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CHARACTERIZATION AND GENETIC ANALYSIS OF THE SELECTED RICE MUTANT POPULATIONS

N. CHOWDHURY[1, 2], S. ISLAM[1, 3], M.H. MIM[1], S. AKTER[1], J. NAIM[1],
B. NOWICKA[4], and M.A. HOSSAIN[1*]

[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Bangladesh Sugar Crop Research Institute, Regional Station, Thakurgaon, Bangladesh
[3]Bangladesh Wheat and Maize Research Institute, Nashipur, Dinajpur, Bangladesh
[4]Department of Plant Physiology and Biochemistry, Jagiellonian University, Kraków, Poland
[*]Corresponding author's email: anwargpb@bau.edu.bd
Email addresses of co-authors: noushinchowdhury1996@gmail.com, shafiul44385@bau.edu.bd,
mimuha691@gmail.com, shahnajakter121296@gmail.com, jannatulmithila406@gmail.com,
beatrycze.nowicka@uj.edu.pl

SUMMARY

The development of mutant populations, followed by their characterization, offers a significant opportunity to isolate genotypes and genes with desired traits of interest. This paper assessed the agronomic performance, genetic variability, and yield-related characteristics of 22 M_3 generation mutants (gamma ray-irradiated) of rice derived from a promising local rice genotype (Fatema dhan). The seeds of the selected mutants, the original parent, and three cultivars were grown in a randomized complete block design at the research farm of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Few mutants exhibited superior quantitative phenotypic traits compared with parental genotypes and check varieties. Mutant lines 1 and 83 required minimum days to reach maturity, and mutant lines 9, 17, and 80 exhibited significantly higher yield per plant than the parent and check varieties. Heritability analysis and genetic parameters revealed that genetic components mostly controlled all observed traits, with a minor influence on the environment. The higher phenotypic and genotypic coefficient of variation, heritability, and genetic gain confirmed possible rice yield improvement through phenotypic selection. The traits, including days to first flowering and maturity, plant height, and panicle length, showed a significant positive correlation with yield. The principal component analysis revealed that the first two components explained 69% of the total variation between genotypes. Thus, the promising mutant lines (1, 9, 17, 80, and 83) isolated in this study can serve for the development of high-yielding and early-maturing rice varieties.

Keywords: Gamma irradiation, mutagenesis, yield-attributing traits, genetic variability, genetic parameters

Key findings: Few promising rice mutants with higher yield potential got identified. The selected mutants can serve in a varietal development program for obtaining high-yielding rice variety.

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INTRODUCTION

Climate change and the additional three billion mouths to feed by 2050 raise serious concerns about global food security (Marsh *et al*., 2021). Rice (*Oryza sativa* L.) is the most consumed cereal grain in the world, constituting the dietary staple food for more than half of the planet's human population, especially in Asia. It is the most indispensable food crop, accounting for 95% of the cereals consumed in Bangladesh, with approximately 36.4 million MT produced from almost 11.5 million ha (28.5 million acres) of land (BBS, 2020). Rice contributes half of the agricultural gross domestic product and one-sixth of the national income in Bangladesh (BBS, 2018). The global rice consumption was about 490 million tons in 2018, projected to reach 550 million tons by 2030 and 590 million tons by 2040 (Humnath, 2019). The rising demand for this crop due to population increase, a saturation of cultivable fields, and low gross domestic production of rice will cause a supply shortage soon. Additionally, the climate change model predicts a 33% decrease in rice production in Bangladesh in near future (Karim *et al*., 2012). Therefore, to meet future increased requirements needs a speedy momentum to boost productivity, break down the yield barriers, and provide food security against unstable, adverse climatic conditions.

Genetic variability is a prerequisite for any crop improvement program initiation and adoption of appropriate selection techniques. High selection pressure application in rice breeding has occurred for thousands of years since its domestication, resulting in the narrowing down of its genetic variability (Viana *et al*., 2019). Additionally, rice has a comparatively smaller genome than other cultivated cereals (Moin *et al*., 2017). Consequently, due to repeated use, elite rice cultivars show narrow genetic variability. Therefore, the improvement of rice genetic variability has gained the attention of breeders and research groups. Obtaining genetic variations can come through natural mutation, hybridization, and/or artificially induced alteration. In higher plants, variations obtained from spontaneous mutation are low, ranging from 10^{-5} to 10^{-8} kb (Jiang and Ramachandran, 2010; Viana *et al*., 2019). In most cases, variation produced by hybridization is also insufficient to achieve the desired traits of interest (Ismachin and Sobrizal, 2006). Recently, mutation breeding has been promoted as a quick technique to increase genetic variability, enhance agricultural output, and improve crop quality (Kozgar *et al*., 2014).

Mutation breeding has a unique position in breaking the bottleneck of germplasm resources. Thought to create more beneficial resources than cross-breeding, it confers higher yields in rice (Tiwari *et al*., 2018; Kato *et al*., 2020; Andrew-Peter-Leon *et al*., 2021). Induced mutations are valuable sources of genetic variability (Oladosu *et al*., 2016). The basis for mutagenesis protocols comes from various physical, chemical, and biological agent applications (Serrat *et al*., 2014). Physical agents, especially gamma (γ) radiation, are the most often used mutagens in rice. Approximately 91.6% of the rice mutants obtained with physical agents have 92% generated with γ-rays (FAO/IAEA, 2019). Gamma rays can generate several allelic series containing small and large (70–500 kb) deletions in genomes in a very short time (Wang *et al*., 2013).

Previous studies confirmed the capability of this method to improve genetic variability in a wide range of rice genotypes, making it an effective tool for rice breeding (Kole *et al*., 2008; Harding, 2012; da Luz *et al*., 2020). Reports stated gamma rays had created mutants tolerant to abiotic stress, such as salinity and drought-tolerant mutants in rice (Song *et al*., 2012; Joshi *et al*., 2016; Huang *et al*., 2019). Further reports said variations in terms of different agronomic traits, such as plant height, tiller number, shoot and root weight, total biomass, and panicle length, occurred due to γ-irradiation (Oladosu *et al*., 2014; Joshi *et al*., 2016; Sharifi, 2019; Kato *et al*., 2020; Andrew-Peter-Leon *et al*., 2021).

Recently, a farmer named Fatema Begum from the Bagerhat District of Bangladesh isolated a super panicle (Figure 1) of rice from her rice field. By planting the seeds of the selected panicle, it recorded a few exceptional rice traits, such as a very long panicle producing more than 1,000 grains per panicle, a large-size flag leaf, and strong and stout culm. However, this genotype has some drawbacks (i.e., lack of uniformity in flowering and maturity time, higher percentage of spikelet sterility, excessive plant height, and presence of long awn) that restrict it from being released as a variety. If defect alteration proceeds in a positive direction through advanced breeding approaches, such as mutation breeding, this genotype could be an excellent source of material for rice breeding programs aimed at developing high-yielding rice varieties. Therefore, the presented study

Figure 1. Phenological appearance of panicles of (a) 'Fatema dhan' and (b) high-yielding mutant line 80.

aimed to (i) develop a large number of mutant lines and characterize them for yield and yield-attributing traits, (ii) revert the drawbacks of 'Fatema dhan' through physical mutagenesis, (iii) isolate a few promising lines for use to release a super-rice variety or as a source of materials for functional genomic research, including the discovery of new genes conferring higher yield and adaptability.

MATERIALS AND METHODS

Experimental site and soil conditions

The studies took place from July 2018 to January 2020 at the experimental farm field of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The land belongs to the agroecological zone-9 (Old Brahmaputra Flood Plain) with a medium-high land classification. The texture of the soil was sandy loam, and the pH of the soil ranged from 6.5 to 6.7.

Gamma irradiation and mutant lines development

The 'Fatema dhan' seeds came from the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, Bangladesh. Four batches of 200 g seeds (approximately 10,000 seeds) received treatments with 200, 250, 300, and 350Gy of γ-rays at BINA. After completing the treatments, germinating the seeds continued in Petri dishes. Germinated seeds followed sowing in the seedbed and subsequently in the field to raise the M_1 plant population. Ten healthy seeds of self-pollinated fertile M_1 plants got collected from the main panicle and bulked according to the radiation dose applied. Bulk seeds were used to grow the M_2 population. Phenotypic characteristics of each plant of the M_2 population underwent critical evaluation through visual assessment at three different stages, viz., seedling, vegetative, and reproductive stages, following the Distinctness, Uniformity, and Stability (DUS) test guidelines. Based on this evaluation, 90 individual mutants gained selection, with their seeds harvested separately. Later on, based on yield and yield-attributing traits, a collection of 22 mutant plants ensued to develop M_3 mutant lines. The seeds from the selected plants proceeded with separate harvesting.

Characterization of the mutant lines

A total of 22 mutants (M_3 generation) isolated from 'Fatema dhan', two check varieties (Binadhan-10 and BRRI dhan28), one promising newly introduced rice genotype (Begunipata) and the parents of 'Fatema dhan' underwent phenotypic characterization during the Aman (wet) season of 2019. The details describing this experiment follow accordingly. Sowing the seeds of the selected mutant lines and the check varieties progressed to the seedbed on 2 July 2019, with the seedlings grown according to the standard method. Transplanting the 25-day-old seedlings continued in the main field.

Land preparation and fertilization

The experimental field preparation comprised plowing and cross-plowing with a power tiller, removing the weeds and debris during the land preparation. Proper laddering succeeded in bringing the soil to the correct tilth and leveling. Fertilizer doses applied were Urea-TSP-MP-Gypsum at 165-110-70-50 kg/ha and cow dung at 10 t/ha. The whole amount of decomposed cow dung, TSP and MP, and Gypsum and half the amount of Urea application occurred at the time of final land preparation. The rest of the urea application took two installments, with the first installment given 15 days after transplanting and the second a week before flowering.

Design and layout of the experiment

The experimentation set out in a randomized complete block design (RCBD) with three replications. Each block consisted of 26 experimental units. The unit plot size was 4 m^2 (2 m × 2 m), with nine rows contained in a plot, and 11 plants in each row, accommodating 99 plants per plot. The seedlings transplanted to each plot kept 20 cm of a plant-to-plant distance and 25 cm of row-to-row spacing. The distance between the two experimental units was 50 cm. The genotypes randomly arranged within the experimental unit used a table of random numbers.

Intercultural operation

The different agronomic practices, such as weeding and irrigation, properly continued to ensure the adequate growth of rice plants. When necessary other intercultural operations were carried out.

Data collection and analysis

Data recording on seven quantitative phenotypic traits (days to first flowering, DFF; days to maturity, DM; plant height, PH; the number of effective tillers plant^{-1}, NET; panicle length, PL; 100-seed weight, 100-SW; yield plant^{-1}, YPP) on 10 randomly selected plants from each replication advanced, using the means of the 10 plants for each trait for statistical analysis.

Data analysis

The performed statistical analysis on the collected data used Minitab 18 Statistical Software, applying analysis of variance (ANOVA) for each trait to test for variations among the genotypes. The means separation followed Tukey's test at the 5% probability level. Additionally, estimating different genetic parameters helped to determine the genetic variability between genotypes and to evaluate the genetic and environmental effects on the characteristics studied.

Estimation of genotypic and phenotypic variances

Genotypic and phenotypic variances of each trait estimation used the formula suggested by Singh and Chaudhary (1985).

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

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) $\text{Genotypic coefficient of variations, GCV} = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$

Where, σ^2_g= Genotypic variance, and $\bar{X}$ = Population mean

(iv) $\text{Phenotypic coefficient of variations, PCV} = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$

Where, σ^2_p = Phenotypic variance, and $\bar{X}$ = Population mean

The GCV and PCV values classified as low (0%–10%), moderate (10%–20%), and high (≥20%) was, according to Sivasubramanian and Madhavamenon (1973).

Estimation of heritability

Estimated heritability in the broad sense (h^2_b) was 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 the broad sense
σ^2_g = Genotypic variance; and
σ^2_p = Phenotypic variance

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

Estimation of genetic advance

Estimation of genetic advance followed the formula given by Johnson *et al.* (1955).

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

Where,
h^2_b = Heritability in the 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 as a percentage of mean, GA (%)

The formula of Comstock and Robinson (1952) was applied for calculating the genetic advance in percent of the mean:

$$\text{GA (\%)} = \frac{GA}{\bar{X}} \times 100$$

Where,
GA = Genetic advance and $\bar{X}$ = Population mean

The genetic advance categorized as low (0%–10%), moderate (10%–20%), and high (>20%) was, according to Johnson *et al.* (1955).

Estimation of the correlation coefficient

Estimating phenotypic correlations used the formula suggested by Miller *et al.* (1958):

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

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 proceeded using the Minitab 18 statistical software package (Minitab Inc. State College, Pennsylvania) to investigate the association between genotypes under treatment.

RESULTS

The results of the analysis of variance for the yield and the yield-contributing traits of the genotypes studied are in Table 1. The results showed a highly significant ($p < 0.001$) variation between genotypes (check varieties, parents, and mutants) for the seven traits studied, indicating the presence of high genetic variability among the mutants.

Mean performance of rice mutants

A recorded wide variation in DFF among the mutants studied emerged (Table 2), with an average value of 115.55 days. The mutant line 1 required a minimum number of days to flower (101.67 days), whereas the mutant lines 6 and 56 required the maximum number of days for first flowering (119 days). Mutant line 1 and Begunipata were the early maturing genotypes, as they required the minimum number of days to mature (151.67 and 152.00 days, respectively), but parents 'Fatema dhan' needed the maximum number of days (165 days) to mature. The lowest PH showed for the genotypes Begunipata and BRRI dhan28 (55.63 and 59.48 cm, respectively), and the highest PH (82.25 cm) appeared in mutant line 6 and statistically different from all other genotypes studied. The maximum NET (14.80) emerged in the variety BRRI dhan28, while other mutants ranged from five to six. The observed highest PL (32.10 cm) was in mutant line 6, followed by line 62 (32.08 cm), line 9 (31.81 cm), and line 7 (31.42 cm), respectively. The lowest PL (20.88 cm) resulted in Begunipata. The noted highest 100-SW occurred in mutant lines 17 and 58 (2.93 and 2.87 g, respectively); however, the lowest value (2.12 g) was in mutant line 82. The highest YPP transpired for mutant line 80 (49.92 g; Figure 1; Table 2), followed by line 9 (46.72 g) and line 17 (42.84 g). Inversely, the lowest YPP came from Begunipata (26.30 g).

Table 1. Analysis of variance (mean square) for different characteristics of 26 genotypes of rice.

Sources of Variation	DF	DFF	DM	PH	NET	PL	100-SW	YPP
Replication	2	2.7821	3.8590	4.049	0.0373	0.1309	0.0003	5.059
Genotypes	25	48.105***	43.1513***	91.562***	17.7395***	24.635***	0.0877***	68.094***
Error	50	0.2221	0.2990	3.539	0.0796	0.2588	0.0011	5.207

*** indicates significant difference at $p < 0.001$; DFF = days to first flowering; DM = days to maturity; PH = plant height; NET = number of effective tillers per plant; PL = panicle length; 100-SW = 100-seed weight; YPP = yield per plant.

Genetic parameters

Table 3 presents data on phenotypic and genotypic variances, PCV and GCV, heritability, GA, and GA% of the studied traits. The characteristics that showed a high magnitude of σ^2_G and σ^2_P were PH (32.88 and 29.34, respectively) and YPP (26.17 and 20.96, respectively). Other traits showed a low magnitude of σ^2_G and σ^2_P. The values of PCV were higher than those of GCV for all traits. Recording of high GCV and PCV were for NET (35.84% and 35.60%, respectively). Meanwhile, the recording of moderate GCV and PCV values were for PL (10.03 and 9.88, respectively) and YPP (13.43–12.02, respectively). Higher differences between the GCV and PCV values resulted for the traits YPP (13.43% and 12.09%, respectively) and PH (8.00% and 7.56%, respectively), with the rest showing lower differences between GCV and PCV. In general, a recorded high h^2_b (>60%) showed for all the traits studied; however, NET gave the highest heritability (98.67%), with the lowest (80.10%) for YPP. Genetic advances ranged from 0.34 to 10.54. Genetic advance as a percentage of the mean ranged from 4.79% (DM) to 72.84% (NET) (Table 3). Significantly, high heritability and GA% were observed for NET, PL, and YPP.

Relationship between yield-attributing traits

The Pearson correlation coefficient among the yield and yield-attributing morphological traits that attribute the yield is available in Table 4. Out of 21 associations, 15 showed statistically significant. Among them, eight associations were positive and highly significant at a 0.1% level of probability, while three associations were positively significant at 1% and 5% levels of probability. Three associations revealed negative and highly significant at the 0.1% probability, with one negatively significant at the 5% level of probability. On the other hand, five associations resulted as positive and non-

Table 2. Mean performance for seven morphological traits of 26 mutants of rice.

Genotypes	DFF (days)	DM (days)	PH (cm)	NET (no)	PL (cm)	100-SW (g)	YPP (g)
Fatema dhan	117.67abc	165.00a	72.42cde	6.20d-g	27.70ijk	2.57def	34.54de
BRRI dhan28	110.33h	154.33f	59.48fg	14.80a	24.32m	2.35jk	36.78cd
Binadhan-10	118.33ab	157.33e	69.74e	13.80b	22.92m	2.74c	37.69cd
Begunipata	114.33g	152.00g	55.63g	10.87c	20.88n	2.81bc	26.30f
Line 1	101.67j	151.67g	71.67cde	6.33def	26.04l	2.29k	36.71cd
Line 4	116.33c-f	161.00cd	70.10de	5.53fg	30.20cdef	2.54d-h	36.75cd
Line 5	116.33c-f	161.33cd	72.79cde	5.73efg	31.20abc	2.58def	41.10bcd
Line 6	119.00a	161.00cd	82.25a	5.43g	32.10a	2.61de	39.20cd
Line 7	115.33efg	161.00cd	73.83b-e	5.53fg	31.42abc	2.56d-g	40.11bcd
Line 9	117.33bc	161.00cd	79.52ab	5.67efg	31.81ab	2.60de	46.72ab
Line 10	115.33efg	161.33cd	69.52e	5.53fg	30.92abcd	2.58de	40.48bcd
Line 11	116.67cde	162.67bc	73.79b-e	6.30d-g	30.15c-g	2.59de	34.49de
Line 13	118.33ab	161.00cd	72.23cde	6.20d-g	29.97c-g	2.63d	40.03bcd
Line 15	117.33bc	160.33d	72.09cde	6.47de	30.51a-e	2.51e-h	34.34de
Line 17	117.67abc	160.00d	73.04cde	6.70d	30.85abcd	2.93a	42.84abc
Line 24	115.33efg	163.33ab	71.86cde	5.70efg	29.42d-h	2.44hij	37.94cd
Line 56	119.00a	164.00ab	71.60cde	6.30d-g	26.73kl	2.55d-h	35.82cd
Line 58	117.33bc	163.67ab	76.03bcd	6.50de	28.19h-k	2.87ab	41.51bcd
Line 62	116.67cde	164.00ab	73.95bcde	6.37def	32.08a	2.54d-h	37.95cd
Line 66	115.33efg	164.33ab	73.29cde	5.83d-g	30.41bcde	2.59de	38.57cd
Line 68	118.33ab	164.33ab	76.29abc	5.63efg	28.59ghij	2.47f-i	36.15cd
Line 76	117.33bc	163.33ab	69.01e	5.80efg	29.17e-i	2.40ij	40.11bcd
Line 77	115.67d-g	163.33ab	72.56cde	6.10d-g	28.79f-j	2.46g-j	38.15cd
Line 80	117.00bcd	163.67ab	74.52bcde	5.83d-g	31.19abc	2.53d-h	49.92a
Line 82	115.00fg	162.67bc	62.16f	6.33def	27.42jkl	2.12l	37.51cd
Line 83	105.33i	154.667f	73.88bcde	5.70efg	26.93kl	2.38ijk	28.24ef
Average	115.55	160.858	71.663	6.815	28.843	2.548	38.075
Min.	101.67	151.67	55.63	5.43	20.88	2.12	26.3
Max.	119	165	82.25	14.8	32.1	2.93	49.92

DFF = days to first flowering; DM = days to maturity; PH = plant height; NET = number of effective tillers per plant; PL = panicle length; 100-SW = 100-seed weight; YPP = yield per plant.

Table 3. Genetic parameters of different characters of 26 rice genotypes.

No.	Traits	Phenotypic variance (σ^2p)	Genotypic variance (σ^2g)	PCV (%)	GCV (%)	Heritability (h^2_b%)	GA	GA (%)
1	DFF	16.18	15.96	3.48	3.46	98.63	8.17	7.07
2	DM	14.58	14.28	2.37	2.35	97.95	7.71	4.79
3	PH	32.88	29.34	8.00	7.56	89.24	10.54	14.71
4	NET	5.97	5.89	35.84	35.60	98.67	4.96	72.84
5	PL	8.38	8.13	10.03	9.88	96.93	5.78	20.03
6	100-SW	0.031	0.028	6.79	6.66	96.33	0.34	13.48
7	YPP	26.17	20.96	13.43	12.02	80.10	8.44	22.17

DFF = days to first flowering; DM = days to maturity; PH = plant height; NET = number of effective tillers per plant; PL = panicle length; 100-SW = 100 seed weight; YPP = yield per plant; GCV = Genotypic co-efficient of variation; PCV = Phenotypic co-efficient of variation; GA = Genetic advance; GA (%) = Genetic advance in the percentage of the mean.

Table 4. Phenotypic correlation co-efficient between yield and yield-contributing traits.

Traits	DM	PH	NET	PL	100-SW	YPP
DFF	0.706***	0.239*	-0.122	0.350**	0.465***	0.335**
DM		0.456***	-0.551***	0.618***	0.040	0.423***
PH			-0.598***	0.689***	0.184	0.406***
NET				-0.735***	0.124	-0.237*
PL					0.028	0.559***
100-SW						0.127

*, **, and *** indicate significance at 5%, 1%, and 0.1% level probability; 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; YPP = yield per plant.

significant, which refer to the information on inherent relationships among the pairs of combinations. However, one association was negative and non-significant, which referred to a complex link in the relation among the pair of combinations. DFF showed a significant positive correlation with DM, PH, PL, 100-SW, and YPP but a non-significant negative correlation with NET. DM showed a significant positive correlation with PH, PL, and YPP yet, a significant negative correlation with NET. PH showed a significant positive correlation with PL and YPP, whereas it showed a significant negative correlation with NET. NET showed a significant negative correlation between PL and YPP. PL showed a significant positive correlation with YPP. Crucially, YPP showed a significant positive correlation with DFF, DM, PH, and PL (Table 4).

Principal component analysis

The first two principal components (PC) explained 69% of the total variation for the effect of 26 rice genotypes on seven yield and yield-related traits (Table 5). Two PCs identified based on an eigenvalue greater than one determines a minimum of 10% of the variation. PC1 and PC2 explained 49% and 20.0% of the total variation, respectively (Table 5). The first principal component (PC1) explained the highest variation (49%) of the data, where PL had the highest positive loading (0.48), followed by DM (0.448), PH (0.416), YPP (0.348), DFF (0.329), and 100-SW (0.105). PC1 was highly significant ($p < 0.001$) for genotype (Table 5). The positive coefficients of 100-SW, DFF, ET, YPP, and DM, and the negative coefficients of PH and PL mostly dominated the variation of PC2. PC2 was also highly significant ($p < 0.001$) for the genotype (Table 5).

Table 5. Principal component analysis showing the contribution of seven quantitative traits among genotypes studied.

Variables	PC1	PC2
DFF	0.329	0.554
DM	0.448	0.083
PH	0.416	-0.139
NET	-0.395	0.415
PL	0.480	-0.204
100-SW	0.105	0.668
YPP	0.348	0.086
% Variation explained	49%	20%
ANOVA for PC scores		
Genotype (*p* value)	<0.001	<0.001

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; YPP = yield per plant.

DISCUSSION

The development of high-yielding rice varieties is one of the most important breeding targets in Bangladesh to meet the requirement of a growing population. The current research study aimed to induce desirable mutants from a promising rice genotype named ‘Fatema dhan.’ The visual assessment based on their phenotypic performance helped select 85 promising mutant plants from the M_2 population. The study chose seven quantitative traits to assess the field performance of the selected mutants (22 mutants, M_3 generation), parent line, and check varieties. Analysis of variance revealed a significant ($p < 0.001$) variation between the mutant lines with the parents and the check varieties. It indicates the effectiveness of mutagenic treatment with γ-rays in creating genetic variability. The presence of high variability among the mutant lines provides ample scope for selection to improve different quantitative characters. Reports of similar phenotypic and genotypic variability in rice mutants for yield-contributing traits also came from other researchers (Babaei *et al*., 2011; Shehzad *et al*., 2011; Sharifi, 2019; Kato *et al*., 2020; Andrew-Peter-Leon *et al*., 2021).

The results from the presented investigation indicated that all mutants under the study displayed a considerable difference in their mean performance concerning all the characteristics studied, which selection can exploit. The development of early flowering and maturing rice varieties is one of the priority research for sustainable rice production in the flood-prone lowland areas of Bangladesh to increase the intensity of cropping, as well as, protect rice cultivation against natural calamities. Additionally, plant growth and flowering period highly affect rice productivity (Zhan *et al*., 2015). The study found a significant variation among the mutants for flowering and maturity times. Among the mutant lines, lines 1 and 83 emerged as early flowering and maturing. Notably, these two mutants required approximately less than two weeks to mature compared with the parental line and other commercial varieties. Similarly to these results, several studies reported γ-irradiation induced early flowering and maturing rice mutants in rice (Oladosu *et al*., 2014; Sharifi, 2019; Andrew-Peter-Leon *et al.*, 2021).

Plant height is relevant in rice breeding because it is closely related to the effective utilization of carbon assimilation to improve the plant product. Generally, cereal breeding programs prefer to select short plants because tall plants without thick stems usually have low yield potential and are more susceptible to lodging (Ni *et al*., 2000; da Luz *et al*., 2016). However, families with increased height still need consideration, including other traits like stem thickness. In this study, mutant lines 82, 10, and 76 had the shortest PH compared with other mutants and parents, with their height very close to that of popular mega varieties (BRRI dhan28 and Binadhan-17) of Bangladesh. Results of a decrease in PH in γ-induced mutants in rice (Martins *et al*., 2005; Oladosu *et al*., 2014; Sharifi, 2019; Andrew-Peter-Leon *et al*., 2021) align with the findings of this study.

The number of effective tillers per plant is one of the most crucial traits related to increased yield. Significant variability in NET recorded among the studied mutants, parents, and check varieties ranged from 5.43 to 14.8. The genotype BRRI dhan28 produced the highest NET (14.8). Almost all mutant lines displayed moderate NET, which showed a slight increase or decrease compared with the parent line. Supporting the present results, Andrew-Peter-Leon *et al.* (2021) also reported a slight increase or decrease in NET in γ-mutants of rice. In the recent concept of rice ideotype breeding, moderate NET is expected to produce higher yield as excessive tiller number causes tiller abortions, low percent of grain filling, and ultimately low yield (Pandey *et al*., 2009).

Panicle length positively relates to the number of grains per panicle and consequently associates with grain yield (Ashfaq *et al*., 2012; Aditya and Bhartiya, 2013; Ranawake *et al*., 2013). The present study recorded the highest PL in mutant lines 6, 62, 9, 7, 5, and 80. Studies also reported an increase in PL in response to mutagenic treatment in rice (Oladosu *et al*., 2014; Luz *et al*., 2016; Sharifi, 2019; Kato *et al*., 2020). Contrastingly, a report stated a decrease in PL in γ-mutants of rice (Andrew-Peter-Leon *et al*., 2021). Like other yield-contributing traits, 100-SW is a very weighty trait connected with a higher yield per plant. This study found significant variability in 100-SW. A relevant increase in 100-SW surfaced for a few mutant lines (lines 17 and 58) compared with the parents, with similar results of a study stating an increase in 100-SW in rice (Andrew-Peter-Leon *et al*., 2021).

The yield per plant, the ultimate target of plant breeders, showed a significant increase compared with parents and check varieties. Among the studied mutant lines, lines 80, 9, and 17 displayed the highest YPP. They showed a significantly increased yield capacity versus their parent genotype and other check varieties. Therefore, these mutants need further characterization in the subsequent generation or use in various development programs to obtain high-yielding types. The results agree with others reporting high-yielding γ-induced mutants in rice (Oladosu *et al*., 2014; Sharifi, 2019; Kato *et al*., 2020; Andrew-Peter-Leon *et al.*, 2021).

The extent of variability for any characteristic is very imperative to improve a crop through breeding. Successful crop breeding depends on the presence of genetic variation along with the inheritance of traits under consideration. The mutants studied showed a wide range of variation and provided a broad range of sources to select superior and desired mutants by plant breeders. Therefore, estimating heritable and non-heritable components is essential in adopting appropriate breeding procedures. The heritable portion of the overall observed variation can be clarified by studying the components of variation, such as, coefficient of genotypic and phenotypic variability, heritability, and predicted genetic advance. For all characteristics, PCV was slightly higher than GCV, as expected. An existing narrow gap between PCV and GCV suggested low environmental expression of all traits (Meena *et al*., 2014). The number of effective tillers per plant recorded with high values of phenotypic and genotypic coefficients of variation suggested that genetic control influenced these characteristics. Others also reported higher GCV and PCV for NFT (Ghosal *et al*., 2012; Emi *et al*., 2021) in agreement with these results. Hence, simple selection needs an application to further improve these traits. The observed GCV and PCV indicated moderate for YPP and PL in the presented experiment. Similarly, moderate variation in GCV and PCV resulted in YPP, as reported by Emi *et al*. (2021) and PL by Ghosal *et al*. (2012). Thus, selection based on YPP and PL in terms of phenotypic value may still be effective. Low GCV and PCV recorded for DFF, DM, PH, and 100-SW agree with the results of other studies (Karim *et al*., 2007; Dinesh *et al*., 2011; Sharifi, 2019).

The heritability of a trait is an index of transmission of that character from parents to offspring. The estimated heritability helps the plant breeder select elite genotypes from diverse genetic populations. Hence, prior knowledge about the heritability of 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 estimate the inheritance of a character (Nirmaladevi *et al*., 2015). Heritability helps classify the traits into three categories, viz., highly (>60%), medium (60%–30%), and low heritable (<30%) (Robinson *et al*., 1949). However, heritability values coupled with genetic advances would be more effective and valuable in formulating selection procedures. The study's estimated broad-sense heritability magnitude ranged from 80.10% for YPP to 98.67% for NET. All the traits analyzed in this study were highly heritable, suggesting the greater effectiveness of selection and improvement expected for these characters in future breeding programs, as the genetic variance is often due to additive gene expression. The latest results follow the findings of Sharifi *et al*. (2019) and Dinesh *et al*. (2011). High heritability and high GA showed for NET, PL, and YPP. It indicates the role of additive genes in expressing these traits and suggests that there is a better scope to improve these traits through straight selection. On the contrary, high heritability with low GA resulted in DFF, DM, PH, and 100-SW, indicating non-additive genetic effects and an inadequate scope to advance these traits via a simple selection process. However, hybridization followed by the selection of progeny can be effective (Meitei *et al*., 2014).

A crop-breeding program aims at increasing the yield, but also at other associate components directly or indirectly impacting the crop. Correlation analyses provide information on the relative pattern of genetic variability present in populations—measure the mutual relationship between various plant characters and determine the component characters as a basis of selection for yield improvement. In a breeding program, the selection efficiency depends on the knowledge of relationships among traits. Yield is the totality of many component characters which directly or indirectly contribute to it. In the presented study, DFF showed a significant positive correlation with DM, with other studies reported similar results of a significant positive correlation between DFF and DM (Kumar *et al*., 2018; Lakshmi *et al*., 2014; Emi *et al*., 2021). In addition, DM showed a significant positive correlation with PH and PL. Likewise, Rahman *et al*. (2020) shared a significant positive correlation between DM, PH, and PL. Most of all, YPP was positively correlated with individual DFF, DM, PH, and PL, with a similar positive association of traits with YPP, reported in rice (Habib *et al*., 2005; Rahman *et al*., 2020). Therefore, the correlation study revealed that the DFF, DM, PH, and PL are crucial parameters for consideration as high-yield contributing characters. Hence, these parameters deserve considerable importance in selecting genotypes in the yield-improving breeding scheme of 'Fatema dhan.' The positive and significant association of these traits will provide plant breeders valuable knowledge concerning phenotypic traits and their degree of association that is beneficial in plant breeding schemes and rice germplasm management. The non-significant relationship of some traits associated with yield may be due to their less contribution to yield improvement or might result from genetic constitution differences in the breeding material evaluated. Therefore, a thorough selection program needs implementation to synchronize the enhancement of traits having negative and non-significant correlations.

The employment of PCA analysis in different crops evaluated morphological variation and established a genetic relationship among germplasm. It measures the importance and contribution of each component to the total variance. It can serve for the measurement of the independent impacts of a particular trait on the total variance, with each coefficient of proper vectors indicating the degree of contribution of every original variable where each principal component is associated. The higher the coefficient, regardless of the sign, the more effective it will be in discriminating between accessions (Sanni *et al*., 2012). The current study found two PCs explaining 69% of the total variance, of which the first component was responsible for 49% of the total variation, with mostly accounted for in DFF, DM, PH, PL, 100-SW, and YPP. Meanwhile, the second component was responsible for 20% of the variability, mainly in DFF, DM, ET, 100-SW, and YPP (Table 5). Two PCs accounted for most of the variability observed in rice germplasm collections, as also reported by Maji and Shaibu (2012). So, the results showed that extensive genetic variation existed in the mutants examined in this study. However, such findings are inconsistent with the results of others who reported more than two PCs (Gour *et al*., 2017; Ojha *et al*., 2017).

CONCLUSIONS

A varied selection opportunity for improving yield traits in rice is possible, as significant phenotypic variability observed among the mutant lines developed from 'Fatema dhan' emerged. The apparent variability among the mutants is mainly due to genotypes, as recorded low differences in PCV and GCV resulted for most of the traits studied. Observed high broad sense heritability showed for all the studied traits; however, high heritability and genetic advance as a mean percentage occurred for NET, PL, and YPL. The correlation study indicates that YP showed a significant positive correlation with DFF, DM, PH, and PL. Principal component analysis revealed that the first two components accounted for 69% of the total variation among the genotypes. Three mutant lines (lines 80, 9, and 17) proved superior for grain yield in M_3 generation, based on mean quantitative trait data and genetic parameters. Furthermore, mutant lines 1 and 83 could serve as sources of early maturing genes. Further studies need exploration to assess the suitability of isolated promising mutants in different locations for release as a new high-yielding mutant variety. For obtaining desirable segregants in the subsequent generations, divergent populations could also function for a hybridization program.

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