

Original Research Article

Genetic Divergence and Heritability Study of Some NERICA Mutant Lines and Their Parents Using Microsatellites Marker and Morphological Traits

ABSTRACT

New Rice of Africa (NERICA) is drought tolerant and early maturing inter specific rice variety which was introduced in Bangladesh from Uganda in 2009. But the field record of NERICA was not very promising. In order to develop NERICA varieties suitable for agro-climatic conditions of Bangladesh different variations in yield contributing characters are required. The experiment was conducted to evaluate the genetic variation of NERICA mutants based on 12 yield attributing characters and 3 simple sequence repeat (SSR) markers. Physical mutagen treated (250, 300 and 350 Gy Gamma-rays) 18 NERICA mutant lines were selected from M4 to M5 generations along with 3 parents (NERICA-1, NERICA-4, NERICA-10) for morphological and molecular evaluation. Pearson's correlation co-efficient of 12 morphological traits indicated that yield per plant had significant positive correlation with the number of tiller, number of effective tiller, panicle length, number of filled grains per panicle and 1000 grain weight but negative correlation with Plant height and days to maturity. Broad sense heritability (h_b^2) ranged from 71% to 99%. Yield per plant, unfilled grains per panicle, number of total tiller, number of effective tiller showed high heritability along with genetic advance as percent of the mean (GA%) value. A total of 24 alleles were detected by 3 SSR markers. The mean gene diversity and Polymorphism Information Content (PIC) values was 0.821 and 0.797, respectively. Dendrogram constructed based on SSR markers clustered the genotypes into six distinct clusters. Combining molecular and morphological evaluation data eight mutant lines, N10/300/P-2-3-5, N10/300/P-2-3-5, N10/300/P-2-3-5-2, N1/300/P-2-3-5, N1/300/P-2-3-5, N1/250/P-7-6-4-1, N10/300/P-2(1)-4-1 and N1/250/P-7-3-7-1 were selected as desired mutant lines having good yield attributing characters and could be recommended for further evaluation in rice breeding program.

Keywords: NERICA, Mutant lines, Heritability, Genetic advance, Correlation, SSR markers

1. Introduction

Rice belongs to the genus *Oryza* in the grass family *Gramineae*. There are more than 20 species in the genus, of which only two are cultivated: *Oryza sativa*, domesticated in the humid tropics of South and Southeast to East Asia, and *Oryza glaberrima*, domesticated in the Niger basin in Africa [1]. The cultivated species are diploid ($2n=24$), whereas among the 22 wild species there are both diploids and tetraploids [2]. New Rice of Africa (NERICA) is a new rice variety in Bangladesh which was introduced in 2009 from Uganda. The rice variety NERICA was developed by Dr. Monty Patrick Jones and his colleagues in 1994, at the M'bé research center of Africa Rice Center (WARDA) by crossing (using embryo rescue) between *Oryza sativa* L. (WAB56-104) and *Oryza glaberrima* (CG14) [1,3]. NERICA is a Rice for Africa and adapted to upland condition in Uganda. It is a drought tolerant and early maturing (mature within 30 to 50 days) inter specific rice variety. Inter specific lines are genotypes that are developed through crossing plants from different species. In rice, genotypes developed through crossing two cultivated species like, *O. glaberrima* and *O. sativa* L. or crossing either of the two with any of their relatives generates interspecific genotypes. NERICA rice could be a potential source of novel traits such as genetic resistance to drought, soil acidity, iron toxicity, weed competition, blast and virus diseases [4,5]. In Bangladesh, NERICA is not a released variety it is in trial condition and due to its draught tolerance, high yielding and early maturity it has great importance in our country for increase food security. But due to soil variation and climatic change the field record of NERICA was not very promising [6]. In order to improve NERICA varieties for Bangladesh different variation in yield contributing characters such as, tiller number, panicle length, plant height, days to maturity, filled grain per panicle, yield per plant etc. are needed. But these natural variations of NERICA varieties are unavailable in Bangladesh. In this circumstance Bangladesh Institute of Nuclear Agriculture (BINA) took a step to create variations for those traits using Gamma (γ) rays as physical mutagen. Treating

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seeds with radiation (γ rays, X-rays and other mutagens) is a useful and frequently used technique to introduce trait variations which might be a source of crop improvement [7,8]. Phenotypic evaluation and molecular assessment is required to select superior mutant lines with desirable trait for breeding purpose.

Traditional morphological marker are inexpensive but unreliable tool for genetic diversity assessment as they are influenced by environmental factors. This gives the modern molecular markers (SSR, RAPD, AFLP etc.) an upper hand as they are free from environmental influences. Microsatellite or simple sequence repeat (SSR) markers have been considered as a popular co-dominant marker for their capability to identify large numbers of separate alleles efficiently, repeatedly and accurately [9]. For this reason SSR markers are recognized as an appropriate and popular molecular tool to assess genetic diversity and identify variability [10]. SSR markers are widely used for genetic diversity analysis [11,12,13], checking purity of different varieties [14], DNA fingerprinting [15]. Utilization of microsatellite or simple sequence repeat (SSR) markers and morphological markers in combination are considered a more comprehensive effort for genetic analysis and variability identification in order to select genotypes documentation and breeding programs.

Heritability is predictive instrument that helps the breeders to identify the traits which have greater chance for transmitting to the progenies [16]. Highly heritable, positively correlate and genetically independent yield components traits are most effective and desirable to increase grain yield [17]. Genetic advance is also considered an important indicator to identify and select yield contributing traits [18]. Coupling of high genetic advance with high heritability is considered more reliable trait selection approach and it helps to identify additive genes linked to the trait [19].

The present study was conducted with the following objectives: i) evaluate 12 yield attributing characters of the 18 selected mutant lines and their parents; ii) estimate genetic advance and heritability and determine correlation among the 12 morphological traits; iii) determine genetic diversity of mutant lines by using SSR markers and morphological traits; iv) identify the mutant lines which can be used as parent in future breeding program.

2. MATERIALS AND METHODS

2.1 Planting materials

Eighteen advanced early maturing drought tolerant NERICA rice mutant lines (250, 300 and 350 Gy gamma-ray treated) were selected from M4 to M5 generations along with 3 NERICA parent varieties- NERICA-1, NERICA-4 and NERICA-10 (Table.1) and were evaluated to study morphological and molecular variability. Seeds were collected from the Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Table 1. List of genotypes, their Parent, Used radiation (Gy) and Source of collection.

Serial no.	Symbol	Genotypes	Parents	Gamma ray (Gy)	Source of collection
1.	G1	N10/300/P-2(1)-3-5-2	NERICA-10	300	Bangladesh Institute of Nuclear
2.	G2	N10/350/P-2(1)-3-5-1	NERICA-10	350	Agriculture (BINA)
3.	G3	N1/250/P-7-6-4-1	NERICA-1	250	
4.	G4	N1/250/P-7-6-4	NERICA-1	250	
5.	G5	N10/300/P-2(1)-4-1	NERICA-10	300	
6.	G6	N10/300/P-5-1-1-1	NERICA-10	300	
7.	G7	N1/300/P-9-5-3-1	NERICA-1	300	
8.	G8	N4/350/P-2(1)-32-5	NERICA-4	350	
9.	G9	N1/250/P-7-3-11	NERICA-1	250	
10.	G10	N10/300/P-2-3-5	NERICA-10	300	
11.	G11	N10/300/P-2-3-5	NERICA-10	300	
12.	G12	N10/300/P-2-3-5-2	NERICA-10	300	
13.	G13	N10/300/P-2-3-5-1	NERICA-10	300	
14.	G14	N1/250/P-7-3-7-1	NERICA-1	250	
15.	G15	N1/250/P-7-3-7-2	NERICA-1	250	
16.	G16	N1/250/P-7-3-7-4	NERICA-1	250	
17.	G17	N1/250/P-7-3-7-2-3	NERICA-1	250	
18.	G18	N1/300/P-2-3-5	NERICA-1	300	
19.	G19	NERICA-1 (Parent)	-----	-----	
20.	G20	NERICA-4 (Parent)	-----	-----	

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2.2 Morphological study

The soil of the experimental site belonging under the agro-ecological zone of Old Brahmaputra Floodplain (AEZ-9; Longitude: 24.7232°N, Latitude: 90.4316°E). The field was medium high land. The experiment was conducted following RCBD design. Row to row and plant to plant distances of the plot were 20 cm and 15 cm, respectively. Cultural practices were followed as and when necessary. Data were recorded for days to 1st flowering, days to 50% flowering, days to 80% flowering, days to maturity (DM), plant height, total tillers per hill, effective tillers per hill, filled grains per panicle, unfilled grains per panicle, 1000 grain weight and yield per plant.

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2.3 Statistical analysis

MSTAT-C software was used to analyze all the morphological traits data and generate analysis of variance (ANOVA) table and all the significant means were compared using LSD taste.

Genotypic variances (σ^2g), environmental variances (σ^2e), phenotypic variances (σ^2p), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were estimated following the formulas proposed by [20]:

$$\sigma^2g = (MSg - MSe)/r$$

$$\sigma^2e = MSe$$

$$\sigma^2p = \sigma^2g + \sigma^2e$$

$$GCV = \frac{\sqrt{\sigma^2g}}{\bar{x}} \times 100$$

$$PCV = \frac{\sqrt{\sigma^2p}}{\bar{x}} \times 100$$

Here,

MSg = mean square of populations, MSe = mean square of error, \bar{x} = mean of the trait, r = replication

Broad sense heritability (h_b^2) was estimated following the formula proposed by [21]:

$$\text{Heritability } h_b^2 = \frac{\sigma^2g}{\sigma^2p} \times 100$$

Genetic advance in percent of the mean (GA %) was estimated according to the formula proposed by [22]:

$$GA \% = \frac{K \times h_b^2 \times \sqrt{\sigma^2p}}{\bar{x}}$$

Here, Standardized selection differential constant, $K=2.06$, at 5% selection intensity, \bar{x} : Mean of the traits.

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2.4 Extraction of DNA

DNA extraction was done by using the modified CTAB method [23] from 3" young leaves tissues of 21-day old seedlings. Purity and concentration of genomic DNA was examined by calculating the ratio of the optical density measured at 260 nm using a spectrophotometer and adjusted to a concentration of 10 ng μ l⁻¹.

2.5 Amplification of microsatellite markers

Ten random primers viz. RM32, RM80, RM202, RM215, RM334, RM510, RM562, RM10720, RM28102, and RM28502 were used for surveying and among them, three primers (RM32, RM202 and RM215) showed polymorphisms during parental survey. These three primers were then utilized for amplification of the DNA sequences of segregating 21 genotypes. The sequence and size of the microsatellite markers (SSRs) are mentioned in Table 2. PCR were performed in a thermo cycler (G-STROM, GSI, England). The volume of PCR cocktail for this study was 8 μ l per sample, containing Mg^{2+} free 10Xx PCR buffer (1.5 μ l), *Taq* DNA polymerase (0.20 μ l), dNTPs (1.0 μ l, 10mM), $MgCl_2$ (1.0 μ l), forward and reverse primers (0.50 μ l each), ddH₂O (3.30 μ l). Then 2 μ l genomic DNA was added and total volume of PCR sample was 10 μ l. The PCR application profile comprised of an initial denaturation step at 95°C for 5 min., three cyclic steps- (35 cycles each), denaturation at 94°C for 1 min., annealing at 55°C for 1 min., primer elongation at 72°C for 2min. followed by a single cycle of final elongation at 72°C for 5 min. Amplified PCR products along with 25 bp DNA ladder (3.0 μ l) were separated by electrophoresis on polyacrylamide gel (10%w/v) in 1X TBE [Tris-borate-

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ethylenediaminetetraacetic acid] buffer and the gels were stained using ethidium bromide. Stained gels were then visualized using a gel documentation unit (Uvipro platinum, EU) linked to a pc.

Table 2. Chromosome position, sequence, repeat motif and expected product size of the microsatellite markers (SSRs) used for parental survey.

Locus	Chromosome position	Primer sequence (5' - 3')	Repeat motif	Major allele bp	
RM32	5	Fwd	AGTCTACGTGGTGTACACGTGG	(TC)3A(C T)9(TC)5	170
		Rev	TGCGGCCTGCCGTTTGTGAG		
RM202	11	Fwd	CAGATTGGAGATGAAGTCCTCC	(CT)30	175
		Rev	CCAGCAAGCATGTCAATGTA		
RM215	9	Fwd	CAAAATGGAGCAGCAAGAGC	(CT)16	162
		Rev	TGAGCACCTCCTTCTCTGTAG		

2.6 Data analysis of SSR markers

Molecular weights (in base-pairs) of microsatellite products were estimated with Alpha Ease FC 5.0 software. Number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were calculated using POWER MARKER version 3.23 software [24]. The band profiles of the SSR primer were scored as present (1) or absent (0). Unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was constructed using online dendrogram construction utility Dendro UPGMA (<http://genomes.urv.es/UPGMA>) [25].

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3. RESULTS AND DISCUSSION

3.1 Phenotypic performances of mutant NERICA genotypes based on yield and yield attributes

The analysis of variance (ANOVA) of morphological trait data exhibited significant differences at 5% and 1% level of significance (Table 3). All morphological trait showed significant difference among the different genotypes.

The phenotypic performances in 18 mutants NERICA genotypes of 12 important quantitative characters are shown in Table-4. Among the 18 mutants NERICA genotypes lowest days to 1st flowering (62 days) was observed in N1/300/P-9-5-3-1 and maximum (84 days) in N1/250/P-7-6-4-1 and N4/350/P-2(1)-32-5. Maximum days required for 50% flowering (91) was observed in N4/350/P-2(1)-32-5 while minimum (65 days) in N1/300/P-9-5-3-1. Maximum days to 80% flowering (95) observed in N4/350/P-2(1)-32-5 and minimum (70 days) in N1/300/P-9-5-3-1. Data indicates that days required to maturity also varied significantly among the mutants genotypes ranged from 100 to 119 days. The mutant genotypes N4/350/P-2(1)-32-5 took the highest time for maturity (119 days) while mutant line, N1/250/P-7-3-7-1 (101 days), N10/300/P-2-3-5-2 (102 days), N1/250/P-7-6-4 (102 days) and NERICA-4 Parent (100 days) took the lowest time and the average time for maturity was 107.52±1.25 days. This result is in full agreement with Dutta (2001) [26] who observed days to maturity varied between 100 to 130 days in mutants NERICA genotypes. These variations may be due to different doses of mutagenic effect. Average plant height of 21 genotypes is 110.84±1.601 cm. Among the 21 NERICA genotypes, N4/350/P-2(1)-32-5 has shown the highest plant height (127.2 cm) and the mutant line N1/250/P-7-3-11 had the lowest plant height (97.5 cm). Plant height is an important trait and controlled by genetically manipulated factors [27]. Major modern rice genotypes are semi dwarf, as the breeders focus on lower plant height during improvement of rice genotypes. In rice plant, dwarfism is controlled by gene *sd-1*, which aids to increase lodging resistance due to shortened culm and thus reduce considerable yield losses [28]. So, in order to produce high yielding variety plant height should consider a very crucial trait. Highest total number of tillers per hill (14) was found in N10/300/P-2-3-5-2 and lowest total number of tillers per hill (6) was found in NERICA-4 Parent with an average of 9.52 ±0.46. The highest number of effective tillers per hill (13) was found in N10/300/P-2-3-5 and the lowest number of effective tillers per hill (6) was found in NERICA-4 (Parent) and NERICA-10 (Parent) with an average of 8.86±0.39 tillers per hill. During drought stress fertile tiller number induce approximate 86% of yield changes and hence it is a vital yield attributing character [29]. Panicle length is also considered an important character as higher panicle length could provide higher grain number [30]. The highest panicle length (29.6 cm) was found in N10/300/P-2-3-5-2 and the lowest (21.6 cm) in N10/300/P-2-3-5-1 mutant genotype with an average of 25.89 ±0.473. The

highest number of grains per panicle (189) was found in N10/300/P-2-3-5 whereas, the lowest grains per panicle (129) was found in N10/350/P-2(1)-3-5-1. The height number of unfilled grains per panicle (27) found in N10/300/P-5-1-1-1, N1/250/P-7-3-7-1 and N1/250/P-7-3-7-4 and the lowest number of unfilled grains per panicle (12) were found in N10/300/P-2-3-5-2 and NERICA-4 (Parent). Results showed that there was significant difference of 1000 grain weight among the studied mutant genotypes. Highest 1000 grain weight (29.05 g) was recorded in N10/300/P-2(1)-4-1 and the lowest 1000 grain weight (19.46 g) was recorded in N1/250/P-7-3-7-2. Average 1000 grain weight was 24.68 ± 0.897 g. Grain weight could be a major criteria of selection for the breeders as it is genetically more stable trait and hence less affected by the environmental factors [31]. Results also showed that there was significant difference of yield per plant among the studied genotypes. The highest yield per plant (21.2g) was recorded in N10/300/P-2-3-5. In contrast, the lowest yield per plant was found in N10/350/P-2(1)-3-5-1 mutant line (9.31 g) and the average yield per plant was 14.40 ± 0.76 g.

3.2 Correlation among the morphological traits data

Correlation co-efficient (Table 5) at phenotypic level indicated that yield per plants had significant positive correlation with number of tiller, number of effective tiller, panicle length, number of filled grains per panicle and 1000 grain weight but negative correlation with Plant height and days to maturity. 1000 grain weight showed significant positive correlation with panicle length, filled grains per panicle and yield per plant but negative correlation with plant height and unfilled grains per panicle. Days to maturation had significant correlation with days to 1st flowering, days to 50% flowering and days to 80% flowering. Plant height had negative correlation with most of the yield attributing characters such as, number of tiller, number of effective tiller, panicle length and number of filled grains per panicle and yield per plant. Such negative association of plant height with the other yield attributing characters indicated that dwarf plants are most desirable in breeding program aimed to develop high yielding rice varieties. These findings of our study correlated with the findings of [32,33].

3.3 Genetic parameters of morphological traits

Different genetic parameters like, genotypic variance (σ^2_g), phenotypic variance (σ^2_p), phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), broad sense heritability (h^2_b) and genetic advance as percent of mean (GA%) of 12 morphological traits in our 21 studied genotypes are presented in table-6. Among the 12 morphological traits unfilled grains per panicle showed highest PCV (31.55) and GCV (30.36) value. Yield per plant (24.12 and 22.95), total tiller per hill (21.11 and 18.88), and effective tiller per hill (20.49 and 16.91) also showed high PCV and GCV, respectively. On the other hand, days to maturity (5.15 and 5.10) showed lowest PCV and GCV values, respectively followed by plant height (6.55 and 6.39), days to 1st flowering (9.37 and 9.04), days to 80% flowering (9.38 and 9.27), days to 50% flowering (9.43 and 9.29) and panicle length (9.86 and 9.22). Similar PCV and GCV value was also reported by [30] in their study of 31 NERICA mutant lines and their parents. From our study it was found PCV is higher than the corresponding GCV value. This may be an indication environmental interaction on the studied traits [34,35].

Heritability could be classified as low (<30%), medium (30-60%) and high (> 60%) [22]. In the current study broad sense heritability (h^2_b) ranged from 71% (1000 grain weight) to 99% (filled grains per panicle). This result indicated that all the studied traits were highly heritable and had less environmental influences on them [36,32]. In the present study, genetic advances in percent mean (GA%) varied from 10% (Days to maturity) to 60% (Unfilled grains per panicle). In order to achieve consistent results in breeding program combination of heritability results and genetic advance is a very useful method [22]. In our study, yield per plant, unfilled grains per panicle, number of total tiller, number of effective tiller showed high heritability and genetic advance as percent of the mean value. Similar result in the study of NERICA mutant lines was also observed by [30,27]. On the other hand [37] found high heritability and genetic advance as percent of the mean value for yield per plant and [38] found high heritability and genetic advance as percent of the mean value for unfilled grains per panicle.

3.4 Allelic and loci variation within the genotypes

Using 3 SSR markers, a total of 24 alleles were detected among 21 rice genotypes. The average number of allele per locus was 8.0 with a range of 7.0 (RM215) to 10.0 (RM202) (Table 7). Primer RM36 showed 7 allele at 151, 157, 161, 165, 170, 174 and 186 bp length; primer RM 202 showed 10 allele at 141, 146, 147, 149, 152, 155, 157, 171, 175 and 182 bp length; primer RM215 showed 7

allele at 137, 145, 150, 154, 155, 158 and 162 bp length. Amplification profile of primer RM202 is shown in figure 1. Allelic frequency of three primers and their variance with standard deviation are presented in (Table-7). The allelic frequency ranging from 0.19 to 0.33. The higher frequency showed in RM32. An allele observed in less than 5% of the studied genotypes was considered to be rare [39]. During these experiments we got 4 rare alleles for RM32 primer, 4 rare alleles for RM202 primer and 3 rare alleles for RM215 primer. Rare alleles are highly informative in fingerprinting of the varieties [40]. Totally absent of allele indicates null allele. In this experiment the average value of null allele is 0.333. Among the three primers, only RM215 primer generates one null allele and other two primers did not generate any null allele.

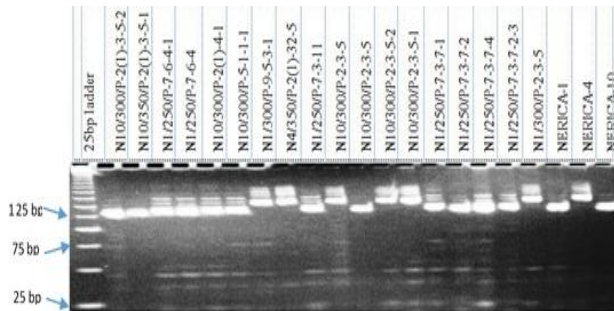


Fig. 1. Amplification profile of primer RM202 for 18 NERICA mutant lines along with their 3 parents.

3.5 Gene diversity and PIC value

According to Nei (1973) [41] the highest level of gene diversity (0.871) was observed in loci RM202 and the lowest level of gene diversity (0.748) was observed in loci RM32 with a mean diversity of 0.821 (Table-7). Similar high level of mean gene diversity (0.8580) were also reported by [6]. The polymorphism information content (PIC) value provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. The PIC values in this study varied from 0.710 (RM32) to 0.857 (RM202) with an average of 0.797 (Table-7). Similar high PIC value were also reported by (0.77) Afiukwa (2016) [42] and (0.75) Borba (2009) [43]. From the gene diversity values and PIC values it can be said that all three SSR primers used in the study were highly informative.

Table 3. Analysis of variance for 12 different morphological traits of 21 studied rice genotypes.

Source	df	1 st flow.	50% flow.	80% flow.	DM	PH	TT	ET	PL	FG	UF G	1000 GW	YP
Replication	2	337	124.	130.2	4.82	231.	71.3	54.6	71.3	731.7	231.	223.0	82.2
Genotypes	20	126.	145.	158.9	91.5	153.	10.5	7.78	17.9	854.2	94.9	21.91	33.9
Error	40	3.00	1.51	1.25	0.58	2.46	0.81	1.05	0.82	4.21	2.46	2.65	1.14

Note- 1st flow.- days to 1st flowering, 50% flow.- days to 50% flowering, 80% flow.- days to 80% flowering, DM- days to maturity, PH- plant height, NT- number of tiller, ET- number of effective titter, PL- panicle length, FG- filled grains per panicle UFG- unfilled grains per panicle, 1000GW- 1000 grain weight, YP- yield per plant, ** = Significant at 1% level, * = Significant at 5% level.

Table 4. Mean performance of 21 NERICA mutant genotypes based on different morphological characters.

Genotypes	1 st flow.	50% flow.	80% flow.	DM	PH (cm)	TT	ET	PL (cm)	FG	UFG	1000 GW. (g)	YP (g)
N10/300/P-2(1)-3-5-2	74	78	84	108	116	10	9	25.4	155	25	21.38	13.9
N10/350/P-2(1)-3-5-1	69	73	77	113	123	7	7	22.5	129	19	23.08	9.13
N1/250/P-7-6-4-1	84	88	91	116	115.5	11	11	26.2	172	13	28.85	16.1
N1/250/P-7-6-4	69	72	74	102	110.5	10	8	28.6	166	13	24.04	15.8
N10/300/P-2(1)-4-1	77	80	84	109	110.8	9	9	27.2	184	16	29.05	17.5
N10/300/P-5-1-1-1	73	77	85	112	115.5	9	9	27.5	160	27	25.51	15.9
N1/300/P-9-5-3-1	62	65	70	108	115	10	10	25.7	158	18	24.57	14.2
N4/350/P-2(1)-32-5	84	91	95	119	127.2	11	8	23.2	139	22	20.13	10.7
N1/250/P-7-3-11	81	86	90	114	97.5	10	9	28.5	161	23	25.75	18.7
N10/300/P-2-3-5	70	72	74	104	102	13	13	29.0	189	15	27.76	21.2
N10/300/P-2-3-5	65	70	73	104	106.5	9	8	26.8	158	15	26.08	17.8
N10/300/P-2-3-5-2	65	68	71	102	104.6	14	12	29.6	160	12	24.09	16.5
N10/300/P-2-3-5-1	65	71	73	110	106.6	9	9	21.6	133	23	21.26	10.11
N1/250/P-7-3-7-1	65	71	73	101	114.6	8	8	28.8	173	27	24.7	18.4
N1/250/P-7-3-7-2	71	74	77	105	113.6	11	10	24.6	142	16	19.46	11.5
N1/250/P-7-3-7-4	71	74	77	107	115	9	9	25.6	130	27	20.19	13.8
N1/250/P-7-3-7-2-3	69	75	79	110	103.6	8	8	23.5	142	10	22.75	12.2
N1/300/P-2-3-5	72	76	80	109	104.5	10	8	25.8	153	14	24.7	15.5
NERICA-1 (Parent)	70	72	74	103	110.8	9	9	27.9	157	20	24.91	13.2
NERICA-4 (Parent)	65	68	71	100	111.7	6	6	22.5	136	12	22.09	9.98
NERICA-10(Parent)	62	65	71	102	103.2	7	6	23.2	144	17	23.26	10.3
Minimum	62	65	70	100	97.5	6	6	21.6	129	12	19.46	9.13
Maximum	84	91	95	119	127.2	14	13	29.6	189	27	29.05	21.2
Mean	70.57	74.57	78.24	107.52	110.84	9.52	8.86	25.89	154.33	18.29	3.98	14.40
CV%	2.45	1.64	1.43	1.56	1.42	9.47	12.38	3.50	1.33	8.51	6.79	7.40
Standard Deviation	6.49	6.96	7.29	5.91	7.16	1.89	1.71	2.44	16.87	5.43	2.70	3.36
Standard Error	1.459	1.53	1.6	1.25	1.601	0.46	0.39	0.473	4.63	1.1	0.897	0.76
LSD _{0.05}	2.86	2.02	1.85	1.84	2.59	1.49	1.69	1.40	3.39	2.50	2.69	1.75

Note- 1st flow.- days to 1st flowering, 50% flow.- days to 50% flowering, 80% flow.- days to 80% flowering, DM- days to maturity, PH- plant height, NT- number of tiller, ET- number of effective titter, PL- panicle length, FG- filled grains per panicle UFG- unfilled grains per panicle, 1000GW- 1000 grain weight, YP- yield per plant.

Table 5. Pearson's correlation coefficients of 12 morphological traits of 21 studied rice genotypes.

	1 st flow.	50% flow.	80% flow.	DM	PH	TT	ET	PL	FG	UFG	1000 GW	YP
1 st flow.	1	.981**	.960**	.605**	.267	.293	.197	.107	.203	.148	.207	.187
50% flow.	0.981**	1	.977**	.676**	.287	.246	.129	.030	.130	.190	.132	.138
80% flow.	.960**	.977**	1	.690**	.308	.194	.075	-.007	.101	.240	.120	.099
PH	.267	.287	.308	.280	1	-.140	-.178	-.315	-.288	.353	-.331	-.415
TT	.293	.246	.194	.081	-	1	.876**	.543*	.453*	-.152	.182	.506*
ET	.197	.129	.075	.069	-.140	.876**	1	.556**	.544*	-.081	.350	.573**
PL	.107	.030	-.007	-.397	-.178	.543*	.556**	1	.795**	.035	.590**	.883**
FG	.203	.130	.101	-.238	-.315	.453*	.544*	.795**	1	-.140	.831**	.870**
UFG	.148	.190	.240	.282	.288	-.152	-.081	.035	-.140	1	-.260	.008**
1000 GW	.207	.132	.120	-.056	-.331	.182	.350	.590**	.831**	-.260	1	.720**
YP	.187	.138	.099	-.221	-.41	.506*	.573**	.883**	.870**	.008	.720**	1

** = Significant at 1% level, * = Significant at 5% level.

Table 6. Genetic parameters of 12 agro-morphological traits in 21 studied rice genotypes.

Character	Mean	MSG	MSE	σ^2g	σ^2p	PCV	GCV	h^2 (%)	GA(%)
1 st flow.	70.57	125.14	3.00	40.71	43.71	9.37	9.04	93	18
50% flow.	74.57	145.37	1.51	47.95	49.46	9.43	9.29	97	19
80% flow.	78.24	158.94	1.25	52.56	53.81	9.38	9.27	98	19
DM	107.52	91.55	0.58	30.32	30.90	5.15	5.10	98	10
PH	110.84	153.02	2.46	50.19	52.65	6.55	6.39	95	13
TT hill-1	9.52	10.51	0.81	3.23	4.04	21.11	18.88	80	35
ET hill ¹	8.86	7.78	1.05	2.24	3.29	20.49	16.91	68	29
PL	25.89	17.91	0.82	5.70	6.52	9.86	9.22	87	18
FG	154.33	854.20	2.21	284.00	286.21	10.96	10.92	99	22
panicle ⁻¹									
UG	18.29	94.91	2.46	30.82	33.28	31.55	30.36	93	60
panicle ⁻¹									
1000 seed wt.	23.98	21.91	2.65	6.42	9.07	12.56	10.57	71	18
Y/ plants	14.40	33.92	1.14	10.93	12.07	24.12	22.95	91	45

Note- MSG- mean square genotypes, MSE-mean square error, σ^2g - genotypic variance, σ^2p -phenotypic variance, PCV- phenotypic coefficient of variance, GCV-genotypic coefficient of variance, h^2 (%) -broad sense heritability, GA(%) -genetic advance as percent of mean.

Table 7. Allele size range, number of alleles, major allele frequency, rare allele, PIC value and gene diversity value of 3 SSR primers.

Locus	Allele size ranges (bp)	No. of allele	Major allele freq.	Rare allele	Null allele	PIC value	Gene diversity
RM32	151-186	7.0	0.33	4	0	0.710	0.748
RM202	141-182	10.0	0.19	4	0	0.857	0.871
RM215	137-162	7.0	0.19	2	1	0.824	0.644
Mean	-----	8.0	0.23	3.67	0.33	0.797	0.821

3.6 UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram.

Dendrogram based on genetic distance [41] using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 21 rice genotypes by 3 markers (Fig. 2). All 21 rice genotypes could be easily distinguished. The UPGMA cluster analysis led to the grouping of the 21 genotypes into six clusters. Cluster I consisted of two mutant lines, N1/250/P-7-6-4-1 and N10/300/P-2(1)-4-1. Both of these two mutant lines showed good morphological characters. Cluster II consisted of only one mutant line, N1/250/P-7-3-7-2-3. Cluster III consisted of six genotypes, out of which three are mutant lines and the other three are the parent genotypes. The mutant lines, N1/250/P-7-3-7-1, N1/250/P-7-3-7-2 and N1/250/P-7-3-7-4, in cluster III formed a separate sub-cluster and the three parent genotypes formed a separate sub-cluster. All the genotypes, except N1/250/P-7-3-7-1 in this cluster did not show very good yield and yield attributing characters. Cluster IV contained five mutant lines, N10/300/P-2-3-5, N10/300/P-2-3-5, N10/300/P-2-3-5-2, N10/300/P-2-3-5-1 and N1/300/P-2-3-5. All the mutant lines, except N10/300/P-2-3-5-1, in this cluster showed very good performance in yield and yield attributing characters. Cluster V consisted of four mutant lines, N10/300/P-2(1)-3-5-2, N10/350/P-2(1)-3-5-1, N1/250/P-7-6-4 and N1/300/P-2-3-5. Mutant line N1/300/P-2-3-5 in cluster V showed high grain yield per plant (18.7 gm.) but lowest plant height (97.5 cm). Performance of other yield attributing character of this mutant line was also good. Finally Cluster VI contained three mutant lines, N10/300/P-5-1-1-1, N1/300/P-9-5-3-1 and N4/350/P-2(1)-32-5. Morphological performance of all the mutant lines in cluster VI were moderate level. In this cluster analysis it was observed that the mutant lines derived from the same parent generally tend to be in same cluster. Though the SSR markers used in the study successfully separated most of the mutant lines, but they could not distinguish between mutant lines: N10/300/P-2(1)-3-5-2 and N10/350/P-2(1)-3-5-1; N10/300/P-2-3-5 and N10/300/P-2-3-5-2; N1/250/P-7-3-7-2 and N1/250/P-7-3-7-4. Mutant lines in these three pairs may be referred as duplicates. This duplicates arise may be because they derived from the same parent genotype. Such duplicates were also reported by [44]. Use of more marker could separate these duplicates. In the end it can be said that, analysis base on SSR markers efficiently distinguished among most of the studied 21 NERICA genotypes and pointed out the desirable mutant lines. Such studies can be used to estimate genetic differences of varieties for their identification.

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Comment [R17]: How was the choice of cluster number made? Did it have any significance or was it subjective? Describe in the text.

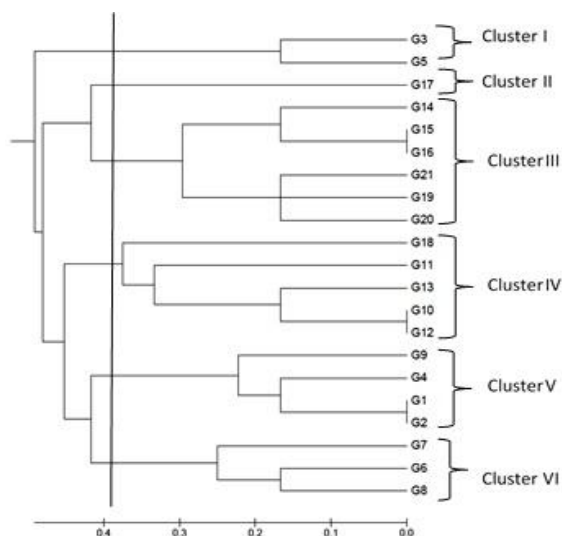


Fig. 2. UPGMA dendrogram based on genetic distance summarizing of 21 rice genotypes according to SSR analysis. Arrow line indicates the scale of genetic distance (0.00-0.49).

4. CONCLUSIONS

Considering the morphological and molecular study it was accomplished that four mutant lines, N10/300/P-2-3-5, N10/300/P-2-3-5, N10/300/P-2-3-5-2, and N1/300/P-2-3-5, in cluster IV had superior morphological characteristics such as, high yielding, lower plant height, early maturity, good panicle characteristics and good grain weights. Mutant line N1/300/P-2-3-5 of cluster V had lowest plant height but high yield and some good yield attributing characters (panicle length and filled grains per panicle). Mutant lines, N1/250/P-7-6-4-1 and N10/300/P-2(1)-4-1, of cluster I had high yield, high grain weight, good panicle length, high filled grains per panicle, low unfilled grains per panicle but not good plant height and maturity time compared to the other mutant lines. On the other hand, mutant line N1/250/P-7-3-7-1 of cluster III had high yield, early maturation, good panicle length, high filled grains per panicle but highest unfilled grains per panicle. Considering the overall performance, eight mutant lines viz. N10/300/P-2-3-5, N10/300/P-2-3-5, N10/300/P-2-3-5-2, N1/300/P-2-3-5, N1/300/P-2-3-5, N1/250/P-7-6-4-1, N10/300/P-2(1)-4-1 and N1/250/P-7-3-7-1 could be suggested as suitable parent for further breeding program. In the present study, yield per plant, unfilled grains per panicle, number of total tiller, number of effective tiller showed high heritability and genetic advance as percent of the mean value and yield per plants had significant positive correlation with number of tiller, number of effective tiller, panicle length, number of filled grains per panicle and 1000 grain weight but negative correlation with Plant height. Selection based on these criteria are suggested for further evaluation in future breeding programs. The results of this study can be used as a baseline for future genetic analysis within mutant rice lines and trace their genetic relationships in Bangladesh. The data will be useful for breeders to select rice lines for development of new varieties.

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