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DEVELOPMENT OF A HIGH-YIELDING EARLY MUTANT FROM FATEMADHAN THROUGH CHEMICAL MUTAGENESIS

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ABSTRACT

The development of mutants and their further characterization offers an ample scope to isolate genes and genotypes with desire trait of interest. Ethyl methanesulphonate (EMS) is a widely used chemical mutagen applied for generating genetic variability in different crop species. This study aimed to assess the agronomic performance, genetic variability and character association of 11 EMS mutants (M_3 generation) of rice derived from a promising local rice genotype (Fatemadhan). Seeds of the selected mutants, original parent and two popular varieties were grown at the field experimental farm of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. Significant inter-population differences for seven yield and yield contributing traits (viz., days to first flowering; days to maturity, DM; plant height, PH; number of effective tiller per plant, ET; panicle length; 100-seed weight, 100-SW; yield per plant) were observed. EMS Line 2 was found to be the most early maturing and high-yielding than those of the other mutants and check varieties. Analysis of heritability and genetic parameters revealed that all the traits are mostly controlled by genetic components while environment has little influence on them. The higher phenotypic and genotypic co-efficient of variation, heritability and genetic advance as percentage of mean confirmed the possibilities of rice yield improvement through phenotypic selection. Yield per plant showed significant positive correlation with ET whereas it showed negative correlation with DM, PH, and 100-SW. The first three components explained 81.4% of the total variation among the genotypes as revealed through principal component analysis. Thus, the promising mutant (EMS Line 2) isolated from this study can be released as an early maturing high-yielding rice variety.

Keywords: Fatemadhan, mutagenesis, rice-yield, morphological traits, genetic parameters

Introduction

Enhanced crop growth and yield are the recurring concerns in agricultural field, considering the soaring world population and climate change. Rice (*Oryza sativa* L.) is the main dietary staple food for more than half of the planet's human population especially in Asia and it is the most consumed cereal grain in the world. The global consumption of rice is expected to grow by 2050 from 500 million to about 750 million tons (Potupureddi *et al*., 2021). It is the most important food crop accounts for 95% of the cereals consumed in Bangladesh, with approximately 36.4 million metric tons produced from almost 28.5 million acres of land (BBS, 2020). Rice contributes one-half of the agricultural gross domestic products and one-sixth of the national income in Bangladesh (BBS, 2018). The rising demand due to population increase, low gross domestic production of rice and decreasing trend of cultivable land and are likely to cause a supply shortage in the near future. Additionally, climate change model predicts 33% decrease in rice yield in 2100 in Bangladesh (Karim *et al*., 2012). Therefore, to meet the increased requirements in the future, a strong momentum is required to boost up productivity, break down the yield barriers and provide security against instabilities in adverse climatic conditions.

Plant breeding continuously works for the development of new plant varieties with desirable traits. It was started at the dawn of civilization with the utilization of spontaneous mutation occurring in nature through simple selection of desirable offspring (Shu *et al*., 2012).In rice breeding, high selection pressure was applied for thousands of years since its domestication which resulted in narrowing down of its genetic variability (Viana *et al*., 2019). Additionally, it has a comparatively smaller genome size than other cultivated cereals (Moin *et al*., 2017). Consequently, due to repeated use, the elite rice cultivars show narrow genetic variability. Therefore, improving the genetic variability of rice has gained the attention of breeders and research groups. Genetic variations can be obtained through natural mutation or by hybridization or by artificially induced mutation. Variations obtained through spontaneous mutation in higher plants are low, ranging from 10^{-5} to 10^{-8} (Jiang & Ramachandran, 2010). Again, the variations produced by hybridization are also insufficient in most cases (Ismachin & Sobrizal, 2006). To overcome these obstacles, mutation breeding is being recommended as a shortcut method to enhance genetic variability and is primarily aimed at increasing the yield and its related traits (Kozgar *et al*., 2014).The two principal methods used to induce mutation in plants are irradiating

with gamma rays, X-rays, ion beam etc. and treating with chemical mutagens. The chemical mutagens which are used mostly to induce mutation in plants are ethyl methanesulfonate (EMS), N-methyl-N-nitrosourea (MNU), methyl methanesulfonate (MMS), sodium azide (SA), hydrogen fluoride (HF),and hydroxylamine (H_3NO) (Parry *et al.*, 2009). Chemical mutagens can be applied easily without any complicated equipment or facilities (Oladosu *et al.*, 2016). Another benefit of using chemical mutagen is that there has the possibility to improve one or two traits by avoiding unwanted changes (Jeng *et al.*, 2011). Although there are a large number of chemical mutagenic compounds available, only a small number has been applied in plant breeding (Wani *et al.*, 2014). According to IAEA, more than 80% of the registered new mutant plant varieties obtained via chemical mutagenesis were induced by alkylating agents of which EMS ranked top most (IAEA, 2015) due to ease of use and potential for producing higher frequency of nucleotide substitution (Talbei *et al.*, 2012; Da Luz *et al.*, 2015). It has been used in many previous studies of rice mutation breeding to induce genetic variability as well as to improve abiotic stress tolerance (Panigrahy *et al.*, 2011; Wattoo *et al.*, 2012; Mohapatra *et al.*, 2014; Xu *et al.*, 2017).

Fatemadhan, a promising rice genotype isolated by a farmer "Fatema Begum" from her rice field in the Bagerhat district of Bangladesh. This rice genotype is featured with robust rice plant ideotype *viz.*, strong and stout culm, large flag leaf, very long panicle with more than one thousand number of grains per panicle (Figure 1). But this promising genotype has some defects such as excessive plant height, lack of uniformity in flowering and maturity times, presence of long awn, and higher percentage of spikelet sterility which restrict it from being released as a variety. However, this genotype could be an excellent source material for rice breeding program if the drawbacks can be overcome through advanced breeding approaches like mutation breeding. Therefore, the present study was carried out to (i) revert the defects of Fatemadhan through chemical mutagenesis; (ii) characterize the mutant lines for a range of traits related to yield; and (iii) find out the suitable mutant lines that can be released as variety or can be used as materials for further breeding programs or functional genomics research including discovery of new genes and alleles in rice.

Materials and Methods

Experimental site, soils and season

The study was carried out at the Field Experimental Farm of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh during the period of July 2018 to December 2020. The land of the experimental area was medium high land belongs to Agro Ecological Zone-9 (Old Brahmaputra Flood Plain). The pH of the soil ranges from 6.5-6.7 and the texture was sandy loam.

EMS mutagenesis and mutant line(s) development

First, 200g (around 10000) seeds were treated with Bavistin-0.2% (a fungicide) for two hours. The seeds were then washed two times with double distilled water and imbibed in double distilled water for 24 hrs. The imbibed seeds were then treated with 0.8% EMS solution at 28°C for 12 hrs with gentle shaking at 60 rpm. The EMS solution was decanted and seeds were rinsed with double-distilled water

for two times. To terminate the residual effect of the mutagen, the seeds were then thoroughly washed with running tap water for two hours. After completion of the treatment, the treated seeds were allowed to germinate in petri dishes. Germinated seeds were sown in the seed bed and subsequently in the field to raise M_1 plant population. Seeds were collected from main panicle of each M_1 plant and bulked. Bulked seeds were used to grow M_2 population. Phenotypic characteristics of the each plant of the M_2 populations were critically evaluated through visual assessment at three different stages, *viz.*, seedling, vegetative and reproductive stages. Only eleven plants were selected based on their superior characteristics to develop M_3 mutant lines. Seeds from the selected plants were harvested separately.

Phenotypic characterization of the selected M_3 Mutant Lines

A total of 11 mutant lines (M_3), parent 'Fatemadhan' along with two Aman seasonal varieties (Binadhan-17 and BRRI dhan 71) were considered for characterization based on morphological traits. Seeds of the selected mutant lines and varieties were sown in the seedbed on 2nd July 2020 and seedlings were grown following standard method. Twenty-five day old seedlings were then transplanted in the main field.

Land preparation and fertilization

The experimental field was prepared through ploughing and cross ploughing with power tiller. All kind of debris and weeds and were removed from the soil during land preparation. To bring the soil to proper tilth and leveling proper laddering was done. Fertilizer doses were Urea-TSP-MP-Gypsum @ 165-110-70-50 kg/ha and cowdung @ 10 t/ha. The required amount of cowdung, MP, TSP and Gypmsum and Urea (half amount) was applied to the soil during final land preparation. Rest of the Urea was applied in two installments. The first installment was given 15 days after transplanting and the second installment was given a week before flowering.

Design and layout of the experiment

A randomized complete block design with three replications was employed in performing the experiment. Each block consisted of fourteen experimental units. The unit plot size was $4m^2$ (2 m x 2 m) with nine row in a plot and 11 plant in each row accommodating 99 plants in each plot. The seedlings were transplanted in each plot keeping plant to plant distance 20 cm and row to row distance 25. The distance between two experimental units was 50 cm. The genotypes were randomly arranged within the experimental unit using table of random numbers.

Intercultural operation

Agronomic practices like weeding and irrigation were performed to provide adequate growth conditions. Other intercultural operations were done whenever necessary.

Data collection

Data on seven yield and yield attributing traits (days to first flowering, DFF; days to maturity, DM; plant height, PH; number of effective tillers per plant, ET; panicle length, PL; 100-seed weight, 100 SW; yield per plant, YP) were recorded from ten randomly selected plants from each replication.

Mean of the ten plants for each traits were used for statistical analysis.

Data analysis

Statistical analysis of the collected data was performed by using Minitab 18 Statistical Software. The analysis of variance (ANOVA) was performed for each traits to test the variations among the genotypes. The separation of means was done following Tukey's test at 5% level of probability. Additionally, the different genetic parameters were estimated to determine the genetic variability among genotypes and evaluate the genetic and environmental effects on the characters studied.

Estimation of genotypic and phenotypic variances

Genotypic and phenotypic variances of each trait were estimated by the formulae suggested by

Singh & Chaudhary (1985).

(i) Genotypic variance: $\sigma^2_G = \dfrac{MSG - MSE}{r}$

where, MSG = mean square of genotypes, MSE = mean square of error, r = number of replications,

(ii) Phenotypic variance: $\sigma^2_P = \sigma^2_G + MSE$

(iii) Genotypic coefficient of variations, $GCV = \dfrac{\sqrt{\sigma^2_g}}{\overline{X}} \times 100$

where, σ^2_g = Genotypic variance; and $\overline{X}$ = Population mean

(iv) Phenotypic coefficient of variations, $PCV = \dfrac{\sqrt{\sigma^2_P}}{\overline{X}} \times 100$

where, σ^2_P = Phenotypic variance; and $\overline{X}$ = Population mean

GCV and PCV values were categorized as low (0-10%), moderate (10-20%) and high (≥20%) according to Sivasubramanian & Madhavamenon (1973).

Estimation of heritability

Heritability in broad sense (h^2_b) was estimated according to the formula suggested by Johnson *et al*. (1955) and Hanson (1961).

$$\text{Heritability, } h^2_b = \frac{\sigma^2_g}{\sigma^2_P} \times 100$$

Where,

h^2_b = Heritability in broad sense
σ^2_g = Genotypic variance; and
σ^2_p = Phenotypic variance

The heritability percentage was categorized as low (0-30%, moderate (30-60%), and high (≥60%) according to Robinson *et al*. (1949).

Estimation of genetic advance

Estimation of genetic advance was done following formula given by Johnson *et al*. (1955).

$$\text{Genetic advance, GA} = h^2_b.K.\sigma_p$$

where,

h^2_b = Heritability in broad sense

K= Selection differential, the value of which is 2.06 at 5% selection intensity

σ_p = Phenotypic standard deviation

Estimation of genetic advance in percentage of mean, GA (%)

The formula of Comstock & Robinson (1952) was applied in calculating the genetic advance in percent of mean.

$$\text{Genetic advance in percentage of mean, } GA(\%) = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic advance and $\overline{X}$ = Population mean

Genetic advance was categorized as low (0-10%), moderate (10-20%), and high (>20%) according to Johson *et al*. (1955).

Estimation of correlation co-efficient

The phenotypic correlations were estimated by the formula suggested by Miller *et al*. (1958).

$$\text{Phenotypic correlation, } rp_{1.2} = \frac{C_0V.P_{1.2}}{\sqrt{\sigma^2p_1 \times \sigma^2p_2}}$$

where,

$C_OV. P_{1.2}$= Phenotypic covariance between the trait X_1 and X_2

σ^2_{p1} = Phenotypic variance of the trait X_1

σ^2_{p2} = Phenotypic variance of the trait X_2

Principal component analysis

Principal component analysis was done using Minitab 18 statistical software package (Minitab Inc. State College, Pennsylvania) to investigate the pattern of quantitative variation with the eigenvector and eigenvalues.

Results

Analysis of variance

The results of analysis of variance for yield and yield contributing traits of the studied genotypes are shown in Table 1. Highly significant variation ($p<0.001$) was recorded among the genotypes (mutant lines, parent and check varieties) for all the quantitative traits studied.

Table 1: Analysis of variance (mean square) for different yield and yield attributing characters of 14 rice genotypes

Source of Variation	df	DFF	DM	PH(cm)	ET	PL(cm)	100- SW (g)	YP (g)
Replication	2	0.6429	1.54	0.403	0.00667	0.3304	0.000802	0.8335
Genotypes	13	84.6026***	116.38***	270.137***	2.75407***	17.9028***	0.087588***	67.7111***
Error	26	0.3608	0.8022	8.753	0.23231	0.5219	0.002192	1.0986

Note: *** indicates significant at 0.1% level of probability
Here, DFF, days to first flowering; DM, days to maturity; PH, plant height; ET, number of effective tillers per plant; PL, panicle length; 100-SW, 100-seed weight; YP, yield per plant

Evaluation of the mean performance of the rice mutants for yield and yield contributing traits

Days to first flowering

Significant variability was recorded among the studied genotypes for DFF. Fatemadhan, EMS Line 10, EMS Line 1, EMS Line 9, EMS Line 11 and EMS Line 2 were the early flowering genotypes as they required minimum days to flower (74.67, 74.67, 75.00, 75.33, 75.33 and 76.33 days, respectively) with no statistical difference. The mutant EMS Line 7 required maximum days (88.33)for flowering(Table 2).

Days to maturity

The mutant EMS Line 2 was the most early maturing genotype as it required minimum days for maturity (110.00; Fig. 1e) followed by BRRI dhan 71(118.00), Binadhan-17 (120.67), Fatemadhan (123.00), EMS Line 3 (125.33), EMS Line 11 (125.67), EMS Line 10 (126.00), EMS Line 8 (127.00), EMS Line 6 (127.33), EMS Line 5 (128.00), respectively. The mutant EMS Line 4 required maximum days (130.00) to mature (Table 2).

Plant height (cm)

Significant variability was recorded for PH among the studied genotypes. The highest PH was observed for EMS Line 6, EMS Line 5 and EMS Line 7 (98.48 cm, 97.60 cm and 97.04 cm, respectively) with no statistical difference. The variety Binadhan -17 showed the shortest PH (67.75 cm), however, it showed a non-significant difference with EMS Line 2 (74.32cm) (Table 2).

Number of effective tillers plant per plant

Maximum number of ET (8.40) was recorded in Binadhan-17 followed by BRRI dhan 71(8.13), EMS Line 10 (7.20), EMS Line 4 (7.00), EMS Line 2 (6.80), EMS Line 7

(6.80), EMS Line 8 (6.60), respectively, whereas the minimum number of ET (5.20) was observed in EMS Line 11(Table 2).

Panicle length (cm)

Significant variability was recorded for PL among the genotypes studied. EMS Line 9, EMS Line 6 and EMS Line 5 had the highest PL (31.25, 31.16 and 30.89cm, respectively) whereas the variety Binadhan-17 was found to produce the smallest PL (22.91 cm) (Table 2). Among the other genotypes, EMS Line 2, EMS Line 3, EMS Line 8, EMS Line 1, EMS Line 7 and EMS Line 10 had an average PL of 29.03, 28.31, 28.04, 27.97, 27.51 and 25.94 cm, respectively.

100-Seed weight (g)

The highest value for 100-SW (2.91g) was observed in EMS Line 5 and the lowest value (2.22g) was observed in Binadhan-17 (Table 2). The mean 100-SW of EMS Line 6, EMS Line 3, EMS Line 9, EMS Line 8, EMS Line 10 and EMS Line 11 was found as 2.53, 2.51, 2.51, 2.50, 2.47 and 2.47g, respectively.

Yield plant per plant

The studied genotypes showed highly significant variation among themselves for the trait YP. The highest YP (28.60g) was recorded in the EMS Line 2 which is significantly differed from other mutants, parent and check varieties (Table 2). The lowest amount of YP (12.47g) was observed in mutant Line 1 (Table 2) whereas Fatemadhan, Binadhan-17, BRRI dhan71, EMS Line 3, EMS Line 4, EMS Line 5, EMS Line 6, EMS Line 7, EMS Line 8, EMS Line 9, EMS Line 10 and EMS Line 11 produced 24.56, 24.28, 25.41, 14.94, 21.15, 16.28, 15.00, 19.01, 16.93, 22.38, 16.47 and 19.13g YP, respectively.

Table 2: Mean performances for different yield and yield attributing characters of 14 rice genotypes

Genotype	DFF	DM	PH	ET	PL	100-SW	YP
EMS Line 1	75.00e	130.00a	93.64ab	5.73def	27.97cd	2.29ef	12.47g
EMS Line 2	76.33e	110.00g	74.32ef	6.80bcde	29.03bc	2.38cde	28.60a
EMS Line 3	82.67c	125.33cd	78.68de	5.73def	28.31c	2.51bc	14.94fg
EMS Line 4	80.33d	130.00a	92.88ab	7.00bcd	25.17e	2.34def	21.15cd
EMS Line 5	86.67ab	128.00abc	97.60a	6.27cdef	30.89ab	2.91a	16.28ef
EMS Line 6	87.67ab	127.33abc	98.48a	5.80def	31.16a	2.53b	15.00fg
EMS Line 7	88.33a	129.67ab	97.04a	6.80bcde	27.51cde	2.38cde	19.01de
EMS Line 8	80.33d	127.00bc	82.16cde	6.60cde	28.04cd	2.50bc	16.93ef
EMS Line 9	75.33e	129.00ab	95.70ab	6.00cdef	31.25a	2.51bc	22.38bc
EMS Line 10	74.67e	126.00c	84.24cd	7.20abc	25.94de	2.47bcd	16.47ef
EMS Line 11	75.33e	125.67cd	93.99ab	5.20f	28.63c	2.47bcd	19.13de
Fatemadhan	74.67e	123.00de	87.94bc	5.47ef	28.91bc	2.30ef	24.56b
Binadhan-17	83.33c	120.67ef	67.75f	8.40a	22.91f	2.22f	24.28b
BRRI dhan71	86.33b	118.00f	90.05abc	8.13ab	25.19e	2.28ef	25.41b

Here, DFF, days to first flowering; DM, days to maturity; PH, plant height; ET, number of effective tillers per plant; PL, panicle length; 100-SW, 100-seed weight; YP, yield per plant

Genetic parameters analysis

Phenotypic and genotypic variances, %PCV and %GCV, heritability in broad sense, GA and GA% of the studied traits are presented in Table 3. The values for phenotypic variance were higher than those of their corresponding genotypic variance for all of the traits studied. The highest phenotypic and genotypic variance was estimated for the character PH (95.88 and 87.13, respectively), whereas the lowest values were observed for 100-SW (0.030 and 0.028, respectively). The highest PCV

and GCV values were recorded for YP (24.43 and 23.84%, respectively), whereas the lowest PCV and GCV values were recorded for DM (4.27 and 4.24%, respectively). Heritability in broad sense was observed higher for most of the traits studied however DFF and DM showed maximum heritability (98.73%) and ET showed minimum value (78.34%) for heritability. The highest GA was estimated for PH (18.33) and the lowest for 100-SW (0.33). GA as percent of mean was the highest for YP (47.96%) and the lowest for DM (8.68%) (Table 3). High heritability coupled with high genetic advance was recorded for PH, ET and YP.

Table 3: Genetic parameters of different traits of 14 rice genotypes

SL No.	Characters	Phenotypic variance (σ^2P)	Genotypic Variance (σ^2G)	PCV (%)	GCV (%)	Heritability (%)	GA	GA (%)
1	DFF	28.44	28.06	6.62	6.58	98.73	10.85	13.47
2	DM	28.44	28.08	4.27	4.24	98.73	10.85	8.68
3	PH	95.88	87.13	11.1	10.59	90.87	18.33	20.79
4	ET	1.07	0.84	15.91	14.08	78.34	1.67	25.68
5	PL	6.32	5.79	9.00	8.62	91.73	4.75	17.01
6	100-SW	0.03	0.03	7.19	6.93	92.85	0.33	13.75
7	YP	23.30	22.20	24.43	23.84	95.29	9.47	47.96

Here, DFF, days to first flowering; DM, days to maturity; PH, plant height; ET, number of effective tillers per plant; PL, panicle length; 100-SW, 100-seed weight; YP, yield per plant; PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation; GA, Genetic advance; GA (%), genetic advance as percentage of mean

Correlation co-efficient analysis

The simple Pearson's correlation coefficients for all of the seven characters measured are presented in Table 4. In the present study, 12 associations were found to be significant out of 21. Days to first flowering showed significant positive correlation with ET (0.336*). Days to maturity showed significant positive correlation with PH (0.617***) whereas it showed significant negative correlation with ET (-0.354*) and YP (-0.729***). Plant height showed significant positive correlation with PL (0.520***) and 100-SW (0.362*) however it showed negative significant association with ET (-0.466**) and YP (-0.390**). Number of effective tillers per plant was showed significant positive relation with YP (0.426**) whereas it showed significant negative correlation with PL (-0.727***). Panicle length showed positive correlation with 100-SW (0.614***) whereas it showed negative significant correlation with YP (-0.411**). Finally, YP showed positive significant correlation with ET (0.426**), however, it showed significant negative correlation with DM (-0.729***), PH (-.390*) and 100-SW (-0.411**) (Table 4).

Table 4: Correlation co-efficient of yield and yield contributing traits

Traits	DFF	DM	PH	ET	PL	100-SW
DM	0.091					
PH	0.171	0.617***				
ET	0.336*	-0.354*	-0.466**			
PL	-0.063	0.192	0.520***	-0.727***		
100-SW	0.238	0.290	0.362*	-0.290	0.614***	
YP	-0.121	-0.729***	-0.390**	0.426**	-0.267	-0.411**

Note: *, ** and *** indicates significant at 5%, 1% and 0.1% level of probability, respectively

Here, DFF, days to first flowering; DM, days to maturity; PH, plant height; ET, number of effective tillers per plant; PL, panicle length; 100-SW, 100-seed weight; YP, yield per plant

Principal component analysis

The PCA that was carried out considering all the seven quantitative traits and the results is presented in Table 5. In the present study, first three components indicated Eigen values of greater than one which cumulatively explained 81.4% of the total variation among 14 rice genotypes. Principal component analysis revealed that the first principal component (PC1) was accounted for 46.1%% of the total variation of which PH had the highest positive loadings (0.431) followed by PL (0.420) and DM (0.404) while ET had highest negative loadings (-0.412) followed by YP (-0.407). The second principal component (PC2) which contributes 20.0% of total variability, the ET (0.334) and YP (0.244)had the highest positive loading. The third principal component (PC3) was responsible for 15.3% of variation mostly contributing to traits 100-SW, PL, YP, DM and DFF (Table 5).

Table 5: Principal components (PCs) for yield and yield-related traits in 14 rice genotypes from PCA with Eigen vectors (loadings) of the first three PCs

Variable	PC1	PC2	PC3
DFF	0.037	-0.706	0.395
DM	0.404	-0.287	-0.496
PH	**0.431**	-0.109	-0.023
ET	-0.412	-0.470	0.078
PL	0.420	**0.334**	0.437
100-SW	0.371	-0.122	**0.518**
YPL	-0.407	0.244	0.363
% Variation explained	46.1%	20.0%	15.3%
Cumulative Variance (%)	46.1%	66.1%	81.4%

Here, DFF, days to first flowering; DM, days to maturity; PH, plant height; ET, number of effective tillers per plant; PL, panicle length; 100-SW, 100-seed weight; YP, yield per plant

Discussion

Evaluation of the mean performance of the rice mutants for yield and yield contributing traits

For creating a novel genetic blend in crop genome, induce mutation technology has been proved to be the best among the available techniques. Additionally, development of high-yielding and early maturing rice varieties is one of the most important breeding targets in Bangladesh to increase rice production, cropping intensity as well as to ensure food security. The present piece of research work was a genuine effort to induce desirable mutants by EMS mutagenesis from a promising rice genotype named Fatemadhan. Eleven promising mutants were selected from M_2 population based on their phenotypic performance as observed by visual assessment. Consequently, field performance of these selected mutants in M_3 generation was carried-out including parent and check varieties based on seven quantitative traits. EMS mutagenesis works through guanine alkylation, mainly GC to AT transition (Talebi *et al.*, 2012). Its mutation frequency is also high, about 2-10 mutation in each Mb (Till *et al.*, 2007). Besides, a large number of point mutation can be tolerated by rice genome. Therefore, it may be possible to achieve multiple changes in a single mutant (Mohapatra *et al.*, 2014). The results of analysis of variance presented (Table 1) showed significant variation among the mutant lines for all traits studied, evidencing the efficacy of EMS mutagenesis. Similar variation in yield and yield attributing traits were also reported by other researchers in rice mutants (Babei *et al.*, 2011; Shehjad *et al.*, 2011; Sharifi, 2019; Kato *et al.*, 2020; Andrew-Peter-Leon, 2021).

The results obtained from the present investigation indicated that all of the mutants under the study displayed considerable amount of differences in their mean performance concerning to all the characters studied which can be exploited through selection. Days to first flowering was either increased or remained similar as the Fatemadhan in the mutants. But no significant coherence was observed between DFF and DM (Table 2). Days to maturity was one of the main concern of our study as early maturing cultivars are more likely to escape from different biotic and abiotic stresses such as drought, flood, heat, cold, pathogen and insect infestations etc. Besides, farmers can use their field for next crop earlier which can increase the cropping intensity. EMS Line 2 was found to be most early maturing mutant line (110 days) which is 13 days earlier than parent genotype Fatemadhan and 8 and 10 days earlier than the check varieties, BRRI dhan 71 and Binadhan- 17, respectively (Table 2). Similar to our results, early flowering and early maturing rice mutants were also reported in different rice mutation breeding studies (Oladosu *et al.*, 2014; Sharifi, 2019; Andrew-Peter-Leon, 2021).

Plant height is important in rice breeding because it is closely related to the effective utilization of assimilation to improve plant product. A significant reduction of PH (about 13cm) was also observed in case of EMS Line 2 while most other mutant lines showed an increasing trend with suggest that possibility of mutagen promoted changes in the alleles that confer short height. Both increase and decrease of PH

due to EMS treatment was reported in earlier studies (Dorosti *et al.*, 2016; De Luz *et al.*, 2016). Generally, rice breeding programs tend to select shorter plant, because tall plants with thin culm usually are susceptible to lodging (Ni *et al.*, 2000). But the taller plants with strong and stout stem should not be discarded immediately as these plants have ability to reduce amount and quality of weeds (De Luz *et al.*, 2016).

Effective tiller is one of the most important yield attributing traits in rice. A significant variability in ET was recorded among the studied mutants, parents and check varieties which ranges from 5.20-8.40. Number of effective tiller was increased parent in all mutants in comparison with their parent EMS Line 11. More number of effective tillers has the capability of producing more yields. But excessive tiller number can cause tiller abortions, low percent of grain filling and ultimately low yield. Therefore, moderate number of effective tiller is expected to produce higher yield (Pandey *et al.*, 2009).

Panicle length is another important character related to yield. Longer PL along with more number of fertile spikelets per panicle can contribute significantly to increase yield. Fatemadhan is characterized with longer panicle than the other rice cultivars. Most of the mutant lines showed similar length with their parent genotype while two mutant populations (EMS Line 6 and EMS Line 9) exceed the value of their parent genotype. Increase in PL in response to mutagenic treatment was also reported in rice (De Luz *et al.*, 2016; Oladosu *et al.*, 2014; Sharifi, 2019; Kato *et al.*, 2020). Like other yield contributing traits, 100-SW is also another most important traits connected with the higher YP. In case of 100-SW, an increasing trend was observed in most of the mutants as compare to their parent. This may indicate the larger grain size and higher percentage of filled grain in mutant lines than their parental line. Similar to our results, an increase in 100-SW was also reported in rice as an effect of EMS mutagenesis (Andrew-Peter-Leon *et al.*, 2021).

Higher yield and better quality is the ultimate target for most of the rice breeding programs. We found significant variability in YP in the studied mutants, parents and checks. However, the EMS Line 2 showed the highest YP (28.60g) which was significantly higher than the parent Fatemadhan (24.56g) and two check varieties, BRRI dhan 71 (25.41g) and Binadhan-17 (24.28g), respectively. However, all other mutants showed a decreasing trend of YP. Similar to our results, high-yielding rice mutants were also reported by others through mutagenesis (Oladosu *et al.*, 2014; Sharifi, 2019; Kato *et al.*, 2020; Andrew-Peter-Leon, 2021). Importantly, we found both increasing and decreasing trend of the studied traits due to EMS mutagenesis. As large number of genes is related in the expression of these quantitative traits and because of random interaction of the mutagen with the target genome, the both directional variation from the parent genotype is likely to happen (Laskar and Khan, 2017).

Genetic variability, heritability and genetic advance

The study of induced mutations need extensive analysis of yield and other yield related attributes by statistical tools for selecting most promising genotype and planning

appropriate breeding strategy. Assessment of genotypic and phenotypic variance, GCV and PCV percentage, heritability and genetic advance (GA%) etc. are required to estimate the maximum genetic advancement for a specific trait that can be achieved through selection. In our present study, the analysis of genetic parameter revealed higher values of heritability for all the seven traits studied resulted from the narrower distance between genotypic and phenotypic variance which indicate the maximum genetic control and the minimum influence of environment on the expression of these traits. Similar to our results, higher GCV and PCV for these traits were also reported by others (Ghosal *et al*., 2012; Emi *et al*., 2021). These findings suggest the successful phenotypic selection as an effective way for improvement of these traits (Bhadru *et al*., 2012).

Heritability of a trait is an index of transmission of that character from parents to off springs. To select elite genotypes from a diverse genetic population, the estimates of heritability significantly helped the breeder for maximizing the genetic gain. Hence, prior knowledge about the heritability of the traits is a prerequisite for the selection program (Singh *et al*., 2011). Broad sense heritability explains both fixable (additive) and non-fixable (dominant and epistatic) variances, which helps in estimating the inheritance of a character (Nirmaladevi *et al*., 2015).On the basis of heritability, the traits can be classified into three categories *viz*., highly heritable (> 60%), medium heritable (60-30%) and low (<30%) (Robinson *et al*. 1949).All the characters in this study were highly heritable which indicated that the genetic variance is mostly due to additive gene expression. Higher heritability of the studied traits was also suggesting the greater effectiveness of selection and improvement. Our results are in accordance with the findings of Sharifi *et al*. (2019) and Dinesh *et al*. (2011). To facilitate effective selection of elite genotypes, considering heritability in association with the GA is recommended (Ogunniyan & Olakojo, 2015). The values of GA% of the studied quantitative traits were not as high as their heritability percentage which implies that high heritability along with high GA can't be expected always. This also indicates non-additive genetic effects and in this case hybridization followed by selection of progeny can be an effective way (Sinha *et al*., 2014).

Character association between yield and yield contributing traits

Yield, being a polygenic character is depended on many inter-related factors. These factors cumulatively influence the quantity and quality of yield. Therefore, in advanced plant breeding programs, the degree of association between yield and yield related attributes is consider a very helpful indicator for the selection of desirable traits. Effective tiller number was reported to have significant positive correlation with YP. Genotypes with higher number of ET are capable of producing more panicle resulting in more yields. This suggests the selection of mutant with higher tillering capability in order to obtain higher yield. The YP showed significant negative correlation with DM, PH and 100-SW. Therefore, early maturing shorter plant type mutants should be selected to gain more yield. The plants with smaller grain size might have less 100-SW. As the 100-SW is negatively correlated with yield, therefore, it can be inferred that high yielding mutants were characterized with smaller grain size. It is obvious from the results that selection of these characters associated with yield will lead towards the higher yield. Therefore, substantial importance should be given to the presence of these traits along with yield while experimenting mutation breeding in rice to achieve maximum genetic gain.

Principal component analysis

Principal component analysis is usually done to explore the relative contribution of each traits and their distribution pattern in observed variation of a population. Moreover, it is an effective tool to identify genotypes with desirable traits for further breeding procedure (Mohammadi & Prasanna, 2003). In the present work, PCA of the studied traits of M_3 mutant lines was done to find out the important characters for further selection in the following generations. We found that the first three components indicated Eigen values of greater than one which cumulatively explained 81.4% of the total variation among 14 rice genotypes. In contrast to our findings, Maji and Shaibu (2012) reported two PCs accounted for most of the variability observed in rice germplasm collections while Gour *et al*. (2017) reported five PCs. The major variation in the mutants was contributed by PH, PL, DM, ET and YP. Therefore, selection based on these traits in the subsequent generation will be helpful to develop more diverse mutant with high yielding capacity.

Conclusion

Significant phenotypic variability was observed in the mutant lines developed from Fatemadhan that signify wider possibility of selection for improving yield traits in rice. Minimum difference in PCV and GCV indicates that the apparent variability primarily due to genotypes. High heritability in broad was recorded for all the traits studied. Correlation study indicates that YP showed significant positive correlation with ET. The first three components accounted for 81.4% of the total variation among the genotypes as revealed through PCA. According to genetic parameter and mean quantitative trait data, one mutagenized population (Mutant line 2) was found superior for grain yield in the M_3 generation (Figure 1). The promising mutants isolated from this study can be further studied in different location to release as a new high yielding mutant variety. Additionally, this mutant can be used in further breeding programs for developing high-yielding elite mutant cultivar. Additionally, the divergent populations developed through chemical mutagenesis could be used for hybridization program for obtaining desirable segregants in the subsequent generations.

Fig. 1 : Phenological appearance of parent (a) Fatemadhan (b) Panicle of Fatemadhan (c) Panicle of EMS Line 2
(d) Early regproductive growth and (e)mautrity of EMS Line 2

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Conflict of Interest

The authors state that they have no conflict of interest.

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