Scholars Journal of Agriculture and Veterinary Sciences (SJAVS) **e-ISSN 2348–1854**
Abbreviated Key Title: Sch. J. Agric. Vet. Sci. **p-ISSN 2348–8883**
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Assessment of Genetic Variability for Short Duration and High Yielding NERICA Rice Mutant Lines

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Original Research Article

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Article History
Received: 11.05.2018
Accepted: 21.05.2018
Published: 30.05.2018

DOI:
10.36347/sjavs.2018.v05i05.003

Abstract: Seven M_3 mutants derived from irradiating the seed of NERICA-10 with carbon ion beam were grown in the experimental farm of BINA, Mymensingh, Bangladesh to select the best performing mutant based on short duration and higher yield. All of the mutants were mature earlier (106 days) compared to its parents, NERICA 10 (127 days). Plant height of mutants (80.4 to 87.4 cm) were less than its parents (94.07cm). Average panicle length ranged from 21.4 and 22.33 cm for mutants 24.87 cm for parent. The number of effective tillers plants^{-1} and filled grain percentage for mutants and its parents were 6.8 to 9.47 and 66-82% and 3.33 and 43% respectively. Thousand grain weight of mutants (22.53-23.20 g) were significantly lesser than their parent (29.25 g). All of the selected mutants were out yielding, the average yield plant^{-1} was 10.6 to 14 g, and their parent was 2.3 g. The genetic assessment revealed that much emphasis should be given on plant height, panicle length, filled grain and thousand grain weight which were the important traits for selection of mutants due to their closest genotypic and phenotypic coefficient of variation, high heritability (90-99.72%) and moderate genetic advance as per cent of mean. The first and second principal component accounted for 83% and 8% of total variance respectively. Among the mutants RM-N_{10}-40(C)-1-5 produced highest yield plant^{-1}(14.05 g) which was attributed to its shorter plant height, longer panicle length, higher number of filled grains and medium thousand grain weight. Thus, suggested mutation breeding is an effective approach for developing desirable varieties.

Key words: Rice, NERICA mutant, Genetic variability, PCA.

INTRODUCTION

Rice (*Oryza sativa* L.) cultivation requires high amount of water. Due to climatic changes water table is depleting day by day in Bangladesh. But it requirement is increasing inversely. In Bangladesh, there is 5.46 million ha. of land prone to drought mainly located in the districts of Chapai Nawabganj, Naogaon, Rajshahi, Natore, Rangpur, Dinajpur, Joypurjhat, Pabna and Bogra [1]. Thus, pragmatic steps must be taken to introduce and develop such varieties that will require less soil moisture and thrive in drought condition.

To mitigate these problems NERICA was brought in Bangladesh in 2009. Because, NERICA rice can survive for around three weeks without water and is therefore considered suitable for cultivation in the dry uplands which do not have irrigation facilities [22]. The introduced NERICA-10 rice varieties produce lower yield even than the local land race of Bangladesh apart from its higher susceptibility to insect, pests and heterogeneous growth mostly at the reproductive stage [2]. Therefore, much attention should be paid to this in terms of improving the characteristics and making more adaptive with changing climate of Bangladesh.

Induced mutation can play vital role in improving desired characteristics of crop plants. Considering this aspect induces mutation was imposed in introduced NERICA rice to make desirable changes in Bangladesh condition. Genetic improvement of any yield attributes either qualitative or quantitative in nature has been successful with this technique [3, 4].

Recently, ion beam irradiation has been utilized as an effective method of inducing mutations. Considering the effectiveness of ion beam irradiation, NERICA-10 variety of rice was exposed to different doses of carbon ion beam irradiations in 2013 from Japan Atomic Energy Agency (JAEA). Unexpectedly, one fixed M_1 plant was evolved from 40 Gy dose of irradiation with changed maturity, pericarp color, seed size and shape, plant stature, etc. [5]. In the following Aman season, 2013, seven M_2 plants were obtained from the seeds of the fixed M_1 plant. The observed character changes in M_1 plant remained mostly unaltered in all the seven M_2 plants. In the present study, the M_3 seed were used to select the best performing mutants for short duration and higher yield. The selected mutants may be used as genetics materials for cultivation in drought prone areas of Bangladesh.

MATERIALS AND METHODS

All the seven mutants of M_3 generation (RM-N_{10}-40(C)-1-1, RM-N_{10}-40(C)-1-2, RM-N_{10}-40(C)-1-3, RM-N_{10}-40(C)-1-4, RM-N_{10}-40(C)-1-5, RM-N_{10}-40(C)-1-6 and RM-N_{10}-40(C)-1-7) were obtained from fixed M_1 plant (obtained from 40 Gy dose of carbon ion beam irradiation) and their parent NERICA-10 were used in this study. After collection, admixtures were separated and the seeds were kept in an incubator oven for five days at 50ºC temperature for breaking seed dormancy.

Seed bed was prepared by raising soil up to 5-10 cm from the field surfaces followed by puddling. Before puddling cowdung was applied at the rate of 2 kgm^{-2}. The entire seed bed was then divided into eight blocks for mutants and the parent. Drainage channels were prepared between seed beds to drain out excess water.

The seeds were soaked into water on 13thMarch, 2014 for 24 hours and incubated in moist cloth sacks for 48 hours to faster germination. The pre-germinated seeds were sown on seedbed. The experimental plot was prepared by ploughing with power tiller followed by laddering. Weeds and stubbles were removed from the field. The land was mudded and leveled well before transplanting. Recommended doses of manures and fertilizers (Urea= 214 Kg/ha, TSP= 182 Kg/ha, MP= 100Kg/ha and Gypsum= 100 Kg/ha) were applied to the soil.

A Randomized Complete Block Design (RCBD) with three replications was used to carry out the experiment. The individual plot size was 1.5 m × 0.8 m. Twenty five days old one (1) seedling $hill^{-1}$ was transplanted to the main plot. The seedlings were transplanted at 15 cm distance within rows of 20 cm apart. Gap filling was done within seven days after transplanting with the seedlings from same source to obtain uniform plant population. Irrigation, drainage, and weeding were done as and when required. Insecticides and fungicides were sprayed once.

Different genotypes matured at different times. The maturity time of different genotypes was not same. Harvesting was done when 80% of the plant population of each plot reached maturity. Data on Plant height, panicle length, number of total tiller, effective tiller, filled grain and yield $hill^{-1}$ were recorded from 5 randomly selected competitive plants. Days to 50% flowering, maturity and 1000 grain weight were recorded as plot basis.

The detail procedure of data recording is as follows

a) **Days to 50% flowering:** Recorded as days from sowing to flowering when 50% of the plant of each plot flowered.
b) **Plant height (cm):** The length of main culms of five randomly selected plants from the ground level to tip of its leaf was measured and averaged.
c) **Total tillers $plant^{-1}$:** The number of tillers were counted from each of the five randomly selected sample plants and averaged.
d) **Effective tillers $plant^{-1}$:** The number of panicle bearing tillers were counted from each of the five randomly selected sample plants and averaged.
e) **Panicle length (cm):** Recorded as the distance from the last node of the rachis to tip of the main panicle which was randomly selected from each plant and averaged.
f) **Filled grains $panicle^{-1}$:** The spikelet with kernel was considered as filled grain and counted from one randomly selected panicle from each plant and averaged.
g) **Per cent filled grains $panicle^{-1}$:** It was calculated by the following formula and data were recorded from the same panicle:

$$\textbf{Per cent filled grains panicle}^{-1}\ (\textbf{PFGPP}) = \frac{\text{FGPP} \times 100}{(\text{TSPP})}$$

Where,

FGPP = Filled grains $panicle^{-1}$
TSPP = Total $spikeletpanicle^{-1}$

h) **Weight of 1000 grains (g):** 1000 of seeds per replication were counted and weighted.
i) **Yield $plant^{-1}$ (g):** Total seed weight of each of the five sample randomly selected plants were taken after proper cleaning and drying for at least two days and averaged.

STATISTICAL ANALYSIS

SAS (university edition) was used for analysis of variance and Minitab 17 for principal component analysis. Means were separated by Duncan's Multiple Range Test (DMRT) [6], genotypic and phenotypic variances [7], heritability in broad sense (h^2_b) [7, 8], genotypic and phenotypic co-efficient of variations [9, 10], genetic advance [7, 11], genetic advance as per cent of mean [12], principal component analysis [13] were estimated in this study.

RESULTS AND DISCUSSION

Table-1: Mean sum of squares for different plant traits of seven mutants along with their parent

Traits	Source of variation		
	Replication (df=2)	Genotype (df=7)	Error (df=14)
DF	0.00	37.50***	0.00
DM	0.00	165.38***	0.00
PH	0.41	67.34***	0.48
TT	1.21	13.84**	3.38
ET	0.60	12.39**	2.11
PL	0.15	3.87***	0.14
FGPP	3.82	582.10***	2.83
PFGPP	6.55	552.61***	23.34
TGW	0.00	15.90***	0.01
YPP	0.37	41.12***	4.17

** and *** indicates significant at 0.01 and 0.001 level of probability respectively

Note: DF= days to 50% flowering, DM= days to maturity, PH= plant height, TT= total tillers plant^{-1}, ET= effective tillers plant^{-1}, PL= panicle length, FGPP= filled grains panicle^{-1}, PFGPP= per cent filled grains panicle^{-1}, TGW= 1000 grains weight, YPP= yield plant^{-1}

Table-2: Mean performance of seven mutants and their parent for yield and yield attributes

Genotypes	DF	DM	PH	TT	ET	PL	FGPP	PFGPP	TGW	YPP
RM-N$_{10}$-40(C)-1-1	82b	106b	80.67e	8.93a	7.93ab	21.40d	88.13b	81.82a	22.78c	11.61a
RM-N$_{10}$-40(C)-1-2	82b	106b	80.40e	9.67a	9.00ab	21.53d	87.07b	82.07a	22.68cd	12.38a
RM-N$_{10}$-40(C)-1-3	82b	106b	87.4b	9.73a	9.20ab	22.20bc	88.07b	76.60a	22.53d	12.00a
RM-N$_{10}$-40(C)-1-4	82b	106b	82.93c	10.07a	9.07ab	22.27bc	88.00b	81.62a	23.08b	12.99a
RM-N$_{10}$-40(C)-1-5	82b	106b	81.13de	9.93a	8.27ab	22.33b	95.00a	81.35a	22.58cd	14.05a
RM-N$_{10}$-40(C)-1-6	82b	106b	81.20de	10.67a	9.47a	21.47d	73.33c	66.16b	22.57d	12.77a
RM-N$_{10}$-40(C)-1-7	82b	106b	82.13cd	8.00a	6.8b	21.67cd	87.07b	79.17a	23.20b	10.61a
NERICA-10	92a	127a	94.07a	3.93b	3.33c	24.87a	51.13d	43.08c	29.25a	2.27b
Minimum	82	106	80.40	3.93	3.33	21.40	51.13	43.08	22.53	2.27
Maximum	92	127	94.07	10.67	9.47	24.87	95.00	82.07	29.25	14.05
Mean	83.25	108.63	83.74	8.87	7.88	22.22	82.22	73.98	23.59	11.08
SEM	1.25	2.63	1.68	0.76	0.72	0.40	4.93	4.80	0.81	1.31
LSD$_{0.05}$	0	0	1.21	3.22	2.54	0.65	2.95	8.46	0.21	3.58

Means with the same letter in a column are not significantly different at $P<0.05$according to DMRT.

Note: DF= days to 50% flowering, DM= days to maturity, PH= plant height, TT= total tillers plant^{-1}, ET= effective tillers plant^{-1}, PL= panicle length, FGPP= filled grains panicle^{-1}, PFGPP= per cent filled grains panicle^{-1}, TGW= 1000 grains weight, YPP= yield plant^{-1}, SEM= Standard error of mean, LSD= Least significant difference.

Table-3: Estimation of genetic variability for yield and yield attributing traits of NERICA rice mutants and its parent

Traits	Genotypic Variance (σ^2_g)	Phenotypic Variance (□^2_p)	Population Mean	Heritability, h^2_b (%)	GCV (%)	PCV (%)	GA	GA (%)
PH	22.29	22.76	83.74	97.91	5.64	5.70	9.62	11.49
TT	3.49	6.87	8.87	50.82	21.06	29.54	2.74	30.92
ET	3.43	5.53	7.88	61.94	23.50	29.86	3.00	38.10
PL	1.25	1.38	22.22	90.00	5.02	5.29	2.18	9.81
FGPP	193.09	195.92	82.22	98.55	16.90	17.02	28.42	34.56
PFGPP	176.43	199.76	73.98	88.32	17.95	19.10	25.71	34.76
TGW	5.29	5.31	23.59	99.72	9.75	9.77	4.73	20.07

Note: PH= plant height, TT= total tillers plant^{-1}, ET= effective tillers plant^{-1}, PL= panicle length, FGPP= filled grains panicle^{-1}, PFGPP= per cent filled grains panicle^{-1}, TGW= 1000 grains weight, YPP= yield plant^{-1}, GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, GA= Genetic advance, GA (%)= Genetic advance as per cent of mean

Table-4: Principal components (PCs) for yield and yield contributing traits under field condition

Trait	**PC1**	**PC2**
DF	0.341	-0.120
DM	0.341	-0.120
PH	0.307	-0.233
TT	-0.288	-0.578
ET	-0.293	-0.543
PL	0.306	-0.244
FGPP	-0.311	0.235
PFGPP	-0.310	0.279
TGW	0.339	-0.096
YPP	-0.322	-0.294
Variation explained	83.50%	8.40%
Cumulative variation explained	83.50%	91.90%
Eigen value	8.3476	0.8412

Note: DF= days to 50% flowering, DM= days to maturity, PH= plant height, TT= total tillers plant^{-1}, ET= effective tillers plant^{-1}, PL= panicle length, FGPP= filled grains panicle^{-1}, PFGPP= per cent filled grains panicle^{-1}, TGW= 1000 grains weight, YPP= yield plant^{-1}

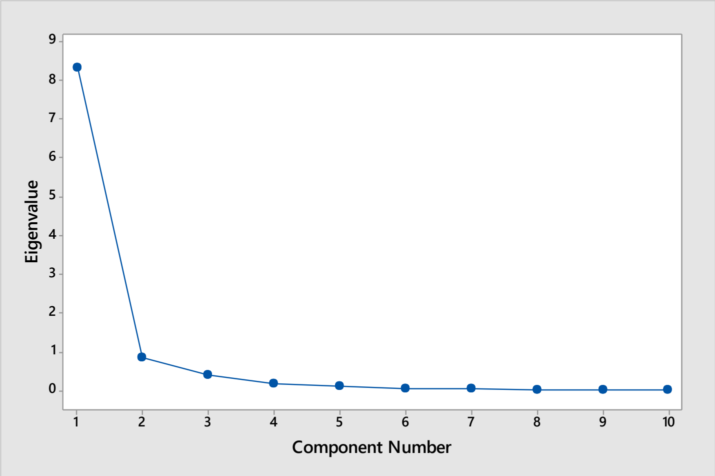

Fig-1: Scree plot of principal component analysis of yield attributing traits with their Eigen values and number of principal components

Analysis of variance (ANOVA)

All the yield and yield contributing characters studied among seven mutants and their parent were highly significant at 0.01 level of probability (Table 1). These results suggest that there is a scope of selecting better mutants than the parent.

Mean performance for yield and yield attributes

Earliness behavior is one of the most important yield contributing traits which is assessed by days to flowering and maturity. In this study, mutants derived from NERICA-10 had 21 days earlier maturity (Table 2). This result indicates that all the mutants had 21 days earlier maturity than their parent NERICA-10. This might help to increase the cropping intensity and to reduce the production cost like labor, fertilizer, irrigation etc. in Bangladesh. From the genetic study, it is evident that much emphasis should be given on plant height, panicle length, filled grain and thousand grain weight for selection of mutants. Because, these four traits exhibited closest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), high heritability and moderate genetic advance as per cent of mean (Table 3).

Plant height is an important character because the taller the plant the more the possibility of lodging. In this study, all the mutants had significantly shorter height than their parent NERICA-10 (Table 2). Among the mutants, plant height of RM-N_{10}-40(C)-1-1, RM-N_{10}-40(C)-1-2, RM-N_{10}-40(C)-1-5 and RM-N_{10}-40(C)-1-6 were shorter than the other mutants. Plant height of these four mutants although did not differ significantly from each other.

Effective tiller is a most important yield attribute in rice. In this study, all the mutant had significantly higher number of effective tillers than their parent NERICA-10. (Table 2). Among the mutants, RM-N_{10}-40(C)-1-6 had the highest number of effective tillers which did not differ significantly from the other mutants.

Longer panicle length with more number of filled grains contributes to yield much. Although panicle length was smaller in the mutants than their parent NERICA-10, but due to significantly higher number of filled grains they had produced significantly higher yield plant^{-1} (Table 2). Among the mutants RM-N_{10}-40(C)-1-5 had the longest panicle length and highest number of filled grains which contributed to its significantly higher yield plant^{-1}. This mutant would be put into further trial for release as a variety.

Similar results have been reported by other researchers for days to 50% flowering, effective tillers plant^{-1}, panicle length, filled grains panicle^{-1}, per cent filled grains panicle^{-1}, 100 grains weight, yield plant^{-1} and other attributes in rice [14, 15].

Estimation of genetic parameters

For any Plant Breeding program, it is very much imperative to assess the genetic behavior of the studied traits. Therefore, genotypic and phenotypic variances, genotypic and phenotypic coefficient of variations, heritability and genetic advance were studied. The magnitude of difference between PCV and GCV was relatively low for all the traits, which indicates less environmental influence on these traits. Similar results have been reported by other researchers working with rice [16].The closest GCV and PCV values with high heritability (90.00-99.72%) and moderate genetic advance as per cent of mean were observed in plant height, panicle length, filled grain and thousand grain weight (Table 3). Therefore, much emphasis should be given on these traits for selection of higher yielding mutants. This result corroborates with the previous findings for different traits in rice [17-18].

Principal component analysis (PCA)

Basically, total variance is combined with every original variable. The contributions of every original variable are indicated by each of the coefficient. 'Proper values' of PCA compute the contribution and involvement of each component to total variance. The higher coefficients are more effective regardless they are either positive or negative. There are no standard tests to prove significance of proper values and the coefficients. In this study, the criterion was used, which suggested that the first two principal components are often the most important in reflecting the variation patterns among genotypes and the traits associated with these are more useful in differentiating genotypes [19]. According to this criterion, the first two components account for more than 91% of total variation (Table 4), giving a clear idea of the structure underlying the variables analyzed. This result was also supported by scree test (Figure 1) indicating that the consideration of first two components which account for more than 91% of total variation. However, the criterion was chosen, which suggested to determine the cutoff limit for the coefficients of the proper vectors; this criterion treated coefficients greater than 0.3 as having a large enough effect to be considered important, while traits having a coefficient less than 0.3 were considered not to have important effects on the overall variation observed [20].

The first principal component accounted for more than 83% of total variance, whereby dominated (excluding less than 0.3 values) by days to 50% flowering, days to maturity, plant height, panicle length, filled grains panicle^{-1}, percent filled grains panicle^{-1}, thousand grains weight and yield plant^{-1}. As a result, the first component differentiated those genotypes that flowered and matured earlier and gave higher yield in that season. Similar results were observed for days to 50% flowering and days to maturity of rice by the earlier researchers [21]. The second principal component accounted for more than 8% of total variance. Variables highly correlated were total tillers plant^{-1} and effective tillers plant^{-1}. Thus, the second principal component differentiated the genotypes with good architecture.

Considering all of the above information, the investigation revealed that, plant height, panicle length, filled grains and thousand grain weight were important yield attributes in rice. Selection based on these traits exhibited that the mutant RM-N_{10}-40(C)-1-5 produced significantly the highest yield plant^{-1}(14.05 g) which was attributed to its shorter plant height, longer panicle length, higher number of filled grains and medium thousand grain weight. The results suggest that, irradiation with carbon ion beam would be an effective mutation breeding approach for developing short duration and high yielding varieties in Bangladesh.

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