997). Rashid, 2013 found significant variations in rind thickness, fruit weight in different types of lemon. Kayesh E. *et al.,* (2017) reported year round, broad and seedless fruits of lemon with early flowering. Al-Mouei and Choumane, 2014 stated that variability of the juice contents may be due to the variation of the genetic potentiality of individual genotype. According to Shrestha *et al.* (2012) a seedless variety of citrus contained highest juice. So, the LVG-1 genotype can be select as a new variety for high yielding, year-round, scented and almost seedless variety.

The incidence of Citrus greening, Gummosis and Scab, and insect (Aphid, Citrus leaf miner and Citrus butterfly) infestation were also studied in different locations under field conditions. The advance line LVG-1was found to be tolerant to Citrus greening, Gummosis and Scab diseases and also showed lower infestation by insects. Overall infestation caused by leaf feeder insects like aphid, Citrus leaf miner and Citrus butterfly were lower in LVG-1 genotype compared to the check varieties. The present results are in agreement with the results of Prasad *et al.* (1997) (Table 3).

Table 3.**Reaction of diseases and insect-pest on lemon genotypes**

Genotypes	Disease			Insect infestation		
	Citrus greening	Gummosis	Scab	Aphid	Citrus leaf miner	Citrus butterfly
LVG-1	T	T	MT	T	MT	T
LVG-2	MT	S	S	MT	S	MT
BAU Lebu-3	MT	MT	MT	MT	S	MT

S = Susceptible, MT = Moderately Tolerant, T = Tolerant.

The overall performance of LVG-1genotype was better in different parameters in the consecutive three years trails at two different locations of Bangladesh. Considering the above parameters BINA apply to the National Seed Board (NSB) of Bangladesh for the registration of this genotype as a high yielding lemon variety proposed as Binalebu-1. The NSB registered LVG-1as new variety Binalebu-1 in 2019 for commercial cultivation in Bangladesh.

The salient characters of this lemon are:

- High yielding, year round, scented and all most seedless variety
- Mostly oval to cylindrical in shape, fruit tip acute and fruit surface smooth
- 2-5 seeds contain in a mature fruit but most of the fruits are seedless

- Average single fruit weight: 100-130 g
- Thickness of fruit skin: 4.1-4.7mm
- 38% juice contain in a mature fruit
- First fruiting duration: 10-11 months
- Vitamin C content is 67 mg/100 g fruit weight
- This variety is tolerant to citrus greening disease

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IN VITRO REGENERATION POTENTIALITY OF STRAWBERRY FROM DIFFERENT EXPLANT UNDER VARIOUS HORMONAL CONCENTRATIONS

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Abstract

An experiment was carried out to examine the effects of explants and different combinations of plant growth regulators *in vitro* micro propagation of strawberry. The research was laid out in 2 factorial experiments in completely randomized design. For shoot induction, six concentrations viz. BAP (0.5, 1.0, 1.5), GA_3 (0.5) and KIN (0.1, 0.5) and for root induction four combinations viz. IBA (0.1, 0.5) and 2, 4-D (0.1, 0.5) were used. The interaction effects between explants and growth regulators showed significant differences for all the parameters used in the experiment. In case of shoot initiation, runner tip explants performed the best for days required to shoot initiation (8.00), number of shoot per plantlet (11.20), number of leaves per plantlet (12.80) and shoot length (4.70 cm) when 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L was used. In case of root initiation, runner tip explants exhibited the best results for days to root initiation (10.00) and number of roots per plantlets (12.00) when 1.0 mg IBA + 0.5 mg 2, 4-D/L was used. Maximum good results observed when 1.0 mg BAP was combined with different concentration of GA_3 and KIN. Low concentration of IBA and 2, 4-D produces significant results. The findings of the present study could be useful to develop protocol to identify the potentiality of exact concentration of different growth regulators.

Key words: Micro propagation, MS media, strawberry, explants, plant growth regulators

Introduction

The strawberry is an important and popular fruit produced in temperate and sub-tropical climates due to its fragrance, taste and nutritional properties. It is the most widely consumed berry fruits throughout the world (Sultana, 2011). It is cultivated in 73 countries world-wide on 2,00,000 hectares and produced 31 lac metric tons strawberry (FAO, 2016). The cultivated strawberry (*Fragariax ananassa* Duch) is a member of the Rosaceaealong with blackberries and raspberries (Sultana, 2011). It contains 90.6 g water, 0.89 g protein, 0.5 g fat, 7.6 g carbohydrates, 1.7 g fiber, 53.0 mg vitamin C and 30.0 g vitamin B per 100 g edible fruit (Rahman, 2011). Strawberry is also qualified as a very good source of iodine as well as a good source of potassium, folate, omega-3 fatty acids, vitamin K, magnesium and

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copper (Sultana, 2011). It contains relatively high quantities of ellagic acid, which is thought to be an anti-carcinogenic (ICAR news, 2005) and a wide range of biological activity (Sakila *et al.*, 2007). Strawberries are growing in many areas of Bangladesh from last few years. In Bangladesh, commercial cultivation has been started with the released varieties of Rajshahi University. Bangladesh Agricultural Research Institute has also released two varieties of strawberry (Rahman, 2011). It has become very popular fruit to the people of the country. As a result, a number of farmers have now taken up their activity.

Tissue culture considered as an *in vitro* aseptic culture of cells, tissues, organs or whole plant below exact nutritional and ecological circumstances (Thorpe, 2007). Through using plant tissue culture methods plants can be attained from several explants over direct or indirect morphogenesis. Direct morphogenesis refers to the manufacture of shoots from explants deprived of passing over callus (unorganised tissue) stage while indirect morphogenesis relates to the generation of shoots over the callus stage (Kadhimi, 2014). The standardization of protocol and procedure of micropropagation of strawberry was successfully attempted by many researchers (Kaur *et al.*, 2005; Sakila *et al.*, 2007; Gantait *et al.*, 2010). Micropropagation of strawberry has been used in horticultural production since 30 years (Boxus, 1974). Some workers reported that high concentration of BAP is the best for strawberry micropropagation (Morozova, 2002) while other authors suggested 1.0 mg/L IAA + 1.0 mg/L BAP + 0.05 mg/L GA_3; 0.5 mg/L BAP + 0.1 mg/L GA_3 + 0.1 mg/L IBA (Boxus, 1999; Litwinczuk, 2004) and 0.5 mg/L BAP + 0.1 mg/L IBA (Bozena, 2001) for strawberry micropropagation.

Micropropagation of strawberry has been reported a large number of disease free plants (Boxus, 1974). Micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases (Biswas *et al.*, 2008). In addition, the storage of tissue cultured propagules requires less space than traditional propagated plant and the *in vitro* storage can be initiated at any time during the production cycle (Litwinczuk, 2004). Virus free shoot tips, runner tips, leaves and nodal segments are commonly used as a source of plant material for regeneration and transformation. Prior experiences with strawberry micropropagation indicate that *in vitro* plants are more uniform, produce higher number of runners and have better survival in the field and the fruit yield increases 24% than plants propagated by the traditional method. Each square meter of growing area of strawberry can produce 40000 plantlets per year (Boxus, 1999), these plants were vigorous and after transplanting in the soil some produced up to 500 new runner plants. Micropropagated strawberry plants were comparatively better in different characters (canopy size, number of runners, flowering time and yield of berries) than conventionally propagated runner plants, which is very labour intensive (Sakila *et al.*, 2007), time consuming and results in the transmission of viral diseases. Moreover, the conventional way of production is not adequate to meet the commercial demand. So, there is an urgent need to develop an efficient protocol for *in vitro* regeneration of strawberry plantlet for mass production of planting materials. Therefore, the present experiment has been planned to identify the suitable explants of strawberry for large scale production and determine the effective concentration and combination of BAP, GA_3, KIN, IBA and 2, 4-D on callus induction and plantlet regeneration of strawberry.

Materials and Methods

The experiment was conducted in the Plant Biotechnology Laboratory, Patuakhali Science and Technology University, Bangladesh during the period from July to October, 2015. The mother parts of the BARI strawberry-1wascollected from Regional Horticultural Research Station, Lebukhali, Patuakhali used as explants such as Runner tip, Shoot tip and Nodal segments.

Healthy, disease free and young runner tips, shoot tip and nodes were cut from the field grown plants of strawberry and were collected in a conical flask. The runner tips, shoot tip and nodes were washed thoroughly under running tap water for 20-25 minutes to remove the dust and then transferred in another conical flask containing distilled water adding with 2-3 drops of tween-80 and a few drops of savlon with constant shaking, then kept them 3-5 minutes and were washed 4-5 minutes with distilled water to remove sterilizing agents. The materials were separated into different segments. Inside the laminar air flow cabinet surface disinfection was done with 0.1% $HgCl_2$ solution by gently shaking for 3-8 minutes. After exposure to the sterilant, the materials were then washed in several times with double distilled water to remove all traces of $HgCl_2$ and the material was ready for inoculation on appropriate nutrient medium.

MS (Murashige & koog, 1962) basal media supplemented with different concentrations and combinations of growth regulators viz. 6-benzyladenine (BA), gibberellic acid (GA_3) and 6-ferfuryl amino purine (KIN) for shoot proliferation andindole butyric acid (IBA) and 2, 4-Dichlorophenoxy Acetic acid (2, 4-D) for root proliferation.

After mixing all stock solutions and growth regulators at appropriate volume, 3% sucrose was added. The pH of the medium was adjusted at 5.8 and then agar (0.7%) was added and dissolved. The media weredispensed in a 20-25 ml glass bottles. The media were sterilized by autoclaving at 121^0C for 15 minutes. Sterilized explants materials were dissected and cultured on MS medium supplemented with BAP (0.5, 1.0 and 1.5 mg/L), GA_3 (0.5 mg/L) and KIN (0.1 and 0.5 mg/L). After 30-35 days of culture inoculated shoots were produces multiple shoots.

When the regenerated shoot apices were reached 3-5 cm in length with 4-5 well developed leaves, they were rescued aseptically from the culture vessel and were separated from each other. Micro-cutting were prepared from these shoots by snapping off the basal leaves and cultured them individually in tubes containing 20 ml of rooting medium with different combinations of auxins (IBA and 2, 4-D). The pH of the medium was adjusted at 5.8 and then agar (0.7%) was added and dissolved. The media were sterilized by autoclaving at 121^0C for 15 minutes.

The data collected on different parameters were statistically analyzed to ascertain the significance of the experimental results. The analyses of variances were performed and the means were compared by DMRT for interpretation of results (Gomez and Gomez, 1984). The significance of the difference between the pair of means was evaluated using the MSTAT-C computer package program.

Results and Discussion

Effect of different explants and growth regulators (BAP, GA_3 and KIN) on in vitro shoot regeneration

Significant variation was observed among the different explants and combination of plant growth regulators regarding the shoot regeneration characteristics.

Among the explants, runner tip took the lowest number of days (13.17) and produced significantly the highest percentage (90.83) of shoot while nodal segment took the highest number of days (17.17) for shoot initiation and lowest percentage (64.67) of shoots (Fig. 1 and 2). This means specific explants took higher or lower percentage to initiate shoot with certain concentrations of BAP, GA_3 and KIN. MS Medium that containing 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) took the least number of days (10.67) for shoot initiation and produced the highest percentage (90.67%) of shoots (Fig. 3 and 4). In contrast, concentration 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) took the highest number of days (19.67) for shoot initiation and produced the lowest percentage of shoot (70.67%). Negi *et al.* (2008) observed that MS medium supplemented with BAP (0.5 mg/L) and KIN (0.5 mg/L) took less time to shoot emergence (9.81 days).

In case of interaction effect, runner tips with 1.0 mg BAP+0.5 mg GA_3+0.5 mg KIN/L (T2) required the lowest number of days (8.00) for shoot initiation and highest percentage of shoot initiation (97.00). In contrast, the lowest (70.00%) percentage and highest number of days required (21.00) for shoot initiation were obtained from nodal segments with the supplement of 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) (Table 1). Murari *et al.* (2003) also found that runner tip with BAP at 4.0 mg/L produced the maximum shoot regeneration (100%) after 7 weeks of incubation.

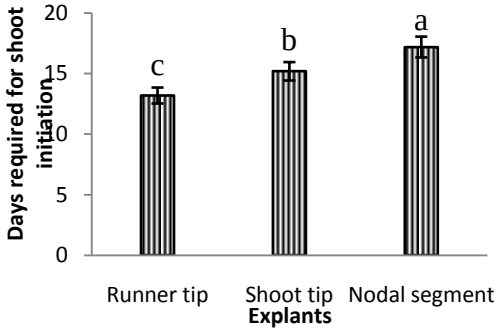

Fig. 1. Effects of different explants on days required for shoot initiation

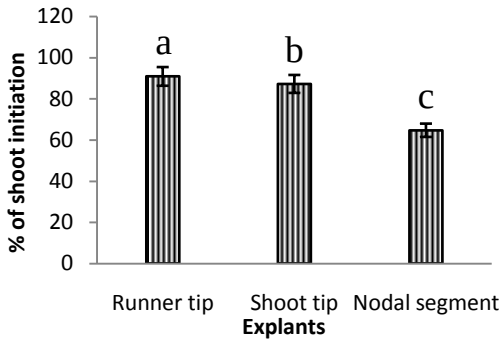

Fig. 2. Effects of different explants on percentage of shoot initiation

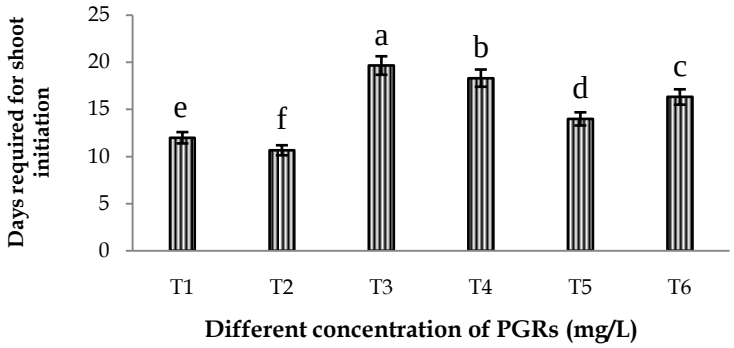

Fig 3. Effects of different concentrations of BAP, GA_3 and KIN on days required for shoot initiation

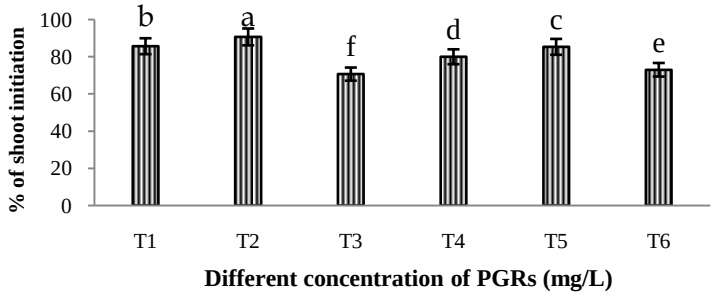

Fig 4. Effects of different concentrations of BAP, GA_3 and KIN on percentage of shoot initiation

Here,

T1= 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2= 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3=1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4= 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5= 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6= 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L

Among the explants, runner tip took the highest number of shoot (7.98), number of leaves (9.31) and longest shoot (3.15 cm) per plantlet (Fig. 5 and 6). In contrast, nodal segment took the lowest number of shoot (4.30), lowest number of leaves (7.28) and shortest shoot (2.65 cm). Rahman *et al.* (2011), Karim *et al.* (2011), Biswas *et al.* (2008), Sakila *et al.* (2007) also observed that the different number of leaves produced from different explants.

Medium that containing 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) took the highest (9.40) number of shoots, maximum number of leaves (11.60) per plantlet and highest shoot length (4.23 cm) while the concentration 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) took the lowest number of shoot per plantlet (3.60), minimum number of leaves (4.93) per plantlet and smallest shoot length (2.06 cm). This observation agreed with the findings of Negi *et al.* (2008), they also observed that MS medium supplemented with BAP (0.5 mg/L) and kinetin (0.5 mg/L) was found best for shoot elongation.

The highest number of shoot per plantlet (11.20), number of leaves per plantlet (12.80) and longest shoot (4.70 cm) were recorded from the combination of runner tip with 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T2) concentration (Table 2). In contrast, the lowest number of shoot per plantlet (2.00), lowest number of leaves per plantlet (4.20) and smallest shoot (2.00 cm) were recorded from the combination of nodal segment with 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L (T3) concentration. Biswas*et al.* (2008) also reported the highest number of shoots per plantlet from runner tip explants of strawberry having 0.5 mg/L BAP.

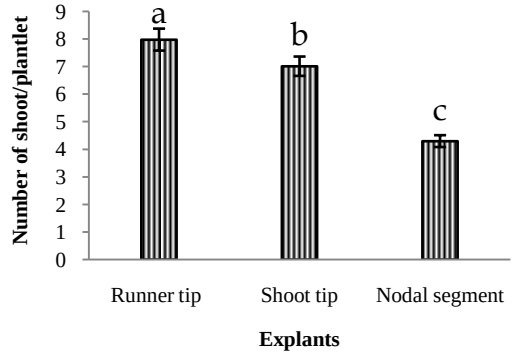

Fig. 5. Effects of different explants on number of shoots per plantlet for shoot initiation

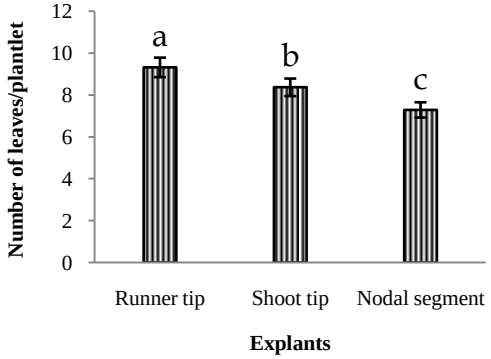

Fig. 6. Effects of different explants on number of leaves for shoot initiation

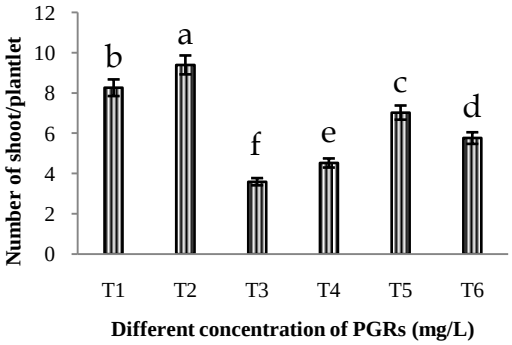

Fig. 7. Effect of different concentrations of BAP, GA_3 and KIN on number of shoot per plantlet of strawberry

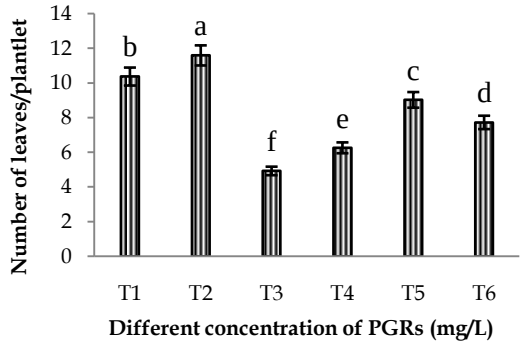

Fig. 8. Effects of different concentrations of BAP, GA_3 and KIN on number of leaves per plantlet of strawberry

Here,

T1= 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2= 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3= 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4= 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5= 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6= 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L

Table 1. Interaction effects between explants and different concentrations of BAP, GA_3 and KIN on days required for shoot initiation, % of shoot initiation, number of shoot per plant, number of leaves per shoot and shoot length

Treatment combination	Days to shoot initiation	% of shoot initiation	No. of shoot per plant	No. of leaves per shoot	Shoot length (cm)
T1P1	9.00k	95.00b	9.80b	11.50b	3.60b
T1P2	12.00i	92.00c	9.00c	10.50c	3.50bc
T1P3	15.00f	70.00l	6.00g	9.13d	3.00d
T2P1	8.00l	97.00a	11.20a	12.80a	4.70a
T2P2	11.00j	95.00b	10.00b	11.60b	4.50a
T2P3	13.00h	80.00j	7.00f	10.40c	3.50bc
T3P1	18.00c	84.00h	5.00h	5.70j	2.10g
T3P2	20.00b	80.00j	3.80i	4.90k	2.10g
T3P3	21.00a	48.00o	2.00k	4.20l	2.00g
T4P1	17.00d	87.00f	6.10g	7.20g	2.40fg
T4P2	18.00c	82.00i	5.00h	6.20i	2.50efg
T4P3	20.00b	50.00n	2.50j	5.40j	2.20g
T5P1	12.00i	92.00c	8.50d	10.10c	3.10cd
T5P2	14.00g	89.00e	7.80e	9.20d	3.10cd
T5P3	16.00e	75.00k	4.80h	7.80f	2.80def
T6P1	15.00f	90.00d	7.30f	8.60e	3.00cd
T6P2	16.00e	85.00g	6.50g	7.80f	2.90df
T6P3	18.00c	65.00m	3.50i	6.80h	2.40fg
$LSD_{0.05}$	0.496	0.331	0.496	0.384	0.447
CV (%)	1.98	0.25	4.90	2.78	9.13

In a column values having different letter (s) differ significantly at 5% level of probability

Here,

T1 = 0.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T2 = 1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T3 = 1.5 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L
T4 = 0.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T5 = 1.0 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L
T6 = 1.5 mg BAP + 0.5 mg GA_3 + 0.1 mg KIN/L and
P1 = Runner tip; P2 = Shoot tip and P3 = Nodal segment

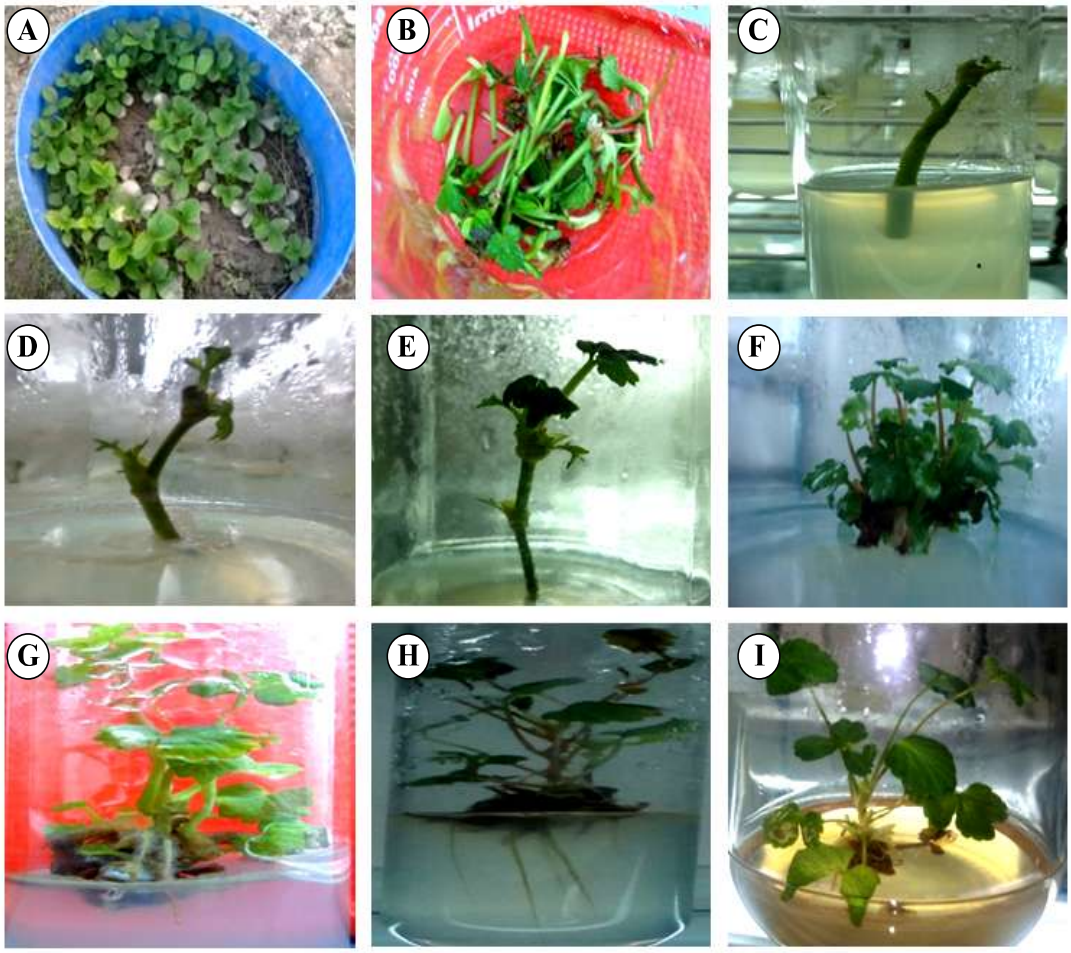

Plate 1. Source of explants for micropropagation (A); washing of explants (B); runner tips proliferation on MS + 1.0 mg BAP + 0.5 mg GA3 + 0.5 mg KIN/L (C); after 7 days (D); after 15 days (E); After 35 days (F) of culture medium. Rooted shoots on 1.0 mg IBA + 0.5 mg 2, 4-D/L after 2 weeks (G); after 4 weeks (H) of culture medium Regenerated plantlets with roots (I).

Effect of IBA and 2, 4-D on in vitro root regeneration

Distinct variations were found in respects of rooting characteristics due to the effect of different explants and different concentrations of growth regulators.

Among the explants, runner tip took the shortest number of days (13.50) for root initiation, highest percentage (93.00) of roots, highest number (8.60) of roots and the longest (2.40 cm) root. In contrast, nodal segment had required the highest days (19.00) for root initiation, lowest (73.50) percentage of roots, lowest (5.34) number of roots and shortest root (2.07 cm) (Table 2). Sakila *et al.* (2007) observed the different number of roots produced from different explants.

Table 2. Effects of different explants on the days to root initiation, percentage of root initiation no. of roots/plantlet and root length (cm)

Explants	Days to root initiation	Percentage of root initiation	No. of root per plantlet	Root length (cm)
Runner tip	13.50c	93.00a	8.60a	2.40a
Shoot tip	16.56b	82.50b	6.78b	2.18b
Nodal segment	19.00a	73.50c	5.34c	2.07c
$LSD_{0.05}$	0.571	0.805	0.629	0.323
CV (%)	2.10	4.50	1.70	2.55

In a column values having different letter (s) differ significantly at 5% level of probability.

MS Medium that containing 1.0 mg IBA + 0.5 mg 2, 4-D/L (T4) took the least number of days (10.00) for root initiation, produced the highest percentage (91.33) of roots, maximum number of roots per plantlet (10.07) and recorded highest roots length (3.33 cm)while required the highest number of days (12.00) for root initiation, lowest percentage of root (80.67), minimum (4.50) number of roots per plantlet and shortest (1.23 cm) roots length were recorded from the concentration 1.0 mg IBA + 0.1 mg 2, 4-D/L (T2) (Table 3). Rahman (2011) found that runner tip explants showed better compared to shoot tip with 1 mg/L BAP whereas for root development, earlier roots could be found from both the explants with 0.5 mg/L IBA. Sakila *et al.* (2007) observed that the maximum frequency of rooting and highest number of roots was produced on medium containing 1.0 mg/L IBA.

Table 3. Effects of different concentrations of IBA and 2, 4-Don the days to root initiation, Percentage of root initiation, no. of roots/plantlet and root length (cm)

Concentrations and combinations of PGRs (mg/L)	Days to root initiation	Percentage of root initiation	No. of root per plantlet	Root length (cm)
T_1 (0.5 mg IBA + 0.1 mg 2, 4-D/L)	10.98b	89.33ab	8.03b	2.65b
T_2 (1.0 mg IBA + 0.1 mg 2, 4-D/L)	10.00c	80.67c	4.50d	1.23c
T_3 (0.5 mg IBA + 0.5 mg 2, 4-D/L)	11.87a	85.67b	6.78c	1.87bc
T_4 (1.0 mg IBA + 0.5 mg 2, 4-D/L)	12.00a	91.33a	10.07a	3.33a
$LSD_{0.01}$ value	0.742	0.531	0.814	0.420

In a column values having different letter(s) differ significantly at and 5% level of probability.

In interaction effect, the lowest number of days (10.00) for root initiation, highest percentage of root initiation (98.00), highest number of root (12.00) and the longest root (3.70 cm) were found from the explant runner tip with 1.0 mg IBA + 0.5 mg 2, 4-D/L (T4). In contrast, highest number of days required (23.00) for root initiation, lowest (68.00) percentage of root initiation and lowest (3.10) root number were recorded from nodal segments with (T2) 1.0 mg IBA + 0.1 mg 2, 4-D/L (Table 4). It observed that rest of the combination showed intermediate results compared to the highest and lowest value of root

length. This result corroborates with that of Biswas *et al*. (2007), Karim *et al.* (2011), they also observed significant interaction effects between explants and concentrations of IBA.

From the above discussion, it is revealed that, different explants and growth regulators with their different concentration and combination provided the best results in *in vitro* plant regeneration of strawberry. Runner tip explants showed the best performance than other explants when MS media supplemented with (1.0 mg BAP + 0.5 mg GA_3 + 0.5 mg KIN/L) for shoot initiation and MS media supplemented with (1.0 mg IBA + 0.5 mg 2, 4-D/L) for root initiation.

Table 4. Interaction effects between explants and different concentrations of IBA and 2, 4-D on days required for root initiation, % of root initiation, number of roots per plantlet and root length

Treatment Combination	Days to root initiation	% of root initiation	No. of roots per plantlet	Root length (cm)
T1P1	15.00f	91.00e	7.50e	1.90de
T1P2	17.00e	89.00f	6.16f	1.40ef
T1P3	20.00b	71.00k	4.16i	2.30d
T2P1	17.00e	88.00g	5.60g	1.50ef
T2P2	19.00c	86.00h	4.80h	1.20f
T2P3	23.00a	68.00l	3.10j	1.00f
T3P1	12.00h	95.00c	9.30c	2.50cd
T3P2	14.00g	93.00d	8.10d	2.40cd
T3P3	18.00d	75.00j	6.10f	2.00de
T4P1	10.00j	98.00a	12.00a	3.70a
T4P2	11.00i	96.00b	10.20b	3.30ab
T4P3	15.00f	80.00i	8.00d	3.00bc
$LSD_{0.05}$	0.3370	0.3370	0.4522	0.6005
CV (%)	1.26	0.23	3.79	16.30

In a column values having different letter (s) differ significantly at 5% level of probability

Here,

T1 = 0.5 mg IBA + 0.1 mg 2, 4-D/L T3 = 0.5 mg IBA + 0.5 mg 2, 4-D/L
T2 = 1.0 mg IBA + 0.1 mg 2, 4-D/L T4 = 1.0 mg IBA + 0.5 mg 2, 4-D/L
and P1= Runner tip, P2= Shoot tip, P3 = Nodal segment

The findings of the present study could be useful to develop protocol to identify the potentiality of exact concentration of different growth regulators. Furthermore, the results could be used to produce large scale production of healthy and disease free planting materials commercially.

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EFFECT OF ROW SPACING AND DATES OF TRANSPLANTING ON YIELD PERFORMANCE OF ADVANCED RICE MUTANT (RM-40(C)-4-2-8) IN BORO SEASON OF BANGLADESH

M.I. Ali[1], M.S. Islam[1], M. Ali[2], A. Mahmud[1] and N. Aktar[1]

Abstract

Three experiments were conducted at BINA HQs farm Mymensingh and BINA sub-station Rangpur during boro season 2018-19 and 2019-20. The objective was to evaluate the mutant line at four different dates of transplanting and three levels of spacing. The mutant line, RM-40(c)-4-2-8 was compared with two check varieties Binadhan-18, BRRI dhan28 with four dates of transplanting were January 3, January 15, February 1 and February 15 and three row spacing were 20 cm × 15 cm, 20 cm × 20cm, 20 cm × 25 cm for both locations. The effect of date, variety and spacing's showed that transplanting date at January 15 the mutant RM-40(c)-4-2-8 produced maximum yield (5.88 t ha^{-1}) at 20 cm × 15 cm spacing during 2018-19 in Rangpur. The effect of dates of transplanting on grain yield showed that of January 15 transplanting produced the highest grain yield (5.82 t ha^{-1}) during 2019-20 at Mymensingh. Overall observation showed that mutant line RM-40(c)-4-2-8 yielded the best at 20 cm × 15 cm when transplanted on January 15 in Bangladesh.

Key words: Spacing; transplanting dates, grain yield, RM-40(c)-4-2-8, rice mutants

Introduction

Rice (*Oryza sativa* L.) is the most important cereal in Bangladesh. It is extensively cultivated throughout the year and also staple food crop in Bangladesh. The environmental condition of Bangladesh favourable for rice cultivation. Bangladesh earns about 13.02% of her gross domestic product (GDP) from crop (AIS, 2019). In Bangladesh, total cultivable land 11.77 million hectare and used for boro rice 20.18 million hectare and average yield 4.24 t ha^{-1} with a total production of about 38.7 million tons (AIS, 2019). In Bangladesh rice yield is lower than other countries like China, Japan, Egypt and Korea where yield is 7.5, 5.9,7.3 and 7.5 t ha^{-1}, respectively (Choi, 2000). The yield of rice may be increased through improved agronomic manipulations such as proper spacing and transplanting dates. The growth, development, yield and yield components of rice in are highly influenced by date of transplanting. Generally boro rice is transplanted from early December to mid-March. Early transplantation of boro rice prolongs field duration due to low temperature and involves high cost of production, particularly for management practices including irrigation, while delayed transplanting reduces the yield in some cases (BRRI, 2004). Generally closer spacing

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hampers intercultural operations, increases competition among the plants for nutrients, air, light, which results in weaker plants, mutual shading thus favours more straw yield than grain yield and wider plant spacing reduces grain yield per unit area. The benefit in respect of rice yield can be obtained where planting is done with optimum spacing. Genotypes are the key components of produce higher yield of rice depending upon their differences in characters, input requirements and response, growth process and off course the prevailing environmental conditions during the growing season. The growth process of rice plants under a given agro-climatic condition differs with genotypes. Closer spacing results, plants become weaker and thinner and consequently, yield is reduced. Importance of determine optimum spacing for maximizing the yield of rice. Number of seedling $hill^{-1}$ is another important factor that influences plant population $unit^{-1}$ area (Islam *et al.,* 2002). Planting density in transplanted rice culture constitutes the number of seedlings $hill^{-1}$. Mizan 2010, found the highest yield at 25 cm × 15 cm spacing for transplanted boro rice. The number of tillers and their growth is greatly affected both qualitatively and quantitatively by number of seedlings $hill^{-1}$. Effect of row and hill spacing influence on yield performance of boro rice (cv. BRRI dhan45) (M. R. Sultana *et al.,* 2012). Optimum number of seedlings $hill^{-1}$ may enable the rice plant to grow properly both in its aerial and underground parts by utilizing maximum solar energy, nutrients, space, water and also could reduce seedling cost of farmers (Azad, 2004). The introduced plant material should be need evaluation under the prevailing climatic conditions of Bangladesh in relation to morphological and physiological characteristics. The highest sterility of spikelet at closest spacing reflects the high competition among the tillers for resources (Rashid, 2009). The normal planting dates show the possibility of day-time heat stress. Adjustment of the planting date is currently limited because high temperature tolerant cultivars are not available in the study region. So, it is also necessary to standardize the optimum planting time and planting spacing for exploiting the potential yield of the advanced mutant line under Bangladesh climatic conditions. Therefore, the research was undertaken to analyze the yield performance of the advanced lines of RM-40(c)-4-2-8 (developed through carbon-ion beam irradiation) under different plant spacing and planting dates for growing under irrigated conditions in Bangladesh.

Materials and methods

Experiments were carried out at the Agronomy field of BINA HQs farm, Mymensingh during boro season 2018-19 in Mymensingh and Rangpur 2019-20 in Mymensingh. The experiment was laid out in split split plot design with three replications. The experimental site was situated between 24.6°N and 90.5°E latitude and at 18 m high from the sea level. The soil of the experimental field was sandy loam type and belonged to the Old Brahmaputra Flood Plain Alluvial Tract in Mymensingh. The advanced mutant lines RM-40(c)-4-2-8 was collected from plant breeding division of BINA and the check variety was Binadhan-18 and BRRI dhan28 with three levels of spacing, viz. 20 cm × 15 cm, 20 cm × 20 cm and 20 cm × 25 cm having four dates of transplanting. The fertilizer doses applied for the experiment were 120 kg N ha^{-1}, 80 kg P ha^{-1}, 100 kg K ha^{-1}, 20 kg S ha^{-1} and 5 kg Zn

ha^{-1}. Nitrogen, phosphorus, potassium, sulphur and zinc were supplied from urea, TSP, MoP, gypsum and zinc sulphate monohydrate respectively while urea was applied in three equal splits. Twenty five days old seedlings were transplanted in a randomized complete block design with three replications with single seedling $hill^{-1}$. The unit plot size was 3m × 4m. The crop was harvested and data on yield and yield components were recorded and analyzed statistically, and the means were compared with LSD (Gomez, K. A. and Gomez A. A. 1984). The advanced mutant lines RM-40(c)-4-2-8 was evaluated with check variety (Binadhan-18) during 2019-20 with three transplanting dates January 15, February 1 and February 15 in Mymensingh were assigned for comparing the performance of advanced mutant line. The application of herbicide (Bensulfuron methyl 4% + Acetachlor 14%) was necessary to keep the field weed free throughout the growing period along with hand weeding at 35 DAT. Furtera 5 G @ 10 kg ha^{-1} was applied to control the infestation of stem borer. The experiment was done under irrigated condition. After attaining 80% physiological maturity the crop was harvested for first, second, third and fourth dates of transplanting respectively. The harvested plants were threshed, cleaned, and processed, and then yield and yield contributing characters were recorded in agronomy laboratory. Weather parameters were also recorded for understanding the growing environment of the crop.

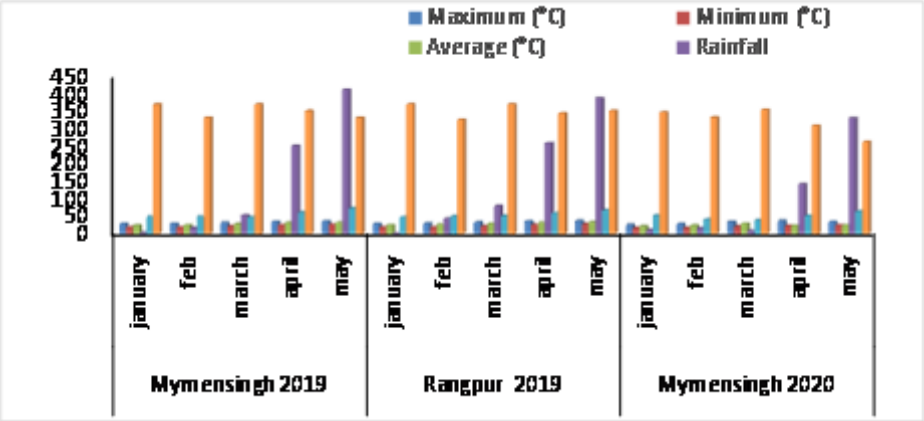

Fig. 1. Weather parameters during experimental period of 2019 in Mymensingh and Rangpur and 2020 in Mymensingh.

Results and discussions

Mymensingh

There was a statistically significant difference in plant height among different spacing. The highest plant height 106.8 cm was observed at (20 cm × 25 cm) spacing and the lowest plant height 105.8 cm at (20 cm × 15 cm) spacing (Table 1). The highest plant height 119.3 was obtained at interaction of mutant RM-40(c)-4-2-8 at spacing (20 cm × 25 cm) and mutant RM-40(c)-4-2-8 at spacing (20 cm × 15 cm) and lowest plant height 97.1 cm at variety BRRI dhan28 (Table 2).

There was a statistically significant difference of total tillers hill^{-1} among spacing. The highest number of total tillers hill^{-1}13.2 was observed at (20 cm × 25 cm) spacing and the lowest number of total tillers hill^{-1}11.5 at (20 cm × 15 cm) spacing (Table 1). There was a statistically significant difference of total tillers hill^{-1} among spacing and genotypes interaction (Table 2). The highest number of total tillers hill^{-1} 13.4 was obtained at interaction of variety Binadhan-18 and spacing (20 cm × 25 cm), mutant RM-40(c)-4-2-8 and spacing (20 cm × 15 cm) and the lowest number of total tillers hill^{-1} 10.8 was at mutant RM-40(c)-4-2-8 at spacing (20 cm × 15 cm) (Table 2).

There was a statistically significant difference of effective tillers hill^{-1} among different spacing. The highest number of effective tillers hill^{-1} 11.7 was observed at (20 cm × 25 cm) spacing and the lowest number of effective tillers hill^{-1} 10.1at (20 cm × 15 cm) spacing (Table 1). Among genotypes the highest number of effective tillers hill^{-1} 11.1 was observed at BRRI dhan28 and the lowest number of effective tillers hill^{-1} 10.6 at genotype RM-40(c)-4-2-8. The highest number of effective tillers hill^{-1} 12 was obtained at interaction of variety Binadhan-18, spacing (20 cm × 25 cm) and lowest number of effective tillers hill^{-1} 9.7 at variety RM-40(c)-4-2-8, spacing (20 cm × 15 cm) (Table 2).

There was a statistically significant difference in panicle length among genotypes the highest panicle length 27.2 cm was recorded inRM-40(c)-4-2-8 and the lowest panicle length 22.4 cm at mutant BRRI dhan28.There was a statistically significant difference of panicle length among spacing and genotype interaction. The highest panicle length 27.3 cm was obtained at interaction of mutant RM-40(c)-4-2-8, spacing (20 cm × 15 cm) and lowest panicle length 22.3 cm at variety BRRI dhan28 at spacing (20 cm × 22 cm) (Table 2).

Among genotypes the highest number of filled grains panicle^{-1} 140.9 was recorded at mutant RM-40(c)-4-2-8 and the lowest number of filled grains panicle^{-1} 117.2 at mutant RM-40(c)-4-2-8. There was a statistically significant difference of filled grain panicle^{-1} among spacing and genotype interaction. The highest number of filled grains panicle^{-1} 145.3 was obtained at interaction of variety RM-40(c)-4-2-8, spacing (20 cm × 20 cm) and lowest number of filled grains panicle^{-1} 116.3 at BRRI dhan28, spacing (20 cm × 25 cm) (Table 2).

Among genotypes the highest number of unfilled grains panicle^{-1} 9 was recorded at mutant Binadhan-18 and the lowest number of unfilled grains panicle^{-1} 30.7 at variety BRRI dhan28.There was a statistically significant difference of unfilled grain panicle^{-1}among spacing and genotype interaction. The highest number of unfilled grains panicle^{-1} 34.2 was obtained at interaction of Binadhan-18, spacing (20 cm × 15 cm) and lowest number of unfilled grains panicle^{-1} 29.4 at mutant RM-40(c)-4-2-8, spacing (20 cm × 25 cm) (Table 2).

Among genotypes, the highest thousand seed weight 22.5g was recorded at variety BRRI dhan28 and the lowest thousand seed weight 19.7gat mutant RM-40(c)-4-2-8. The highest thousand seed weight 22.9 g was obtained at interaction of variety BRRI dhan49, spacing (20 cm × 25 cm) and lowest thousand seed weight 22.1 g at mutant RM-40(c)-4-2-8, spacing (20 cm × 25 cm) (Table 2).

There was a statistically significant difference of grain yield among spacing. The highest grain yield 5.45 t ha^{-1} was recorded at (20 cm × 15 cm) spacing and the lowest grain yield 4.69 t ha^{-1} was at (20 cm × 25 cm) spacing (Table 1). Among the genotype, the highest grain yield 5.67 t ha^{-1} was recorded at mutant RM-40(c)-4-2-8 and the lowest grain yield 4.23 t ha^{-1} at variety Binadhan-18 (Table 1). The highest grain yield 5.86 t ha^{-1} was obtained at interaction of mutant RM-40(c)-4-2-8, spacing (20 cm × 15 cm) and lowest grain yield 3.88 t ha^{-1} at variety Binadhan-18, spacing (20 cm × 25 cm). It might be due to the number of tillers m^{-2}, filled grains $panicle^{-1}$, panicle length was the highest at 20 cm × 15 cm spacing. Optimum (20 cm × 15 cm) spacing for mutant RM-40(c)-4-2-8 proper growth, more effective tiller, panicle initiation, formation of filled grains $panicle^{-1}$ and thousand grain weight and yield was the maximum (Table 1 and Table 2). Optimum plant spacing ensures the plant establishment rates to grow properly with their aerial and underground parts by utilizing more solar radiation interception and nutrients uptake (Miah *et al.*, 1990).

Table 1. Effect of date of transplanting and spacing on yield contributing characters of rice mutant in BINA HQs farm Mymensingh during boro season

Treatments	Plant height (cm)	Total tillers $hill^{-1}$ (no.)	Effective tillers $hill^{-1}$ (no.)	Panicle length (cm)	Filled grains $panicle^{-1}$ (no.)	Unfilled grains $panicle^{-1}$ (no.)	1000 seed wt. (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates of transplanting									
January 15 (D_1)	111.8	12.7	11.2	25.2	127.3	32.4	21.4	5.13	6.35
February 01 (D_2)	109.0	13.3	11.7	25.3	127.9	32.4	21.4	5.06	6.62
February 15 (D_3)	98.0	11.0	9.9	24.6	120.9	30.0	21.4	4.61	6.54
$LSD_{0.05}$	3.3	1.2	1.3	0.6	NS	NS	NS	NS	NS
Genotypes (mutant/varieties)									
RM-40(c)-4-2-8 (V_1)	118.8	11.8	10.6	27.2	140.9	30.9	19.7	5.67	7.35
Binadhan-18 (V_2)	102.3	12.7	11.0	25.5	118.0	33.2	22.1	4.23	5.91
BRRI dhan28 (V_3)	97.7	12.5	11.1	22.4	117.2	30.7	22.5	5.26	6.25
$LSD_{0.05}$	1.8	1.2	NS	0.4	8.0	NS	0.2	0.23	0.38
Spacings									
20 cm × 15 cm (S_1)	105.8	11.5	10.1	25.2	123.4	32.7	21.4	5.45	6.87
20 cm × 20 cm (S_2)	106.2	12.4	10.9	24.9	128.4	31.8	21.5	5.01	6.51
20 cm × 25 cm (S_3)	106.8	13.2	11.7	25.0	124.4	30.3	21.3	4.69	6.13
$LSD_{0.05}$	NS	1.3	0.3	NS	4.5	NS	NS	0.07	0.17

Among genotypes the highest straw yield 7.35 t ha^{-1} was recorded at variety RM-40(c)-4-2-8 and the lowest straw yield 5.91 t ha^{-1}at Binadhan-18. There was a statistically significant difference of straw yield among spacing and genotype interaction. The highest straw yield 7.76 t ha^{-1} was obtained at variety RM-40(c)-4-2-8, spacing (20 cm × 15 cm) and lowest straw yield 5.8 t ha^{-1} at BRRI dhan28, spacing (20 cm × 25 cm) (Table 2). Out of three transplanting dates transplanted on January 15, 2018 produced the highest yield (5.13 t ha^{-1}), whereas that of Feb. 15 transplanting produced the lowest grain yield (4.61 t ha^{-1}). It might be due to the highest plant height, highest panicle length and more filled grains $panicle^{-1}$ (127.3) were produced on January 15 transplanting (Table 1). Available data regarding the effect of spacing in conventionally transplanted rice cultivation in boro season

(Rahman *et al.*, 2008). Among the genotypes, RM-40(c)-4-2-8 produced the highest grain yield (5.67 t ha^{-1}). It might be due to highest plant height, panicle length, more filled grains $panicle^{-1}$ (140.9) produced by mutant line RM-40(c)-4-2-8 (Table 1). Genotypes are the key components of produce higher yield of rice depending upon their differences in characters, input requirements and response, growth process and off course the prevailing environmental conditions during the growing season.

Table 2. Two factor effect on the yield and yield contributing characters of rice mutant/varieties at BINA HQs farm Mymensingh during boro season

Treatments	Plant height (cm)	Total tillers $hill^{-1}$ (no.)	Effective tillers $hill^{-1}$ (no.)	Panicle length (cm)	Filled grains $panicle^{-1}$ (no.)	Unfilled grains $panicle^{-1}$ (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates × Varieties									
D_1V_1	120.9	12.7	11.2	27.1	148.5	29.0	19.4	5.87	7.65
D_1V_2	111.7	11.8	10.5	25.6	117.6	36.8	22.4	3.97	5.46
D_1V_3	102.7	13.7	11.8	22.9	116.0	31.4	22.5	5.12	5.92
D_2V_1	121.2	13.1	12.0	27.3	142.5	33.7	19.6	5.74	7.56
D_2V_2	103.3	13.8	11.3	25.7	121.2	32.9	22.1	4.35	5.89
D_2V_3	102.6	13.0	11.8	22.7	120.0	30.6	22.5	5.11	6.41
D_3V_1	114.4	9.7	8.6	27.0	131.8	30.0	20.0	5.56	6.84
D_3V_2	91.9	12.4	11.1	25.1	115.2	29.9	21.7	4.36	6.36
D_3V_3	87.8	10.9	9.8	21.8	115.7	30.0	22.5	4.96	6.43
$LSD_{0.05}$	3.1	0.8	1.3	0.7	13.8	NS	0.4	0.39	0.65
Dates × Spacings									
D_1S_1	111.2	11.9	10.2	25.4	128.0	34.6	21.5	5.61	6.81
D_1S_2	112.2	12.7	11.3	25.0	128.2	32.8	21.6	5.06	6.42
D_1S_3	111.9	13.6	11.9	25.1	125.8	29.9	21.2	4.72	5.80
D_2S_1	108.9	12.4	10.8	25.7	124.6	32.0	21.4	5.41	6.94
D_2S_2	109.0	13.3	11.6	25.0	131.4	33.7	21.6	4.99	6.59
D_2S_3	109.1	14.2	12.7	25.1	127.8	31.6	21.2	4.80	6.33
D_3S_1	97.2	10.1	9.3	24.5	117.6	31.5	21.5	5.34	6.87
D_3S_2	97.6	11.1	9.8	24.7	125.5	28.8	21.2	4.98	6.52
D_3S_3	99.3	11.8	10.5	24.8	119.6	29.6	21.5	4.56	6.25
$LSD_{0.05}$	1.0	0.2	0.5	0.7	7.8	NS	NS	0.12	0.29
Varieties × Spacings									
V_1S_1	118.2	10.8	9.7	27.1	138.4	32.4	19.7	5.86	7.76
V_1S_2	118.9	11.9	10.8	27.1	145.3	31.0	19.8	5.63	7.35
V_1S_3	119.3	12.8	11.3	27.3	139.1	29.4	19.5	5.51	6.93
V_2S_1	102.0	11.9	10.1	26.0	113.7	34.2	22.2	4.56	6.21
V_2S_2	102.1	12.7	10.8	25.3	122.4	33.8	22.1	4.24	5.86
V_2S_3	102.8	13.4	12.0	25.2	117.8	31.6	21.9	3.88	5.64
V_3S_1	97.1	11.7	10.4	22.6	118.0	31.4	22.4	5.65	6.64
V_3S_2	97.8	12.5	11.1	22.3	117.3	30.5	22.5	5.06	6.32
V_3S_3	98.3	13.4	11.8	22.5	116.3	30.1	22.5	4.69	5.80
$LSD_{0.05}$	NS	0.4	0.5	0.7	7.8	NS	0.4	0.12	0.29
CV%	4.1	10.6	11.4	7.2	10.7	9.4	2.1	7.5	9.7

D_1 = January 15, D_2 = February 01, D_3 = February 15, S_1= 20 cm × 15 cm, S_2 = 20 cm × 20 cm
S_3 = 20 cm × 25 cm, V_1= RM-40(c)-4-2-8, V_2 = Binadhan-18, V_3 = BRRI dhan28.

The grain yield of (20 cm × 15 cm) spacing was the highest (5.45 t ha^{-1}), whereas that of (20 cm × 25 cm) was the lowest (4.69 t ha^{-1}). It might be due statue of the plant and highest panicles m^{-2} in (20 cm × 15 cm) spacing congenial for growth and yield of the mutant (Table 1).

The effect of date and variety showed that RM-40(c)-4-2-8 produced the maximum yield (5.87 t ha^{-1}) when transplanted on January 15 (Table 3). There is a statistically significant different on effect of date and variety the highest filled grains $panicle^{-1}$ (148.5) and lowest (29.4) unfilled grains $panicle^{-1}$ produced on January 15 by RM-40(c)-4-2-8 (Table 2). Transplanting on January 15 produced the maximum yield (5.61 t ha^{-1}) at (20 cm × 15 cm) spacing (Table 2). It might be due to the highest genetic expression for the mutant line in Mymensingh region when the raising of seedling, seedling transplanting. Nonetheless, the bright sunshine at reproductive development phase was also congenial for optimum yield of the mutant line (Table 2). Effect of variety and spacing showed that RM-40(c)-4-2-8 produced maximum yield (5.86 t ha^{-1}) at (20 cm × 15 cm) spacing. It might be due to (20 cm × 15 cm) spacing congenial for suitable growth and development for more panicles produced m^{-2} highest for the mutant RM-40(c)-4-2-8and found maximum yield (Table 2).

Table 3. Combine interaction effect of date of transplanting, mutants and spacing on yield contributing characters of rice mutant at BINA HQs farm Mymensingh during boro season

Treatments	Plant height (cm)	Total tillers $hill^{-1}$ (no.)	Effective tillers $hill^{-1}$ (no.)	Panicle length (cm)	Filled grains $panicle^{-1}$ (no.)	Unfilled grains $panicle^{-1}$ (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates × Varieties × Spacings									
$D_1V_1S_1$	121.1	11.9	10.3	27.2	151.7	33.7	19.3	6.12	8.06
$D_1V_1S_2$	121.1	12.6	11.5	26.9	145.7	26.0	19.6	5.70	7.84
$D_1V_2S_3$	120.4	13.5	11.7	27.3	148.1	27.3	19.4	5.48	7.06
$D_1V_2S_1$	110.5	11.1	9.4	26.4	116.7	40.7	22.7	4.51	6.07
$D_1V_2S_2$	112.5	11.9	10.7	25.4	121.7	37.1	22.4	3.89	5.48
$D_1V_2S_3$	112.2	12.6	11.5	25.1	114.3	32.7	22.0	3.50	4.84
$D_1V_3S_1$	102.1	12.7	10.9	22.7	115.7	29.4	22.4	5.50	6.31
$D_1V_3S_2$	102.9	13.6	11.8	22.8	117.3	35.2	22.7	5.17	5.94
$D_1V_3S_3$	103.2	14.7	12.5	23.1	115.0	29.7	22.3	4.68	5.52
$D_2V_1S_1$	120.1	11.9	10.9	27.4	140.2	34.3	19.5	5.92	8.02
$D_2V_1S_2$	121.3	13.2	12.0	27.3	147.1	34.5	20.0	5.58	7.48
$D_2V_2S_3$	122.1	14.2	13.3	27.3	140.2	32.3	19.3	5.32	7.19
$D_2V_2S_1$	103.2	13.2	10.6	26.5	110.2	32.3	22.3	4.51	6.16
$D_2V_2S_2$	102.8	13.7	10.9	25.3	129.7	35.3	22.3	4.35	5.78
$D_2V_2S_3$	103.8	14.4	12.3	25.4	123.7	31.0	21.7	4.19	5.75

Table 3. Continued

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
D_2V_3 S_1	103.5	12.0	11.0	23.3	123.3	29.3	22.4	5.40	6.65
D_2V_3 S_2	102.9	13.1	11.9	22.3	117.3	31.3	22.4	5.04	6.53
D_2V_3 S_3	101.5	13.9	12.4	22.5	119.3	31.3	22.7	4.88	6.05
D_3V_1 S_1	113.5	8.6	8.0	26.7	123.3	29.3	20.4	5.94	7.21
D_3V_1 S_2	114.3	9.9	8.9	27.1	143.1	32.3	19.8	5.50	6.74
D_3V_2 S_3	115.5	10.7	9.1	27.2	128.8	28.5	19.8	5.24	6.56
D_3V_2 S_1	92.3	11.5	10.4	25.0	114.3	29.7	21.7	4.67	6.41
D_3V_2 S_2	90.9	12.5	10.9	25.2	116.0	29.0	21.5	4.47	6.33
D_3V_2 S_3	92.3	13.2	12.1	25.2	115.3	31.0	22.0	3.93	6.34
D_3V_3 S_1	85.7	10.3	9.4	21.7	115.0	35.5	22.3	5.41	6.97
D_3V_3 S_2	87.5	10.9	9.7	21.7	117.3	25.1	22.4	4.98	6.48
D_3V_3 S_3	90.1	11.5	10.4	22.0	114.7	29.3	22.7	4.49	5.84
$LSD_{0.05}$	1.7	0.6	0.9	5.2	13.5	10.2	0.7	0.20	0.50
CV%	4.1	10.6	11.4	7.2	10.7	9.4	2.1	7.5	9.7

D_1 = January 15, D_2 = February 01, D_3 = February 15, S_1 = 20 cm × 15 cm, S_2 = 20 cm × 20 cm
S_3 = 20 cm × 25 cm, V_1 = RM-40(c)-4-2-8, V_2 = Binadhan-18, V_3 = BRRI dhan28.

The interaction effect of date, variety and spacing showed that the mutant line, RM-40(c)-4-2-8 produced maximum yield (6.12 t ha^{-1}) at (20 cm × 15 cm) spacing in Jan. 15 transplanting dates (Table 3).

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Transplanting on January 15 gave the highest grain yield (5.98 t ha^{-1}) whereas Feb. 15 transplanting date produced the lowest grain yields (4.07 t ha^{-1}) (Table 4). Among the mutant lines/varieties, the mutant RM-40(c)-4-2-8 produced the highest grain yield (4.88 t ha^{-1}) followed by Binadhan-18 (4.81 t ha^{-1}) (Table 4). The grain yield of (20 cm × 15 cm) spacing was the highest (4.94 t ha^{-1}), whereas that of (20 cm × 25 cm) was the lowest (4.66 t ha^{-1}) (Table 4). It might be due statue of the plant and highest panicles m^{-2} in (20 cm × 15 cm) spacing congenial for growth and yield of the mutant (Table 4).

The interaction effect of date and variety showed that the variety BRRI dhan28 produced the maximum yield (6.2 t ha^{-1}) at January 15 followed by the mutant RM-40(c)-4-2-8 (5.48 t ha^{-1}) (Table 5). The interaction effect of spacing and transplanting at January 15 produced the maximum yield (6.06 t ha^{-1}) at (20 cm × 15 cm) which is followed by same transplanting date produced (5.74 t ha^{-1}) at (20 cm × 20 cm) (Table 5). The interaction effect of variety and spacing the mutant RM-40(c)-4-2-8 produced maximum yield (5.09 t ha^{-1} followed by Binadhan-18 (4.98 t ha^{-1}) at same spacing 20 cm × 15 cm (Table 5).

Table 4. Effect of date of transplanting, genotypes and spacing on the yield and yield contributing characters of rice mutant/varieties at different spacings at BINA Sub-station, Rangpur during boro season

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates of transplanting									
January 3 (D_1)	104.4	11.4	10.4	24.0	142.0	23.3	21.3	4.84	6.00
January 15 (D_2)	112.8	13.6	11.8	25.4	153.2	13.5	21.5	5.98	7.01
February 1 (D_3)	110.8	12.2	10.7	24.2	129.2	16.9	21.5	4.22	5.70
February 15 (D_4)	113.1	11.1	9.8	25.2	145.1	20.6	21.4	4.07	5.32
$LSD_{0.05}$	NS	0.7	0.7	0.7	5.9	9.2	NS	0.22	0.19
Mutants/Variety									
RM-40(c)-4-2-8 (V_1)	118.1	11.6	10.4	26.8	166.6	15.4	19.7	4.88	6.10
Binadhan-18 (V_2)	107.0	11.9	10.6	24.5	125.0	22.0	22.2	4.81	6.00
BRRI dhan28 (V_3)	105.8	12.7	11.2	22.8	135.6	18.4	22.4	4.60	5.93
$LSD_{0.05}$	NS	0.8	0.6	0.7	7.5	4.1	NS	0.18	NS
Spacings									
20 cm × 15 cm (S_1)	109.7	10.8	9.7	24.6	143.7	18.8	21.4	4.94	6.15
20 cm × 20 cm (S_2)	110.8	12.3	11.0	24.6	142.1	18.3	21.4	4.74	5.96
20 cm × 25 cm (S_3)	110.4	13.0	11.4	24.9	141.3	18.6	21.5	4.66	5.92
$LSD_{0.05}$	NS	0.7	0.5	NS	NS	NS	NS	0.35	0.19

Table 5. Two factor effect on the yield and yield contributing characters of boro rice mutant/ varieties at BINA sub-station Rangpur

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates × Mutants/Variety									
D_1V_1	116.1	10.8	9.8	25.2	168.1	22.5	19.5	5.48	6.43
D_1V_2	100.3	11.3	10.4	25.5	121.3	27.1	22.2	5.33	6.27
D_1V_3	96.9	12.0	10.9	21.3	136.7	20.5	22.2	3.80	5.30
D_2V_1	119.9	14.3	12.4	27.4	179.0	8.2	19.8	5.59	6.74
D_2V_2	108.5	12.7	11.2	25.2	138.7	14.7	22.3	5.68	7.15
D_2V_3	110.1	13.7	11.9	23.4	141.8	17.6	22.5	6.20	7.15
D_3V_1	115.8	9.6	9.0	25.8	144.9	15.1	19.9	4.17	5.61
D_3V_2	110.4	14.0	11.7	23.9	117.5	20.1	22.2	4.06	5.42
D_3V_3	106.2	13.1	11.4	23.0	125.2	15.4	22.4	4.44	6.08
D_4V_1	120.5	11.6	10.2	28.8	174.3	15.8	19.5	4.38	5.62
D_4V_2	108.7	9.8	8.9	23.5	122.3	26.0	22.3	3.88	5.15
D_4V_3	110.1	11.9	10.4	23.4	138.6	20.1	22.3	3.96	5.21
$LSD_{0.05}$	2.7	1.5	1.1	1.4	15.0	8.0	0.5	0.17	0.65

Table 5. Continued

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates × Spacing									
D_1 S_1	103.3	9.6	8.9	24.4	145.4	22.5	21.2	4.94	5.86
D_1 S_2	104.0	11.6	10.7	23.6	142.0	23.6	21.3	4.69	6.07
D_1 S_3	106.0	12.8	11.6	24.1	138.6	24.0	21.4	4.58	6.07
D_2 S_1	112.2	13.0	11.4	25.5	156.7	13.5	21.5	6.06	7.24
D_2 S_2	113.3	13.5	11.8	25.4	152.9	13.6	21.6	5.74	6.81
D_2 S_3	113.0	14.2	12.3	25.1	149.9	13.4	21.6	5.17	6.99
D_3 S_1	110.2	10.9	9.7	23.6	127.8	19.7	21.6	4.59	5.93
D_3 S_2	111.8	12.6	11.2	24.3	131.2	14.5	21.2	4.16	5.65
D_3 S_3	110.4	13.1	11.2	24.8	128.6	16.4	21.6	3.92	5.53
D_4 S_1	113.1	9.7	8.9	24.9	144.7	19.6	21.2	4.42	5.58
D_4 S_2	113.9	11.6	10.3	25.1	142.5	21.7	21.4	4.12	5.30
D_4 S_3	112.3	11.9	10.4	25.8	148.1	20.6	21.5	3.67	5.09
$LSD_{0.05}$	2.8	1.4	0.9	0.8	12.0	4.4	0.5	0.16	0.39
Mutants/Variety × Spacing									
V_1 S_1	118.0	10.7	9.7	26.8	170.5	14.3	19.5	5.09	6.29
V_1 S_2	117.8	11.4	10.3	26.2	164.6	15.9	19.7	4.75	5.97
V_1 S_3	118.5	12.6	11.1	27.4	164.7	16.0	19.8	4.79	6.05
V_2 S_1	106.0	10.6	9.5	24.4	128.2	22.4	22.2	4.98	6.21
V_2 S_2	107.2	12.4	10.9	24.4	124.6	20.9	22.2	4.91	5.96
V_2 S_3	107.8	12.8	11.3	24.8	122.2	22.5	22.4	4.69	5.82
V_3 S_1	105.2	11.2	9.9	22.6	132.3	19.8	22.4	4.74	5.96
V_3 S_2	107.3	13.2	11.7	23.2	137.3	18.2	22.2	4.57	5.94
V_3 S_3	105.0	13.6	11.8	22.6	137.1	17.3	22.5	4.49	5.90
$LSD_{0.05}$	2.4	1.2	0.8	0.7	10.0	3.8	0.4	0.28	0.34

D_1 = January 3, D_2 = January 15, D_3 = February 01, D_4 = February 15, S_1 = 20 cm ×15 cm, S_2 = 20 cm × 20 cm S_3 = 20 cm × 25 cm, V_1= RM-40(c)-4-2-8, V_2 = Binadhan-18, V_3 = BRRI dhan28.

The interaction effect of date, variety and spacing showed that transplanting date at January 15 the mutant RM-40(c)-4-2-8 produced maximum yield (5.88 t ha^{-1}) at 20 cm × 15 cm spacing. The lowest (3.40 t ha^{-1}) yield was reduced in all interaction at D_4V_2 S_3 (Table 6).

The effect of dates of transplanting on grain yield showed that of Jan. 15 transplanting produced the highest grain yield (5.82 t ha^{-1}) whereas Feb. 15 transplanting date produced the lowest grain yield (5.49 t ha^{-1}) (Table 7). Between two genotypes, Binadhan-18 produced the highest grain yield (5.67 t ha^{-1}) (Table 7). The interaction effect of date and variety showed that Binadhan-18 produced the maximum yield (6.39 t ha^{-1}) at January 15 transplanting followed by Feb. 1 (6.29 t ha^{-1}) (Table 7).

Table 6. Combine interaction effect of date of transplanting and row spacing on the yield and yield contributing characters of boro rice mutant/varieties at BINA Sub-station Rangpur

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Dates × Mutants/Variety× Spacing									
$D_1V_1S_1$	116.5	8.9	8.4	25.5	174.0	18.7	19.1	5.82	6.03
$D_1V_1S_2$	113.6	10.7	9.7	23.0	156.9	24.7	19.6	5.67	6.72
$D_1V_2S_3$	118.1	12.8	11.3	27.1	173.4	24.1	19.7	5.32	6.53
$D_1V_2S_1$	99.3	9.3	8.5	25.4	124.3	27.4	22.1	5.27	6.40
$D_1V_2S_2$	100.6	11.8	10.9	25.5	122.9	24.8	22.3	5.37	5.91
$D_1V_2S_3$	101.0	12.8	11.9	25.5	116.7	29.0	22.2	5.37	6.50
$D_1V_3S_1$	94.2	10.7	9.7	22.1	137.9	21.4	22.3	4.00	5.14
$D_1V_3S_2$	97.8	12.5	11.5	22.1	146.3	21.2	22.1	3.80	5.58
$D_1V_3S_3$	98.8	12.9	11.7	19.7	125.8	18.8	22.2	3.60	5.18
$D_2V_1S_1$	118.9	14.1	12.3	27.5	182.3	9.3	19.8	5.88	7.13
$D_2V_1S_2$	120.2	13.7	12.2	27.4	179.3	7.9	20.2	4.97	6.25
$D_2V_2S_3$	120.6	15.1	12.9	27.2	175.3	7.5	19.6	5.53	6.83
$D_2V_2S_1$	106.9	11.7	10.5	25.3	145.3	15.3	22.2	5.37	7.16
$D_2V_2S_2$	109.0	13.2	11.3	25.1	141.3	14.9	22.1	5.67	7.14
$D_2V_2S_3$	109.5	13.2	11.9	25.4	129.5	13.9	22.5	5.30	7.16
$D_2V_3S_1$	110.9	13.2	11.4	23.8	142.5	16.1	22.5	5.70	7.43
$D_2V_3S_2$	110.7	13.6	12.0	23.7	137.9	17.9	22.4	5.78	7.03
$D_2V_3S_3$	108.7	14.2	12.2	22.8	144.9	18.8	22.7	5.67	6.98
$D_3V_1S_1$	115.2	10.3	9.4	25.9	149.8	17.3	20.2	4.67	5.89
$D_3V_1S_2$	116.6	9.4	9.1	25.9	143.9	13.0	19.5	4.03	5.43
$D_3V_2S_3$	115.7	9.1	8.6	25.7	141.1	15.1	19.9	3.80	5.52
$D_3V_2S_1$	109.3	11.9	10.1	23.9	118.9	19.7	22.3	4.33	5.77
$D_3V_2S_2$	110.1	14.5	12.2	23.3	115.5	19.1	22.2	4.13	5.65
$D_3V_2S_3$	111.9	15.5	12.8	24.5	118.1	21.5	22.3	3.70	4.83
$D_3V_3S_1$	106.1	10.5	9.6	21.1	114.9	22.1	22.4	4.77	6.14
$D_3V_3S_2$	108.7	14.0	12.2	23.6	134.1	11.3	22.0	4.30	5.85
$D_3V_3S_3$	103.7	14.7	12.3	24.1	126.6	12.6	22.7	4.27	6.24
$D_4V_1S_1$	121.1	9.3	8.7	28.3	176.0	12.0	19.1	5.03	6.09
$D_4V_1S_2$	120.9	11.9	10.3	28.5	178.1	18.0	19.7	4.33	5.46
$D_4V_2S_3$	119.6	13.4	11.4	29.7	168.9	17.4	19.7	3.77	5.30
$D_4V_2S_1$	108.5	9.6	8.8	23.1	124.1	27.4	22.2	4.37	5.50
$D_4V_2S_2$	109.1	10.0	9.3	23.6	118.5	24.8	22.2	3.87	5.15
$D_4V_2S_3$	108.6	9.8	8.7	23.8	124.3	25.7	22.5	3.40	4.79
$D_4V_3S_1$	109.5	10.3	9.1	23.3	133.9	19.4	22.2	3.87	5.14
$D_4V_3S_2$	111.9	12.9	11.3	23.2	130.8	22.2	22.4	4.17	5.31
$D_4V_3S_3$	108.8	12.5	10.9	23.8	151.1	18.8	22.4	3.83	5.18
$LSD_{0.05}$	4.8	2.4	1.6	1.5	21.0	7.7	0.8	0.56	0.68
CV%	5.4	12.3	9.2	5.7	10.6	25.2	2.2	7.43	10.96

D_1 = January 3, D_2 = January 15, D_3 = February 01, D_4 = February 15, S_1 = 20 cm × 15 cm, S_2 = 20 cm × 20 cm S_3 = 20 cm × 25 cm, V_1= RM-40(c)-4-2-8, V_2 = Binadhan-18, V_3 = BRRI dhan28.

Table 7. Effect of and dates of transplanting, genotypes and spacing on yield contributing characters of RM-40(c)-4-2-8rice mutant during boro season 2019-20

Treatments	Plant height (cm)	Total tillers hill^{-1} (no.)	Effective tillers hill^{-1} (no.)	Panicle length (cm)	Filled grains panicle^{-1} (no.)	Unfilled grains panicle^{-1} (no.)	1000 seed weight (g.)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Crop duration (days)
Dates of transplanting										
January 15 (D_1)	107.4	10.2	9.4	26.2	106.1	26.7	21.2	5.82	6.77	
February 01 (D_2)	107.8	10.0	8.9	25.3	111.4	25.8	21.2	5.72	6.76	
February 15 (D_3)	106.6	10.3	9.2	25.3	96.7	27.7	20.5	5.49	6.65	
$LSD_{0.05}$	2.5	1.1	1.2	0.9	8.1	3.6	0.7	0.17	0.15	
Mutant/Varieties										
RM-40(c)-4-2-8 (V_1)	99.9	10.6	9.7	24.3	81.3	21.9	23.0	5.12	6.18	142
Binadhan-18 (V_2)	114.7	9.7	8.6	27.0	128.2	31.6	19.0	6.23	7.28	145
T value	1.8	1.2	NS	0.4	8.0	NS	0.2	0.23	0.38	
Dates × Varieties										
D_1V_1	100.5	10.8	10.0	25.4	81.1	19.5	23.1	5.25	6.30	
D_1V_2	114.4	9.5	8.7	27.0	131.2	33.9	19.2	6.39	7.25	
D_2V_1	100.3	10.1	8.8	23.6	91.2	20.6	23.5	5.15	6.13	
D_2V_2	115.3	9.9	8.9	27.0	131.7	30.9	19.0	6.29	7.39	
D_3V_1	98.9	11.0	10.3	23.8	71.7	25.6	22.3	4.98	6.11	
D_3V_2	114.3	9.5	8.1	26.9	121.6	29.9	18.7	6.00	7.20	
$LSD_{0.05}$	2.6	2.0	1.9	1.0	12.4	3.0	0.8	0.16	0.49	
CV (%)	2.9	7.0	8.1	2.2	5.9	8.5	2.2	1.91	3.62	

Conclusion

It was concluded from the findings that grain yield at 20 cm × 15 cm, spacing, the mutant RM-40(c)-4-2-8 produced maximum yield 5.88 t ha^{-1} in Rangpur followed by 5.86 t ha^{-1} in Mymensingh when transplanted on January 15. To ensure satisfactory yield of mutant line of RM-40(c)-4-2-8 might be express full potentialities in boro season at 35 days age seedling, if edaphic condition, favorable weather parameters and management practices of 20 cm × 15 cm spacing on January 15 transplanting.

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EFFECT OF SOWING TIME AND SPACING ON GROWTH, YIELD AND YIELD CONTRIBUTING CHARACTERS OF MUNGBEAN MUTANTS IN THREE LOCATIONS OF BANGLADESH

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Abstract

Field experiments were conducted with mungbean mutants to evaluate their yield performance as affected by time of sowing and spacing. The mutant lines were MBM-656-51-2 and MBM-427-87-3, and the check varieties were BARI Mung8 and Binamung-8. Total four experiments were conducted of which two experiments were executed at Bangladesh Institute of Nuclear Agriculture (BINA) substations, Barishal and Ishurdi during 2018 and other two experiments at BINA substations, Ishurdi and Magura during 2019. Four different sowing times were Jan. 15, Feb. 1, Feb. 15, and Mar. 1 during 2018 and Feb. 15, Feb. 28, Mar. 15 and Mar. 30 during 2019. Three levels of row spacing were 20 cm, 25 cm and 30 cm. During 2018, the interaction effect of sowing time, variety and row spacing showed that Binamung-8 produced the maximum seed yield (1157 kg ha^{-1}) at 20 cm row spacing when the sowing date was Feb 15; followed by MBM-656-51-2 with the same sowing date and spacing. The advanced mutant line MBM-656-51-2 required the least average crop duration of 72 days) and BARI Mung8 required the maximum average duration of 79 days) during 2018; which required 75 days and 78 days during 2019, respectively. During 2019, the interaction effect of sowing date, variety and row spacing showed that MBM-656-51-2 produced the maximum seed yield (843.3 kg ha^{-1}) followed by BARI Mung-8 (828.6 kg ha^{-1}) at 25 cm row spacing when the sowing time was Feb 18. Overall results suggest that to ensure satisfactory yield of mungbean, Feb. 15 to Feb. 28 sowing at 20-25 cm row spacing is needed for all the studied locations.

Key words: Mungbean, spacing, mutant, sowing time, optimum yield

Introduction

Mungbean (*Vigna radiate* L.*)* is an important grain legume in Bangladesh. Pulses are central to many culinary traditions in many countries, and they are a cornerstone of food and nutritional security. With rapid increase in global food needs on the horizon, the role of pulses will become even more significant, especially with regard to dietary protein and micronutrients. It holds the 4th in both acreage (24%) and production in Bangladesh (Alam, 2015). Mungbean play an important role in solving the protein malnutrition in Bangladesh. The country is facing an acute shortage of mungbean due to low yield per unit area and less production. Mungbean cultivated almost throughout the year in the diverse agro-climatic condition of Bangladesh. Its edible seed is characterized by higher digestibility, flavour, high protein content and absence of any flatulence effects (Ahmad *et al.*, 2008). The total

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production of pulses in Bangladesh is only 0.65 million tons against the requirement 2.7 million tons (MoA, 2013). This means the shortage is almost 76% of the total requirement and this is mostly due to low yield (MoA, 2013). Mungbean seed contains about 1-3% fat, 5.4% carbohydrates, 25.67% protein, 3.5-4.5% fibers and 4.5-5.5% ash while calcium and phosphorus are 132 and 367 mg per 100 grams of seed, respectively *(*Haider *et al., 2018*). Mungbean, in particular, is rich in easily digestible form of protein. It contains a high degree of proteins, minerals and vitamins. Genetic potential of legume is not obtained at farmer's field due to poor soil nutrient status, mineral deficiency etc. (Crews *et al*., 2004). Farmers cultivated mungbean by one ploughing and hardly use minimum fertilizers and irrigation. There is a huge scope to increase the mungbean yield through proper fertilizers management practices. The practical implications of packaging mutant line/varieties with optimal management practices are better understood. Climate change is an important new driver for genetic improvement that anticipates future shift of temperature and precipitation. Therefore, the present study was undertaken to find out the optimum sowing date and spacing of advanced mutant line of mungbean for better growth and yield.

Materials and methods

The experiment was carried out at the Field Laboratory of the Department of Agronomy, Bangladesh Institute of Nuclear Agriculture, BINA sub-station Ishurdi and Barishal during Rabi season of 2018 and at BINA substation, Ishurdi and Magura during 2019. The climatic parameters during the growing period of mungbean in different time and location are presented in Fig. 1. The experiment was laid out in a split-split design with three replications. The unit plot size was 3m x 4m. The treatments were two advance mutant lines MBM-656-51-2 and MBM-427-87-3, and the check varieties were BARI Mung8 and Binamung-8 randomly distributed to the plots within a block. A drain of one meter wide provided for watering around the whole experimental plot and between the blocks.

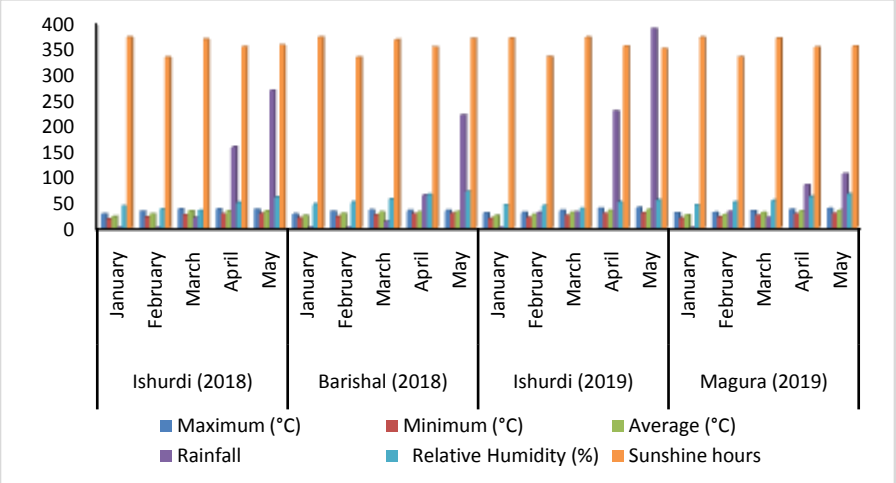

Fig. 1. The climatic parameters during the growing period of mungbean during 2018 and 2019 in different locations.

The plots of Mungbean were fertilized with N, P, K, Zn and Boron respectively according to the recommendation of BARC (2012). The whole amount at triple super phosphate, muriatic of potash, gypsum, Boron and zinc sulphate (separately) were applied to the soil at the time of final land preparation. Mungbean bio-fertilizer was used during sowing of seeds. The maturity of crops was determined when about 70% of the seeds attain their characters color. Seed yields plot were recorded after threshing and sun drying properly. Data recorded for different parameters were subjected to analysis of variance (ANOVA) using statistix-10 software and the treatment means were compared using the least significant different test.

Results and discussions

Results of 1st year (2018)

Mean effects

The results of first year (2018) showed that in case of mean effect of sowing date on seed yield (irrespective of cultivar and spacing), the highest seed yield was produced at February 15 sowing (988 kg ha^{-1}) whereas March 1 sowing produced the lowest seed yield (503 kg ha^{-1}) (Table 1). A substantial higher number of branches per plant and good number of pod per plant contributed to the highest seed yield at Feb. 15 sowing. In early sowing at Jan. 15, there was low soil temperature which caused less germination of seeds and less crop stand. In case of late sowing, due to continuous rainfall overall growth was not up to the mark and higher weed competition was occurred. The mean seed yield of 25 cm row spacing was the highest (792.6 kg ha^{-1}) whereas 30 cm row spacing produced the lowest seed yield (720 kg ha^{-1}). The results are in par with the finding of Miah *et al.,* (2009). Soomro (2003) also reported that delay in sowing caused a substantial decrease in all the growth and development parameters of mungbean.

Table 1. Mean effects of locations, sowing date, mutants/variety and spacing on yield and yield contributing characters during 2018

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $Plant^{-1}$ (no)	Pods $Plant^{-1}$ (no)	Pod Length (cm)	Seeds Pod^{-1} (no)	1000 seed wt. (g)	Seed yield (t ha^{-1})
Location(s)								
Barishal	22.3	44.3	0.4	20.8	8.6	10.6	48.2	664.0
Ishurdi	52.9	55.9	2.6	33.5	8.0	10.1	41.1	853.3
Level of sig.	*	*	*	*	*	*	*	*
Date of Sowing								
Jan. 15 (D_1)	30.3	37.6	1.0	12.6	8.1	10.3	46.2	647.1
Feb. 01 (D_2)	35.4	44.8	0.7	14.6	8.4	10.4	45.3	896.4
Feb. 15 (D_3)	38.6	54.4	4.4	37.5	8.2	10.1	44.8	988.0
Mar. 01 (D_4)	46.0	63.6	0.1	43.8	8.5	10.4	42.4	503.3
$LSD_{0.05}$	2.4	2.0	1.1	4.7	0.2	0.2	1.1	117.1
MBM-656-51-2 (V_1)	37.4	49.9	1.7	25.9	8.8	10.3	43.1	794.0
MBM-427-87-3 (V_2)	37.4	48.0	1.3	27.8	8.5	10.3	48.2	710.9
BARI Mung8 (V_3)	37.8	51.7	1.4	26.4	8.4	10.3	43.4	752.0
Binamung-8 (V_4)	37.8	50.8	1.7	28.4	8.5	10.4	43.9	762.8
$LSD_{0.05}$	NS	1.7	NS	NS	0.2	NS	0.9	NS

Table 1. Continued

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $Plant^{-1}$ (no)	Pods $Plant^{-1}$ (no)	Pod Length (cm)	Seeds Pod^{-1} (no)	1000 seed wt. (g)	Seed yield (t ha^{-1})
Row spacing								
20 cm (S_1)	44.3	50.2	1.5	27.9	8.3	10.3	44.8	763.1
25 cm (S_2)	37.6	50.5	1.6	26.9	8.3	10.2	44.6	792.6
30 cm (S_3)	31.0	49.6	1.6	26.7	8.3	10.4	44.5	720.4
$LSD_{0.05}$	1.8	NS	NS	NS	NS	NS	NS	NS
CV (%)	12.3	9.3	7.6	11.3	7.0	4.2	5.6	13.4

Among the mutant lines and varieties, MBM-656-51-2 produced the highest seed yield (794 kg ha^{-1}) followed by Binamung-8 and BARI Mung8 (Table 1). The canopy coverage, growth and development of mutant line MBM-656-51-2 were good at 25 cm row spacing. These findings closely resembles to those obtained by BINA (2007), BINA (2006), Siddique *et al.*, (2006), Mondal (2004) and Patil *et al.*, (2003).

Interaction effects (2 factors)

In case of sowing date × mutants/variety, the mutant MBM-656-51-2 produced the maximum seed yield (1002 kg ha^{-1}) followed by BARI Mung8 (953 kg ha^{-1}) at same sowing date of Feb. 15 (Table 2). For sowing date × row spacing, sowing at Feb. 15 with 20 cm row spacing produced the maximum seed yield (1066 kg ha^{-1}) followed by sowing at Feb. 15 with 20 cm row spacing (993 kg ha^{-1}). For variety × row spacing, Binamung-8 produced maximum seed yield at 25 cm row spacing (850 kg ha^{-1}) followed by BARI Mung8 at 20cm row spacing (850 kg ha^{-1}) (Table 2). In case of early sowing, less germination and less crop establishment were resulted and ultimately less seed yield was found. Tomar *et al*., (1994) and Miah *et al*., (2009) also reported the similar findings. They found that the interactions of Binamung-5 × 2 March sowing, Binamung-6 × 20 February sowing, Binamung-6 × 2 March sowing and Binamung-7 × 22 March sowing produced similar and the second highest seed yield.

Table 2. Interaction effects of two factors on yield and yield contributing characters during 2018

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $Plant^{-1}$ (no)	Pods $Plant^{-1}$ (no)	Pod Length (cm)	Seeds Pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
Date of Sowing × Mutant/variety								
D_1V_1	29.7	36.1	1.0	12.6	7.8	10.2	46.3	518.9
D_1V_2	29.7	36.4	1.0	10.7	7.9	10.2	47.5	585.5
D_1V_3	31.6	37.7	1.1	13.7	8.4	10.4	44.5	678.9
D_1V_4	30.2	40.3	1.0	13.3	8.5	10.6	46.5	805.2
D_2V_1	35.9	44.6	0.6	14.2	7.9	10.3	43.9	966.2
D_2V_2	34.6	44.0	0.7	14.5	8.6	10.6	46.6	870.5
D_2V_3	35.6	45.8	0.6	14.7	8.6	10.4	45.0	889.9
D_2V_4	35.7	45.0	0.8	15.1	8.5	10.5	45.5	859.0
D_3V_1	39.2	52.9	5.0	38.0	7.4	10.2	42.1	1002.1
D_3V_2	37.7	52.0	4.3	37.4	8.7	10.1	50.1	909.3
D_3V_3	38.2	57.9	4.7	36.3	8.4	10.4	43.6	953.5

Table 2. Continued

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $Plant^{-1}$ (no)	Pods $Plant^{-1}$ (no)	Pod Length (cm)	Seeds Pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
D_3V_4	39.4	54.7	3.4	38.5	8.4	9.9	43.2	947.0
D_4V_1	44.7	66.1	0.3	38.8	8.1	10.3	40.0	509.0
D_4V_2	47.6	59.6	0.9	48.7	8.8	10.4	48.7	478.5
D_4V_3	45.9	65.4	0.7	41.0	8.4	10.1	40.5	515.8
D_4V_4	45.9	63.3	1.7	46.8	8.6	10.5	40.2	509.9
$LSD_{0.05}$	5.4	4.3	2.4	10.4	0.5	0.4	2.3	256.6
Date of Sowing × Row spacing								
D_1S_1	34.8	37.7	0.9	11.5	8.1	10.3	46.0	560.2
D_1S_2	30.5	38.2	1.0	12.6	8.1	10.4	46.6	698.8
D_1S_3	25.5	37.0	1.1	13.6	8.2	10.3	45.9	682.3
D_2S_1	41.4	43.8	0.6	13.2	8.4	10.4	45.6	921.3
D_2S_2	35.0	45.0	0.7	15.5	8.4	10.4	44.7	906.8
D_2S_3	30.0	45.7	0.7	15.2	8.3	10.5	45.6	861.0
D_3S_1	44.8	54.7	4.9	40.2	8.3	10.1	45.0	1066.9
D_3S_2	38.9	53.3	3.9	35.2	8.1	9.9	44.9	993.4
D_3S_3	32.2	55.1	4.2	37.3	8.3	10.4	44.3	903.6
D_4S_1	56.0	64.6	-0.6	46.5	8.3	10.3	42.7	503.9
D_4S_2	45.8	65.7	0.7	44.3	8.7	10.2	41.9	571.1
D_4S_3	36.3	60.6	0.3	40.8	8.4	10.6	42.5	434.8
$LSD_{0.05}$	4.6	3.8	2.1	9.0	0.5	0.3	2.0	222.2
Mutant/variety × Row spacing								
V_1S_1	42.9	48.8	1.4	24.7	7.7	10.4	42.6	771.4
V_1S_2	37.9	50.9	1.5	25.7	7.8	10.1	43.1	777.8
V_1S_3	31.2	50.0	2.3	27.3	7.9	10.3	43.6	697.9
V_2S_1	43.3	50.2	1.3	32.2	8.5	10.2	49.0	700.1
V_2S_2	37.3	47.7	1.1	24.7	8.5	10.3	47.5	778.1
V_2S_3	31.6	46.0	1.4	26.6	8.5	10.4	48.2	654.6
V_3S_1	44.7	50.2	1.2	24.6	8.5	10.3	43.7	806.8
V_3S_2	37.8	53.9	1.4	28.3	8.5	10.2	43.7	763.6
V_3S_3	30.9	50.9	1.7	26.4	8.4	10.5	42.8	775.7
V_4S_1	46.1	51.4	1.9	29.9	8.5	10.3	43.9	774.0
V_4S_2	37.1	49.6	2.3	28.8	8.6	10.3	43.9	850.8
V_4S_3	30.2	51.4	0.9	26.6	8.4	10.5	43.8	753.5
$LSD_{0.05}$	3.7	3.0	NS	7.1	0.4	0.3	1.5	175.7
CV (%)	12.3	9.3	7.6	11.3	7.0	4.2	5.6	13.4

Interaction effects (3 factors)

From the interaction effect of sowing date, cultivar and row spacing (Table 3), the highest seed yield was recorded for Binamung 8 with 20 cm row spacing sown in Feb.15 followed by MBM 656-51-2 with same spacing and sowing date. The result complies with the findings of Ahmad *et al.,* (2021). The seed yield is the interplay of yield components. The maximum seed yield in the early sown at the end of January crop can be due to maximum emergence count, longer pods with more seeds and seed weight as compared to the other SD (Khattak *et al.,* 2006; Ahmad *et al.,* 2008).

Table 3. Interaction effect of three factors on yield and yield contributing characters of mungbean during 2018

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $Plant^{-1}$ (no)	Pods $Plant^{-1}$ (no)	Pod Length (cm)	Seeds Pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
Sowing date × Mutant/variety × Row spacing								
$D_1V_1S_1$	34.2	35.0	0.8	11.2	7.3	10.3	45.1	423.6
$D_1V_1S_2$	29.3	37.8	1.0	12.1	8.0	10.1	47.6	536.8
$D_1V_1S_3$	25.5	35.6	1.2	14.5	8.2	10.2	46.2	596.2
$D_1V_2S_1$	34.5	37.5	1.0	10.9	8.3	10.2	47.3	508.3
$D_1V_2S_2$	28.8	37.1	0.9	10.4	7.5	10.4	46.6	635.0
$D_1V_2S_3$	25.7	34.5	1.0	10.8	7.9	9.8	48.5	613.1
$D_1V_3S_1$	35.8	36.4	0.9	11.7	8.1	10.2	45.2	600.8
$D_1V_3S_2$	33.2	37.9	1.1	14.4	8.4	10.6	44.8	693.7
$D_1V_3S_3$	25.7	38.7	1.2	15.0	8.5	10.4	43.5	742.1
$D_1V_4S_1$	34.8	41.7	1.0	12.4	8.6	10.6	46.4	708.2
$D_1V_4S_2$	30.8	39.9	1.1	13.6	8.6	10.7	47.5	929.9
$D_1V_4S_3$	25.0	39.2	1.1	14.0	8.2	10.6	45.5	777.5
$D_2V_1S_1$	41.5	44.3	0.5	12.8	7.8	10.2	43.6	1023.3
$D_2V_1S_2$	37.0	43.4	0.6	15.4	7.8	10.5	43.1	966.9
$D_2V_1S_3$	29.2	46.1	0.6	14.4	8.1	10.3	45.0	908.2
$D_2V_2S_1$	40.8	43.1	0.6	13.1	8.6	10.5	46.6	921.3
$D_2V_2S_2$	34.8	45.1	0.6	15.5	8.7	10.5	46.1	904.0
$D_2V_2S_3$	28.2	43.7	0.8	15.0	8.5	10.7	47.2	786.1
$D_2V_3S_1$	40.3	45.0	0.6	13.0	8.6	10.4	45.9	839.1
$D_2V_3S_2$	34.2	45.9	0.7	15.8	8.8	10.4	44.4	932.9
$D_2V_3S_3$	32.3	46.4	0.7	15.3	8.2	10.4	44.8	897.8
$D_2V_4S_1$	42.8	42.8	0.7	14.1	8.5	10.4	46.1	901.4
$D_2V_4S_2$	33.8	45.6	1.0	15.2	8.3	10.4	45.1	823.6
$D_2V_4S_3$	30.3	46.6	0.7	16.1	8.6	10.6	45.3	852.1
$D_3V_1S_1$	43.7	54.0	4.9	39.9	7.6	10.1	42.0	1055.5
$D_3V_1S_2$	40.0	52.3	4.5	35.7	7.3	10.0	42.2	1010.4
$D_3V_1S_3$	33.8	52.2	5.6	38.5	7.4	10.5	42.1	940.2
$D_3V_2S_1$	41.3	53.0	4.5	43.0	8.7	9.8	51.8	940.6
$D_3V_2S_2$	39.3	48.7	3.4	34.1	8.7	10.2	50.0	956.9
$D_3V_2S_3$	32.3	54.4	4.9	35.2	8.7	10.4	48.5	830.5
$D_3V_3S_1$	45.0	58.4	4.7	34.3	8.4	10.6	43.6	1157.1
$D_3V_3S_2$	39.0	58.0	4.8	35.5	8.3	10.2	44.4	953.4
$D_3V_3S_3$	30.7	57.4	4.7	39.1	8.5	10.3	42.8	920.1
$D_3V_4S_1$	49.2	53.3	5.5	43.7	8.5	9.9	42.6	1014.6
$D_3V_4S_2$	37.3	54.3	2.9	35.5	8.3	9.4	43.1	1052.7
$D_3V_4S_3$	31.8	56.4	1.7	36.3	8.5	10.3	44.0	923.6
$D_4V_1S_1$	52.4	62.0	0.8	35.0	8.0	10.8	39.8	583.1
$D_4V_1S_2$	45.4	70.2	0.1	39.6	8.3	10.0	39.3	596.9
$D_4V_1S_3$	36.4	66.2	1.9	42.0	8.0	10.2	41.0	346.9
$D_4V_2S_1$	56.4	67.4	1.1	62.0	8.3	10.2	50.4	430.3
$D_4V_2S_2$	46.4	60.0	0.4	39.0	9.1	10.2	47.2	616.4
$D_4V_2S_3$	40.0	51.5	1.1	45.3	9.0	10.9	48.5	388.6
$D_4V_3S_1$	57.7	61.2	1.1	39.3	8.6	10.0	40.2	530.3
$D_4V_3S_2$	45.0	73.8	1.1	47.6	8.3	9.7	41.4	474.2
$D_4V_3S_3$	35.0	61.2	0.2	36.0	8.3	10.7	40.0	542.8
$D_4V_4S_1$	57.7	67.8	0.6	49.6	8.3	10.2	40.4	472.0
$D_4V_4S_2$	46.4	58.8	4.2	51.0	9.1	10.8	39.7	596.9
$D_4V_4S_3$	33.7	63.4	0.2	40.0	8.3	10.5	40.5	460.8
$LSD_{0.05}$	9.2	7.5	4.2	18.0	0.9	0.7	4.0	444.4
CV (%)	12.3	9.3	7.6	11.3	7.0	4.2	5.6	13.4

Interaction effects (4 factors)

From the interaction effect of location, date of sowing, mutant/variety, and row spacing (Table 4), the highest seed yield (1198.6 kg ha^{-1}) was found in Barishal at Feb. 15 sowing for the variety BARI Mung8 with 20 cm spacing; which is followed by the mutant line MBM-656-51-2 (1173.6 kg ha^{-1}) at same sowing date and spacing in Ishurdi.

Table 4. Interaction effects of locations, sowing dates, cultivars and spacing on yield and yield contributing characters of mungbean during 2018

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $plant^{-1}$ (no)	Pods $plant^{-1}$ (no)	Pod length (cm)	Seeds pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
Location × Sowing time × Mutant/variety × Row spacing								
$L_1D_1V_1S_1$	9.7	25.0	1.2	11.2	7.3	10.4	46.9	202.8
$L_1D_1V_1S_2$	9.7	27.8	1.3	12.9	8.2	10.4	49.6	257.5
$L_1D_1V_1S_3$	8.7	27.5	1.6	16.4	8.0	10.3	47.4	243.9
$L_1D_1V_2S_1$	8.7	30.3	1.4	10.9	8.5	10.5	48.3	245.8
$L_1D_1V_2S_2$	7.7	29.6	1.3	8.8	7.9	10.4	47.2	236.7
$L_1D_1V_2S_3$	10.0	25.5	1.2	9.0	8.4	10.4	49.9	161.1
$L_1D_1V_3S_1$	11.3	29.4	1.1	11.2	8.6	10.8	49.2	323.6
$L_1D_1V_3S_2$	13.7	29.6	1.5	15.7	8.4	10.6	48.6	314.4
$L_1D_1V_3S_3$	10.7	32.2	1.5	16.3	9.1	10.6	47.8	403.7
$L_1D_1V_4S_1$	14.0	32.3	1.5	13.4	8.8	10.8	49.9	534.6
$L_1D_1V_4S_2$	15.3	30.1	1.3	15.0	8.9	10.8	50.0	661.2
$L_1D_1V_4S_3$	12.3	30.2	1.4	15.4	8.8	10.6	49.1	637.1
$L_1D_2V_1S_1$	24.7	38.0	0.7	13.0	7.9	10.5	46.9	988.9
$L_1D_2V_1S_2$	23.3	36.3	0.6	16.8	8.3	10.5	47.4	864.4
$L_1D_2V_1S_3$	18.7	39.9	0.8	14.9	8.5	10.5	50.8	761.1
$L_1D_2V_2S_1$	22.7	37.6	0.5	13.4	8.8	10.5	50.2	787.3
$L_1D_2V_2S_2$	18.3	39.4	0.7	15.7	8.7	10.8	51.1	697.2
$L_1D_2V_2S_3$	17.7	35.8	0.8	14.9	8.6	10.7	52.9	641.6
$L_1D_2V_3S_1$	24.0	37.7	0.7	13.3	9.1	10.5	48.6	618.5
$L_1D_2V_3S_2$	18.3	39.7	0.8	18.9	9.0	10.7	45.0	671.3
$L_1D_2V_3S_3$	21.7	39.3	0.8	16.2	8.6	10.5	47.1	587.5
$L_1D_2V_4S_1$	25.7	37.9	0.8	15.4	8.7	10.4	49.8	913.9
$L_1D_2V_4S_2$	17.0	41.0	1.1	17.3	8.7	10.4	48.1	787.5
$L_1D_2V_4S_3$	21.3	41.0	0.8	18.5	8.8	10.6	48.3	787.5
$L_1D_3V_1S_1$	29.7	51.1	0.5	15.1	8.6	10.7	48.9	1152.7
$L_1D_3V_1S_2$	26.7	45.7	0.7	16.7	8.0	10.7	49.3	1173.6
$L_1D_3V_1S_3$	24.0	49.2	0.8	16.3	7.6	10.5	49.2	930.5
$L_1D_3V_2S_1$	23.0	49.7	0.7	17.6	9.3	10.5	54.9	1047.2
$L_1D_3V_2S_2$	27.7	47.3	0.7	17.1	9.2	10.5	52.5	1191.6
$L_1D_3V_2S_3$	23.7	48.9	0.5	16.8	8.8	10.4	51.6	1036.1
$L_1D_3V_3S_1$	28.3	54.7	0.7	14.5	8.6	10.4	46.4	1115.3
$L_1D_3V_3S_2$	27.0	57.7	0.7	17.0	8.6	10.6	47.5	1073.6
$L_1D_3V_3S_3$	21.7	53.7	0.8	17.2	8.9	10.6	45.8	1076.4
$L_1D_3V_4S_1$	35.0	50.8	0.4	17.7	8.7	10.5	48.4	1126.4
$L_1D_3V_4S_2$	26.3	53.4	0.5	19.0	8.6	10.7	49.0	1118.1
$L_1D_3V_4S_3$	23.3	54.9	0.8	18.6	8.6	10.6	50.0	1013.8
$L_1D_4V_1S_1$	67.7	67.8	0.3	41.3	7.7	10.6	36.2	677.7
$L_1D_4V_1S_2$	60.7	76.0	1.0	46.0	8.0	10.0	35.7	691.6
$L_1D_4V_1S_3$	51.7	72.0	3.0	48.3	7.7	9.7	37.4	441.6
$L_1D_4V_2S_1$	71.7	73.2	0.0	68.3	8.0	10.6	46.8	525.0
$L_1D_4V_2S_2$	61.7	65.8	0.7	45.3	8.8	10.0	43.6	711.1
$L_1D_4V_2S_3$	55.3	57.3	0.0	51.7	8.7	9.9	44.9	483.3
$L_1D_4V_3S_1$	73.0	67.0	0.0	45.7	8.3	10.5	36.6	625.0
$L_1D_4V_3S_2$	60.3	79.6	0.0	54.0	8.0	9.8	37.8	568.9

Table 4. Continued

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $plant^{-1}$ (no)	Pods $plant^{-1}$ (no)	Pod length (cm)	Seeds pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
$L_1D_4V_3\ S_3$	50.3	67.0	1.3	42.3	8.0	9.5	36.4	637.5
$L_1D_4V_4\ S_1$	73.0	73.6	1.7	56.0	8.0	10.5	36.9	566.6
$L_1D_4V_4\ S_2$	61.7	64.6	5.3	57.3	8.8	10.3	36.1	691.6
$L_1D_4V_4\ S_3$	49.0	69.2	1.3	46.3	8.0	9.9	36.9	555.5
$L_2D_1V_1S_1$	58.7	45.0	0.5	11.3	7.3	10.2	43.2	644.4
$L_2D_1V_1\ S_2$	49.0	47.8	0.7	11.3	7.7	10.2	45.6	816.1
$L_2D_1V_1\ S_3$	42.3	43.5	0.8	12.6	8.3	9.8	44.9	948.6
$L_2D_1V_2\ S_1$	60.3	45.4	0.6	10.9	8.0	9.9	46.3	770.8
$L_2D_1V_2\ S_2$	50.0	43.7	0.6	12.0	7.0	9.3	45.9	1033.3
$L_2D_1V_2\ S_3$	41.3	43.5	0.8	12.6	7.3	10.4	47.2	1065.2
$L_2D_1V_3\ S_1$	60.3	43.4	0.6	12.1	7.7	10.0	41.2	878.0
$L_2D_1V_3\ S_2$	52.7	46.1	0.7	13.1	8.3	9.8	41.0	1073.0
$L_2D_1V_3\ S_3$	40.7	45.2	0.9	13.8	8.0	10.5	39.2	1080.5
$L_2D_1V_4\ S_1$	55.7	51.0	0.5	11.3	8.3	10.6	45.1	881.9
$L_2\ D_1V_4\ S_2$	46.3	49.7	0.8	12.1	8.3	10.6	43.0	1198.6
$L_2D_1V_4\ S_3$	37.7	48.0	0.7	12.7	7.7	10.3	41.8	918.0
$L_2D_2V_1S_1$	58.3	50.5	0.4	12.6	7.7	10.0	40.2	1057.7
$L_2D_2V_1\ S_2$	50.7	50.4	0.5	14.0	7.3	9.8	38.8	1069.4
$L_2D_2V_1\ S_3$	39.7	52.3	0.5	14.0	7.7	10.5	39.3	1055.4
$L_2D_2V_2\ S_1$	59.0	48.5	0.7	12.7	8.3	10.6	43.0	1055.3
$L_2D_2V_2\ S_2$	51.3	50.8	0.6	15.3	8.7	10.5	41.0	1110.9
$L_2D_2V_2\ S_3$	38.7	51.6	0.8	15.1	8.5	10.3	41.4	930.5
$L_2D_2V_3\ S_1$	56.7	52.2	0.4	12.7	8.2	10.2	43.3	1059.7
$L_2D_2V_3\ S_2$	50.0	52.0	0.6	12.7	8.7	10.2	43.8	1194.4
$L_2D_2V_3\ S_3$	43.0	53.5	0.6	14.5	7.8	10.1	42.6	1108.1
$L_2D_2V_4\ S_1$	60.0	47.6	0.7	12.8	8.3	10.7	42.4	888.8
$L_2D_2V_4\ S_2$	50.7	50.1	0.8	13.1	7.8	10.4	42.1	859.7
$L_2D_2V_4\ S_3$	39.3	52.3	0.6	13.8	8.3	10.4	42.3	916.6
$L_2D_3V_1S_1$	57.7	56.8	9.3	64.7	6.7	9.5	35.1	958.3
$L_2D_3V_1\ S_2$	53.3	58.8	8.3	54.7	6.6	9.4	35.2	847.2
$L_2D_3V_1\ S_3$	43.7	55.2	10.3	60.7	7.2	10.4	34.9	950.0
$L_2D_3V_2\ S_1$	59.7	56.2	8.3	68.3	8.2	10.3	48.6	834.0
$L_2D_3V_2\ S_2$	51.0	50.1	6.0	51.0	8.2	9.9	47.5	722.2
$L_2D_3V_2\ S_3$	41.0	59.8	9.3	53.7	8.6	9.0	45.5	625.0
$L_2D_3V_3\ S_1$	61.7	62.0	8.7	54.0	8.2	10.6	40.8	998.9
$L_2D_3V_3\ S_2$	51.0	58.2	9.0	54.0	8.0	10.1	41.3	833.3
$L_2D_3V_3\ S_3$	39.7	61.0	8.7	61.0	8.0	9.9	39.8	763.8
$L_2D_3V_4\ S_1$	63.3	55.8	10.7	69.7	8.3	9.9	36.9	902.8
$L_2D_3V_4\ S_2$	48.3	55.0	5.3	52.0	8.0	9.3	37.2	801.3
$L_2D_3V_4\ S_3$	40.3	57.9	2.7	54.0	8.3	8.3	37.9	833.3
$L_2D_4V_1S_1$	67.7	67.8	0.3	41.3	7.7	10.6	36.2	677.7
$L_2D_4V_1\ S_2$	60.7	76.0	1.0	46.0	8.0	10.0	35.7	691.6
$L_2D_4V_1\ S_3$	51.7	72.0	3.0	48.3	7.7	9.7	37.4	441.6
$L_2D_4V_2\ S_1$	71.7	73.2	0.0	68.3	8.0	10.6	46.8	525.0
$L_2D_4V_2\ S_2$	61.7	65.8	0.7	45.3	8.8	10.0	43.6	711.1
$L_2D_4V_2\ S_3$	55.3	57.3	0.0	51.7	8.7	9.9	44.9	483.3
$L_2D_4V_3\ S_1$	73.0	67.0	0.0	45.7	8.3	9.8	36.6	625.0
$L_2D_4V_3\ S_2$	60.3	79.6	0.0	54.0	8.0	9.5	37.8	568.9
$L_2D_4V_3\ S_3$	50.3	67.0	1.3	42.3	8.0	10.5	36.4	637.5
$L_2D_4V_4\ S_1$	73.0	73.6	1.7	56.0	8.0	10.5	36.9	566.6
$L_2D_4V_4\ S_2$	61.7	64.6	5.3	57.3	8.8	10.3	36.1	691.6
$L_2D_4V_4\ S_3$	49.0	69.2	1.3	46.3	8.0	9.9	36.9	555.5
$LSD_{0.05}$	9.9	11.2	0.5	5.8	2.78	1.5	5.5	111.7
CV (%)	12.3	9.3	7.6	11.3	7.0	4.2	5.6	13.4

Results of 2nd year (2019)

Mean effects

The mean effect of sowing date on seed yield showed that the highest seed yield (752.8 kg ha^{-1}) was produced at Feb. 28 sowing while March 30 sowing produced the lowest (658.3 kg ha^{-1}) (Table 5). The seed yield of 25 cm row spacing was the highest (764.7 kg ha^{-1}) whereas 30 cm row spacing produced the lowest (705 kg ha^{-1}). Singh *et al.,* (2010) and Singh *et al.,* (2013) also reported similar results.

Among the mutant lines/varieties, MBM-656-51-2 produced the highest seed yield (794.5 kg ha^{-1}) followed by MBM-427-87-3 and BARI Mung8. The potential yield was not found due to the reason that second picking could not be done for early rainfall which caused germination of seed in pod and rotten thereafter. So, lower yield was found in second year compared to first year experiments. These results are in line with those of Sarkar *et al.,* (2004) and Khan and Malik (2001).

Table 5. Mean effects of location, dates of sowing, cultivars and spacing on yield and yield contributing characters of mungbean during 2019

Treatment	Populations m^{-2} (no.)	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Pod length (cm)	Seeds pod^{-1} (no.)	1000 seed wt. (g)	Seed yield (t ha^{-1})
Location(s)								
Ishurdi (L_1)	39.6	44.3	0.8	10.8	7.4	10.2	47.1	740.2
Magura (L_2)	40.6	51.4	0.8	10.5	7.4	10.3	41.4	739.7
T value	NS	*	NS	NS	NS	NS	*	NS
Sowing date								
Feb. 15 (D_1)	38.9	26.8	0.8	8.6	7.4	9.7	45.1	679.6
Feb. 28 (D_2)	40.1	40.6	0.5	12.0	8.1	10.1	44.2	752.8
Mar. 15 (D_3)	41.7	54.3	1.1	9.7	7.0	10.2	43.7	709.2
Mar. 30 (D_4)	39.7	69.6	0.7	12.2	7.3	10.9	42.4	658.3
$LSD_{0.05}$	NS	21.9	NS	NS	NS	NS	1.3	42.5
Mutant/varieties								
MBM-656-51-2 (V_1)	39.5	46.1	0.7	10.2	7.4	10.3	43.2	794.5
MBM-427-87-3 (V_2)	40.7	48.6	0.8	10.7	7.3	10.3	48.1	763.8
BARI Mung-8 (V_3)	40.5	48.4	0.8	11.0	7.4	10.2	43.2	705.6
Binamung-8 (V_4)	39.6	48.3	0.8	10.6	7.5	10.3	43.6	696.0
$LSD_{0.05}$	NS	3.1	NS	NS	NS	NS	0.6	20.6
Row spacing								
20 cm (S_1)	41.1	47.4	0.7	10.0	7.4	10.2	43.7	729.3
25 cm (S_2)	40.0	49.1	0.8	10.7	7.4	10.3	44.2	764.7
30 cm (S_3)	39.2	47.0	0.8	11.1	7.5	10.2	43.4	705.8
$LSD_{0.05}$	1.1	1.7	NS	0.9	NS	NS	NS	15.6
CV (%)	10.1	12.3	17.4	10.0	14.4	11.2	8.1	7.2

Interaction effect (2 factors)

The interaction effect of sowing date and mutants/variety showed that MBM-656-51-2 produced the maximum seed yield (817.8 kg ha^{-1}) followed by BARI Mung8 (799.3 kg ha^{-1}) at same sowing date of Feb 28 (Table 6). The sowing date × row spacing showed maximum seed yield (783.9 kg ha^{-1}) at Feb. 28 sowing with 25 cm row spacing followed by Feb 15 sowing with 25 cm row spacing (756.7 kg ha^{-1}). The cultivar × row spacing showed that Binamung-8 produced maximum seed yield (809 kg ha^{-1}) at 25 cm row spacing followed by BARI Mung-8 (791.8 kg ha^{-1}) at 25cm row spacing. Sadeghipour, (2008) also found similar results.

Table 6. Mean effects of two factors on yield and yield contributing characters of mungbean during 2019

Treatment	Populations m^{-2} (no.)	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Pod length (cm)	Seeds pod^{-1} (no.)	1000 seed wt. (g)	Seed yield (t ha^{-1})
Sowing date × Mutant/varieties								
D_1V_1	39.2	26.3	0.7	8.3	7.2	9.5	46.4	784.4
D_1V_2	39.9	25.6	0.8	8.5	7.3	9.9	47.5	755.0
D_1V_3	38.1	26.5	0.8	9.4	7.5	9.6	44.3	711.1
D_1V_4	38.3	28.9	0.9	8.4	7.7	10.0	46.5	700.0
D_2V_1	37.8	37.4	0.4	12.1	8.3	10.0	43.4	817.8
D_2V_2	40.7	41.5	0.4	11.3	8.1	10.2	46.1	763.0
D_2V_3	41.5	41.8	0.6	11.8	7.9	10.2	45.3	799.7
D_2V_4	40.4	41.9	0.6	12.8	8.0	10.1	45.5	699.4
D_3V_1	40.1	50.4	0.9	8.7	6.9	10.6	42.1	764.1
D_3V_2	42.6	56.7	1.2	10.7	6.9	10.2	50.1	775.6
D_3V_3	42.6	55.7	1.2	9.5	6.9	9.9	43.4	708.9
D_3V_4	41.6	54.5	1.0	9.8	7.2	10.0	43.2	694.4
D_4V_1	40.9	70.4	0.7	11.6	7.3	11.0	40.2	776.1
D_4V_2	39.8	70.5	0.6	12.2	7.1	10.7	48.7	761.7
D_4V_3	40.0	69.5	0.6	13.4	7.4	11.0	40.1	693.3
D_4V_4	38.2	67.9	0.8	11.5	7.3	11.0	40.1	690.0
$LSD_{0.05}$	3.5	2.8	0.3	1.9	NS	NS	2.1	41.2
Sowing date × Row spacing								
D_1S_1	39.5	25.6	0.8	8.4	7.0	9.9	46.3	750.4
D_1S_2	38.1	29.0	0.9	8.3	7.7	10.0	46.1	756.7
D_1S_3	38.9	25.9	0.8	9.3	7.6	9.3	45.9	705.8
D_2S_1	41.5	41.7	0.5	11.1	8.6	10.0	45.2	753.1
D_2S_2	40.5	40.6	0.5	12.7	7.7	10.2	44.7	783.9
D_2S_3	38.4	39.6	0.5	12.2	7.9	10.2	45.5	711.3
D_3S_1	43.0	53.1	1.0	9.4	7.0	10.0	45.5	755.8
D_3S_2	41.6	56.9	1.0	9.3	6.8	10.2	44.4	742.5
D_3S_3	40.6	53.1	1.2	10.2	7.1	10.4	44.3	719.2
D_4S_1	40.4	69.3	0.7	11.2	7.2	11.0	42.5	739.6
D_4S_2	39.9	70.1	0.6	12.6	7.3	10.9	41.4	734.2
D_4S_3	38.8	69.3	0.7	12.7	7.2	10.9	42.1	687.1
$LSD_{0.05}$	2.3	1.7	NS	NS	0.6	0.5	1.6	31.3

Table 6. Continued

Treatment	Populations m^{-2} (no.)	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Pod length (cm)	Seeds pod^{-1} (no.)	1000 seed wt. (g)	Seed yield (t ha^{-1})
Mutant/variety × Row spacing								
V_1S_1	39.8	46.6	0.7	9.9	7.5	10.2	42.7	775.7
V_1S_2	39.2	46.4	0.6	10.0	7.2	10.3	43.2	809.4
V_1S_3	39.6	45.5	0.7	10.6	7.6	10.3	43.5	760.8
V_2S_1	42.0	47.8	0.8	10.4	7.2	10.3	49.1	770.8
V_2S_2	40.8	49.0	0.8	10.3	7.4	10.3	47.5	777.7
V_2S_3	39.5	49.0	0.7	11.3	7.4	10.2	48.2	712.9
V_3S_1	42.0	48.7	0.8	10.4	7.5	10.2	43.4	735.8
V_3S_2	40.4	49.1	0.8	11.3	7.5	10.5	43.7	791.8
V_3S_3	39.2	47.3	0.8	11.3	7.2	9.8	42.9	660.0
V_4S_1	40.7	46.7	0.7	9.4	7.5	10.3	43.9	719.6
V_4S_2	39.7	52.1	0.8	11.3	7.5	10.1	43.7	703.8
V_4S_3	38.5	46.1	0.9	11.2	7.6	10.5	43.2	664.6
$LSD_{0.05}$	2.3	3.4	NS	1.8	0.6	0.5	1.3	31.3
CV (%)	10.1	12.3	17.4	10.0	14.4	11.2	8.1	7.2

Interaction effects (3 factors)

The interaction effect of date of sowing, mutant/variety and row spacing showed that the highest seed yield (843.3 kg ha^{-1}) was found for the mutant line MBM-656-51-2 sowing at Feb. 28 with 25 cm row spacing; which is followed by the BARI Mung-8 (828 kg ha^{-1}) at same sowing date and spacing (Table 7). This result agrees with the findings of Yoldas and Esiyok (2007) and Corokalo *et al.*, (1992).

Table 7. Mean effects of different dates of sowing and spacing on yield and yield contributing characters of mungbean mutants/variety during 2019

Treatment	Populations m^{-2} (no.)	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Pod length (cm)	Seeds pod^{-1} (no.)	1000 seed weight (g)	Seed yield (t ha^{-1})
Sowing date× Mutant/variety × Row spacing								
$D_1V_1S_1$	38.7	25.8	0.7	7.8	6.3	9.7	45.1	776.7
$D_1V_1S_2$	36.7	25.9	0.7	8.2	7.5	9.8	47.6	783.3
$D_1V_1S_3$	42.1	27.2	0.8	9.0	7.7	9.1	46.2	773.3
$D_1V_2S_1$	41.4	25.9	0.9	9.3	6.6	10.1	47.3	781.7
$D_1V_2S_2$	39.3	26.5	0.9	8.5	7.8	10.0	46.6	773.3
$D_1V_2S_3$	38.9	24.5	0.6	7.7	7.5	9.5	48.5	710.0
$D_1V_3S_1$	38.8	26.1	0.7	9.0	7.7	10.0	45.2	726.7
$D_1V_3S_2$	38.5	25.6	0.8	7.8	7.7	9.8	44.8	730.0
$D_1V_3S_3$	36.9	27.8	0.9	11.4	7.2	8.9	43.3	676.7
$D_1V_4S_1$	39.2	24.6	0.9	7.6	7.3	9.9	46.4	716.7
$D_1V_4S_2$	38.0	37.8	1.0	8.6	7.8	10.5	47.5	720.0
$D_1V_4S_3$	37.8	24.3	0.8	9.0	7.8	9.7	45.2	663.3
$D_2V_1S_1$	38.9	38.1	0.3	11.1	9.6	9.7	43.6	805.8

Table 7. Continued

Treatment	Populations m^{-2} (no.)	Plant height (cm)	Branches $plant^{-1}$ (no.)	Pods $plant^{-1}$ (no.)	Pod length (cm)	Seeds pod^{-1} (no.)	1000 seed weight (g)	Seed yield (t ha^{-1})
$D_2V_1S_2$	39.7	36.3	0.4	12.6	7.5	10.1	43.1	843.3
$D_2V_1S_3$	34.9	37.7	0.6	12.4	7.8	10.2	45.3	790.0
$D_2V_2S_1$	41.5	43.4	0.6	11.0	8.1	10.4	46.6	783.3
$D_2V_2S_2$	42.0	40.8	0.5	11.7	8.0	10.2	46.1	790.7
$D_2V_2S_3$	38.7	40.4	0.2	11.2	8.2	10.0	47.1	715.0
$D_2V_3S_1$	43.2	43.1	0.5	11.1	8.3	10.0	45.9	726.7
$D_2V_3S_2$	40.4	40.8	0.6	12.2	7.8	10.7	44.3	828.6
$D_2V_3S_3$	41.0	41.3	0.6	12.3	7.7	10.0	44.8	660.0
$D_2V_4S_1$	42.3	42.2	0.4	11.2	8.4	10.1	46.1	696.7
$D_2V_4S_2$	40.0	44.4	0.6	14.3	7.7	9.7	45.1	721.7
$D_2V_4S_3$	39.0	38.9	0.6	13.0	8.0	10.6	45.3	680.0
$D_3V_1S_1$	41.0	52.4	0.8	9.2	6.9	10.2	42.2	806.7
$D_3V_1S_2$	40.0	54.0	0.7	7.9	6.6	10.7	42.2	821.3
$D_3V_1S_3$	39.4	44.9	1.0	9.0	7.3	11.0	42.1	817.3
$D_3V_2S_1$	42.3	54.9	1.0	9.6	6.9	10.0	51.8	803.3
$D_3V_2S_2$	43.2	58.4	1.3	9.3	6.5	10.1	50.0	773.3
$D_3V_2S_3$	42.4	56.8	1.4	13.1	7.2	10.6	48.5	730.0
$D_3V_3S_1$	44.8	53.0	1.3	9.6	6.8	9.6	43.6	760.0
$D_3V_3S_2$	42.2	59.2	1.3	10.2	7.1	10.3	44.4	706.7
$D_3V_3S_3$	40.8	54.9	1.2	8.8	6.7	9.7	42.8	660.0
$D_3V_4S_1$	44.0	52.0	1.0	9.3	7.3	10.2	42.6	733.3
$D_3V_4S_2$	41.0	55.9	0.8	10.0	7.0	9.6	43.1	686.7
$D_3V_4S_3$	39.7	55.7	1.2	10.0	7.3	10.2	44.0	663.3
$D_4V_1S_1$	40.4	69.8	0.9	11.3	7.1	11.1	39.8	801.7
$D_4V_1S_2$	40.5	69.2	0.6	11.5	7.2	10.7	39.3	770.0
$D_4V_1S_3$	41.8	72.2	0.5	12.0	7.4	11.2	41.3	756.7
$D_4V_2S_1$	42.6	66.9	0.6	11.7	7.1	10.7	50.4	815.0
$D_4V_2S_2$	38.7	70.3	0.5	11.7	7.4	11.0	47.2	773.3
$D_4V_2S_3$	37.9	74.3	0.6	13.2	6.9	10.6	48.5	696.7
$D_4V_3S_1$	41.4	72.7	0.5	12.0	7.3	11.0	40.4	730.0
$D_4V_3S_2$	40.6	70.6	0.5	15.2	7.5	11.3	41.4	706.7
$D_4V_3S_3$	38.0	65.2	0.7	12.9	7.3	10.6	40.0	643.3
$D_4V_4S_1$	37.3	67.8	0.6	9.7	7.2	11.0	40.4	731.7
$D_4V_4S_2$	39.9	70.4	0.8	12.1	7.3	10.6	39.7	686.7
$D_4V_4S_3$	37.5	65.4	0.9	12.7	7.4	11.4	40.4	651.7
$LSD_{0.05}$	4.5	6.9	0.5	3.6	1.3	1.1	3.2	62.5
CV (%)	10.1	12.3	17.4	10.0	14.4	11.2	8.1	7.2

Interaction effects (4 factors)

The interaction effects of location, date of sowing, mutant/variety and row spacing showed that the highest seed yield (841.3 kg ha^{-1}) was produced in Ishurdi at Feb. 28 sowing, for the mutant line MBM-656-51-2 at 25 cm spacing; which is followed by the mutant line MBM-656-51-2 (829.7 kg ha^{-1}) at same sowing date and at 20cm row spacing in Magura (Table 8).

Table 8. Interaction effects of location, cultivar, sowing date and spacing on yield and yield contributing characters of during 2019

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $plant^{-1}$ (no)	Pods $plant^{-1}$ (no)	Pod length (cm)	Seeds pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
Location × Sowing time × Mutant/variety × Row spacing								
$L_1D_1V_1S_1$	34.3	20.7	1.1	7.2	6.8	9.6	37.5	726.7
$L_1D_1V_1S_2$	33.8	20.7	1.1	7.5	6.8	9.0	34.5	803.3
$L_1D_1V_1S_3$	42.5	20.8	1.3	8.9	6.7	9.5	39.9	723.3
$L_1D_1V_2S_1$	41.8	21.1	1.5	9.0	6.8	9.7	40.0	740.0
$L_1D_1V_2S_2$	43.0	20.8	1.2	7.8	6.9	9.3	41.2	773.3
$L_1D_1V_2S_3$	42.0	21.1	0.9	9.0	6.9	9.0	37.2	690.0
$L_1D_1V_3S_1$	44.0	21.3	1.1	8.7	7.0	9.5	41.3	693.3
$L_1D_1V_3S_2$	38.3	19.9	0.9	6.1	7.0	9.7	37.8	753.3
$L_1D_1V_3S_3$	37.0	21.6	1.1	8.4	7.0	9.0	34.7	693.3
$L_1D_1V_4S_1$	44.0	20.9	1.1	6.2	6.7	10.1	37.5	700.0
$L_1D_1V_4S_2$	39.3	45.4	1.3	8.7	7.1	9.7	36.3	753.3
$L_1D_1V_4S_3$	30.5	19.1	1.2	8.3	7.1	10.0	34.4	663.3
$L_1D_2V_1S_1$	45.2	31.3	0.5	11.0	7.3	8.9	39.3	785.0
$L_1D_2V_1S_2$	38.0	28.0	1.2	14.5	8.1	10.0	46.7	841.3
$L_1D_2V_1S_3$	39.3	31.8	0.7	12.6	7.5	9.3	33.2	756.7
$L_1D_2V_2S_1$	42.3	36.1	0.8	10.7	7.7	10.4	40.6	743.3
$L_1D_2V_2S_2$	45.0	34.6	0.7	11.0	8.0	10.4	41.8	808.0
$L_1D_2V_2S_3$	33.3	34.3	0.3	12.3	8.2	10.5	36.0	700.0
$L_1D_2V_3S_1$	42.5	37.3	0.8	13.3	8.1	9.8	44.0	693.3
$L_1D_2V_3S_2$	38.5	36.5	0.6	14.7	7.3	10.2	40.5	773.3
$L_1D_2V_3S_3$	34.8	38.1	0.7	10.9	7.6	9.7	38.3	660.0
$L_1D_2V_4S_1$	35.3	36.3	0.6	11.1	8.2	10.0	39.4	660.0
$L_1D_2V_4S_2$	38.3	38.5	0.7	13.3	7.2	9.4	36.7	756.7
$L_1D_2V_4S_3$	33.5	34.3	0.6	13.3	7.5	10.2	39.2	696.7
$L_1D_3V_1S_1$	33.0	47.1	0.5	10.7	7.6	10.0	44.2	826.7
$L_1D_3V_1S_2$	31.8	46.8	0.6	12.2	7.4	10.7	43.3	803.3
$L_1D_3V_1S_3$	38.0	44.2	0.8	11.4	8.0	11.3	40.3	823.3
$L_1D_3V_2S_1$	36.0	45.8	0.9	11.1	7.7	10.7	41.6	821.2
$L_1D_3V_2S_2$	35.7	47.3	0.8	12.3	7.2	10.7	40.8	773.3
$L_1D_3V_2S_3$	36.2	42.5	1.1	14.9	7.7	10.5	44.2	730.0
$L_1D_3V_3S_1$	37.5	45.9	0.8	11.6	7.6	10.8	45.0	760.0
$L_1D_3V_3S_2$	36.8	50.6	0.5	11.7	8.0	11.2	42.5	706.7
$L_1D_3V_3S_3$	32.3	47.1	1.1	12.3	7.5	10.7	43.3	660.0
$L_1D_3V_4S_1$	33.3	47.7	0.7	9.8	7.8	10.5	44.2	733.3
$L_1D_3V_4S_2$	31.3	47.3	0.8	13.4	7.9	9.9	42.5	686.7
$L_1D_3V_4S_3$	39.3	46.0	0.9	13.6	7.5	9.8	41.7	663.3
$L_1D_4V_1S_1$	35.0	72.9	0.3	9.6	7.5	10.9	44.1	816.9
$L_1D_4V_1S_2$	30.0	74.8	0.2	11.1	7.6	10.9	41.1	803.3
$L_1D_4V_1S_3$	30.0	74.6	0.9	11.4	7.6	11.3	42.2	812.3
$L_1D_4V_2S_1$	35.0	71.1	0.6	12.1	7.2	11.1	42.8	825.3
$L_1D_4V_2S_2$	42.5	72.7	0.5	10.0	7.8	11.1	37.9	773.3
$L_1D_4V_2S_3$	32.5	75.7	0.3	11.2	7.6	10.9	35.7	730.0
$L_1D_4V_3S_1$	44.5	76.5	0.5	12.2	7.7	11.4	38.6	760.0
$L_1D_4V_3S_2$	36.5	72.9	0.4	12.2	7.8	10.9	39.5	706.7
$L_1D_4V_3S_3$	30.0	68.4	0.7	10.4	8.0	10.7	40.4	660.0
$L_1D_4V_4S_1$	35.8	73.0	0.2	8.7	7.3	11.3	35.8	733.3
$L_1D_4V_4S_2$	30.0	75.5	0.7	12.1	7.6	10.5	36.2	686.7
$L_1D_4V_4S_3$	27.5	67.1	0.5	11.3	7.4	10.5	35.8	663.3

Table 8. Continued

Treatment	Populations m^{-2} (no)	Plant height (cm)	Branches $plant^{-1}$ (no)	Pods $plant^{-1}$ (no)	Pod length (cm)	Seeds pod^{-1} (no)	1000 seed weight (g)	Seed yield (t ha^{-1})
$L_2D_1V_1S_1$	38.0	31.0	0.9	8.5	5.9	9.7	40.0	826.7
$L_2D_1V_1S_2$	40.0	31.1	0.3	8.9	8.2	10.7	38.8	803.3
$L_2D_1V_1S_3$	31.0	33.5	0.3	9.1	8.8	8.7	44.3	823.3
$L_2D_1V_2S_1$	44.2	30.7	0.4	9.5	6.3	10.5	42.8	823.3
$L_2D_1V_2S_2$	38.0	32.1	0.7	9.1	8.7	10.8	37.3	773.3
$L_2D_1V_2S_3$	35.6	27.9	0.2	6.4	8.1	9.9	40.7	730.0
$L_2D_1V_3S_1$	45.0	30.9	0.3	9.3	8.3	10.4	36.3	760.0
$L_2D_1V_3S_2$	37.5	31.3	0.7	9.5	8.4	9.9	39.2	706.7
$L_2D_1V_3S_3$	37.5	34.1	0.7	14.4	7.4	8.8	39.2	660.0
$L_2D_1V_4S_1$	40.0	28.3	0.6	9.0	7.9	9.7	40.8	733.3
$L_2D_1V_4S_2$	37.5	30.3	0.6	8.4	8.6	11.3	39.7	686.7
$L_2D_1V_4S_3$	42.5	29.5	0.5	9.7	8.6	9.3	41.2	663.3
$L_2D_2V_1S_1$	45.0	45.0	0.2	11.3	11.9	10.4	38.5	829.7
$L_2D_2V_1S_2$	40.0	44.7	0.2	15.7	7.6	10.3	42.7	803.3
$L_2D_2V_1S_3$	38.5	43.6	0.5	12.3	8.1	11.0	36.7	823.3
$L_2D_2V_2S_1$	42.5	50.7	0.3	11.3	8.5	10.4	42.5	823.3
$L_2D_2V_2S_2$	45.0	47.0	0.3	12.4	7.9	10.0	42.1	773.3
$L_2D_2V_2S_3$	40.0	46.4	0.1	10.1	8.1	9.5	41.3	730.0
$L_2D_2V_3S_1$	42.5	48.9	0.3	8.9	8.5	10.3	42.3	760.0
$L_2D_2V_3S_2$	47.5	45.1	0.5	9.7	8.3	11.1	40.3	706.7
$L_2D_2V_3S_3$	47.5	44.5	0.5	13.7	7.8	10.3	43.6	660.0
$L_2D_2V_4S_1$	42.5	48.2	0.2	11.2	8.5	10.2	45.1	733.3
$L_2D_2V_4S_2$	42.5	50.3	0.5	15.4	8.3	9.9	43.3	686.7
$L_2D_2V_4S_3$	47.5	43.5	0.7	12.7	8.4	11.1	38.8	663.3
$L_2D_3V_1S_1$	45.0	57.1	1.2	7.7	6.3	10.4	37.8	826.7
$L_2D_3V_1S_2$	40.0	61.1	0.9	3.5	5.7	10.6	36.6	803.3
$L_2D_3V_1S_3$	40.8	45.5	1.3	6.6	6.6	10.6	38.6	823.3
$L_2D_3V_2S_1$	39.8	64.1	1.1	8.2	6.2	9.4	43.0	823.3
$L_2D_3V_2S_2$	40.0	69.4	1.8	6.3	5.7	9.5	45.6	773.3
$L_2D_3V_2S_3$	42.5	71.1	1.6	11.3	6.6	10.7	40.6	730.0
$L_2D_3V_3S_1$	47.5	60.1	1.7	7.6	6.0	8.5	44.6	760.0
$L_2D_3V_3S_2$	42.5	67.7	2.1	8.7	6.1	9.4	41.8	706.7
$L_2D_3V_3S_3$	42.5	62.6	1.3	5.3	5.9	8.7	38.3	660.0
$L_2D_3V_4S_1$	47.5	56.3	1.4	8.7	6.8	9.9	43.8	733.3
$L_2D_3V_4S_2$	42.5	64.5	0.9	6.7	6.1	9.3	39.6	686.7
$L_2D_3V_4S_3$	42.5	65.4	1.4	6.5	7.1	10.7	37.8	663.3
$L_2D_4V_1S_1$	47.5	66.8	1.5	13.1	6.7	11.4	36.7	776.7
$L_2D_4V_1S_2$	42.5	63.7	1.0	11.9	6.9	10.6	40.0	736.7
$L_2D_4V_1S_3$	40.0	69.8	0.5	12.6	7.3	11.1	41.4	690.0
$L_2D_4V_2S_1$	45.0	62.7	0.7	11.3	7.0	10.3	42.5	806.7
$L_2D_4V_2S_2$	42.5	67.9	0.6	13.3	7.0	10.8	39.5	773.3
$L_2D_4V_2S_3$	42.5	72.9	0.9	15.1	6.1	10.3	40.2	663.3
$L_2D_4V_3S_1$	47.5	68.9	0.6	11.8	6.9	10.7	44.2	700.0
$L_2D_4V_3S_2$	42.5	68.3	0.6	18.2	7.2	11.7	41.7	706.7
$L_2D_4V_3S_3$	40.0	61.9	0.7	15.3	6.6	10.5	39.8	626.7
$L_2D_4V_4S_1$	40.0	62.7	1.0	10.7	7.1	10.7	38.8	730.0
$L_2D_4V_4S_2$	45.0	65.2	0.9	12.2	6.9	10.7	39.3	686.7
$L_2D_4V_4S_3$	42.5	63.8	1.3	14.1	7.5	12.3	39.2	640.0
$LSD_{0.05}$	8.9	11.0	0.6	5.14	1.78	1.85	6.5	106.7
CV (%)	10.1	12.3	17.4	10.0	14.4	11.2	8.1	7.2

Conclusions

The total yield and yield attributing features of mungbean reveal that the advance mutant lines MBM-656-51-2 produced better yield during a two-year experiment in three agro-ecological zones. Overall results suggest that to ensure satisfactory yield of mungbean mutants sowing at Feb.15 to Feb. 28 sowing at 20 to 25 cm row spacing should be maintained at Barishal, Ishurdi and Magura regions.

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STUDY ON EFFICACY OF TRICHODERMA IN BIOLOGICAL CONTROL AGAINST PURPLE BLOTCH OF ONION

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Abstract

The efficacy of the antagonistic microorganism, *Trichoderma harzianum* in controlling purple blotch of onion (*Alternaria porri)* was investigated in the laboratory and field of Plant Pathology Division of the Bangladesh Institute of Nuclear Agriculture, Mymensingh. The antagonistic activity of three isolates of *T. harzianum* was observed against *A. porri* through dual culture technique and the superior one was used in the field experiment. Five treatments were given viz. T_1: Seed treatment with *T. harzianum*, T_2: Seedling treatment with *T. harzianum*, T_3: Foliar application with *T. harzianum*, T_4: Seed treatment and seedling treatment with *T. harzianum* and T_5: Control (*A. porri* in absence of *T. harzianum*). The variety BARIpiaj-1 was used as tested crop. All treatments significantly reduced purple blotch disease intensity compared to the control. The least disease intensity on leaf and flower stalk was found in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*). The highest disease reduction over control (63.1% on leaf and 47.18% on flower stalk) was observed in the treatment T_4 (seed and seedling treatment with *T. harzianum*) followed by the seed treatment only (T_1) which gave disease reduction 58.7% on leaf and 37.03% on flower stalk over control. Therefore, application of *T. harzianum* both in seed and seedling was more effective in reducing disease intensity than individual application of *T harzianum* in seed or seedling.

Key words: *Trichoderma harzianum*, biological control, onion, purple blotch

Introduction

Onion (*Allium cepa* L.), family Alliaceae is economically an important horticulture crop cultivated all over the world. In Bangladesh it is one of the most important spices crop and an integral part of Bangladeshi diet. It is known as protective food because of its special nutritive value. Onions are rich in sulphur, fibres, potassium, iron, calcium, vitamin B, vitamin C but low in fat, cholesterol. Apart from being used as food it is also famous for its medicinal values. Onion contains anticancer compounds that assist in inhibiting the growth of cancer cells and thus protect against the development of colon and liver cancer (Rahim, 1991). Antioxidants are also provided by onion with its sweet flavor and distinct aroma.

In Bangladesh, onion is commercially cultivated in the greater district of Faridpur, Rajshahi, Jessore, Pabna and Kushtia. The average yield of onion is 9.71 t ha^{-1} (BBS, 2020) which is very low as compared to other leading onion growing countries like China, India,

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Egypt and USA. Onion may suffer from 22 fungal diseases among which purple blotch caused by *Alternaria porri* (Ellis) is noted as the major disease in many onion growing countries including Bangladesh (Meah and Khan, 1987). Purple blotch can infect all above ground parts of the plant as well as the bulb. Initial symptoms appear on older leaves as small water soaked lesions that quickly develop purple center with yellow margin under favorable conditions (Verma and Sharma, 1999). In Bangladesh, it is reported that the disease can reduce bulb yield up to 41-44% (Islam *et al.*, 2020). In favorable condition there may cause complete failure of onion bulb and seed production due to the disease (Sharma, 1986). Damage of foliage and breaking of floral stalks due to purple blotch is considered as the major factor for the reduction of true seed production of onion in our country.

The foliar fungicide Rovral (dicarboximide group) was found to be effective against the disease (Rahman, 1990). However synthetic fungicides have adverse effect on environment, human health and these also have negative impact on the beneficial microorganisms in the soil (Mimbs *et al*, 2016). Thus it is appropriate to minimize the use of the fungicides in crop production. Biological control for the management of plant pathogens is considered as an alternative environment friendly strategy for sustainable agriculture where *Trichoderma* sp. has gained attention as an effective fungal antagonist against foliage and soil borne pathogens (Amin *et al.*, 2010). The antagonist is able to produce compounds that can induce resistance in host plant against the pathogen. In addition to disease suppression, treatment with the antagonistic organisms can increase root growth, uptake of nutrients, productivity of plants (Harman, 2006). The efficacy of *T. harzianum, T. pseudokoningii* and *T. virens* on the inhibition of mycelial growth and spore germination of *A. porri* were observed by other researchers (Imtiaz and Lee, 2008 and Tyagi *et al.*, 1990). With this view, the present study was undertaken to investigate the efficacy of the antagonistic microorganism *T. harzianum* in controlling purple blotch of onion.

Materials and Methods

The experiment was conducted in the laboratory and field at the Plant Pathology Division of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during 2017-18.

Sources and maintenance of *Trichoderma harzianum* and *Alternaria porri*

The isolates of *T. harzianum* were obtained from the Plant Pathology Division, BINA. Pure culture of *T. harzianum* was made in PDA plates following hyphal tip culture technique (Tuite, 1969) and preserved at 5ºC for further use. The isolate of *A. porii* was obtained from onion leaf showing typical symptom of purple blotch collected from the experimental field of BINA at Mymensingh. Infected plant parts were cut into 3 mm segments including the advancing margins of infection. The segments were surface disinfected with 0.5% sodium hypochlorite solution for 2 minutes, washed thoroughly with sterilized water and dried between folds of filter paper. Then the segments were transferred in PDA plates and incubated for 7 days at 28ºC. Pure culture was obtained by sub-culturing

for three times. Pathogenicity test on the crop was carried out following the method as described by Abdel Hafez (2014). Pure culture of the final isolate was maintained on PDA plates and kept in the refrigerator (5ºC) until used.

In vitro evaluation of *T. harzianum* against *A. porri*

The antagonistic activity of three isolates of *T. harzianum* was evaluated against *A. porri* through dual culture technique. Both *T. harzianum* and *A. porri* were cultured individually on PDA media in petridishes. A disc (5mm diameter) of five days old culture of *T. harzianum* was inoculated on one side of PDA plate (9 cm) and another disc of *A. porri* of the same size from ten days old culture was inoculated at the opposite side in the same plate. The distance between the discs was approximately 5 cm. In the control treatment, a sterile PDA disc (5mm diameter) was placed instead of *T. harzianum* only. The plates were incubated at 27±1ºC for 7 days. The design used in *in vitro* experiment was Completely Randomized Design (CRD) with five replications. The percentage of inhibition by *T. harzianum* in the growth of the pathogen was calculated according to the following formula (Rini and Sulochana, 2007):

$$\text{Percentage growth inhibition} = (C-T)/C \times 100$$

Here,

C = the radial mycelia growth of *A. porri* in control plate (mm)

T = the radial mycelia growth of *A. porri* in presence of *T. harzianum* (mm)

Preparation of mass inocula of *T. harzianum* and *A. porri*

Inoculum of *T. harzianum* was made in chickpea bran following the method of Dubey and Patel (2002). Chickpea bran was soaked in water for 12 hours. Around 20g chickpea bran was taken in a conical flask of 500 ml and was autoclaved at 120ºC under 15 lbs for 30 minutes. The sterilized substrate in the conical flask was inoculated with 5 mycelial discs (5mm diameter) from 3 days old culture of *T. harzianum* previously grown on PDA. The flasks were incubated at 25ºC for 15 days with intermittent hand shaking at 5 days. For the inoculum of *A. porri,* the pathogen was grown on petri plates (9 cm diameter) containing PDA for ten days at 26ºC. The mycelial growth was carefully scraped with a sterilized needle after adding 10 ml sterilized water to each plate. The obtained conidial suspension was used for inoculation.

Field experiments

To test the efficacy of *T. harzianum* against *A. porri* in the field condition, an experiment was conducted during rabi season of 2017-18 at BINA farm, Mymensingh. The variety BARIpiaj-1 was used in the experiment. Five treatments were given viz. T_1: Seed treatment with *T. harzianum*, T_2: Seedling treatment with *T. harzianum*, T_3: Foliar application with *T. harzianum*, T_4: Seed and seedling treatment with *T. harzianum* and T_5: Control (*A. porri* in absence of *T. harzianum*).

Seed treatment with *T. harzianum* was done following the method of Begum *et al.* (1998). The surface of seeds was moistened with sterilized water. The seeds were taken in petri dishes having 7 days old culture of *T. harzianum* growing in PDA. The seeds were stirred gently with a sterilized glass rod so that the whole surface of the seeds was coated with the culture of *T. harzianum*. Then the coated seeds were air dried for 1 hour. The number of conidia on treated seeds was counted in a haemacytometer and $2x10^6$ conidia $seed^{-1}$ was estimated. For seedling treatment, the inoculum of *T. harzianum* (grown in chickpea bran) was suspended in water (@ 4g inocula L^{-1} water) and sieved. Seedlings of one month old were dipped in the inocula suspension (10^8conidia ml^{-1}) for half an hour before transplanting. For foliar treatment the same inocula suspension (10^8conidia ml^{-1}) was applied at 30 days after transplanting. The onion plants were inoculated by spraying with conidial suspension of *A. porri* (10^6 ml^{-1}) after two days of application of *T. harzianum* inoculants.

The soil in the seed bed was well prepared and levelled. The bed size was 1.5m x 3m and it was 6 cm high from the field level. Urea 95g, TSP 75g and 10 kg cowdung were applied in the bed during final land preparation. About 120g seeds of BARIpiaj-1 were soaked in water for 12 hours. The wet seeds were taken in cotton bag and kept it at room temperature (24ºC) for 48 hours. The sprouted seeds were sown in the seed bed. Beds were covered with gunny bags for 6 days and after that the bags were removed. The field was prepared by four ploughings and cross ploughings. The experiments were laid out in a randomized complete block design with three replications. The unit plot size was 1.5m x 1.0m. The row to row and plant to plant spacing was 25 cm and 15 cm, respectively. The recommended dose of fertilizer and cowdung were applied in the field. One month old seedlings were transplanted to the well prepared plot. Inoculation of *T. harzianum* and *A. porri* was done as mentioned earlier. Weeding was done two times during the growing period. Irrigation was given to maintain the soil moisture as and when necessary. Disease assessment was done on leaf and flower stalk at 15 days intervals started just after the onset of disease symptoms in the experimental plots. Disease scoring was recorded according to 0-5 scale (Sharma, 1986):

Score 0	No symptom of disease
Score 1	A few spots towards the tip covering less than 10% of leaf area/ flower stalk
Score 2	Several dark purplish brown patches covering less than 20% of leaf area/ flower stalk
Score 3	Several dark purplish brown patches covering less than 40% of leaf area/ flower stalk
Score 4	Long streaks, covering up to 75% of leaf area/flower stalk or breaking of the leaves/ flower stalk from the center
Score 5	Complete drying of the leaves/flower stalk or breaking of the leaves/flower stalk from the base

The percent disease intensity (PDI) was calculated by using the following formula (Wheeler, 1969):

$$PDI = (TNR \times 100) \div (TIL \times MDR)$$

Where, PDI = percent disease intensity, TNR = Total sum of numerical ratings, TIL = total number of infected leaves observed and MDR = maximum disease rating.

Data on plant height (cm), bulb diameter (cm), bulb fresh weight (g) and yield (t ha^{-1}) were also recorded.

Results and Discussion

Table 1. Percent inhibition of growth of *A. porri* induced by *T. harzianum* in PDA plates

Trichoderma isolates	Inhibition (%)		
	3DAI	5DAI	7DAI
TI-1	16.5a	53.2a	62.7a
TI-2	8.7b	33.0c	45.8c
TI-3	9.8b	35.2b	48.5b
LSD (P≥0.05)	2.04	1.96	1.61

DAI= Days after inoculation. In a column data followed by the same letter are statistically similar at 5% level of significance.

In the *in vitro* evaluation of *T. harzianum* against *A. porri*, the three isolates of the antagonist inhibited the mycelial growth of the pathogen (Table 1). The isolate TI-1 inhibited the growth of *A. porii* up to 62.7% at 7 days after inoculation (DAI) followed by TI-3 (48.5%). The least inhibition was found in TI-2 (45.8%). The *in vitro* test indicated that the isolate TI-1 was superior to other ones in suppressing the growth of *A. porri* in PDA media. Prakasam and Sharma (2012) recorded significant inhibition of *A. porri* by *T. harzianum* up to 61.5% in the *in vitro* evaluation. In another study, among five biocontrol agents *T. harzianum* gave maximum inhibition (79.5%) of *A. porri* (Chethana *et al.*, 2013). The inhibition of the pathogen by *Trichoderma* on PDA medium may be due to diffusible antibiotic production and mycoparasitism (Kumar, 2013). Protease and fungal cell wall degrading enzymes make *Trichoderma* an attractive biocontrol agent for plant pathogenic fungi (Elad, 2000).

In the field experiment the disease intensity of purple blotch of onion on leaf and flower stalk due to the application of different treatments is shown in the Table 2 and the Table 3. The percent disease intensity (PDI) on leaf was recorded from 45 days after transplanting (DAT) to 90 DAT and it was found that PDI gradually increased in all treatments with time (Table 2). However, all treatments significantly reduced purple blotch disease intensity compared to the control. At 90 DAT, the disease intensity on leaf ranged from 29.21-79.16%. The least PDI (29.21%) was found in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*). The highest disease reduction over control (63.1%) was observed in the treatment T_4 (Seed and seedling treatment with *T. harzianum*) followed by the treatment T_1 (Seed treatment with *T. harzianum*) which was 58.7%. On flower stalk the percent disease intensity (PDI) was recorded from 90 DAT up to 120 DAT. At 120 DAT, the disease intensity ranged from 41.36-78.30% (Table 3). At 90 DAT, The least percentage of disease intensity (41.36%) and the highest disease reduction over control (47.18%) on flower stalk was recorded with the application of *Trichoderma* in seeds and subsequently seedlings. The

disease intensity on leaf at 90 DAT and on flower stalk at 120 DAT was higher in the treatment T_3 (foliar application with *Trichoderma*) than other treatments. The result of the present study indicates that application of *T. harzianum* in different methods was able to reduce purple blotch disease intensity significantly over control. This is in agreement with the study of other researchers where *T. harzianum* showed potential biocontrol activity against *A. porri* (Mishra, 2019 and Shahnaz *et al.*, 2012). Prakasam and Sharma (2012) reported that an isolate of *T. harzianum* was able to reduce onion purple blotch disease 67.7% under greenhouse and 64.8% under field condition. *Trichoderma* spp. might act as bio-control agent by growing and parasitizing towards the pathogen, coiling and penetrating the pathogen hyphae resulting lysis of the cytoplasm of pathogens (Howell, 2003).

Table 2. Percent disease intensity (PDI) on leaf with the various treatments at different days after transplanting

Treatments	45DAT	60DAT	75DAT	90DAT
T_1 (Seed treatment with *T. harzianum*)	12.36c (48.21%)	20.05d (55.98%)	28.41d (57.22%)	31.42d (58.70%)
T_2 (Seedling treatment with *T. harzianum*)	18.40b (22.92%)	23.08c (49.32%)	31.05c (53.25%)	36.56c (53.82%)
T_3 (Foliar application with *T. harzianum*)	16.93b (29.07%)	29.38b (35.40%)	42.47b (36.06%)	50.31b (36.45%)
T_4 (Seed and seedling treatment with *T. harzianum*)	10.06c (57.86%)	18.48d (59.60%)	26.63d (59.91%)	29.21e (63.10%)
T_5 (Control)	23.87a	45.54a	66.42a	79.16a
LSD (P≥0.05)	2.52	2.13	2.04	2.10

DAT = Days after transplanting, In a column data followed by the same letter are statistically similar at 5% level of significance. Data in the parentheses indicate percent disease reduction over control.

Table 3. Percent disease intensity (PDI) on flower stalk with the various treatments at different days after transplanting

Treatments	90DAT	105DAT	120DAT
T_1 (Seed treatment with *T. harzianum*)	22.35c (20.52%)	46.74c (24.89%)	52.42d (33.05%)
T_2 (Seedling with *T. harzianum*)	22.46c (20.13%)	48.70c (21.74%)	57.50c (26.56%)
T_3 (Foliar application with *T. harzianum*)	23.56b (16.23%)	51.39b (17.41%)	61.24b (21.79%)
T_4 (Seed and seedling treatment with *T. harzianum*)	17.40d (38.12%)	38.54d (38.07%)	41.36e (47.18%)
T_5 (Control)	28.12a	62.23a	78.30a
LSD (P≥0.05)	1.02	2.12	2.32

DAT = Days after transplanting, In a column data followed by the same letter are statistically similar at 5% level of significance. Data in the parentheses indicate percent disease reduction over control.

The growth parameters like, plant height, bulb diameter, bulb fresh weight and yield were increased by the application of *T. harzianum* in all the treatments compared to the control (Table 4). The highest plant height (41.30cm), bulb diameter (4.3cm), bulb fresh weight (23.74g) and yield (9.90 t ha^{-1}) were provided in the combined application of soil and seed treatment with *T. harzianum*. In a study, Altintas and Bal (2008) found that yield and quality characters of onion were improved with the application of *Trichoderma* sp. *Trichoderma* spp. can colonize root surface, interact with plant and exchange compounds which bring substantial changes in plant metabolism and thus plant growth and crop yield are enhanced (Gajera *et al.*, 2013).

Table 4. Yield and yield contributing characters of onion as influenced by different treatments

Treatments	Plant height (cm)	Bulb diameter (cm)	Bulb fresh weight (g)	Yield (t ha^{-1})
T_1 (Seed treatment with *T. harzianum*)	40.32ab	3.20b	21.96b	7.84b
T_2 (Seedling treatment with *T. harzianum*)	39.05b	3.60b	18.70c	7.41b
T_3 (Foliar application with *T. harzianum*)	40.37ab	3.81b	19.89c	7.03b
T_4 (Seed and seedling treatment with *T. harzianum*)	41.30a	4.30a	23.74a	9.90a
T_5 (Control)	34.61c	2.51c	13.47d	5.20c
LSD (P≥0.05)	1.50	0.70	1.23	1.41

In a column data followed by the same letter are statistically similar at 5% level of significance.

Conclusion

The results revealed that *T. harzianum* was a potential biocontrol agent in reducing the disease intensity of purple blotch of onion and application of *T. harzianum* could increase the yield of onion. However, the application of *T harzianum* in seed and subsequently seedling was more effective in disease reduction than the individual application of *T harzianum* in seed or seedling.

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FRUIT DIVERSITY OF TEN MANGO (*Mangifera indica* L.) GERMPLASM OF CHAPAINAWABGANJ DISTRICT IN BANGLADESH

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Abstract

An experiment was conducted to study ten mango (*Mangifera indica* L.) germplasm during July 2018 to May 2019 in Shibganj, Chapainawabganj. Fruit characteristics varied greatly among the studied mango germplasm. The germplasm, Fazli was the largest fruit (Length 14.47 cm, Breadth 8.40 cm, Thickness 7.80 cm) having maximum fruit weight 743.33 g and the highest pulp quantity 614.67 g with highest pulp/peel & stone ratio (4.80) among the germplasm. Ripen mango contained the highest TSS (°Brix) in the germplasm Langra (20.67%) which was better than Fazli. The largest stone was recorded in Fazli (Length 11.60 cm, Breadth 4.87 cm, Thickness 2.70 cm) having maximum weight 66 g whereas minimum in Khirsapat 39.13 g among the germplasm. From biplot analysis it was recorded that there was a positive correlation between fruit weight and pulp weight in the germplasm Fazli. From genetic diversity analysis it was revealed that Fazli had higher genetic similarity and Ashina had lower genetic similarity with another germplasm. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 10 mango cultivars into four major clusters. The cluster size varied from 1 to 5. Cluster I was the largest cluster comprising of five germplasm. The tendency of clustering among mango cultivars revealed that they have strong affinity towards further breeding program.

Key words: Germplasm, multivariate analysis, biplot, correlation, refractometer, UPGMA

Introduction

Mango (*Mangifera indica* L.) is one of the most important cultivated commercial and widely distributed fruits of tropical and subtropical countries of the world belonging to the family Anacardiaceae. It is popular in the world and being important for its excellent flavor, attractive color and delicious taste. Records suggest that it has been in cultivation in the Indian subcontinent from 4000 years ago (Candole, 1984). It is also reported that mango has an origin from the Indo-Myanmar region, especially the North-Eastern part of India (Iyre, 1991).

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In Asia, especially in Bangladesh mango is the most popular fruit. Mango is the national fruit of Bangladesh, India, Pakistan, and the Philippines. It is also the national tree of Bangladesh since 2012. Mango has got a unique position in respect of nutritional quality, taste, consumer's preference etc. among the different kinds of fruits grown in Bangladesh. The major mango producing countries in the world are India, Pakistan, Mexico, Brazil, Haiti, Bangladesh, Philippines and USA. India is the single largest producer of mango with approximately 66% of the world mango production (Jacobi *et al*., 2001) and Bangladesh is in 7^{th} position in terms of worldwide mango production. According to BBS (2020) Bangladesh produced about 1222368 metric tons of mango in the year of 2019-2020 from 235348 acres of land. The main mango growing districts are Chapainawabganj, Rajshahi, Dinajpur, Naogaon and Kushtia in Bangladesh. In the year of 2019-2020 in Chapainawabganj 187174 metric tons of mangoes were produced from 62800 acres of land. The main commercial part of mango is its pulp. Quality of mango pulp depends on the varietal superiority and environmental susceptibility of a region. Fibrous pulp has low economic potentiality than non-fibrous. Sometimes magnetic resonance and fluorescence are used for controlling the quality of Mango (Bureau, 2009). In most cases higher fruit weight has comparatively more pulp to peel and stone ratio than the lower one (Rahman *et al*., 2016). The gemplasm having heavy fruit weight has more edible portion (Barua *et al*., 2013). So, high pulp containing mango germplasm are very much important to meet up food demands and for industrial purposes. Mechanical properties of mango fruits such as firmness and elasticity are also very important attributes for fruit handling, transport, storage, and consumer acceptability (Elsheshetawy *et al*., 2016). This study helps to assess variations and to establish genetic relationships among the mango the germplasm at morphological level.

Materials and Methods

The experiment was conducted to study ten mango (*Mangifera indica* L.) germplasm during July 2018 to May 2019 in Shibganj, Chapainawabganj. The experimental site enjoyed a tropical climate characterized by comparatively low rainfall, high humidity, high temperature, long day and clear sunshine period during the month of April to September and scanty rainfall, low temperature, low humidity, short day and long clear sun shine period during the rest of the year. The experiment was conducted under Randomized Complete Block Design (RCBD) where each mango germplasm was considered as individual treatment of the experiment with three replications and the age of each germplasm was in between 25 to 30 years. Data on the following parameters were recorded from all the studied plants of 10 local germplasm of mango (Table 1) to compare the pulp quantity according to the plant descriptors of mango (IPGRI, 2006).

Table 1. Germplasm list and location of the collected germplasm

Serial No	Germplasm Name	Source Location
1	Fazli	Shibganj, Chapainawabganj
2	Shurma Fazli	Shibganj, Chapainawabganj
3	Tota Fazli	Shibganj, Chapainawabganj
4	Ashina	Shibganj, Chapainawabganj
5	Langra	Shibganj, Chapainawabganj
6	Bombai	Shibganj, Chapainawabganj
7	Khirsapat	Shibganj, Chapainawabganj
8	Gopalvog	Shibganj, Chapainawabganj
9	Lakkhonvog	Shibganj, Chapainawabganj
10	Kachmithi	Shibganj, Chapainawabganj

Parameters studied as follows:

i. Fruit Weight (g)
ii. Fruit Length (cm)
iii. Fruit Breadth (cm)
iv. Fruit Thickness (cm)
v. Peel Weight (g)
vi. Stone Weight (g)
vii. Stone Length (cm)
viii. Stone Breadth (cm)
ix. Stone Thickness (cm)
x. Pulp Weight (g)
xi. Pulp/Peel & Stone Ratio
xii. Total soluble solids (°Brix)

For estimation of pulp weight following formula was used:

Pulp weight (g) = Fruit weight (g) – {Peel weight (g) + Stone weight (g)}

For calculation of pulp to peel and stone ration following formula was used:

$$\text{Ratio of pulp to peel and stone} = \frac{\text{Pulp weight (g)}}{\text{Peel weight (g)} + \text{Stone weight (g)}}$$

For determination of TSS (°Brix) content following instrument was used:

Three samples of each treatment were taken. A drop of juice squeezed from the sample was placed on the surface of the prism of the refractometer and percent total soluble solids (°Brix) was obtained from direct reading by a BRIX model hand-held refractometer made by ERMA INC-Tokyo, Japan.

Statistical Analysis

The recorded data of the study for all characters was analyzed statistically using Wasp Web Agri Stat package program. The mean for all treatments were calculated and analysis of variance was performed by F variance test. The mean differences were evaluated by least significant different (LSD) test (Gomez and Gomez, 1984). Correlation study was done by Past and R studio software. Principal component analysis was done by Origin pro 2021 software.

Results and Discussion

Fruit weight

The fruit weight was varied significantly among the germplasm. It ranged from 183.33g to 743.33g. The highest fruit weight was observed in the germplasm Fazli 743.33g followed by Shurma Fazli, Tota Fazli and others. And the lowest fruit weight was recorded in the germplasm Gopalvog 183.33g (Table 2). Five mango germplasm were evaluated by (Bhuyan and Islam, 1989) where fruit weight ranged from 208g to 654.44g. The variation occurred mainly due to the differences in the genotypic constitution of the germplasm and the environment where they are grown.

Table 2. Variation in fruit characteristics of the collected mango germplasm

Germplasm	Fruit Weight (g)	Fruit Length (cm)	Fruit Breadth (cm)	Fruit Thickness (cm)	Peel Weight (g)
Fazli	743.33 a	14.47 a	8.40 a	7.80 a	62.67 b
Shurma Fazli	570.00 b	14.07 a	7.87 cd	6.80 d	60.00 bc
Tota Fazli	535.00 b	13.20 b	7.03 f	6.23 e	52.00 d
Ashina	384.00 d	11.80 c	7.60 de	7.10 c	43.00 ef
Langra	346.00 e	10.00 d	7.50 e	6.90 cd	40.20 f
Bombai	420.00 cd	10.20 d	8.30 ab	7.70 a	78.67 a
Khirsapat	407.00 cd	9.03 e	8.03 bc	7.40 b	58.67 c
Gopalvog	183.33 g	8.70 e	6.63 g	6.07 ef	42.33 ef
Lakkhonvog	430.00 c	11.60 c	8.53 a	6.97 cd	57.43 c
Kachmithi	263.33 f	11.63 c	6.90 fg	5.97 f	44.67 e
CV (%)	5.10	2.17	2.20	2.27	3.53
LSD (0.05)	37.46	0.43	0.29	0.27	3.28

In a column, figure (s) with same letter do not differ significantly at 5% level

Fruit length

There was significant variation among the mango germplasm in relation to length of fruit. It ranged from 8.70 cm to 14.47 cm. The longest fruit length was observed in the germplasm Fazli 14.47 cm and the shortest fruit length was recorded in the germplasm Gopalvog 8.70 cm (Table 2). Five mango germplasm were evaluated by (Bhuyan and Islam, 1989) where fruit length ranged from 8.30 cm to 13.87 cm and stone length ranged from 6.88 cm to 12.22 cm.

Fruit breadth

It ranged from 6.63 cm to 8.40 cm. The highest fruit breadth was observed in the germplasm Fazli 8.40 cm and the shortest fruit breadth was recorded in the germplasm Gopalvog 6.63 cm (Table 2). Five mango germplasm were evaluated by (Bhuyan and Islam, 1989) where fruit breadth ranged from 6.38 cm to 9.55 cm.

Fruit thickness

Fruit thickness ranged from 5.97 cm to 7.80 cm. The highest fruit thickness was observed in the germplasm Fazli 7.80 cm and the lowest one was recorded in the germplasm Kachmithi 5.97 cm (Table 1).

Peel weight

Peel weight varied significantly among the mango germplasm. It ranged from 40.20g to 78.67g. The highest peel weight was observed in the germplasm Bombai 78.67g and the lowest peel weight was recorded in the germplasm Langra 40.20g (Table 2).

Stone weight

Stone weight ranged from 39.13g to 66.00g. The growth of the fruit is directly associated with the growth of the seed (Saini *et al*., 1971). The highest stone weight was observed in the germplasm Fazli 66.00g and the lowest stone weight was recorded in the germplasm Khirsapat 39.13g (Table 3).

Stone length

The stone length differed significantly among the mango germplasm. It ranged from 7.03 cm to 11.60 cm. The longest stone length was observed in the germplasm Fazli 11.60 cm and the shortest stone length was recorded in the germplasm Khirsapat 7.03 cm (Table 3).

Table 3. Variation in stone characteristics of the studied mango germplasm

Germplasm	Stone Weight (g)	Stone Length (cm)	Stone Breadth (cm)	Stone Thickness (cm)
Fazli	66.00 a	11.60 a	4.87 a	2.70 a
Shurma Fazli	60.33 ab	10.23 c	4.47 b	2.50 a
Tota Fazli	55.67 b	9.83 d	4.13 d	2.23 bc
Ashina	48.00 c	10.20 c	3.80 e	2.10 bcd
Langra	40.30 d	7.97 f	3.03 f	1.90 d
Bombai	41.30 d	8.73 e	3.90 e	1.93 d
Khirsapat	39.13 d	7.03 g	4.30 c	2.03 cd
Gopalvog	41.33 d	7.20 g	3.83 e	2.17 bc
Lakkhonvog	39.67 d	8.73 e	4.07 d	1.33 e
Kachmithi	43.67 cd	10.93 b	4.47 b	2.27 b
CV (%)	7.75	1.54	1.89	5.94
LSD (0.05)	6.32	0.25	0.14	0.22

In a column, figure (s) with same letter do not differ significantly at 5% level

Stone breadth

Highly significant variation was manifested among the mango germplasm. It ranged from 3.03 cm to 4.87 cm. The highest stone breadth was observed in the germplasm Fazli 4.87 cm and the lowest stone breadth was recorded in the germplasm Langra 3.03 cm (Table 3).

Stone thickness

Stone thickness ranged from 1.33 cm to 2.70 cm. The highest stone thickness was observed in the germplasm Fazli 2.70 cm and the lowest one was recorded in the germplasm Lakkhonvog 1.33 cm (Table 3).

Pulp weight

Pulp weight varied significantly among the mango germplasm. It ranged from 175.00g to 614.67g. The highest pulp weight was observed in the germplasm Fazli 614.67g followed by Shurma Fazli, Tota Fazli and others. And the lowest pulp weight was recorded in the germplasm Kachmithi 175.00g (Table 4). BARI Aam-4 had heavy fruit weight and higher edible portion (78.66%) (Barua *et al*., 2013).

Table 4. Pulp characteristics and TSS (°Brix) of the collected mango germplasm

Germplasm	Pulp Weight (g)	Pulp/Peel & Stone Ratio	TSS (°Brix)
Fazli	614.67 a	4.80 a	17.33 b
Shurma Fazli	449.67 b	3.73 bc	15.67 cd
Tota Fazli	427.33 b	3.97 b	14.67 d
Ashina	293.00 de	3.24 d	15.67 cd
Langra	265.50 e	3.30 d	20.67 a
Bombai	300.03 cd	2.50 e	17.33 b
Khirsapat	309.20 cd	3.17 d	17.33 b
Gopalvog	99.67 g	1.17 g	19.67 a
Lakkhonvog	332.90 c	3.42 cd	12.67 e
Kachmithi	175.00 f	1.98 f	16.33 bc
CV (%)	6.03	6.54	4.76
LSD (0.05)	33.76	0.35	1.36

In a column, figure (s) with same letter do not differ significantly at 5% level

Pulp/peel & stone ratio

Pulp/peel & stone ratio of the fruits varied from 1.17 to 4.80 among mango germplasm. The highest ratio was observed in Fazli 4.80. The germplasm Kachmithi had the lowest 1.17 ratio (Table 4).

Total soluble solids (°Brix)

Total soluble solids contents of mango fruits were measured at ripen stage. It was observed there were variations in TSS (°Brix) among 60 mango genotypes ranged from 16.90 to 28.26 (Majumder *et al*., 2011). Ripen mango contained the highest TSS (°Brix) in the germplasm Langra 20.67 followed by Gopalvog, Khirsapat and Fazli. And the lowest in the germplasm Lakkhonvog 12.67 (Table 4).

Correlation among fruit weight and other selected traits

Correlation study indicated that there was a significant positive correlation between fruit weight and pulp weight along with fruit length, stone weight and pulp/peel & stone ratio. But fruit weight had a significant negative correlation with TSS (°Brix). Pulp weight, fruit length, stone length and stone breadth had also negative correlation with TSS (°Brix) (Fig. 1).

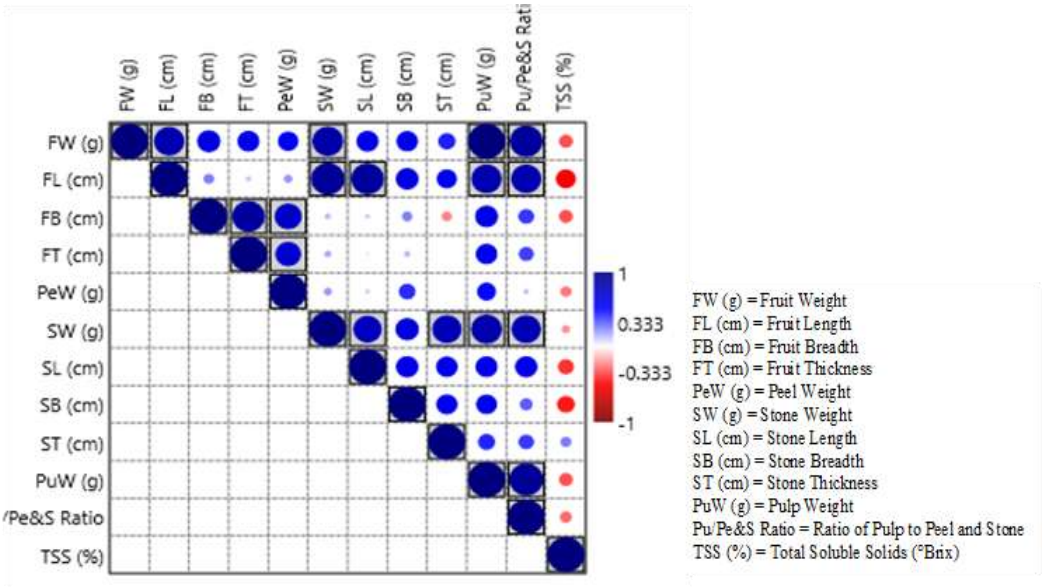

Fig. 1. Correlation among the studied traits of the selected mango germplasm.

Genotype by trait biplot for the selected traits

Biplot analysis mostly used to determine varietal stability in the multi-environmental trial (Farshadfar *et al*., 2013). Genotype by Trait (GT) biplot analysis describes the association among the traits across different genotypes (Yan and Reid, 2008). Biplot can also be used to determine gene expression of plants (Chapman and Smith, 2002). The Principal Component Analysis (PCA) identified a total of 10 Principal Components (PCs) for the selected traits. Among the PCs three having Eigen value greater than 1. The first two PCs explained about 74.54% of the total variation (Fig. 2). The association between selected

traits among the germplasm were visualized by the genotype by trait biplot analysis. The acute angle between two traits represents positive correlation while the obtuse angle between two traits represents negative correlation (Yan and Reid, 2008). Acute angle was found between fruit weight (FW) and pulp weight (PuW) along with fruit length (FL), fruit breadth (FB), fruit thickness (FT), peel weight (PeW), stone weight (SW), stone length (SL), stone breadth (SB), stone thickness (ST) and pulp-peel & stone ratio (Pu/Pe&S) indicating all of them had positive correlations.

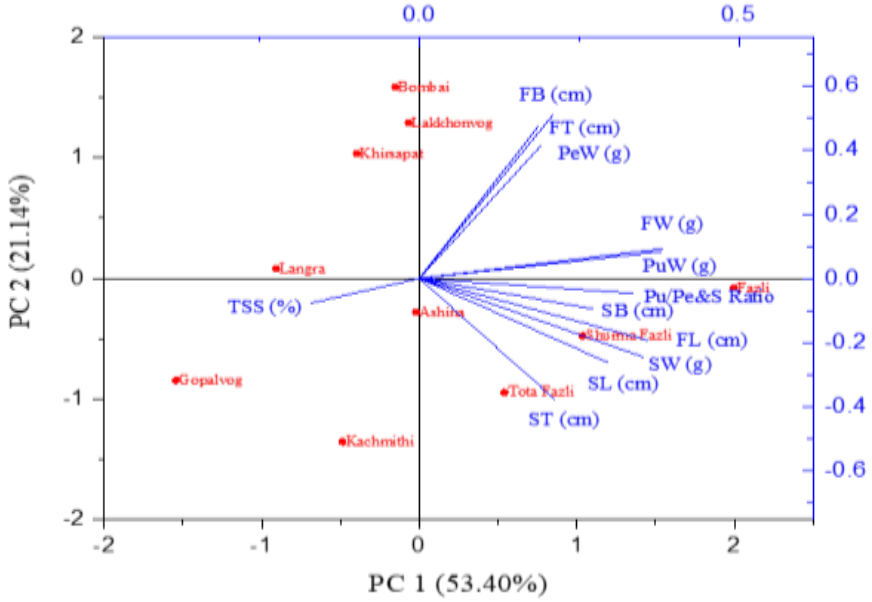

Fig. 2. Biplot analysis explaining the correlation and genotype by trait relationship of the selected mango germplasm

Obtuse angle was observed between TSS (°Brix) and fruit weight (FW) along with pulp weight (PuW), peel weight (PeW), stone weight (SW), pulp-peel & stone ratio (Pu/Pe&S) indicating they had negative correlations (Fig. 2). All these results indicating that direct selection for any trait would give positive rewards for the other traits which were positively correlated while it would bring negative results for the negatively related traits. Again, biplot analysis showed the trait profiles of the genotypes, especially those genotypes positioned far away from the origin (Yan and Reid, 2008).

Genetic Dissimilarity Analysis

Blue colored point showed lowest genetic dissimilar pair while red colored point indicated maximum genetic dissimilar pair. In Gower's matrix the germplasm Ashina was found to be the most dissimilar accession with others and the germplasm Fazli showed higher amount of similarity with another germplasm (Fig. 3). In this study genetic distance ranged from 0.1670 to 0.7942.

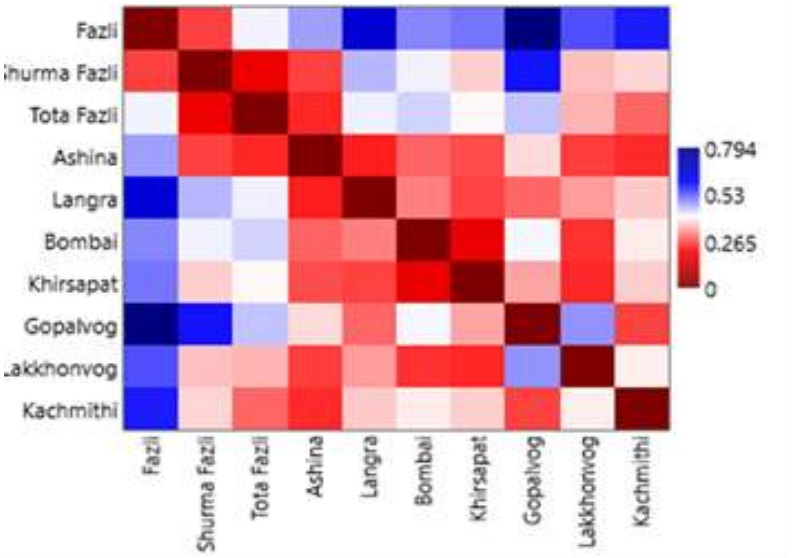

Fig. 3: Genetic dissimilarity matrix of the studied mango germplasm

Genetic similarity analysis using UPGMA

Dendrogram based on Gower similarity index generated from these 10-mango germplasm. The Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis lead to the grouping of the 10 germplasm into four major clusters (Figure 4). Based on genetic distances different mango genotypes grouped into different sub-cluster (Molla *et al*., 2019).

Fig. 4. Dendogram showing the clusters among the studied germplasm

Cluster I included Ashina, Langra, Bombai, Khirsapat and Lakkhanvog. Cluster II included Gopalvog and kachmithi. Shurma Fazli, Tota Fazli were in cluster III. And cluster IV consisted of Fazli. From this study, the dendrogram revealed that the germplasm that were derivatives of genetically similar type form cluster together. Maximum mango germplasm (5) were included in cluster I and minimum (1) was in cluster IV.

Conclusion

Mango has been considered as an important fruit crop in many countries for its unmatchable taste, characteristic flavors, nutritional values and economic importance. World mango production is now spread over 100 countries and mango industry is the 6th largest tropical fruit industry in the world. Considering Fruit weight (743.33 g), Pulp weight (614.67 g), Pulp/peel & stone ratio (4.80) the germplasm Fazli was the best one among them. But in case of TSS (°Brix) Langra (20.67) performed better than Fazli. These variations can be used for selection of superior germplasm for cultivation at farmer's level as well as future breeding programme of mango in Bangladesh. Further collection of mango germplasm should be continued for getting more variability in respect of desired traits.

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ADOPTION OF MODERN SESAME PRODUCTION TECHNOLOGIES IN SELECTED AREAS OF BANGLADESH

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Abstract

The study was conducted to assess the extent of adoption along with examine the associated factors and also identify the constraints in adopting modern sesame production technologies by the farmers of five villages of five districts i.e., Kushtia, Chuadanga, Faridpur, Jashore and Narail. Data were obtained from 100 randomly selected farmers in five selected villages of five districts by using an interview schedule. Data were collected during 15 September to 25 October, 2014. Five improved practices were selected to measure the adoption level. The results showed that adoption level of five practices such as adoption of improved seed, adoption of recommended dose of fertilizer, weeding and thinning, irrigation and pesticide use were 38.94, 38.75, 38.15, 35.26 and 32.98 percent, respectively. Considering overall adoption level, it was revealed that more than half (51 percent) of the farmers had low adoption while 37 percent had medium and 12 percent had high adoption of modern sesame production technologies. Results indicated that there were 13 problems which hindered the adoption of modern sesame production technologies by the farmers. Among the problems, three major problems were (i) probability of being caused harm by heavy rainfall or drought (77.3 percent), (ii) problem in harvesting and processing for heavy rainfall (65.3 percent) and (iii) irrigation problem (40.7 percent). It was also found that 37 percent of the farmers faced low problem, while 63 percent faced medium problem and there was no farmer who faced high problem regarding the adoption of modern sesame production technologies.

Key words*:* Adoption, modern technologies, suitability of technology, profitability of technology

Introduction

Edible oils play a very important role in human nutrition. It is not only a high energy food but also a carrier for fat soluble vitamins (A, D, E and K) in the body. Oils are not only important for human diets but also services as important raw material for industrial use such as making soaps, paints, varnishes, hair oils, lubricants, textile auxiliaries, pharmaceuticals etc. Oil cakes and meals are used as animal feeds and manures. The major oilseed crops grown in Bangladesh are mustard, sesame, groundnut and linseed. The major contribution of oil comes from mustard (65%) followed by sesame (10.71%) and groundnut (invisible oil 10.5%) (BBS, 2016).

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Sesame is the second largest source of edible oil in Bangladesh next to mustard both in respect of acreage and production. Sesame is one of the world's oldest spice and oilseed crop grown mainly for its seeds that contain approximately 35-50% oil, 20-25% protein, 20% sugar, 6% fibre and many kinds of minerals. Sesame oil is quality edible oil. The oil is tasteless, odourless and also used as hair oil and as a component of cosmetics. The seed is used in making various food items like cakes, *khaja*, biscuits, etc. Dry plants and leaves are used as fuel and oilcakes as cattle feeds and manures. Sesame has also been used as folk medicine (Brar and Ahuja, 1979) in India and Bangladesh and its oil have been used traditionally to cure various ailments, such as asthma, in "*ayurveda*" since ancient times. It is well known that sesame has nutritive, laxative, demulcent, emollient, diuretic and lactagogue properties (Tylor *et al*., 1988). It may also be employed in the preparation of liniments, plasters, ointments and soaps (Weiss, 1971). The roots, usually unused parts of sesame, contains antifungal compound such as chlorosesamone, hydroxysesamone and 2-3 epoxysesamone (Hasan *et al*, 2000 and 2001). The climate of Bangladesh is more suitable for sesame cultivation and it is grown in almost all districts but grows well in greater Khulna, Faridpur, Pabna, Barisal, Rajshahi, Jashore, Kushtia, Cumilla, Dhaka, Rangpur, Sylhet, and Mymensingh districts. Due to increase of area under cereal crops for meeting the increasing demand of food-stuff, land under oilseed crops has declined and price of oil has gone up. Cultivation of traditional varieties, imbalance use of fertilizers, inability to seed sowing in proper time, non adoption of other production technologies, natural calamities, socio-economic barrier, large yield gap (20-60%), nutrient mining in existing cropping pattern, unavailability of seeds of suitable HYV varieties etc. are the main constraints of maximizing yield of oilseeds. The government of Bangladesh has, therefore, provided priority to the agriculture sector to increase the production of oilseeds by giving subsidy to the farmers on different inputs such as fertilizer, irrigation etc. to achieve self sufficiency in oilseeds. In view of the foregoing discussion, the researcher undertook a study entitled, "adoption of modern sesame production technologies in some selected areas" along with the following objectives - (i) to assess the extent of adoption of modern sesame production technologies (ii) to find out the degree of relationship of different factors with the adoption of modern sesame production technologies, and (iii) to identify the constraints in adopting modern sesame production technologies by the farmers in some selected areas.

Materials and Methods

Study areas and source of data

Considering the sesame growing area the study was conducted in five villages of Sadar upazila of Kushtia; Alamdanga of Chuadanga; Modhukhali of Faridpur; Bagharpara of Jashore and Lohagora of Narail district. All the farmers of selected five villages of project areas who cultivated sesame constituted the population of the study. A list of sesame growers of selected villages was prepared with the help of local Sub-Assistant Agriculture Officer (SAAO) of Department of Agricultural Extension of the concerned area. The list

comprised of 305 farmers which served as the population of the study of each selected villages. Out of them, 33% of the farmers were selected following random sampling method. Thus, 100 sesame growers were the sample of the study. These 100 growers were considered as the representative of the five villages of respective districts.

Variables of the study and their measurement

Age, education, family size, farm size, cropping intensity, family annual income, training exposure, extension media contact, innovativeness, cosmopoliteness, organizational participation, agricultural knowledge on sesame cultivation, credibility of extension agents, risk orientation, suitability of technology and profitability of technology were consisted as the independent variables whereas 'adoption of modern sesame production technologies in some selected areas' was considered as the dependent variable of the study. The selected modern technologies were consisted of recommended package of five practices. The five practices were adoption of improved seed, adoption of recommended rate of fertilizer, adoption of weeding and thinning, adoption of irrigation and adoption of pesticide use. These five practices were selected to measure the adoption level. It was measured on the basis of the extent of adoption of modern sesame production technologies by the farmers for a period of two years (2013 & 2014). An Adoption Index (AI) for modern sesame production technologies was computed by using of Chattapadhyay (1963) and simplified by Ray (1998). The adoption score was expressed in percentage. The Adoption Index (AI) of sesame grower could range from 0 to 100, where 0 indicate no adoption and 100 indicate highest adoption.

Data collection and statistical analysis

Data were collected through using the interview schedule from the respondents during Sep 15 to Oct 25, 2014. Data were collected by the researcher himself through interview schedule from the farmers of the selected villages. The interview was conducted with the respondents individually in their respective houses. The SPSS (Statistical Package for Social Science) computer package was used to perform data management. Descriptive analytical parameters such as mean, range, number and percentage, standard deviation and rank order were used whenever necessary. Pearson's Product Moment Correlation Coefficient (r) was computed to explore the relationships between the dependent and independent variables.

Results and Discussion

Adoption of Modern Sesame Production Technologies by the Farmers

Adoption level on five practices have been computed separately and presented in Table 1. These practices were adoption of improved seed, adoption of recommended rate of fertilizer, adoption of weeding and thinning, adoption of irrigation and adoption of pesticide use.

Table 1. Adoption level of five recommended package of practices for adoption of modern sesame cultivation technologies

Recommended practices	Measuring unit	Score Ranges	Category	Farmers (%)	Mean	SD
Adoption of improved seed	Percentage	7.26 – 93.07	Low (up to 33)	50	38.94	22.28
			Medium (34-66)	36		
			High (above 66)	14		
Adoption of recommended dose of fertilizer	Percentage	7.26 – 93.07	Low (up to 33)	50	38.75	22.02
			Medium (34-66)	37		
			High (above 66)	14		
Adoption of weeding and thinning	Percentage	7.26 – 88.67	Low (up to 33)	50	38.15	21.37
			Medium (34-66)	36		
			High (above 66)	14		
Adoption of irrigation	Percentage	7.26 - 82.44	Low (up to 33)	53	35.26	18.00
			Medium (34-66)	39		
			High (above 66)	8		
Adoption of pesticide use	Percentage	0 – 80.51	Low (up to 33)	55	32.98	15.62
			Medium (34-66)	44		
			High (above 66)	1		
Overall adoption	Percentage	6.95 – 80.51	Low (up to 33)	51	36.58	19.21
			Medium (34-66)	37		
			High (above 66)	12		

Discussion on five recommended package of practices

Adoption of improved seed

The adoption of improved seeds of the respondents ranged from 7.26 to 93.07 against the possible range of 0 to 100. The average adoption was 38.94 with a standard deviation of 22.28. Based on the adoption scores the respondents were classified into three categories: “low adoption” (up to 33), “medium adoption” (34-66), and “high adoption” (above 66). The distribution of respondents according to their adoption of improved seeds has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (50 percent) of farmers fell under the low adoption category, while 36 percent had medium adoption and 14 percent had high adoption. Thus an overwhelming majority of the farmers had medium to high adoption. It is a good signal for the programme of yield maximization of sesame.

Adoption of recommended rate of fertilizer

The adoption of recommended rate of fertilizer of the respondents ranged from 7.26 to 93.07 against the possible range of 0 to 100. The average adoption was 38.15 with a standard deviation of 22.02. Based on the adoption scores the respondents were classified into three categories: “low adoption” (up to 33), “medium adoption” (34-66), and “high

adoption" (above 66). The distribution of respondents according to their adoption of recommended rate of fertilizer has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (50 percent) of farmers fell under the low adoption category, while 37 percent had medium adoption and 13 percent had high adoption. Thus an overwhelming majority of the farmers had low to medium adoption.

Adoption of weeding and thinning

The adoption of recommended rate of fertilizer of the respondents ranged from 7.26 to 88.67 against the possible range of 0 to 100. The average adoption was 38.15 with a standard deviation of 21.37. Based on the adoption scores the respondents were classified into three categories: "low adoption" (up to 33), "medium adoption" (34-66), and "high adoption" (above 66). The distribution of respondents according to their adoption of weeding and thinning has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (50 percent) of farmers fell under the low adoption category, while 36 percent had medium adoption and 14 percent had high adoption. Thus an overwhelming majority of the farmers had low to medium adoption.

Adoption of irrigation

The adoption of irrigation of the respondents ranged from 7.26 to 82.44 against the possible range of 0 to 100. The average adoption was 35.26 with a standard deviation of 18.00. Based on the adoption scores the respondents were classified into three categories: "low adoption" (up to 33), "medium adoption" (34 to 66), and "high adoption" (above 66). The distribution of respondents according to their adoption of irrigation has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (53 percent) of farmers fell under the low adoption category, while 39 percent had medium adoption and 8 percent had high adoption. Thus an overwhelming majority of the farmers had low to medium adoption.

Adoption of pesticide use

The adoption of recommended rate of fertilizer of the respondents ranged from 0 to 80.51 against the possible range of 0 to 100. The average adoption was 32.98 with a standard deviation of 15.62. Based on the adoption scores, the respondents were classified into three categories: "low adoption" (up to 33), "medium adoption" (34 to 66), and "high adoption" (above 66). The distribution of respondents according to their adoption of pesticide use has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (55 percent) of farmers fell under the low adoption category, while 44 percent had medium adoption and only 1 percent had high adoption. Thus an overwhelming majority of the farmers had low to medium adoption. The findings indicate that many farmers in the study area did not regularly use balanced fertilizer. This might be due to fact that like other crops, farmers in general, were not serious about using balanced fertilizer.

Overall adoption of modern sesame production technologies

The adoption of modern sesame production technologies of the respondents ranged from 6.95 to 80.51 against the possible range of 0 to 100. The average adoption was 36.58 with a standard deviation of 19.21. Based on the adoption scores the respondents were classified into three categories: "low adoption" (up to 33), "medium adoption" (34 to 66), and "high adoption" (above 66). The distribution of distribution of respondents according to their adoption of improved seeds has been shown in Table 1. Data contained in Table 1 revealed that the highest proportion (51 percent) of farmers fell under the low adoption category, while 37 percent had medium adoption and 12 percent had high adoption. Thus an overwhelming majority of the farmers had low to medium adoption. For clarity of understanding a bar diagram has been presented in Fig. 1.

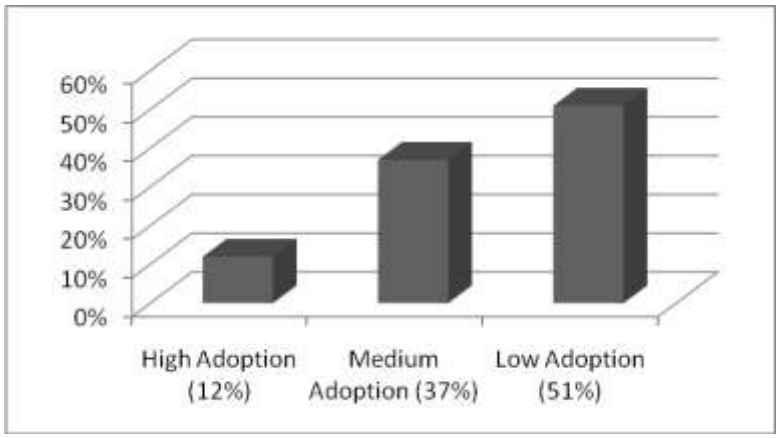

Fig.1. Farmers' adoption of modern sesame production technologies measured according to their extent of adoption.

Factors related to adoption

Relationship between the selected sesame growers' characteristics & other factors (16 factors) and their adoption of modern sesame production technologies were ascertained by the Pearson's product moment coefficient of correlation and the summary of the result has been presented in Table 2.

Out of sixteen characteristics, thirteen namely: education of the farmers, farm size of the farmers, cropping intensity, family annual income, training exposure of the farmers, extension media contact, innovativeness, cosmopoliteness, agricultural knowledge on sesame cultivation, credibility of extension agents, risk orientation, suitability of technology, profitability of technology had significant and positive relationship with their adoption of modern sesame production technologies and rest three factors namely age, family size and organizational participation shown no significant relationship with their adoption.

Table 2. Co-efficient of correlation of the selected characteristics of the respondents and their adoption of modern sesame production technologies (N = 100)

Selected characteristics of the farmers and others factors	Co-efficient of correlation ('r')
Age	0.047
Education	0.209*
Family Size	- 0.166
Farm Size	0.252*
Cropping Intensity	0.466**
Family Annual Income	0.203*
Training Exposure	0.580**
Extension Media Contact	0.574**
Innovativeness	0.376**
Cosmopoliteness	0.223*
Organizational Participation	0.154
Knowledge on sesame cultivation	0.307**
Credibility of the extension agents	0.401**
Risk Orientation	0.365**
Suitability of the technology	0.206*
Profitability of the technology	0.522**

***** = Correlation is significant at the 0.05 level (2-tailed).
****** = Correlation is significant at the 0.01 level (2-tailed).

Constraints faced by the Farmers in adoption of modern sesame production technologies

As many as 13 constraints were included in constraints confrontation scale. The constraints score ranged from 9 to 25 against the possible range of 0 to 39. The mean and standard deviation of the score were 14.92 and 3.11, respectively (Table 3).

Table 3. Distribution of sesame growers according to their constraint confrontation

Categories	No. of Farmers	Mean	SD
Low (up to 13)	37		
Medium (14-26)	63	14.92	3.11
High (>26)	0		

Rank order of the constraints confrontation by the sesame growers

The extent of constraints faced by the farmers in adopting modern sesame production technologies in terms of Constraints Facing Index (CFI) along with their rank order based on the CFI have been presented in the Table 4. Farmers gave their responses as high, medium, low and not at all against each problem included in problem confrontation scale.

Table 4. Ranked order of the constraints faced by the farmers in adopting modern sesame production technologies

Sl No.	Constraints	High (3)	Medium (2)	Low (1)	Not at all (0)	CFI	RO
1.	Irrigation problem	3	37	39	21	40.67	3
2.	Input cost	0	2	73	25	25.67	9
3.	Problem in getting credit	0	3	36	61	14.00	12
4.	Problem in getting technical information	0	0	62	38	20.67	10
5.	Lack of suitable land for sesame cultivation	0	33	38	29	34.67	4
6.	Lack of availability of improved seeds	1	7	62	30	26.33	8
7.	Problem in marketing	0	0	28	72	9.33	13
8.	Problem for low market demand	0	3	54	43	20.00	11
9.	Constraints in harvesting and processing for heavy rainfall.	26	50	18	6	65.33	2
10.	Problem for low market price	4	7	61	28	29.00	6
11.	Probability of being caused harm by heavy rainfall or drought	43	49	5	3	77.33	1
12.	Attack of insects and diseases	0	0	100	0	33.33	5
13.	Lack of farm labours	3	10	60	27	28.67	7

Notes: CFI= Constraints Facing Index, RO = Rank order

Data presented in the Table 4 indicated that among the problems, "probability of being caused harm by heavy rainfall or drought" had the highest score and accordingly it has been ranked in the first position. "Constraints in harvesting and processing for heavy rainfall" was the second most crucial constraints of the farmers in adopting modern sesame production technologies. These two constraints occur due to natural causes on which no control of human being. Human being could avoid these two constrains by the following of early cultivation practices. The third cited problem was "irrigation problem". There were not sufficient deep tube wells and available water in the rivers which was the cause of these constraints. Other constraints according to rank order are lack of suitable land for sesame cultivation, attack of insects and diseases, problem for low market price, lack of farm labours, lack of availability of improved seeds, input cost, problem in getting technical information, problem for low market demand, problem in getting credit, problem in marketing.

Conclusion

Findings of the study revealed that the highest proportion (51 percent) of the farmers had low adoption of modern sesame production technologies and an average adoption quotient was 36.58. Again 37 percent of the respondents were in medium category and only

12 percent respondents were in high adoption category. It could be concluded from the findings that there remains an ample scope to improve farmers' level of adoption regarding modern sesame production technologies. Among the thirteen identified constraints, probability of being caused harm by heavy rainfall or drought and constraints in harvesting and processing for heavy rainfall are the main constraints. As these constraints are natural and out of jurisdiction of intervening agencies, these may hinder the adoption of modern sesame production technologies. Some factors played a very significant role in adopting modern sesame production technologies. These factors were: suitability of technology, profitability of technology, risk orientation, credibility of extension agents and cropping intensity. All of these factors contributed positively and significantly to the adoption of modern sesame production technologies. These factors could be called as key to the success of any extension efforts.

Based on the above findings the following recommendations are put forward for maximizing production of modern sesame:

- Training exposure and extension media contact of the sesame growers showed high significant and positive relationship with their adoption of modern sesame production technologies. Farmers' level of knowledge should be increased through training, extension contact and other extension methods, in order to develop clear understanding about the use and benefit of technologies.
- Frequent contact with extension media can make farmers more innovative and cosmopolitan which will ultimately lead to their adoption of modern sesame production technologies. Hence, the concern authorities should take cognizance of these facts and take necessary steps to increase the frequency of extension contact of the farmers' and to provide necessary training sessions to the farmers.
- Increased adoption rate of modern sesame production technologies are important for meeting the national demand of edible oil. To achieve higher degrees of adoption of modern sesame production technologies, the farmers' knowledge, attitude and perception have to be increased. Henceforth, DAE and other extension service providing organizations should be given more emphasis to take necessary steps to increase knowledge and perception level of farmers for dissemination and adoption of modern sesame production technologies. For this regard Government and non-government organizations should provide effective training program on modern sesame production packages for the farmers at regular intervals to build their farming skills.
- DAE should strengthened the field level services by the field workers (SAAOs) to give farmers proper information, suggestions and advice regarding adoption of modern sesame production technologies.

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FARMERS LEVEL ADOPTION OF BINA DEVELOPED CROP VARIETIES IN MYMENSINGH REGION OF BANGLADESH

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Abstract

The study was conducted in Mymensingh agricultural region remaining four districts of Bangladesh to examine the farmers' level adoption of BINA developed crop varieties. Primary data were used and those were collected from four districts through concerned DAE office and sub-stations of Bangladesh Institute of Nuclear Agriculture (BINA). Both tabular and descriptive statistical analysis was used to fulfill the objectives. The results showed that among the overall farmers level adoption of BINA developed Aus, Aman and Boro varieties, the highest area coverage was found in Aman 4.65% followed by Aus 0.26% and Boro 0.10%, respectively, The overall farmers level adoption of BINA developed pulse and oilseed varieties were 0.63% and 1.44%, respectively. The results also revealed that among the eight cropping pattern in Mymensingh agricultural region Boro-Fellow-Aman was the highest. It was found that farmers' level adoption was highest by Binadhan-7 (15.86%) followed by Binadhan-17 (15.17 %), Binadahn-10 (10.74%), Binadhan-11 (10.69%), BRRIdhan-28 (9.97%), Binadhan-19 (7.07 %), Binadhan-14 (5.76%) and the lowest area was for BRRIdhan-29 (5.28%). Binasharisha-9 and vegetables were cultivated 15.55% and 3.91%, respectively. 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.

Key words: Adoption, BINA varieties, Mymensingh agricultural region

Introduction

Agriculture is the largest sector in Bangladesh, making up 13.02 percent of total GDP and employing about 40.60 percent of the workforce (BBS, 2020). Agricultural development is one of the most powerful tools to end extreme poverty, boost shared prosperity and feed a projected 9.7 billion people by 2050 (World Bank, 2020). In Bangladesh, Agriculture plays a leading role in the development and stability of the economy. The arable land in Bangladesh is 15.92 million hectares about 55 percent of the total land area which is contributing to feed 160 million people (BBS, 2019). 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, 11.5 percent triple cropping, 0.10 percent quadruple cropping and 2.86 percent currently fallow land (BBS, 2019).

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Population of Bangladesh is increasing but cultivable land is decreasing day by day. Bangladesh has faced many factors in recent years that driving land use and land cover changes such as population dynamics, rapid changes in economic growth, climate change, construction of roads and highways, electrification, more advanced agriculture technology and irrigation facilities, extended education, improved health services, new residential infrastructure etc. (Hasan *et al.,* 2017). Rapid land use and land cover change (LUCC) induced land degradation, together with climate change and human activities, is thought to be a threat worldwide (Wu, X. *et al*., 2008, Biro *et al.*, 2013, Leh *et al.*, 2013). Keeping this in mind scientists of different agricultural research institutes developed modern/high yielding varieties.

Until recently, the choice of technologies available to farmers was largely determined by the need to increase production, profits and productivity. The main constraints were the availability of capital, knowledge of how to use the technology and market risks that in many countries policies were shielded by government policies. In the past, "good policy practices" was therefore rather straightforward, relating primarily to increasing output and the aim of agricultural policies was to increase productivity in agriculture. Agricultural research and extension services could concentrate for example, on improving the productivity of small farms.

As a result, Bangladesh agriculture is now transforming from a traditional to a modern agricultural system. Now, the country has been successful in maintaining most of its food demand for the existence of the fertile soils on the few vast floodplains that are annually refilled by siltation during the annual flood (Rahman and Islam, 2014), though there are considerable imports of some agricultural commodities. Area coverage of high yielding modern variety is increasing by replacing traditional variety. Here, Cropping Intensity increases up to 216% (MOA, 2018) from 183% in 2008 (BBS 2021). The specific objectives of the present study were: i) to examine the farmers' level adoption of BINA developed crop varieties; and ii) to suggest some policy guidelines**.**

Materials and Methods

The study was conducted in Mymensingh agricultural region of Bangladesh remaining four districts namely Mymensingh, Sherpur, Netrokona and Jamalpur (Fig. 1). Among these four districts eight upazilas were selected purposively remaining two upazilas of each district. In total 120 farmers were randomly selected, 30 from each district and 15 from each upazilas to collect primary data for conducting the research. Primary data were used for this study which was collected through pre-designed interview schedule using structural questionnaire from the farmers. In the questionnaire per hectare area of BINA developed rice (Aus, Aman and Boro), pulses and oilseed were included to fulfill the objectives. Besides, secondary data from various issues 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 2019-2020.

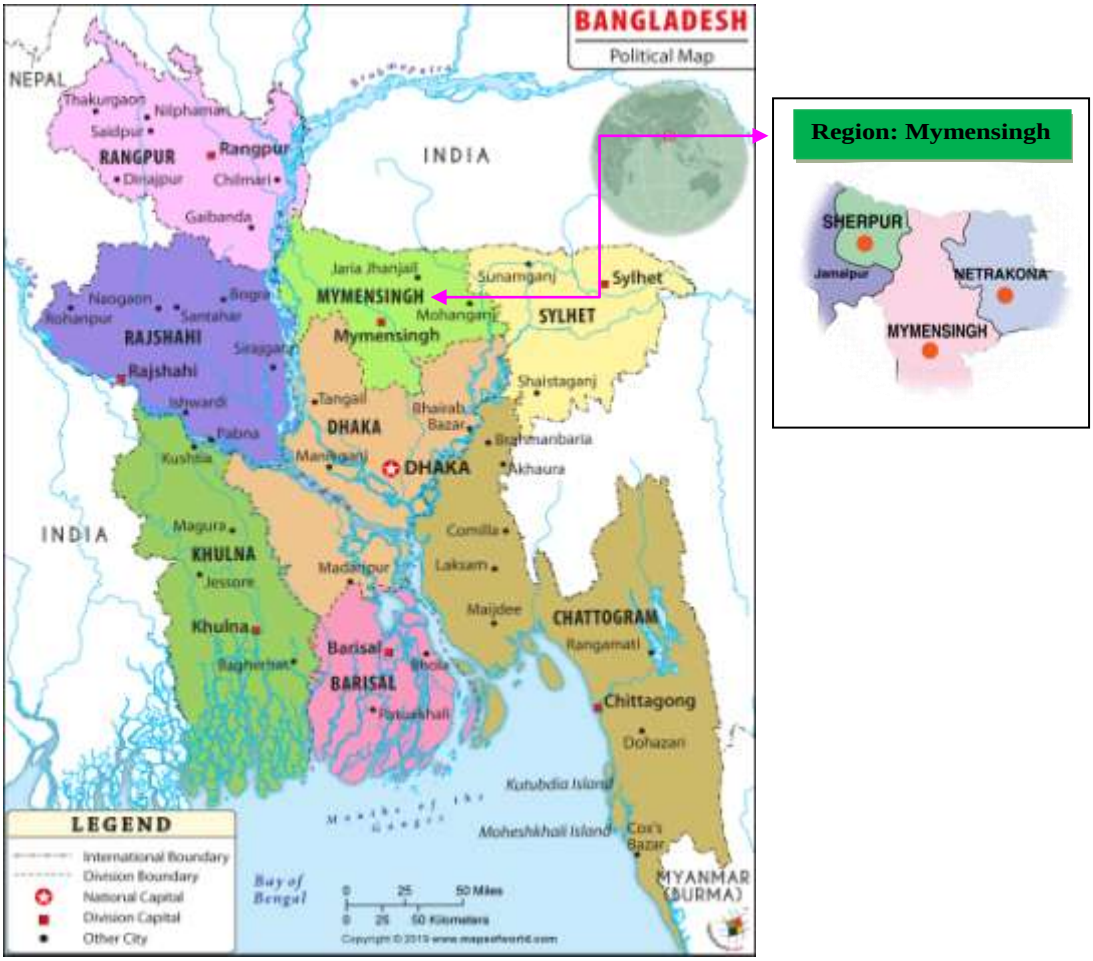

Fig. 1. Map of the Study area of Bangladesh

Results and Discussion

The results presented in Table 1 depicted that adoption of the rice varieties among three rice seasons (Aman, Boro, Aus), Aman was the highest coverage by 4.65% followed by Aus 0.78 % and the lowest for Boro i.e. 0.10 % in the Mymensingh agricultural region. In Aus season, the highest area coverage was found 7.44 % in Netrokona district followed by Jamalpur (1.76%) Sherpur (0.93%) and Mymensingh (0.07%) district. In Aman season, the highest area coverage was found 8.95% in Netrokona district followed by Sherpur (8.21%), Jamalpur (4.26%) and Mymensingh (2.05%) district. The area coverage found 0.38% in Jamalpur district and 0.02% in Mymensingh district of Boro season. In Aman season, the highest area (7741 ha) was found in Netrokona district and the lowest was found (2847 ha) in Jamalpur district.

The overall area coverage of BINA developed pulse and oilseed varieties were 4.62% and 5.82%, respectively (Table 2). Amongst the four districts the highest area coverage for BINA developed pulse varieties was found in Sherpur (20.62%) followed by Netrokona (19.32%). In case of oilseed varieties, the highest area coverage was in Netrokona (46.67%) followed by Mymaensingh (6.17%), Sherpur (5.61%) and Jamalpur (1.49%).

From Table 3, it was observed that there are ten cropping pattern in Mymensingh region. Among 120 farmers and eight cropping pattern the Boro-fallow-Aman was the highest as well as its rank was I, followed by Aman-Vegetables-Boro-Fallow, Aman-Mustard-Boro, Potato-Boro-Fallow-Fallow/Aman-vegetables-fallow-fallow, Boro-Aus-Aman/Boro-Jute-Aman, Aman-Mustard-Boro-Fellow and Vegetable-Fellow-Aman was the lowest which was ranked as VIII.

From Table 4, it was observed that farmers' level adoption was highest by Binadhan-7 (15.86%) followed by Binadhan-17 (15.17 %), Binadahn-10 (10.74%), Binadhan-11 (10.69%), BRRI-28 (9.97%), Binadhan-19 (7.07 %), Binadhan-14 (5.76%) and the lowest area was for BRRI-29 (5.28%). The area of Binasharisha-9 was 15.55% and vegetables (3.91%) in Mymensingh agricultural region among the studied farmers.

Table 1. District wise adoption of BINA developed rice varieties in Mymensingh region.

District	Rice (ha)						BINA Cultivated Area (%)		
	Total Cultivated Area (HYV)			BINA Cultivated Area					
	Aus	Aman	Boro	Aus	Aman	Boro	Aus (%)	Aman (%)	Boro (%)
Mymensingh	16591.00	214185.00	585760.00	12.00	4396.00	97.00	0.07	2.05	0.02
Sherpur	2150.00	58850.00	66116.00	20.00	4833.00	90.00	0.93	8.21	0.14
Netrokona	1545.00	86510.00	160357.00	115.00	7741.00	370.00	7.44	8.95	0.23
Jamalpur	1137.00	66840.00	106517.00	20.00	2847.00	398.00	1.76	4.26	0.38
Mymensingh region	21423.00	426385.00	918750.00	167.00	19817.00	955.00	0.78	4.65	0.10

Source: Field Survey, 2019-20

Table 2. District wise adoption of BINA developed pulse and oilseed varieties in Mymensingh region

(Area in hectare)

Region	Pulse		Oilseed		BINA Cultivated Area in %	
	Total Cultivated Area	BINA Cultivated Area	Total Cultivated Area	BINA Cultivated Area	Pulse (%)	Oilseed (%)
Mymensingh	545	17	2737	169	3.12	6.17
Sherpur	97	20	2175	112	20.62	5.61
Netrokona	88	17	1052	491	19.32	46.67
Jamalpur	700	12	10047	150	1.71	1.49
Mymensingh region	1430	9	16011	231	4.62	5.82

Source: Field survey, 2019-20.

Table 3. Cropping patterns with number of farmers selection in Mymensingh region

Patterns	Mymensingh	Jamalpur	Sherpur	Netrokona	All Areas	Rank
Boro-Fellow-Aman	15	-	-	20	35	I
Boro-Vegetables-Aman	03	-	-	06	09	V
Boro-Aus-Aman	06	-	-	-	06	VI
Boro-Jute-Aman	04	-	-		04	VII
Vegetable-Fellow- Aman	-	-	-	02	02	VIII
Boro-Aman-Mustard	02	-	08	02	12	III
Boro-Vegetable-Fellow-Aman	-	22	04	-	26	II
Boro-Potato-Fellow-Fellow	-	08	03	-	11	IV
Aman-Vegetables-Fellow-Fellow	-	-	11	-	11	IV
Boro-Mustard-Aman-Fellow	-	-	04	-	04	VII
Total	30	30	30	30	120	

Source: Field survey, 2019-20.

Table 4. Area wise farmers level adoption among the farmer's land of crop varieties in Mymensingh region

Varieties	Mymensingh		Jamalpur		Sherpur		Netrokona		All	
	In hectare	(%)	In hectare	(%)	In hectare	(%)	In hectare	(%)	In hectare	(%)
Binadahn-10	2.01	13.09	1.87	8.40	2.24	14.12	1.79	8.87	7.91	10.74
Binadhan-7	2.78	18.11	2.05	9.21	2.91	18.35	3.94	19.53	11.68	15.86
Binadhan-11	1.78	11.60	2.45	11.01	1.17	7.38	2.47	12.25	7.87	10.69
Binadhan-17	2.86	18.63	2.92	13.12	2.80	17.65	2.59	12.84	11.17	15.17
Binadhan-14	0.21	1.37	2.45	11.01	0.92	5.80	0.66	3.27	4.24	5.76
Binadhan-19	0.58	3.78	0.79	3.55	2.28	14.38	1.56	7.73	5.21	7.07
Binasarisha-9	3.11	20.26	4.58	20.58	1.17	7.38	2.59	12.84	11.45	15.55
BRRI-28	1.03	6.71	1.88	8.45	0.51	3.22	3.92	19.43	7.34	9.97
BRRI-29	0.93	6.06	1.98	8.89	0.65	4.10	0.33	1.64	3.89	5.28
Vegetables	0.06	0.39	1.29	5.80	1.21	7.63	0.32	1.59	2.88	3.91
Total	15.35	100	22.26	100	15.86	100	20.17	100	73.64	100

Source: Field survey, 2019-20.

From Figure 2, it was found that in Mymensingh district in case of rice, the highest area covered by Binadhan-17 (18.63%) followed by Binadhan-7 (18.11%), Binadahn-10 (13.09%), Binadhan-11 (11.60%), BRRI-28 (6.71%), BRRI-29 (6.06%), Binadhan-19 (3.78%) and the lowest area was for Binadhan-14 (1.37%). It was also found that Binasharisha-9 and vegetables covered 20.26% and 0.39%, respectively.

From Figure 3, it was found that in Jamalpur district in case of rice, the highest area covered by Binadhan-17 (13.12%) followed by Binadhan-11 (11.01%), Binadhan-14 (11.01%), Binadhan-7 (9.21%), BRRI-29 (8.89%), BRRI-28 (8.45%), Binadahn-10 (8.40%) and the lowest area was for Binadhan-19 (3.55%). It was also found that area of Binasharisha-9 (20.58%) and Vegetables (5.80%).

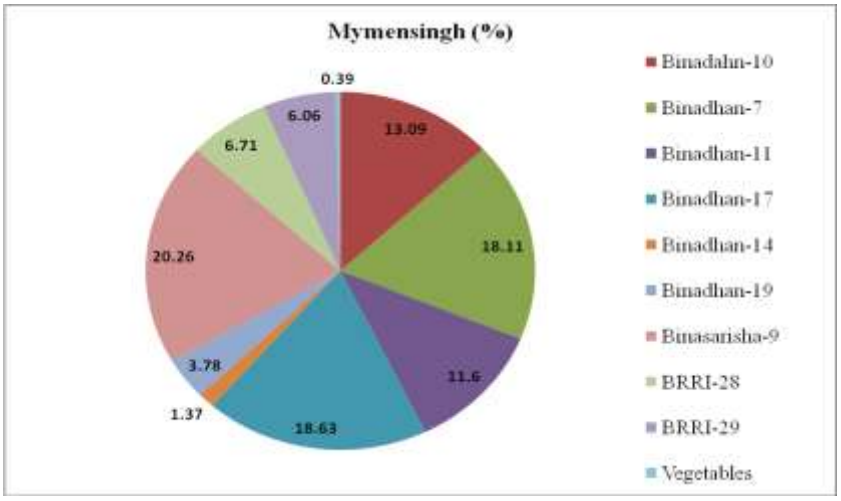

Fig. 2. Farmers level adoption among the farmer's land of crop varieties in Mymensingh district (in %)

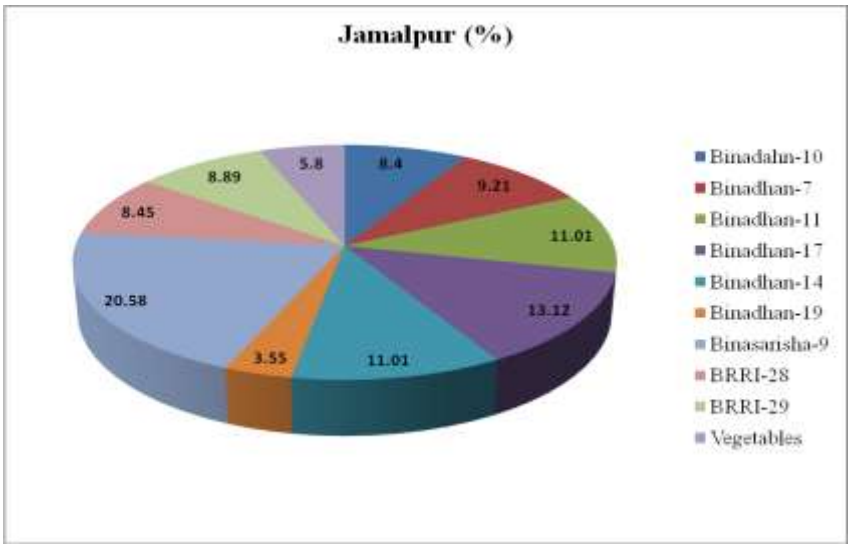

Fig. 3. Farmers level adoption among the farmer's land of crop varieties in Jamalpur district (in %)

From Figure 4, it was found that in Sherpur district in rice, the highest area covered by Binadhan-7 (18.35%) followed by Binadhan-17 (17.65%), Binadhan-19 (14.38%), Binadahn-10 (14.12%), Binadhan-11 (7.38%), Binadhan-14 (5.80%), BRRI-29 (4.10%) and the lowest area was for BRRI-28 (3.22%). It was found that area of Binasharisha-9 (7.38%) and Vegetables (7.63%).

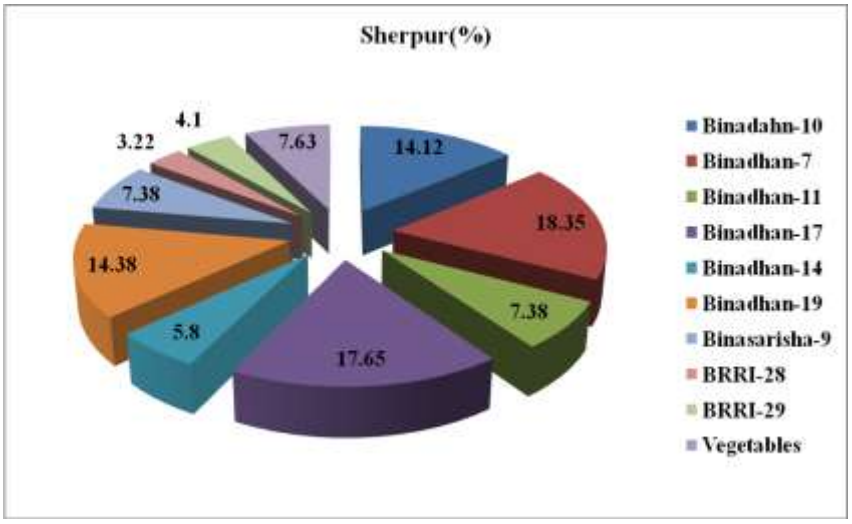

Fig. 4. Farmers level adoption among the farmer's land of crop varieties in Sherpur district (in %)

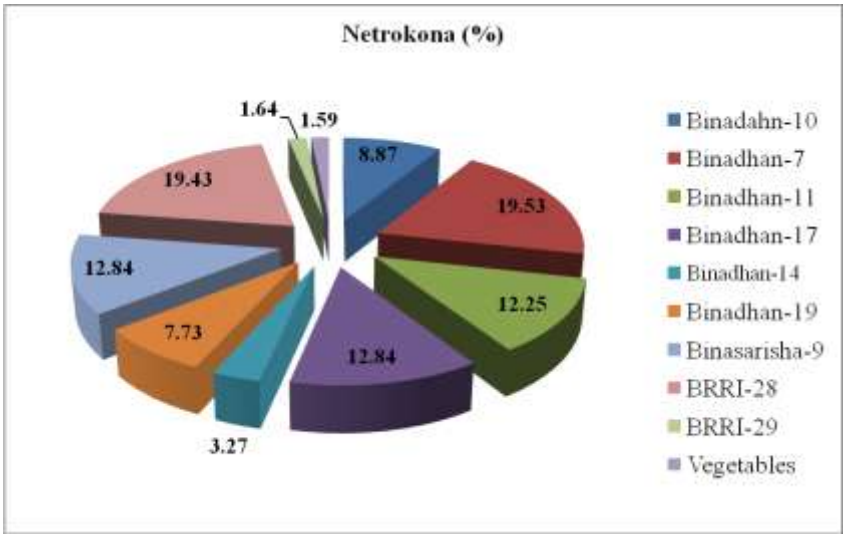

Fig. 5. Farmers level adoption among the farmer's land of crop varieties in Netrokona district (in %)

From Figure 5, it was found that in Netrokona district in rice, the highest area covered by Binadhan-7 (19.53%) followed by BRRI-28 (19.43%), Binadhan-17 (12.84%), Binadhan-11 (12.25%), Binadahn-10 (8.87%), Binadhan-19 (7.73%), Binadhan-14 (3.27%), and the lowest area was for BRRI-29 (1.64%). It was found that area of Binasharisha-9 (7.38%) and Vegetables (7.63%).

Table 5. Area wise farmers level yield among the farmers land in Mymensingh region.

Varieties	Mymensingh (t ha^{-1})	Jamalpur (t ha^{-1})	Sherpur (t ha^{-1})	Netrokona (t ha^{-1})	Average (t ha^{-1})
Binadhan-10	5.09	4.98	5.19	5.08	5.09
Binadhan-7	3.5	5.52	4.76	4.26	4.51
Binadhan-11	4.09	4.26	4.19	5.2	4.44
Binadhan-17	4.75	4.62	4.58	4.62	4.64
Binadhan-14	4.95	4.39	4.75	4.72	4.70
Binadhan-19	3.58	4.53	3.78	4.88	4.19
Binasarisha-9	1.53	1.72	1.67	1.53	1.61
BRRI-28	3.61	5.12	5.49	4.87	4.77
BRRI-29	5.05	4.97	5.01	5.05	5.02

Source: Field data, 2019-20.

From Table 5, it was found that in Mymensingh region, Binadahn-10 showed the best performance among the Boro varieties i.e. the highest average yield of Bindhan-10 was 5.09 t ha^{-1}, followed by BRRI dhan-29 (5.02 t ha^{-1}), BRRI dhan-28 (4.77 t ha^{-1}) and the lowest was for Binadhan-14 (4.70 t ha^{-1}). For aman rice, Binadhan-17 showed the best performance among the Aman varieties i.e. the highest average yield of Bindhan-17 was 4.64 t ha^{-1} followed by Bindhan-7 (4.51 t ha^{-1}) and the lowest was for Bindhan-11 (4.44 t ha^{-1}) in the study areas. For Aus rice, the highest yield of Binadhan-19 was found in Netrokona district (4.88 t ha-1) and the lowest yield was found in Jamalpur district (4.53 t ha^{-1}). The average yield of Binadhan-19 was 4.19 t ha^{-1} among the study areas.

In case of oilseed variety, the highest yield of Binasarisha-9 was found in Jamalpur district (1.72 t ha^{-1}) and the lowest yield was found in Mymensingh and Netrokona district (1.53 t ha^{-1}). The average yield of Binasarisha-9 was 1.61 t ha^{-1} in the Mymensingh agricultural region.

Constraints to BINA developed varieties at farm level

The farmers in the study areas encountered some constraints to BINA developed varieties/technologies (Table 6). The first rank problem was inadequate supply of seeds to the farmers, lack of motivation to the farmer's to cultivate the BINA released varieties, lack of coordination of BINA, DAE and farmers, lack of training facilities to the farmers about BINA technologies, farmer didn't got risk to accept new technologies or varieties and lack of supervision to the farmer's field.

Table 6. Constraints to BINA released varieties at farm level in Mymenshingh region

SL. No.	Constraints	Rank
1.	Inadequate supply of seeds to the farmers	1
2.	Lack of coordination of BINA, DAE and farmers	3
3.	Lack of motivation to the farmer's to cultivate the BINA released variety	2
4.	Lack of training facilities to the farmers about BINA technology	4
5.	Farmer didn't get risk to accept new technology or variety	5
6.	Lack of supervision to the farmer's field	6

Conclusion and recommendations

Increasing yield as well as agricultural productivity is urgent for economic growth and development for any country in the world. The study found that in Mymensingh region, farmers level adoption percentages are worthwhile in BINA developed varieties in all the seasons.

Based on study findings and field experience, the following recommendations are put forwarded for wider adoption of BINA developed varieties.

a) Seeds of BINA developed varieties should be made locally available to the farmers. For this reason the institute should encourage private seed companies to come forwarded for improvement seed production.

b) Motivational campaign through providing training, booklets and other supporting materials to farmers should be continued.

c) Existing extension services and field demonstration of BINA developed varieties should be strengthen for higher diffusion.

d) Ensure market facilities and higher prices of BINA developed varieties.

If we could increase extension activities then the area coverage of BINA varieties will be increase. On that case country's total production will be increased, which will support in achieving food security as well as Sustainable Development Goals (SDGs).

Conclusion

Farmers level adoption of BINA developed variety is viable and the continuation for variety expansion, it should be ensured the seed demand at proper time. To facilitate the dissemination more training, demonstration, collaboration with DAE and BADC as well as research and its budget should be increased which would support in production as well as will support in achieving food security.

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Short Communication

WATER STRESS TOLERANCE AT THE REPRODUCTIVE PHASE IN SELECTED LENTIL GENOTYPES

M.T. Islam[1]

Abstract

Lentil (*Lens esculenta* Medik.) is an important pulse crop with high protein content, has the potential capacity to combat nutritional deficiencies in developing countries. An experiment was carried out with six mutants *viz*., LMI-3, LMM-4, LMM-5 LMM-6, LMM-7, LMM-9 and a check variety (Binamasur-8) of lentil at the pot-yard of Bangladesh Institute of Nuclear Agriculture, Mymensingh during November 2020-March 2021. Seedling were grown in plastic pot filled with field soil and three water stress treatments *viz.* control, 45 and 30% FC were imposed at flowering stage and continued up to maturity. The experiment was laid out following a completely randomized design with three replications. Data on morpho-physiological attributes *viz*., plant height, number of branches plant-^{1}, total dry matter plant-^{1}, harvest index, yield and yield attributes were recorded. Results revealed that almost all the traits decreased significantly in response to water stress. The mutants LMM-7 and LMM-4 were considered as water stress tolerant as they showed better yield performance under stress.

Key words: Water stress, total dry matter, yield, lentil

Lentil (*Lens esculenta* Medik.) is an important pulse crop with high protein content, has the potential capacity to combat nutritional deficiencies in developing countries. High temperature and water stress are significant abiotic stresses that limit production worldwide (Sehgal *et al*. 2017; Gaur *et al*. 2015). Lentil is commonly grown under rain fed condition, conserves moisture from preceding monsoon season and usually faces water stress (Islam and Ferdousi, 2006; Helai *et al*. 2002). Water stress is one of the most common environmental factors affecting plant growth and yield. In general, lentil is relatively tolerant to drought, severe drought stress experienced during flowering can cause yield and quality losses. Even though lentils are a moderately drought tolerant crop and can grow in reduced water supply, plant productivity can decrease under a range of drought stress conditions. Water stress affects plants at different growth stages, including vegetative (intermittent drought) and reproductive (terminal drought) stages. Terminal drought can suppress nearly all the processes of lentil growth and metabolism, causing heavy yield losses (Bhandari *et al*. 2016), as it reduces flower production, pod number, and seed number (Shrestha *et al*. 2005). During seed filling, sucrose metabolism is crucial in leaves and seeds, as it plays an important role in the hexose-sucrose balance that regulates essential aspects of seed development (Weschke *et al*. 2000). High temperature affects crops through either:

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(i) above-optimum temperatures for an extended period, which increases supply of assimilates but reduces grain filling period and yield; or (ii) heat wave responses, which is a short period of high temperature (>32^{o}C) that causes non-recoverable reduction in grain set and yield potential (Vadez *et al*. 2012). Lentil requires low temperatures during vegetative growth, while at maturity, warm temperatures required; the optimum temperature for its best growth has been reported to be 18-30^{o}C (Roy *et al*. 2012). Lentil is particularly sensitive to high temperature (>30^{o}C) during the reproductive phase, causing pod and flower abortion and significant reduction in grain yield and quality (Sita *et al*. 2017). Yield was reduced by 87% for lentils grown in pots under field conditions with high temperature during the reproductive phase (38^{o}C day time, 23^{o}C night) (Bhandari *et al*. 2016), and grain set was observed to be the most sensitive yield component (Bhandari *et al*. 2016; Gaur *et al*. 2015). In Bangladesh, lentil sowings occasionally get postponed because of the delayed harvest of the preceding crop, mostly T. Aman rice. The lentil crop is then adversely affected by the high approaching summer temperatures, leading to low grain yields and poor grain quality (Islam and Haque 2020; Tickoo *et al*. 2005). Identifying the plant species resistant/tolerant to drought stress and understanding the tolerance mechanisms can play an important role in coping with drought conditions. Efforts can be given to increase area as well as yield of lentil crops by the use of water stress tolerant variety. The lentil mutants were previously selected on the basis of yield performance and those might have some tolerance to water stress. The present paper reports our results on variation for morpho-physiological attributes *viz*., plant height, number of branches plant-^{1}, total dry matter plant-^{1}, harvest index and yield and yield attributes in lentil mutants under different water regimes to identify water stress tolerant mutants.

A pot experiment was conducted at the pot yard of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. The experimental site falls under the AEZ (Agro-Ecological-Zone)-9 (Old Brahmaputra Floodplain) of Bangladesh and situated at latitude 24.75°N and longitude of 90.50°E. The soils of the experiment were collected from the field of BINA Farm. The top soil was non-calcareous Dark Grey Floodplain with loamy texture belonging to the AEZ Old Brahmaputra Floodplain. The collected soil was pulverized, inert materials, visible insect pest and plant propagules were removed. Pots are filled with top soils. The pot was 25 cm deep with 27 cm diameter at the top. The soil moisture stresses were calculated based on field capacity (FC). Gravimetric Method determined FC. FC of the soil was treated as 100% FC and 60% of FC (control), 45 and 30% were used as drought stress. Each pot contained 12 kg soil. All soils pots were fertilized with urea, TSP, MP and gypsum @ 0.41, 0.55, 0.30 and 0.26 g pot^{-1} corresponding @ 32, 77, 32 and 50 kg ha^{-1}, respectively. All fertilizers were applied as basal dose. The experiment was carried out with six mutants *viz*., LMI-3, LMM-4, LMM-5 LMM-6, LMM-7, LMM-9 and a check variety (Binamasur-8) of lentil. Seeds were sown in pots on 24 November 2020. Five seeds were sown in each pot and finally one plant was allowed to grow for treatment imposition and data collection. The experiment was set in a two factorial RCBD with three replications. The first factor was lentil genotypes and the second factor

was irrigations: control (60% FC) and drought (45 and 30% FC) stress treatments. Treatments were imposed at flowering stage and continued up to maturity. Cultural practices were followed as and when required. At maturity, data on plant height, number of branches plant^{-1}, pods plant^{-1}, seeds pod^{-1}, 1000-seed weight, seed weight plant^{-1}, straw weight plant^{-1}, total dry matter plant^{-1} and harvest index were recorded. Statistical analysis was done and DMRT test adjusted the means.

Results showed that plant height, number of branches plant^{-1}, number of pods plant^{-1} and yield plant^{-1} decreased with increasing water stress (Table 1). The highest values of those parameters were found in control plants and the lowest was recorded in 30% water stress condition. The results conforms the findings of many authors (Gaur *et al*. 2014; Islam *et al*. 1998; Salam and Islam 1994; Islam *et al*. 1993). Seeds plant^{-1}, 1000-seed wt. and HI were significantly reduced by 30% FC compared to control. Under the treatments, LMM-9 produced the longest plant (38.67 cm) and LMM-6 produced the lowest (31.33 cm) (Table-2). LMM-7, LMM-4, LMM-9 and Binamasur-8 had higher number of branches (3.08-3.22). LMM-6 produced the lowest number of branches (2.61). The highest seed weight plant^{-1} was found in LMM-7 (2.47 g) followed by LMM-4 (2.38 g) and LMM-9 (2.38 g) and the lowest in Binamasur-8 (2.01 g). The higher yields of those mutants are due to their higher number of pods plant^{-1}, seeds pod^{-1} and 1000-seed weight. The highest TDM were recorded in LMM-7 (6.27 g) and the lowest in LMI-3 (5.31 g). LMM-7 produced the highest yield (2.97 g) and TDM (6.87 g) under control (Table 3). On the other hand, the lowest yield (1.56 g) and TDM (4.76 g) were found in Binamasur-8 at 30% FC. Plant height, branches plant^{-1} and pods plant^{-1} were decreased up to 36.48, 26.53 and 38.33%, respectively due to water stress compared to control (Table 1). Water stress decreased seeds pod^{-1} and 1000-seed weight up to 4.16 and 2.66%, respectively and seed weight, straw weight, TDM and HI 37.9, 19.48, 19.93 and 21.95%, respectively. The results agree with the findings of Shrestha *et al*. (2005); Salam and Islam (1994). Water stress decreased seed yield and TDM plant^{-1}of LMM-7 and LMM-4 less compared to other genotypes (Table 3). Reduced cell division under water stress may result in shorter plants and less branch number. High biomass production is almost always associated to higher yield and biomass production may be decreased due to lower photosynthesis under stress (Islam and Haque 2020).

Table 1. Effect of different soil moisture levels on morpho-physiological and yield attributes of lentil genotypes during 2020-21

Mutants /variety	Plant height (cm)	Branches plant^{-1} (no.)	Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	1000-seed wt. (g)	Seed wt. plant^{-1} (g)	Straw wt. plant^{-1} (g)	TDM plant^{-1} (g)	HI
Control	41.61 a	3.43 a	77.39 a	1.68 a	21.35 a	2.77 a	3.85 a	6.62 a	0.41 a
45% FC	36.48 b	3.08 b	63.39 b	1.66 ab	21.28 a	2.25 b	3.10 c	5.35 b	0.41 a
	(12.32)	(10.20)	(18.09)	(1.19)	(0.32)	(18.77)	(19.48)	(19.18)	(0)
30% FC	26.77 c	2.52 c	47.72 c	1.61 b	20.78 b	1.72 c	3.58 b	5.30 b	0.32 b
	(35.66)	(26.53)	(38.33)	(4.16)	(2.66)	(37.90)	(7.01)	(19.93)	(21.95)

Values having common letter(s) in a column do not differ significantly at 5% level as per DMRT.
Figures within parenthesis indicate % decrease at 45 and 30 % FC compared to control.

Table 2. Morpho-physiological and yield attributes of lentil genotypes under water stress during 2020-21

Mutants /variety	Plant height (cm)	Branches plant^{-1} (no.)	Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	1000 seed wt. (g)	Seed wt. plant^{-1} (g)	Straw wt. plant^{-1} (g)	TDM plant^{-1} (g)	HI
LMI-3	34.64 c	2.83 b	63.00 cd	1.59 b	20.14 c	2.11 c	3.21 e	5.31 e	0.39 a
LMM-4	34.07 c	3.20 a	64.67 bc	1.65 ab	21.60 b	2.38 ab	3.75 a	6.13 b	0.38 abc
LMM-6	31.33 d	2.61 c	60.78 d	1.71 a	19.96 c	2.11 c	3.40 c	5.51 d	0.37 bc
LMM-7	34.10 c	3.22 a	67.11 ab	1.72 a	21.80 b	2.47 a	3.81 a	6.27 a	0.39 ab
LMM-9	38.67 a	3.08 a	69.33 a	1.64 b	20.16 c	2.38 b	3.61 b	5.99 c	0.39 a
Binamasur-8	36.91 b	3.09 a	52.11 e	1.59 b	23.16 a	2.01 d	3.31 d	5.32 e	0.37 c

Values having common letter(s) in a column do not differ significantly at 5% level as per DMRT.

Table 3. Combined effect of genotype and water stress (45 and 30% FC) with control on morpho-physiological and yield attributes in six lentil genotypes during 2020-21

Mutants /variety	Plant height (cm)	Branches plant^{-1} (no.)	Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	1000 seed wt. (g)	Seed wt. plant^{-1} (g)	Straw wt. plant^{-1} (g)	TDM plant^{-1} (g)	HI
V_1 x T_0	42.43 ab	3.34 abcd	79.66 b	1.64 a	20.40 d	2.66 b	3.68 d	6.35 c	0.41 ab
V_1 x T_1	36.03 d	2.83 ef	63.66 de	1.62 ab	20.34 de	2.05 d	2.76 h	4.81 gh	0.42 a
	(15.08)	(15.26)	(20.08)	(1.21)	(0.29)	(22.93)	(25.00)	(24.25)	(-2.43)
V_1 x T_2	25.46 hi	2.32 gh	45.66 h	1.50 b	19.67 de	1.60 f	3.17 f	4.78 h	0.33 e
	(39.99)	(30.53)	(42.68)	(8.53)	(3.57)	(39.84)	(13.85)	(24.72)	(19.51)
V_2 x T_0	39.19 c	3.59 a	79.33 b	1.65 a	21.74 bc	2.86 a	3.95 b	6.81 ab	0.41 abc
V_2 x T_1	35.98 d	3.23 cd	67.00 d	1.65 a	21.7 bc	2.46 c	3.40 e	5.87 de	0.41 abc
	(8.19)	(10.02)	(15.54)	(0)	(0.18)	(13.98)	(13.92)	(13.80)	(0)
V_2 x T_2	27.04 h	2.77 f	47.66 gh	1.65 a	21.36 c	1.81 e	3.88 bc	5.69 ef	0.31 ef
	(31.00)	(22.84)	(39.92)	(0)	(1.74)	(36.71)	(1.77)	(16.44)	(24.39)
V_3 x T_0	38.37 cd	3.06 de	76.33 bc	1.73 a	20.24 de	2.68 b	3.95 b	6.63 b	0.40 bcd
V_3 x T_1	32.61 ef	2.60 fg	60.66 ef	1.71 a	20.14 de	2.03 d	2.96 g	5.00 g	0.40 bcd
	(14.98)	(15.03)	(20.52)	(1.15)	(0.49)	(24.25)	(25.06)	(24.58)	(0)
V_3 x T_2	22.99 i	2.17 h	45.33 h	1.67 a	19.48 e	1.61 f	3.29 ef	4.90 gh	0.32 ef
	(29.50)	(29.08)	(40.61)	(3.46)	(3.75)	(39.92)	(16.70)	(26.09)	(20.00)
V_4 x T_0	39.06 c	3.53 ab	78.66 b	1.73 a	21.80 bc	2.97 a	3.88 bc	6.86 a	0.42 a
V_4 x T_1	35.45 de	3.30 bcd	65.00 de	1.73 a	21.79 bc	2.55 bc	3.42 e	5.98 d	0.42 a
	(9.24)	(6.51)	(17.36)	(0)	(0.04)	(14.14)	(11.85)	(12.82)	(0)
V_4 x T_2	27.79 gh	2.84 ef	57.66 f	1.70 a	21.79 bc	1.87 e	4.11 a	5.98 d	0.30 f
	(28.85)	(19.54)	(26.69)	(1.73)	(0.04)	(37.03)	(-5.92)	(12.82)	(28.57)
V_5 x T_0	45.42 a	3.46 abc	85.00 a	1.65 a	20.29 de	2.86 a	3.80 cd	6.66 ab	0.42 a
V_5 x T_1	40.40 bc	3.11 de	72.00 c	1.63 a	20.26 de	2.45 c	3.19 f	5.64 f	0.43 a
	(11.05)	(10.11)	(15.29)	(1.21)	(0.14)	(14.33)	(16.05)	(15.31)	(-2.38)
V_5 x T_2	30.19 fg	2.65 f	51.00 g	1.62 ab	19.92 de	1.82 e	3.82 bc	5.65 f	0.32 ef
	(33.53)	(23)	(40.00)	(1.81)	(1.82)	(36.36)	(-0.52)	(15.16)	(23.80)
V_6 x T_0	45.19 a	3.55 ab	65.33 d	1.64 a	23.61 a	2.55 bc	3.84 bc	6.39 c	0.39 cd
V_6 x T_1	38.41 cd	3.35 a-d	52.00 g	1.61 ab	23.43 a	1.91 de	2.87 gh	4.79 h	0.39 d
	(15.00)	(5.63)	(20.40)	(1.82)	(0.76)	(25.09)	(25.26)	(25.03)	(0)
V_6 x T_2	27.12 h	2.35 gh	39.00 i	1.51 b	22.43 b	1.56 f	3.20 f	4.76 h	0.32 ef
	(39.98)	(33.80)	(40.30)	(7.92)	(4.99)	(38.82)	(16.66)	(25.50)	(17.94)

Values having common letter(s) in a column do not differ significantly at 5% level as per DMRT.

Where, V_1: LMI-3, V_2: LMM-4, V_3: LMM-6, V_4: LMM-7, V_5: LMM-9, V_6: BINA-8 and T_0: Control, T_1: 45% FC, T_2: 30% FC. Figures within parenthesis indicate % decrease at 45 and 30 % FC compared to control.

Plant height, number of branches plant^{-1}, pods plant^{-1}, seeds pod^{-1}, 1000-seed weight, seed weight plant^{-1}, straw weight plant^{-1}, total dry matter plant^{-1} and harvest index of the lentil genotypes decreased significantly in response to water stress. Plant height, branches plant^{-1} and pods plant^{-1} were decreased up to 36.48, 26.53 and 38.33%, respectively due to water stress compared to control. Water stress decreased seeds pod^{-1} and 1000-seed weight up to 4.16 and 2.66%, respectively and seed weight, straw weight, TDM and HI 37.9, 19.48, 19.93 and 21.95%, respectively. The mutants LMM-7 and LMM-4 were considered as water stress tolerant as they showed better yield performance under water stress.

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Short Communication

YIELD PERFORMANCE OF BINA DEVELOPED LENTIL VARIETIES

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Abstract

The field experiment was conducted at research farm of BINA sub-station Ishurdi during the winter (rabi) season, 2016-2017 to find out the best yield performance of BINA developed lentil varieties that are able to increase lentil production in Bangladesh within short maturity period. BINA developed nine lentil varieties (Binamasur-1, Binamasur-2, Binamasur-3, Binamasur-4, Binamasur-5, Binamasur-6, Binamasur-7, Binamasur-8 and Binamasur-9) were tested in Randomized Complete Block Design (RCBD) with three replications to evaluate their morphological and yield contributing characters. The performance of Binamasur-8 is the best for maximizing seed yield with short maturity period. Binamasur-8 produced 2.37 t ha^{-1} yields with 88 maturity days. Primary branch production is more important than plant height in achieving higher seed yield in lentil. More primary branches ensure more pod number which also increase seed number and finally produce more seed yield. Seed size was also responsible for high yield achievement. Among all the BINA developed lentil varieties Binamasur-8 was found pioneer followed by Binamasur-5 in respect of seed yield with earliest maturity period. Therefore, it will be possible to increase lentil production in Bangladesh by cultivating Binamasur-8.

Key word: Lentil, morphological and yield contributing characters

Legumes are used worldwide as an inexpensive meat alternative and are considered the second most important food source after cereals (Kouris-Blazos *et al.*, 2016). Major legume crops grown in Bangladesh are lentils, chickpea, mungbeans, blackgrams, grass peas. Lentil (*Lens culinaris* Medik) contributes largest production percentage (64%) which ranks the first position regarding area and production in Bangladesh (BBS, 2019). Lentil grain contains 10% protein, 34.57% carbohydrate, 0.63% free amino acid and 2% polyphenol content (Khatun *et al.*, 2021). These polyphenol compounds are most bioactive component for human health. In addition to protein, lentil is a rich source of minerals and vitamins as human food, while the straw serves as high-value animal feed (Rasheed *et al.*, 2010). Not only that, its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter, which promotes sustainable crop production system (Mondal *et al.* 2013a).

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Lentil is an important legume crop that plays a significant role in nutritional security of our growing population. So, it is necessary to increase its production. In South Asia, the yield of lentil remains low and average seed yield on a country basis is below 1.0 t ha^{-1} (SAIC, 2018. Further, the area under lentil cultivation in South Asia has been decreasing at a faster rate because of increasing demand for staple grains like rice and wheat (Rahman and Ali, 2011). Lentil has been identified as a narrow adapted crop and the principal constraint of lentil production is its low yield potential because of undesirable plant type (Mondal *et al.,* 2013b). Several causes are responsible for low yield of lentil such as the use of traditional local cultivars; low plant density, weed infestation and poor crop management practices constitute the major ones. The main cause of decreasing lentil production in Bangladesh is to cultivate traditional low yielding long duration variety. Therefore, it needs to increase its production with cultivating high yielding short duration lentil variety.

Bangladesh Institute of Nuclear Agriculture (BINA) has developed nine lentil varieties through chemical and physical mutagenesis and different cultivars contain different special characters. Therefore, the present research work was conducted to find out the best yield performance of BINA developed lentil varieties that are able to increase lentil production in Bangladesh within short maturity period.

The study was conducted at research farm of BINA Sub-station, Ishurdi during the winter (rabi) season, 2016-2017. The land is medium high having sandy loam textured soil with soil pH 7.65 (Annonymous, 2012). The experiment was laid out in Randomize Complete Block Design (RCBD) with three replications. The unit plot size was 2.5 m × 2 m and seeds were sown in rows with spacing 30 cm. Nine BINA developed lentil varieties such as Binamasur-1, Binamasur-2, Binamasur-3, Binamasur-4, Binamasur-5, Binamasur-6, Binamasur-7, Binamasur-8, Binamasur-9 were used as planting material. Recommended intercultural practices such as weeding, thinning, irrigation and application of pesticides were done as and when necessary for proper growth and development of the plants. Nitrogen, phosphorus and potassium were provided during final land preparation at the rate of 25, 77 and 32 kg ha^{-1} in the form of Urea, Triple Super Phosphate (TSP) and Muriate of Potash (MP), respectively and 4 kg ha^{-1} zinc sulphate.

At maturity, ten plants were randomly sampled for recording quantitative traits such as plant height, number of main branch, number of pods per plant, number of seeds per pod, 1000 seed weight of each variety following IBPGR Descriptors (Anonymous, 2013). Grain yield was recorded through whole plot harvesting. All collecting data were analyzed statistically to one-way analysis of variance (ANOVA) using PROC GLM in SAS program (SAS Institute, 1989). The values were expressed as the mean ± standard deviation (SD) calculated using Microsoft Excel 2010. All assay data were subjected 3 mean values were compared with Duncan's Multiple Range Test at 0.05 level of Type I error.

Morphological Characters

Lentil varieties show significant differences in morphological characters such as plant height, days to maturity, and number of branch per plant were shown in Table 1. Among all BINA developed lentil varieties the Binamasur-2 was shown the highest (48.93 ± 1.21 cm) plant height that was statistically similar with Binamasur-7 (46.00 ± 2.55 cm), Binamasur-8 (47.07 ± 2.69 cm) and Binamasur-9 (47.73 ± 2.15 cm). Whereas, Binamasur-5 was the shortest (34.93 ± 2.52 cm) that was statistically different from other varieties. Maturity duration is lowest in Binamasur-8 (88 days) followed by Binamasur-6 (94 days). Maximum maturity duration was observed in Binamasur-1 (104 days). Binamasur-8 produced highest number of primary branches (3.27 ± 0.30 branches plant^{-1}) followed by Binamasur-6 (3.20 ± 0.80 branches plant^{-1}). But the number of primary brunch was statistically similar in all varieties.

Table 1. Morphological characters of BINA developed lentil varieties

Variety	Plant height (cm)	Days to maturity	No of branch plant^{-1}
Binamasur-1	40.83 ± 1.64^{b}	104	2.50 ± 0.30^{a}
Binamasur-2	48.93 ± 1.21^{a}	96	3.03 ± 0.60^{a}
Binamasur-3	41.90 ± 2.13^{b}	100	3.10 ± 0.30^{a}
Binamasur-4	41.90 ± 0.60^{b}	97	3.10 ± 0.90^{a}
Binamasur-5	34.93 ± 2.52^{c}	96	2.77 ± 0.90^{a}
Binamasur-6	39.50 ± 1.01^{b}	94	3.20 ± 0.80^{a}
Binamasur-7	46.00 ±2.55^{a}	96	2.87 ± 0.60^{a}
Binamasur-8	47.07 ± 2.69^{a}	88	3.27 ± 0.30^{a}
Binamasur-9	47.73 ± 2.15^{a}	98	2.80 ± 0.40^{a}

In a column figures with same letter (s) do not differ significantly whereas figures with dissimilar letter (s) differ significantly (as per DMRT, $p \leq 0.05$),

Yield attributes of BINA developed lentil varieties

Yield attributes of BINA developed lentil varieties also showed significant differences that was elucidated in Table 2. Maximum number of pod was produced in Binamasur-8 (109.31 ± 1.00 pods plant^{-1}) that was statistically different from other varieties and followed by Binamasur-5 (103.93 ± 1.10 pods plant^{-1}). In contrast minimum number of pod was produced by Binamasur-3 (67.97 ± 1.80 pods plant^{-1}). Similarly highest number of seed per pod was counted in Binamasur-8 (1.86 seed pod^{-1}) followed by Binamasur-5 (1.83 ± 0.06 seeds pod^{-1}) and Binamasur-9 (1.83 ± 0.06 seeds pod^{-1}) and all are statistically similar. Binamasur-8 showed bolder seed thus 1000 seed weight found maximum (22.08 ± 0.26g) followed by Binamasur-5 (21.31 ± 0.43 g) and both were statistically different. On the other hand, the lowest 1000 seed weight was recorded in Binamasur-1 (14.31 ± 0.89g) followed by Binamasur-2 (15.84 ± 0.61 g) and Binamasur-3 (15.61 ± 0.93 g). Highest amount of seed yield was collected from Binamasur-8 (2.37 ± 0.06 t ha^{-1}) that was statistically different from other varieties.

Table 2. Yield attributes of BINA developed lentil varieties

Variety	Pods plant^{-1} (no.)	Seeds pod^{-1} (no)	1000-seed weight (g)	Seed yield (t ha^{-1})
Binamasur-1	105.23 ± 1.20^{b}	1.53 ± 0.31^{bc}	14.31 ± 0.89^{e}	1.63 ± 0.12^{e}
Binamasur-2	102.74 ± 0.57^{c}	1.67 ± 0.12^{ab}	15.84 ± 0.61^{de}	1.87 ± 0.05^{bc}
Binamasur-3	67.97 ± 1.80^{g}	1.37 ± 0.06^{c}	15.61 ± 0.93^{de}	1.83 ± 0.05^{cd}
Binamasur-4	83.30 ± 1.11^{e}	1.43 ± 0.06^{bc}	17.31 ± 1.77^{d}	1.90 ± 0.06^{bc}
Binamasur-5	103.93 ± 1.10^{b}	1.83 ± 0.06^{a}	21.31 ± 0.43^{b}	2.07 ± 0.06^{b}
Binamasur-6	67.40 ± 0.79^{g}	1.60 ± 0.20^{abc}	19.43 ± 1.21^{c}	2.03 ± 0.15^{b}
Binamasur-7	76.63 ± 2.32^{f}	1.47 ± 0.15^{bc}	16.60 ± 0.86^{d}	2.03 ± 0.20^{b}
Binamasur-8	109.31 ± 1.00^{a}	1.86 ± 0.05^{a}	22.08 ± 0.26^{a}	2.37 ± 0.06^{a}
Binamasur-9	88.70 ± 1.47^{d}	1.83 ± 0.06^{a}	17.33 ± 0.47^{d}	2.02 ± 0.11^{b}

In a column figures with same letter(s) do not differ significantly whereas figures with dissimilar letter (s) differ significantly (as per DMRT, $p \leq 0.05$),

Maximum number of pods per plant, no of seeds per pod and seed size contributed to maximum yield production on Binamasur-8. Minimum yield production was observed in Binamasur-1 (1.63 ± 0.12 t ha^{-1}). Though maturity duration was shortest among all BINA developed lentil variety but production of yield shown highest in Binamasur-8. This result indicating that primary branch production is more important than plant height in achieving higher seed yield in lentil. More primary branches ensure more pod number which also increase seed number and finally produce more seed yield. Seed size was also responsible for high yield achievement. So, it can be concluded that seed yield is positively correlated with branch production as well as number of pod per plant and seed size. Similar result was also reported by many workers in lentil (Khatun *et al.,* 2016, Mondol *et al.,* 2013a, Karadavut, 2009, Anzam *et al*., 2005, Kakde *et al*., 2005, Yadav *et al*., 2003) all were reported that seed yield was positively and significantly correlated with branch number as well as number of pod per plant and seed size.

It may be concluded that Binamasur-8 is the best for maximizing seed yield with short maturity period. Binamasur-8 produced 2.37 t ha^{-1} yields with 88 maturity days. Among all the BINA developed lentil varieties Binamasur-8 was found pioneer followed by Binamasur-5 in respect of seed yield with earliest maturity period. So, Binamasur-8 may be cultivated countrywide for increase lentil production.

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