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

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Authors' contributions

This work was carried out in collaboration among all authors. Author MMI designed and coordinated this research. Authors SCD and MAI carried out this experiment. Author TRA analyzed the data. Authors MMI and TRA drafted the manuscript. All authors read and approved the final manuscript.

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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.In the experiment, 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 in orderto identify desired mutant linesusing 12 yield

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attributing characters and 3 simple sequence repeat (SSR) markers. We also estimated heritability, genetic advance and correlation among the studied morphological traits to identify besttraits based on which further selection could be made. 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 grainsper 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% while genetic advances in percent mean (GA%) varied from 10% to 60%. 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-7-3-7-1were selected as desired mutant lines having good yield attributing characters and could be recommended for further evaluation in rice breeding program.

Keywords: NERICA (Oryza glaberrima); genetic advance; correlation; SSR markers; UPGMA.

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 collogues 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 to 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, yieldper 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 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 is 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 [10,11,12,13], 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 for documentation and breeding programs.

On the other hand, 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 twelve yield attributing characters of the 18 selected mutant lines and their parents; ii) estimate genetic advance and heritability and determine correlation among the twelve 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.

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 during the aus (Kharif-1) cropping season. The temperature ranged from 27.4°C to 36°C while relative humidity ranged from 55% to 90%. Seeds were heat-treated in a convection oven for 24 hours at 50°C to break the dormancy. Then seeds were soakedin fresh water for 24 hours.

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 Agriculture (BINA)
2.	G2	N10/350/P-2(1)-3-5-1	NERICA-10	350	
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)	----------	----------	
21.	G21	NERICA-10(Parent)	----------	----------	

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	$(TC)_3A(CT)_9(TC)_5$	170
		Rev	TGCGGCCTGCCGTTTGTGAG		
RM202	11	Fwd	CAGATTGGAGATGAAGTCCTCC	$(CT)_{30}$	175
		Rev	CCAGCAAGCATGTCAATGTA		
RM215	9	Fwd	CAAAATGGAGCAGCAAGAGC	$(CT)_{16}$	162
		Rev	TGAGCACCTCCTTCTCTGTAG		

After socking the seeds were placed into petridishes with moistened filter papers and incubated for 48 hours at 30°C to germinate and then transferred to a nursery for proper establishment. Seedlings (two week old) were transplanted in the field following RCBD (Randomize complete block design) with three replications. Twenty plants per replicate were showed and row to row and plant to plant distances of the plot were 20 cm and 15 cm. Weeding and drainage were done when required. No artificial irrigation was given. Fungicide and insecticide were applied once only. Data were recorded for days to 1st flowering, days to 50% flowering, days to 80% flowering, days to maturity (DM), plant height (Cm.), total tillers per hill (Counted tillers at maturity stage), effective tillers per hill (Counted number of tillers with panicles), panicle length (Cm), filled grains per panicle, unfilled grains per panicle, 1000 grain weight and yield per plant traits.

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 test. The Pearson correlation coefficient between morphological traits was calculated using IBM SPSS Statistics 20 software.

Genotypic variances (σ²g), environmental variances (σ²e), phenotypic variances (σ²p), genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) ware estimated following the procedure proposed by Burton and De Vane[20]:

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

$$\sigma^2 e = MSe$$

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

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

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

Here,

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

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

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

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

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

Here, Standardized selection differential constant, K=2.06, at 5% selection intensity, ¯: Mean of the traits.

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 µl-1.

2.5 Amplification of Microsatellite Markers

Ten rice primers obtained from Gramene (http://www.gramene.org) viz. RM32, RM80, RM202, RM215, RM334, RM510, RM562, RM10720, RM28102, and RM28502 were used

for surveying and among them, three primers (RM32, RM202 andRM215) showed polymorphisms during parental survey. These three primers were then utilized for amplification of the DNA sequences to segregate 21 genotypes. The sequence and size of the microsatellite markers (SSRs) are mentioned in Table 2 above. PCR were performed in a thermo cycler (G-STROM, GSI, England). The volume of PCR cocktail for this study was 8 µl per sample, containing Mg^{2+} free 10X× PCR buffer (1.5 µl), *Taq* DNA polymerase (0.20 µl), dNTPs (1.0 µl, 10mM), $MgCl_2$ (1.0 µl), forward and reverse primers (0.50 µl each), ddH_2O (3.30 µl). Then 2 µl genomic DNA was added and total volume of PCR sample was 10 µl. 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 µl) were separated by electrophoresis on polyacrylamide gel (10%w/v) in 1X TBE [Tris-borate-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.

2.6 Data Analysis of SSR Markers

Molecular weights (in base-pairs) of micro-satellite 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) in order to convert them into binary data. UPGMA dendrogram was constructed using Jaccard's coefficient with the aid of online dendrogram construction utility Dendro UPGMA (http://genomes.urv.es/UPGMA) [25].

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

The phenotypic performances in 18 mutants NERICA genotypes of twelve important quantitative characters are shown in Table-4. Among the 18 mutants NERICA genotypes lowest days to 1st flowering (62 days) ware 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) ware 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) ware observed in N4/350/P-2(1)-32-5 and minimum (70 days) in N1/300/P-9-5-3-1. From the above data it was clear that flowering time varied substantially among the mutant lines.Mutant lines that flowered earlier matured early while mutant lines that flowered late also mature late. Early flowering is considered a very important character as it is an indication of short life span cycle and is considered a positive character for rice improvement [26]. Data indicated that days required to maturity also varied significantly among the mutant 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 [27] 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. Early maturation minimize the water utilization and weed control cost [28]. If drought occurs during the reproductive stage such as, pollination, fertilization and grain filling production could be severely hamper. In this case, fast growth during vegetative stage could be a better solution and this is a characteristics of early maturing rice varieties [29]. So, early maturating varieties are especially suitable for the rain-fed low land and drought prone areas. Average plantheight of 21 genotypes is 110.84±1.601 cm. Among the 21 NERICA genotypes, N4/350/P-2(1)-32-5 had 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 [30]. 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 [31]. So, in order to produce high yielding variety plant height should consider a very crucial trait. Highest total number of tillers per hill (14) ware found in N10/300/P-2-3-5-2 and lowest total number of tillers per hill (6) ware found in NERICA-4 Parent with an average of 9.52 ±0.46. Fewer tillers causes fewer panicles while excess tillers results in high tiller abortions, small panicles, poor grain filling, and hence reduce grain yield [32]. In our study, tiller number ranged from moderate to low. The highest number of effective tillers per hill(13) ware found in N10/300/P-2-3-5 and the lowest number of effective tillers per hill (6) ware 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 [33]. Moreover, panicle length is also considered an important character as higher panicle length could provide higher grain number [34]. 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) ware found in N10/300/P-2-3-5 whereas, the lowest grains per panicle (129) ware found in N10/350/P-2(1)-3-5-1. The height number of unfilled grains per panicle (27) ware 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) ware found in N10/300/P-2-3-5-2 and NERICA-4 (Parent). Results showed that there were significant difference in 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 ±0.897g. 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 [35]. 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 several author [36,37].

3.3 Genetic Parameters of Morphological Traits

Different genetic parameters like, genotypic variance (σ^2g), phenotypic variance (σ^2p), phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), broad sense heritability (h_b^2) and genetic advance as percent of mean (GA%) of twelve morphological traits in our 21 studied genotypes are presented in Table 6. Among the twelve morphological traits unfilled grainsper 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. Thismay be an indication environmental interaction on the studied traits [38,39].

Heritability could be classified as low (<30%), medium (30-60%) and high (> 60%) [22]. In the current study, broad sense heritability (h_b^2) ranged from 71% (1000 grain weight) to 99% (filled grainsper panicle). This result indicated that all the studied traits were highly heritable and had less environmental influences on them [40,36]. In the study, genetic advances in percent mean (GA%) varied from 10% (Days to maturity) to 60% (Unfilled grainsper 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 [34,31]. On the other hand, [41] found high heritability and genetic advance as percent of the mean value for yield per plant and [42] 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 and162 bp length. Amplification profile of primer RM202 is shown is Fig. 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 [43]. 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 [44]. 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.

3.5 Gene Diversity and PIC Value

According to Nei, [45] 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) was also reported by Hasan et al. [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 was also reported by (0.77) Afiukwa (2016) [46] and (0.75) Borba (2009) [47]. From the gene diversity values and PIC values it can be said that all three SSR primers used in the study were highly informative.

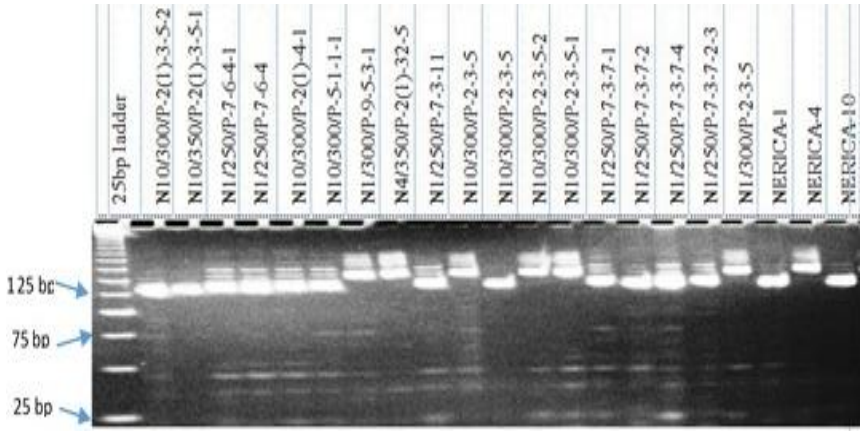

Fig. 1. Amplification profile of primer RM202 for 18 NERICA mutant lines along with their 3 parents

3.6 UPGMA (Unweighted Pair Group Method of Arithmetic Means) Dendrogram

Dendrogram was constructed from the binary data obtained from SSR marker based DNA profile using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated segregation of the 21 rice genotypes by three markers (Fig. 2). Cophenetic correlation coefficient (CP) value was 0.50.All 21 rice genotypes could be easily distinguished from the dendrogram. The UPGMA cluster analysis led to the grouping of the 21 genotypes into six clusters at the coefficient of 0.39. 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 characters of this mutant line ware 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 ware moderate level. In this cluster analysis, it was observed that the mutant lines derived from the same parent generally tend to be in the 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 arose may be because they derived from the same parent genotype. Such duplicates were also reported by [48]. 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.

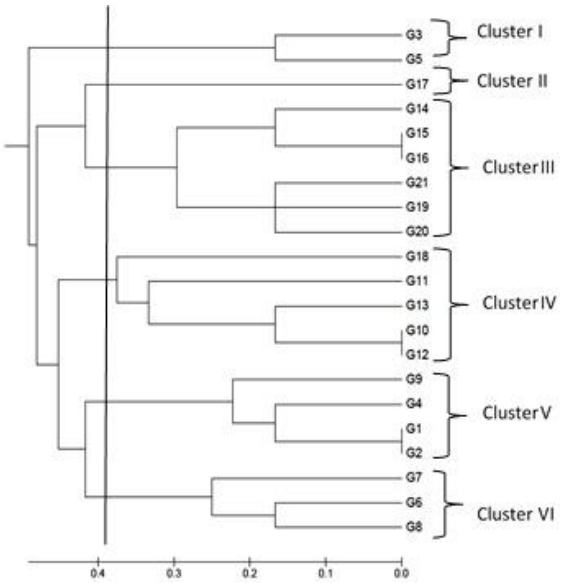

Fig. 2. UPGMA dendogram based on genetic distance summarizing of 21 rice genotypes according to SSR analysis. Cophenetic Correlation Coefficient (CP) value was 0.50

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

Source	df	1st flow.	50% flow.	80% flow.	DM	PH	TT	ET	PL	FG	UFG	1000 GW	YP
Replication	2	337	124.91	130.29	4.82	231.44	71.30	54.62	71.30	731.76	231.44	223.0	82.28
Genotypes	20	126.14**	145.37**	158.94**	91.55**	153.02**	10.51**	7.78**	17.91**	854.20**	94.91**	21.91**	33.92**
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- 1-st 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 grainsper 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	1st 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

Genotypes	1st flow.	50% flow.	80% flow.	DM	PH (cm)	TT	ET	PL (cm)	FG	UFG	1000 GW. (g)	YP (g)
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- 1-st 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

	1st flow.	50% flow.	80% flow.	DM	PH	TT	ET	PL	FG	UFG	1000GW	YP
1st 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	-.140	1	.876**	.543*	.453*	-.152	.182	.506*
ET	.197	.129	.075	.069	-.178	.876**	1	.556**	.544*	-.081	.350	.573**
PL	.107	.030	-.007	-.397	-.315	.543*	.556**	1	.795**	.035	.590**	.883**
FG	.203	.130	.101	-.238	-.288	.453*	.544*	.795**	1	-.140	.831**	.870**
UFG	.148	.190	.240	.282	.353	-.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	σ2g	σ2p	PCV	GCV	hβ (%)	GA(%)
1st 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 panicle^{-1}	154.33	854.20	2.21	284.00	286.21	10.96	10.92	99	22
UG panicle^{-1}	18.29	94.91	2.46	30.82	33.28	31.55	30.36	93	60
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, σ2g- genotypic variance, σ2p-phenotypic variance, PCV- phenotypic coefficient of variance, GCV-genotypic coefficient of variance, hβ (%)-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

4. CONCLUSION

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 grainsper panicle, low unfilled grainsper 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 the development of new varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nassirou TY, He Y. NERICA: A hope for fighting hunger and poverty in Africa. Mol Plant Breed. 2011;2:75-82.

2. Chaudhary RC, Mahyuddin S. Development and use of rice production technologies in 21st century and stabilization of rice availability for Consumption and export. In: Syam M, Chaudhary RC, editors. Asia International Rice Conference. 5th ed. Bali, Indonesia. 2000;17-19.
3. Jones MP, Dingkuhn M, Aluko K, Mande S. Interspecific *Oryza sativa* L. X *O. glaberrima* Steud. progenies in upland rice improvement. Euphytica. 1997;94(2):237-246.
4. Dingkuhn M, Jones MP, Johnson DE, Sow A. Growth and yield potential of *Oryza sativa* and *O. glaberrima* upland rice cultivars and their interspecific progenies. Field Crops Res. 1998;57(1):57-69.
5. Diagne A. The diffusion and adoption of NERICA rice varieties in Côte d´Ivoire. Develop Econ. 2006;44(2):208-231.
6. Hasan MN, Bhuiyan MJH, Begum SN, Raffi SA, Razia S. Morphological and molecular characterization of some NERICA mutant lines under drought condition. Prog Agric. 2015;26:15-21.
7. Ahloowalia BS, Maluszynski M. Induced mutations – A new paradigm in plant breeding. Euphytica. 2001;118:167-173..
8. Borba TCO, Brondani RPV, Rangel PHN, Brondani C. Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. Genetica. 2009;137(3):293-304.
9. Varshney RK, Graner A, Sorrells ME. Genic microsatellite markers in plants: Features and applications. Trends Bio-technol. 2005;23(1):48-55.
10. Ijaz S. Microsatellite markers: An important fingerprinting tool for characterization of crop plants. Afr J Biotechnol. 2011;10(40): 7723-7726.
11. Ren F, Lu BR, Li S, Huang J, Zhu Y. A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD and SSR markers. Theor Appl Genet. 2003;108:113-120.
12. Juneja S, Das A, Joshi SV, Sharma S, Vikal Y, Patra BC, et al. *Oryzanivara* (Sharma et Shastry) the progenitor of *O. sativa* (L.) subspecies *indica* harbors rich genetic diversity as measured by SSR markers. Curr Sci. 2006;91:1079-1085.
13. Kibria K, Nur F, Begum SN, Islam MM, Paul SK, Rahman KS, Azam SMM. Molecular marker based genotypic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. Int J Sust Crop Prod. 2009;4:23-34.
14. Nandakumar N, Singh AK, Sharma RK, Mohapatra T, Prabhu KV, Zaman FU. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica. 2004;136:257-264.
15. Ma H, Yin Y, Guo ZF, Chen LJ, Zhang L, Zhong M, Shao GJ. Establishment of DNA fingerprinting 323 of Liaojing series of japonica rice. Middle-East J Sci Res. 2011; 8(2):384-392.
16. Ali SS, Jafri SJH, Khan TZ, Mahmood A, Butt MA. Heritability of yield and yield components of rice. Pak J Agr Res. 2000; 16:89-91.
17. Surek H, Beser N. Selection for grain yield and yield components in early generations for temperate rice. Philippine J Crop Sci. 2003;28:3-15.
18. Seedek SEM, Hammoud SAA, Ammar MH, Metwally TF. Genetic variability, heritability, genetic advance and cluster analysis for some physiological, traits, and grain yield and its components in rice (*Oryza sativa* L.). J Agric Res. 2009; 35:858-877.
19. Ogunniyan DJ, Olakojo SA. Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L.). Nigeria J Genet. 2014;28:24-28.
20. Burton GW, De Vane EH.Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. Agro J. 1953;45:478-481.
21. Allard RW. Principles of plant breeding. John Wiley and Sons, Inc., New York; 1960.
22. Johnson HW, Robinson HP, Comstoc RE. Estimation of genetic and environmental variability in soybeans. Agron J. 1995; 47:314-318.
23. Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, et al. Saturated molecular map of the rice genome based on an inter specific backcross population. Genetics. 1994;138:1251-1274.
24. Liu K, Muse SV. Power Marker: Integrated analysis environment for genetic marker data. Bioinformatics. 2005;21:2128-2129.

25. Garcia-Vallvé S, Palau J, Romeu A. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in *Escherichia coli* and *Bacillus subtilis*. Mol Biol Evol. 1999;16:1125-1134.
26. Oladosu Y, Rafii MY, Abdullah N, Malek MA, Rahim HA, Hussin G, Latif MA, KareemI. Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits. Scientific World J. 2014; 19053.
27. Dutta RK. Assessment of the advanced mutant lines/varieties of BINA with emphasis on physiological criteria. Report on ARMP Project No. 512, BARC, Farmgate, Dhaka-1215; 2001.
28. Khush GS. Modern varieties-their real contribution to food supply and equity. Geo Journal. 1995;35(3):275-284.
29. Haefele SM, Bouman BAM. Drought-prone rainfed lowland rice in Asia: Limitations and management options. In: Serraj J, Bennett J, Hardy B, editors. Drought frontiers in rice: Crop improvement for increased rain-fed production. World Scientific Publishing, Singapore. 2009;211-232.
30. Rana MM, Islam MA, Imran S, Rubani S, Hassan L. Morphological characterization of NERICA mutant lines and their parents. Asian J Med Biol Res. 2018;4(2):207-221.
31. Spielmeyer W, Ellis MH, Chandler PM. Semi dwarf (sd-1), "green revolution" rice, contains adefective gibberellin 20-oxidase gene. Plant Biol. 2002;99:9043-9048.
32. Pandey P, John AP, Tiwari DK, Yadav SK, Kumar B. Genetic variability, diversity and association of quantitative traits with grain yield in rice (*Oryza sativa* L.). J. Bio-Sci. 2009;17(1):77-82.
33. Miller BC, Hill JE, Roberts SR. Plant population effects on growth and in water seeded rice. Agron J. 1991;83:291-297.
34. Nuruzzaman M, Hassan L, Begum SN, Huda MM. Morphological characterization and assessment of genetic parameters of NERICA mutant rice lines under rain fed condition. Asian J Med Biol Res. 2016; 2(4):532-540.
35. Rafiee M. Effects of planting date on yield of some cultivars in Khorramabad condition. J Agric Res. 2008;37:251-263.
36. Ahmad F, Hanafi MM, Hakim MA, Rafii MY, Arolu IW, Akmar ASN. Genetic divergence and heritability of 42 Coloured upland rice genotypes (*Oryza sativa*) as revealed by microsatellites marker and agro-morphological traits. Plos One. 2015;10(9):e0138246.
37. Osman KA, Mustafa AM, Ali F, Yonglain Z, Fazhan Q. Genetic variability for yield and related attributes of upland rice genotypes in semi-arid zone (Sudan). Afr J Agric Res. 2012;7:4613-4619.
38. Prajapati M, Singh CM, Suresh BG, Lavanya GR, Jadhav P. Genetic parameters for grain yield and its component characters in rice. Electron J Plant Breed. 2011;2:235-238.
39. Bhadru D, Rao VT, Mohan YC, Bharathi D. Genetic variability and diversity studies in yield and its component traits in rice (*Oryza sativa* L.). SABRAO J Breed Genet. 2012;44:129-137.
40. Patel A, Chaudhari PR, Verulkar SB. Analysis of genetic variability, heritability and genetic advance for yield and yield components in rice (*Oryza sativa* L.) under different water regimes. Plant Arch. 2012; 12:425-435.
41. Bisne R, Sarawgi AK, Verulkar B. Study of heritability, genetic advance, and variability for yield contributing characters in rice. Bangladesh J Agric Res. 2009;34:175-179.
42. Babu VR, Shreya K, Dangi KS, Usharani G, Nagesh P. Genetic variability studies for qualitative and quantitative traits in popular rice (*Oryza sativa* L.) hybrids of India. Int J Sci Res Publ. 2012;2:1-5.
43. Jayamani P, Negrao S, Martins M, Macas B, Oliveira MM. Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci. 2007;47:879-886.
44. Jain S, Jain RK, McCouch SR. Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theor Appl Genet. 2004;109:965-977.
45. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA. 1973;70:3321-3323.
46. Afiukwa CA, Faluyi JO, Atkinson CJ, Ubi BE, Igwe DO, Akinwale RO. Screening of some rice varieties and landraces cultivated in Nigeria for drought tolerance based on phenotypic traits and their

association with SSR polymorphisms. Afr J Agric Res. 2016;11(29):2599-2615.

47. Borba TCO, Brondani RPV, Rangel PHN, Brondani C. Microsatellite marker-mediated analysis of the EMBRAPA rice core collection genetic diversity. Genetica. 2009;137(3):293-304.

48. Sajib AM, Musharaf MH, Mosnaz ATMJ, Hossain H, Islam MM, Ali MS, Prodhan SH. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landreces of rice (*Oryza sativa* L.). J. Biosci. Biotechnol. 2012;1(2):107-116.

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