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Genetic Diversity and Population Structure in Hashemi Rice (*Oryza sativa* L.) Mutants Revealed by Morphological and Molecular Markers

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INTRODUCTION

Mutagenesis has been one of the effective methods for creating genetic diversity and plant mutants can be significant bio-resources for crop breeding and functional genomics studies. The genetic and phenotypic diversity of 95 selected mutants from 17 mutant populations, obtained from an EMS mutagenized rice Hashemi variety, were phenotypically and molecularly assessed in M_3 generation. Phenotypic variation of these mutants showed that grain yield components varied among the selected mutants compared to the control plants. In parallel, genetic diversity assessed by 13 Inter-Simple Sequence Repeats（ISSR) primers showed that the number of amplified fragments per primer varied from 4 (pr1-7) to 11 (ISSR-7, ISSR-11). In general, 13 primers amplified 99 fragments, 50 of which were polymorphic (52.92%). The genetic variation created by ISSR markers within 17 populations varied from 11.11% in HM9 (Hashemi Mutant Line number 9) to 45.45% in HM2. The average molecular polymorphism value was 0.27. In the total genetic variance, 95% of differences were attributed to within-population diversity, and 5% were related to among-populations. The Unweighted Pair-Group Method with Arithmetic mean (UPGMA) trees illustrating ISSR diversity classified the rice mutant population into seven groups, which were further supported by model-based STRUCTURE analysis. In general, the studied mutant genotypes revealed desirable genetic characteristics in populations 13 and 17, with em_3h204 and em_3h280 genotypes being the most divergent.

Keywords: Ethyl methane sulfonate, ISSRs, Phenotypic variation, Mutagenized rice.

INTRODUCTION

About 20% of the world's dietary energy supply is provided by rice (*Oryza sativa* L.) grain and more than three billion people all around the world feed on rice daily (Birla *et al.*, 2017). Consequently, enhancement in the production of rice varieties with high quality and quantity is considered an important goal. In this regard, the improvement of rice varieties depends on accessibility, genetic variability, and the use of new biotechnological tools. Mutation is considered and applied as a suitable method in many crops, especially rice, and has an important function in development of novel varieties with favorite characters (Sharma and Singh, 2013). The induction of rice mutants has confirmed that utilizing this method could be an impressive research tool for genetic and physiological appraisal of yield-limiting factors in rice. Hence, EMS (Ethyl Methane Sulfonate), an alkylating agent, works as a chemical mutagen for the induction of transitional point mutations in nucleotide sequences by reacting with DNA

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(Kanra and Brunner, 1970).

In plant breeding, DNA markers are used as effective tools for illustrating and evaluating genetic assortment within and between varieties and populations. In contrast to other DNA markers, Inter-Simple Sequence Repeats (ISSR) markers have great advantages for employing technical simplicity, speed of assay, requiring minimal DNA contents and low cost approach in identification of genetic variations. Numerous ISSR markers in rice have been identified and are employed for assessment of rice genetic diversity (Lapitan *et al.,* 2007; Pervaiz *et al.,* 2010; Das *et al.,* 2013; Yadav *et al.,* 2013). A model-based approach by STRUCTURE for population analysis has been frequently implemented for studying population structure by many researchers (Jin *et al*., 2010; Liakat Ali *et al*., 2011; Zhang *et al*., 2011; Chakhonkaen *et al*., 2012; Courtois *et al*., 2012; Das *et al*., 2013). They identified two subcategories in the investigated population and categorized rice varieties into two groups with few admixture lines. Das *et al*. (2013) classified a set of 91 accessions of rice landraces from eastern and north- eastern India into four classes.

Therefore, we evaluated morphological characteristics by means of economic agronomic traits as well as genetic diversity using 13 ISSR markers to gain a better understanding of the amount of genetic variation among mutant populations, following population structure assessment to produce novel cultivars carrying the genes related to yield increment and yield components.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of Hashemi local variety of rice were obtained from the germplasm center maintained by the Department of Plant Breeding and Agronomy, Rice Research Institute of Iran (Rasht, Gilan Province, Iran). Then, 500 g of seeds were used to be treated by EMS.

EMS Treatment

To induce point mutations, first, 500 g seeds of Iranian local rice Hashemi cultivar, as wild type, were soaked in distilled water for 24 hours at room temperature in the central laboratory of Faculty of Agricultural Sciences of University of Guilan (Dr. Shirzadian-Khorramabad 's research project Number 714: Use of EMS mutagen to create a population of mutant plants in one of Iranian rice cultivars). Then, the water was drained and the seeds were treated in a solution of eight millimolar (0.8%) Ethyl Methane Sulfonate (EMS) for 6 hours (Mohapatra *et al*., 2014). After measurement of the germination percentage (results not shown), the treated seeds were conserved for sowing in rice field. Next, the M_1 treated and control seeds were planted in the field to develop M_1 plant population. At the end of the growing period, the seeds of each plant were harvested separately (labeled as 1-18). Whenever the mutant population were generated, due to identification for mutation properties, the seeds from the mutant plant comprising the next generation were collected for phenotype investigation (Henry *et al*., 2014) (Figure 1).

Evaluation of M_2 and M_3 Mutant Plants

Then, 309 M_2 seeds along with non-mutated Hashemi local rice seeds were sown in the following year, and the desirable mutants (95 genotypes from 17 mutant populations) were selected based on the observed diversity in the field. Later, the M_3 selected plants were grown in paddy fields at the Rice Research Institute of Iran (RRII), for DNA analysis and, subsequently, the M_4 seeds were collected for analysis of morphological traits (Table 1). During the growth period, all the data concerning vegetative traits, yield, and its components

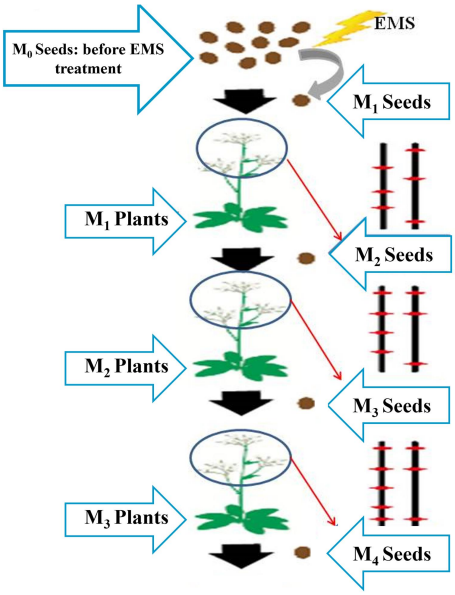

Figure 1. The employed scheme for gaining various mutant populations in local rice Hashemi cultivar. The figure has been made by the manuscript authors.

were recorded. The measured morphological traits included 50% flowering, plant height, panicle number per plant, panicle length, filled grain number per panicle and unfilled grain number per panicle, 100-seed weight, grain length, grain width, and seed yield.

Genomic DNA Isolation and PCR Amplification

For genetic diversity assessment of the studied mutant population, 13 ISSR markers were utilized (Fernandez *et al*., 2002; Jabbarzadeh *et al*., 2013; Aalami and Karimi, 2017) (Table 2). ISSR primers with the highest amplifications were selected for ISSR evaluation.

The CTAB method (Murray and Thompson, 1980) was used to extract DNA from fresh leaves of plant mutants (95 selected M3 mutants plus control parent plants) (Table 3). Then, a similar annealing temperature was optimized for ISSR primers (Table 2). PCR amplification was accomplished on a thermal cycler (Model T-Gradiant, Bio-metra, USA) at an initial

Table 1. List of mutant genotypes used with the control parent (Hashemi accession).[a]

SN	Mutant genotype	SN	Mutant genotype	SN	Mutant genotype	SN	Mutant genotype	SN	Mutant genotype
1	em_3h7	21	em_3h53	41	em_3h116	61	em_3h201	81	em_3h272
2	em_3h9	22	em_3h57	42	em_3h122	62	em_3h204	82	em_3h275
3	em_3h10	23	em_3h58	43	em_3h127	63	em_3h208	83	em_3h280
4	em_3h12	24	em_3h61	44	em_3h130	64	em_3h219	84	em_3h281
5	em_3h13	25	em_3h65	45	em_3h135	65	em_3h223	85	em_3h284
6	em_3h17	26	em_3h68	46	em_3h140	66	em_3h224	86	em_3h287
7	em_3h21	27	em_3h69	47	em_3h145	67	em_3h227	87	em_3h290
8	em_3h22	28	em_3h75	48	em_3h151	68	em_3h229	88	em_3h294
9	em_3h25	29	em_3h78	49	em_3h157	69	em_3h232	89	em_3h296
10	em_3h29	30	em_3h81	50	em_3h163	70	em_3h233	90	em_3h297
11	em_3h30	31	em_3h83	51	em_3h164	71	em_3h236	91	em_3h298
12	em_3h31	32	em_3h88	52	em_3h166	72	em_3h243	92	em_3h303
13	em_3h32	33	em_3h93	53	em_3h177	73	em_3h246	93	em_3h307
14	em_3h35	34	em_3h94	54	em_3h178	74	em_3h251	94	em_3h309
15	em_3h36	35	em_3h96	55	em_3h181	75	em_3h256	95	em_3h313
16	em_3h39	36	em_3h97	56	em_3h183	76	em_3h259	96	Parent (Hashemi rice)
17	em_3h42	37	em_3h104	57	em_3h184	77	em_3h260	-	-
18	em_3h44	38	em_3h106	58	em_3h189	78	em_3h263	-	-
19	em_3h50	39	em_3h111	59	em_3h194	79	em_3h266	-	-
20	em_3h51	40	em_3h112	60	em_3h198	80	em_3h270	-	-

[a] em_3hn: em showing EMS, 3: Is third generation, h: Hashemi variety, and n: Is the genotype number.

Table 2. Primers used in the ISSR analysis for molecular evaluation of the selected mutants.

ISSR primer	Sequence (5′–3′)[a]	Annealing temperature (ºC)	Fragment size range (bp)
ISSR440	*CACACACACACACACACAG*	53	103-932
ISSR425	*ACACACACACACACACACC*	52	218-1588
PR1-1	*CAGCAGCAGCAGCAG*	55	240-1046
PR1-4	*CTCTCTCTCTCTCTCTT*	45.9	257-1051
PR1-5	*CCACCACCACCACCA*	55	267-1000
PR1-7	*CAACAACAACAACAA*	46	392-1176
PR1-9	*ACTGACTGACTGACTG*	45.9	141-1224
ISSR-2 (UBC808)	*AGAGAGAGAGAGAGAGC*	53.6	382-757
ISSR-5 (UBC841)	*GAGAGAGAGAGAGAGAC*	50	169-800
ISSR-7 (UBC823)	*TCTCTCTCTCTCTCTCC*	52.4	216-1067
ISSR-9 (UBC828)	*TGTGTGTGTGTGTGTGA*	45.9	163-1029
ISSR-11	*AGGAGGAGGAGGAGGCC*	57	141-1224
ISSR-12	*AAGAAGAAGAAGAAGAAGC*	40	316-1211

Table 3. DNA quality and average DNA yield obtained using different DNA extraction methods.

Solution name/Buffer	Reactive material	
TE buffer	Tris (1M)	10M
	EDTA (0.5M)	2 ml
Tris (1M)	Tris	121/14 g
EDTA[a] (0.5M)	EDTA($2H_2O$)	186/7 g
	NAOH	20 g
Nacl (5M)	Nacl	292/2 g
CTAB[b] 2%	CTAB	2 g
	Nacl (5M)	14 mL
Extraction buffer	EDTA (0.5M)	5 mL
	Tris (1M)	5 ml
	Nacl (5M)	6 mL
SDS[c] (20%)	SDS	20 g
Chloroform Isoamylalcohl	Cloroform	24 mL
	Isoamylalcohl	1 mL

[a] Ethylen Diamine Tetra Acetic acid, [b] Cetyl Trimethyl Ammonium Bromide, [c] Sodium Dodecyl Sulphate.

temperature of 94°C for 4 minutes, followed by 35 cycles at 94°C for 40 seconds, 40-55°C for 40 seconds (depending on TM of the primers), 72°C for 1 minute, and a final cycle of 72°C for 5 minutes. Then, it was stored at 4°C. The ISSR PCR products were separated by electrophoresis based on their molecular weight on 2% (w/w) agarose gel submerged in 1X TBE and subsequently stained with ethidium bromide (10 mg mL^{-1}) solution for 20 minutes. Since ISSR primers are dominant molecular markers, amplified bands were scored 1 for presence or 0 for lack of bands. All the computation analyses were carried out by means of NTSYS-pc $version_{2.02}$ software package (Rohlf, 2002). Polymorphism Information Content (PIC) for every ISSR marker was separately calculated in the selected mutant M_3 population using the following formula:

$$PIC= 1-\sum(Pi)^2$$

Where, Pi shows the frequency of i^{th} allele. Analysis Of Molecular Variance (AMOVA) and Principal Coordinates Analysis (PCoA) were performed by GenAlEx software version 6.5 (Peakall and Smouse, 2006).

Parameters in accordance with the mutant population including the number of effective alleles, Shannon diversity index, and Neiṡ gene diversity index were calculated by POPGENE 32 software version 1.31. Structure of the mutant population was assessed by a model-based method in Structure software v. 2.3.4 (Pritchard *et al*., 2000). The number of populations was tested from *K*= 1-10 by the admixture and correlated allele frequencieṡ models. Ten individual runs were arranged for each *K* with a burn-in period of 100,000 and a run of 500,000. Optimum *K* value was determined from log probability of data [ln *P*(*D*)] and ad-*hoc* statistic Δ*K* proposed by Evanno *et al*. (2005) using the structure harvester website and software (Earl and Vonholdt, 2012). The number of putative populations (K), assumed according to a set of allele frequencies at each locus can provide the degree of admixture of mutant genotypes. Phenotypic data were analyzed by SPSS software version 22.0 package (SPSS Inc., Chicago, IL, USA). Afterwards, multivariate analysis was run for separation of families using cluster analysis by the Ward's method.

RESULTS AND DISCUSSION

Evaluation of Phenotypic Variation

The results of phenotypic assessment showed that all ten measured quantitative traits were diverse among the selected mutant plants in population compared to the control plants, demonstrating the presence of high genetic diversity among the mutant plants, which can be used to select suitable mutant lines for further breeding of Hashemi local variety (Table 4). The grain number per plant ranged from 57 to 147, with a mean of 91.9. Moreover, the 100-grain weight ranged from 2.16 to 3.89 g with a mean of 2.78 g. The mean grain yield of mutants per hectare was equal to 3,204.89

Table 4. Analysis of variability in phenotypic traits within the 95 M_3 mutant genotypes with non-mutant parent cultivar (control).

Trait	Hashemi var.	Mean±SEM	Range	amount of variation	Genotypes with lowest–highest means	Standard deviation
Days to 50% flowering	78	82.3 ± 0.14	73 – 87	14	em_3h298 - em_3h75	1.33
Plant height	142	132.9 ± 0.62	143 – 155	52	em_3h272 - em_3h201	6.01
Panicle number per plant	16	12.6 ± 0.2	6 – 23	17	em_3h88- em_3h307	1.99
Panicle length	30.5	29.1 ± 0.16	37.5 – 21	16.5	em_3h303 - em_3h166	1.56
Filled grain number per panicle	95	91.9 ± 1.33	147 – 57	90	em_3h256 - em_3h104	12.95
Unfilled grain number per panicle	7	7.01 ± 0.31	29 – 1	28	em_3h303 - em_3h232	2.99
100-Seed weight	2.57	2.78 ± 0.01	3.89 - 2.16	1.73	em_3h280 - em_3h208	0.11
Grain length	9.8	10 ± 0.03	10.97 - 8.8	2.17	em_3h275 - em_3h163	0.34
Grain width	2.2	2.62 ± 0.01	3.1 - 2.06	1.04	em_3h224 - em_3h227	0.11
Seed yield	3906.4	3204.98 ± 70.1	5950 – 1361	4589	em_3h251 - em_3h75	683.71

kg (Table 4). Results revealed that the number of tillers and filled grains per plant were extremely elevated in contrast to the other traits. Luz *et al*. (2016) discovered that inducing chemical mutation with EMS increased the number of main panicle grains and panicle grain weight in rice genotypes.

In general, distributions of phenotype frequencies displayed an approximately normal distribution (Figure 2), although only frequencies of panicle length and filled-grain number per panicle were normally distributed. Grain size was determined by grain length and grain width, and the grain length to width ratio was considered as a major determining factor of grain appearance quality and grain weight (Edzesi *et al*., 2016). Three principal components of grain yield include the number of panicles per plant, number of grains per panicle, and grain weight, among which grain weight calculated as 1000-grain weight was a determinant trait for grain yield (Huang *et al*., 2013; Edzesi *et al*., 2016). To achieve

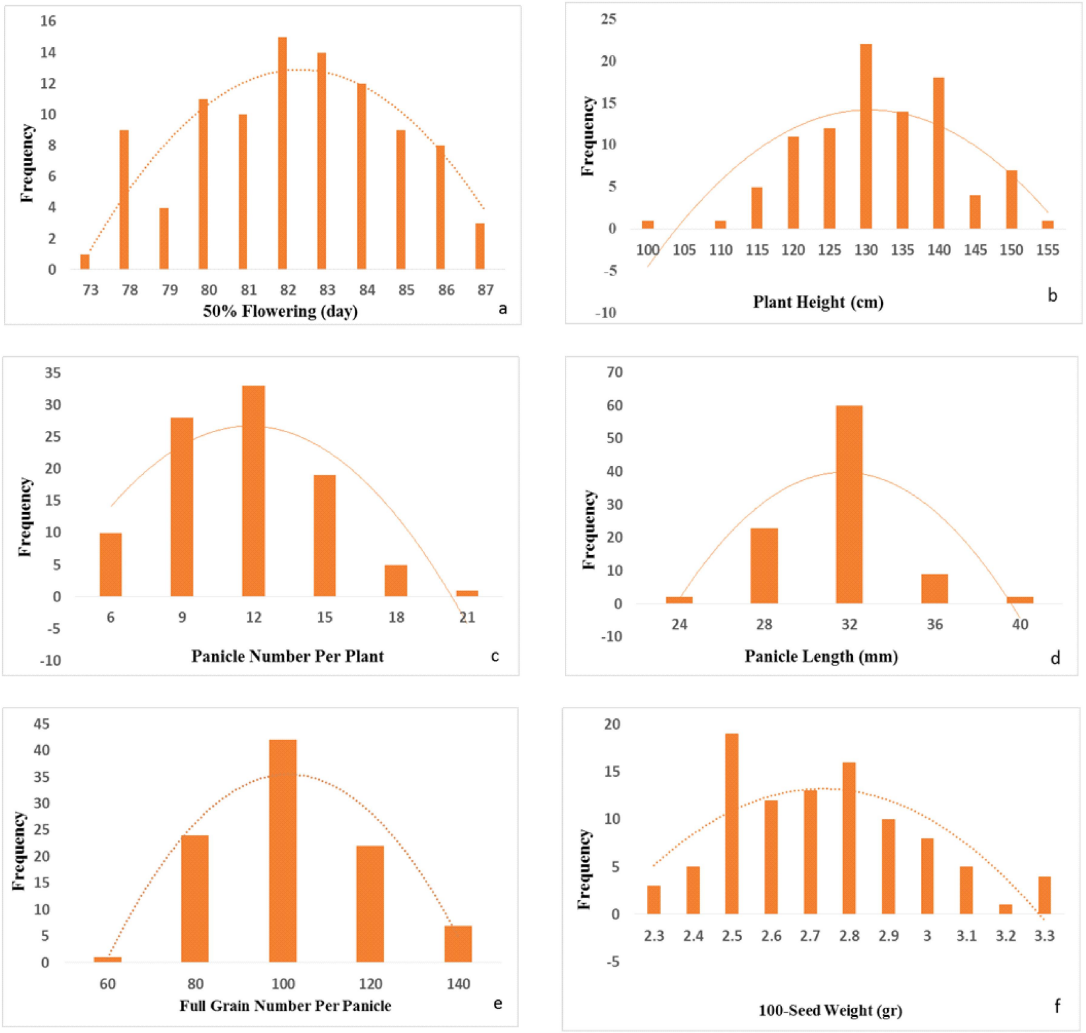

Figure 2. Phenotypic distributions for the studied agronomic traits: (a) Days to 50% flowering, (b) Plant height, (c) Panicle number per plant, (d) Panicle length, (e) Filled grain number per panicle and (f) 100-Seed weight. Histograms show data of all genotypes used in the assays. The resulting curves from fitting the data to a normal distribution are shown.

dwarf and long grain varieties, it is necessary to use suitable traits for plant height and long grain. Early maturity is the best characteristic of these genotypes that can be utilized in breeding programs. The results showed that all the studied genotypes comprising Hashemi local variety and mutant genotypes varied in their growth duration, yield, and yield components. Viana *et al.* (2019) presented the documentary results that the use of EMS enhances agronomically important traits.

Plant height reduction, increases in panicle number, grain number, and 100-seed weight are desirable and optimal traits for increasing seed yield. Plants were thus identified based on one or more appropriate traits.

Genetic Diversity

A total of 13 ISSR primers consisting of di and tri-repeat motifs were utilized to obtain distinct, reproducible, and well-resolved fragments in 95 selected mutant genotypes in M_3 populations. Primers were found to confer a clear polymorphic pattern and were subsequently used to analyze the entire set of 95 mutant genotypes (Table 1, Figure 3).

The ISSR amplified fragments were placed within a range of 103 to 1,588 bp. The fragments amplified per primer varied from 4 (pr1-7) to 11 (ISSR-7 and ISSR-11). 13 ISSR primers, in total, generated 99 fragments, 50 of which (52.92%) were polymorphic. The average number of loci and polymorphic loci produced per primer were equal to 7.62 and 3.85, respectively. In this study, the genetic variation detected by ISSR markers in 17 populations varied from 11.11% in HM9 (Hashemi Mutant 9) to 45.45% in HM2 (Hashemi Mutant 2). Analysis of genetic variation of total population is represented in Table 5.

Evaluation of genetic diversity is an important factor in identification and preservation. In this research, Nei's gene diversity index was utilized for evaluation of markers, along with the PIC and Shannon index. PIC value is a principal factor for recognizing the ratio of polymorphism of a marker at a particular locus, so, the higher

Table 5. Analysis of genetic variations in seventeen studied populations generated by ISSR makers (HM).[a]

Population	% Polymorphic Bands (PPB)	Allele number (no)	Effective allele number (ne)	Nei's gene diversity (He)	Shannon's Index (I)
HM1	37.37	1.3737	1.2673	0.1495	0.2179
HM2	45.45	1.4545	1.3568	0.1941	0.2789
HM3	20.2	1.202	1.1486	0.0822	0.1194
HM4	35.35	1.3535	1.2616	0.1445	0.2093
HM5	39.39	1.3939	1.2866	0.1573	0.2285
HM6	26.26	1.2626	1.1807	0.1028	0.1511
HM7	22.22	1.2222	1.1712	0.093	0.1342
HM8	18.18	1.1818	1.1256	0.0716	0.1053
HM9	11.11	1.1111	1.0786	0.046	0.0672
HM10	26.26	1.2626	1.2126	0.1134	0.1625
HM11	32.32	1.3232	1.2508	0.138	0.1986
HM12	26.26	1.2626	1.1914	0.1053	0.1534
HM13	38.38	1.3838	1.2636	0.1487	0.2179
HM14	32.32	1.3232	1.2367	0.1321	0.1921
HM15	35.35	1.3535	1.2679	0.1457	0.2104
HM16	35.35	1.3535	1.2573	0.1431	0.2081
HM17	33.33	1.3333	1.2236	0.1268	0.1866

[a] Overall Gst (Genetic differentiation)= 0.37 and overall Nm (gene flow from Gst)= 0.87. H: Hashemi parent, M: Mutant, Number: Number of populations.

the PIC value, the higher the percentage of polymorphism (Anupam *et al*., 2017). PIC values varied from 0.09 to 0.27, with a mean value of 0.16. The maximum PIC value (0.27) was illustrated in PR1-5 primer; while the minimum PIC value (0.09) was demonstrated in PR1-4 primer. PIC confirms that the employed ISSR loci are highly informative to identify genotype variations (Maclean, 2013). This result is in close agreement with the findings reported in the previous studies (Muthusamy *et al*., 2008; Reddy *et al*., 2009; Kshirsagar *et al*., 2014; Khaled *et al*., 2015) in rice genotypes. Among the populations, the HM2 population showed the highest variability with 45.45% of Polymorphic Bands (PPB), an Allele number (Ao) of 1.45, an effective Allele number (Ae) of 1.36, Nei's genetic diversity (He) of 0.19 and a Shannon's Index (I) of 0.28. Meanwhile, the least variability was found in HM9 with 11.11% of Polymorphic Bands (PPB), an Allele number (Ao) of 1.11, an effective Allele number (Ae) of 0.046, Nei's gene diversity (He) of 0.067, and a Shannon's Index (I) of 0.28. In this study, the Global genetic diversity (Gst) among populations was 0.37, indicating that 37% of genetic diversity was allocated among the populations. The Number of gene flow (Nm) was anticipated to be 0.87 per generation amongst the populations. The high Shannon's index of this primer showed that it could better represent genetic diversity and is suitable for evaluating within-population diversity. The estimates of Nei's (1973) genetic identities and genetic distances from pairwise comparisons between population pairs are shown in Table 6. According to the results, the highest identity (0.97) was observed between HM2 and HM5 populations and the lowest identity (0.82) was found in local cultivar Hashemi and HM8 populations (Table 6). Similarity coefficients revealed the genetic relationship between the mutants and their parents.

Principal component analysis separated 95 individual trees into 17 distinct groups (Figure 4). The distribution of various individuals related to the two principal axes of variation could be found in Figure 4. The first seven components showed that 90.8% of the changes were related to the 13 studied primers. Based on the results, 85.1% of the total variation was described by PC1 and PC2. Among 32 rice genotypes, 82.7% of the explained variation by PC1 was also shown by Lasalita-Zapico *et al*. (2010) in their study. Also, 68.6% of variation explained by PC1 and PC2 was reported by Caldo *et al.* (1996). The results show that the sites and applied markers are independent and distributed in various locations in the genome (Figure 4)

STRUCTURE analysis showed that the maximum peak value for Delta K (0.56) was identified at an optimum K value (K= 7), implying that among the selected mutant populations, at least seven distinct groups (G1-G7) could be recognized (Figure 5). An UPGMA cluster classified the cultivars into seven groups based on genetic distance. The distribution of sub-populations was similar to the classification presented in the UPGMA dendrogram (Figure 6), performed based on ISSR markers. Sub-populations 1 and 4 contained 8 and 9 genotypes, respectively, whereas sub-population 6 consisted of 44 genotypes. The majority of the mutant genotypes were included in sub-population 6, whereas Hashemi rice (non-mutant parent cultivar) and other mutants were located in an admixture of sub-populations (Figure 7). Non-mutant parent (Hashemi rice) and 31 mutant genotypes did not fall into any sub-population contribution among the 95 mutant genotypes categorized by molecular markers in parallel with STRUCTURE results.

Analysis of Molecular Variation (AMOVA) was conducted at three hierarchical levels in the present study to assess the total genetic variance. The significance level was found at 1,000 random permutations of sequences amongst the populations. The significant genetic differences ($P< 0.001$) among and within the populations were identified. From the total molecular variance, 95% of differences were

Table 6. Measurement of genetic identity (above diagonal) and genetic distance (below diagonal) between the seventeen studied mutant populations and Hashemi parent cultivar.

Pop	HM1	HM2	HM3	HM4	HM5	HM6	HM7	HM8	HM9	HM 10	HM 11	HM 12	HM 13	HM 14	HM 15	HM 16	HM 17	Hashemi rice
HM1	**	0.98	0.93	0.96	0.96	0.95	0.93	0.88	0.90	0.92	0.94	0.90	0.93	0.93	0.90	0.95	0.94	0.87
HM2	0.02	**	0.95	0.98	0.98	0.97	0.95	0.93	0.93	0.95	0.98	0.95	0.97	0.96	0.95	0.97	0.97	0.90
HM 3	0.08	0.06	**	0.93	0.94	0.95	0.96	0.91	0.91	0.93	0.94	0.92	0.95	0.94	0.94	0.93	0.93	0.84
HM 4	0.04	0.02	0.08	**	0.97	0.97	0.95	0.93	0.92	0.94	0.97	0.93	0.97	0.95	0.94	0.97	0.96	0.88
HM 5	0.04	0.02	0.07	0.03	**	0.97	0.96	0.92	0.92	0.97	0.97	0.92	0.96	0.94	0.94	0.96	0.95	0.86
HM 6	0.06	0.03	0.05	0.03	0.03	**	0.94	0.90	0.91	0.95	0.95	0.92	0.96	0.91	0.92	0.96	0.95	0.88
HM 7	0.07	0.05	0.04	0.05	0.05	0.06	**	0.95	0.95	0.95	0.96	0.95	0.95	0.93	0.95	0.96	0.96	0.87
HM 8	0.13	0.07	0.10	0.07	0.08	0.11	0.05	**	0.93	0.92	0.93	0.93	0.95	0.93	0.93	0.92	0.91	0.83
HM 9	0.11	0.07	0.09	0.08	0.08	0.09	0.05	0.07	**	0.93	0.93	0.92	0.94	0.93	0.93	0.92	0.91	0.83
HM 10	0.09	0.05	0.08	0.06	0.03	0.05	0.05	0.09	0.07	**	0.95	0.93	0.95	0.92	0.94	0.94	0.92	0.85
HM 11	0.06	0.02	0.06	0.03	0.04	0.05	0.04	0.07	0.07	0.05	**	0.95	0.98	0.96	0.97	0.96	0.95	0.90
HM 12	0.10	0.05	0.08	0.07	0.08	0.08	0.05	0.07	0.08	0.07	0.05	**	0.94	0.93	0.93	0.93	0.96	0.91
HM 13	0.07	0.03	0.05	0.03	0.04	0.04	0.05	0.05	0.06	0.05	0.02	0.06	**	0.98	0.97	0.95	0.93	0.88
HM 14	0.08	0.04	0.06	0.05	0.07	0.09	0.07	0.08	0.08	0.09	0.04	0.07	0.02	**	0.97	0.92	0.92	0.85
HM 15	0.11	0.05	0.06	0.06	0.06	0.08	0.06	0.07	0.07	0.06	0.03	0.07	0.03	0.03	**	0.93	0.93	0.85
HM 16	0.05	0.03	0.07	0.03	0.04	0.04	0.04	0.08	0.08	0.06	0.04	0.07	0.05	0.09	0.07	**	0.97	0.91
HM 17	0.07	0.03	0.07	0.04	0.05	0.05	0.04	0.09	0.09	0.08	0.05	0.04	0.07	0.08	0.07	0.03	**	0.90
Hashemi rice	0.14	0.10	0.18	0.13	0.15	0.13	0.13	0.19	0.19	0.16	0.10	0.09	0.13	0.16	0.16	0.10	0.10	**

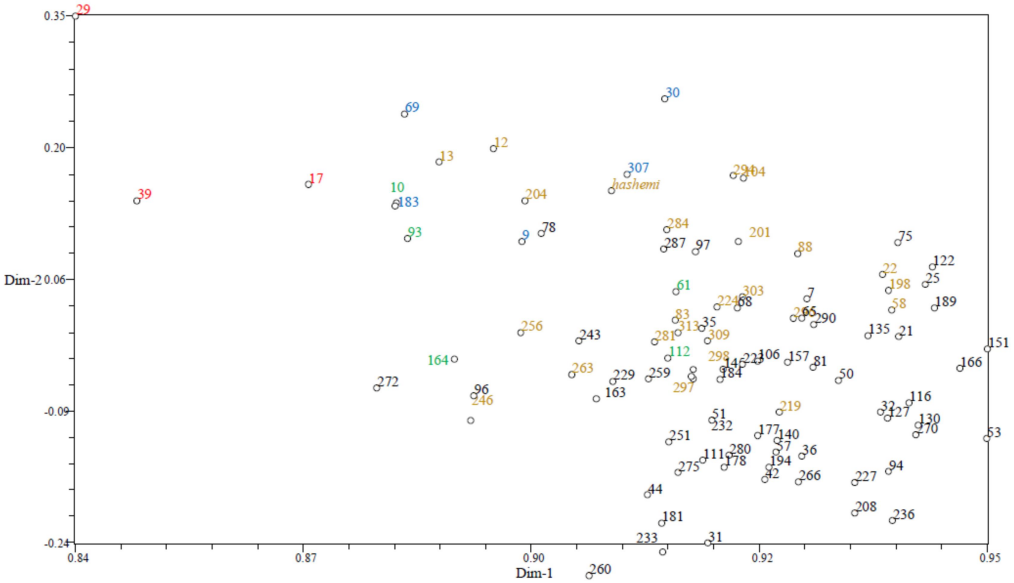

Figure 4. Principle component analysis based on 13 ISSR markers for 95 M_3 mutated genotypes (selected from 17 populations) with Hashemi parent cultivar.

ascribed to within-population diversity, while 5% of differences were related to among-population diversity (Table 7). As a result, AMOVA results revealed a highly significant genetic differentiation between and within groups. Recent studies have illustrated a higher level of diversity within groups and within populations compared to genetic diversity among groups in non-mutated species (Thangjam, 2014; Islam *et al*., 2017; Tu Anh *et al*., 2018; Zhang *et al*., 2022).

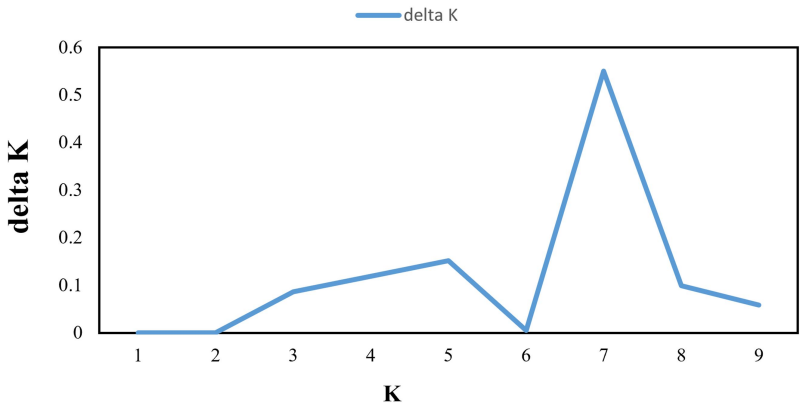

Figure 5. Two-dimensional scatter for determining optimum value of K.

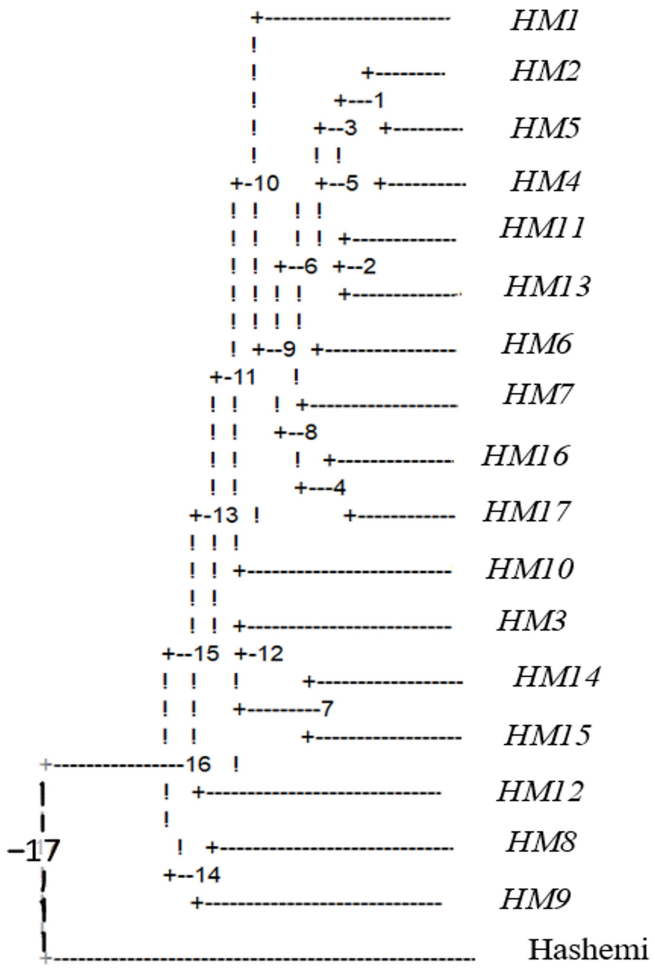

Figure 6. Dendrogram of 95 M_3 mutant genotypes with Hashemi parent cultivar (control). Individual trees belonging to seventeen populations based on UPGMA analysis of ISSR polymorphism.

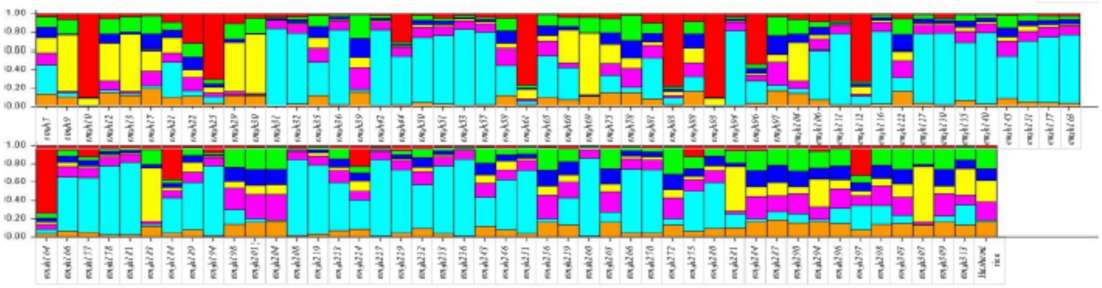

Figure 7. The diagrammatic representation shows the distribution of M_3 selected mutant genotypes with Hashemi parent cultivars in different sub-populations.

Table 7. Result of the Analysis Of Molecular Variance (AMOVA) of 95 M_3 mutated genotypes with Hashemi parent cultivar (control) individual trees representing seventeen populations based on ISSR analysis.

Source	df	SS	MS	Est. var.	%
Among Pops	17	197.643	11.626	0.498	5%
Within Pops	78	701.982	9.000	9.000	95%
Total	95	899.625		9.498	100%

Choudhary *et al.* (2013) reported a high-level genetic diversity within populations (96%) and a very low genetic diversity among populations (4%) in four rice populations. More diversity within populations demonstrates that there is more differences than what could be detected in mutant populations for further use in breeding programs. Moreover, AMOVA results attributed to a greater percentage of molecular variance (95%) to within-group diversity, representing a higher magnitude of genetic differentiation within group individuals than among groups (5%), which could be due to the effect of local domestication preferences of rice varieties (Figure 8).

There is also evidence from various studies that populations maintain high levels of variability both within their own species and within themselves, and that this genetic diversity is relatively low compared to that of non-mutated varieties (Thangjam, 2014; Tu Anh *et al.,* 2018; Ab Razak *et al.,* 2020). Like many other outcrossing species, the mutated population is also heterozygous with most of its morphological, physiological, and biochemical properties, presenting continuous variation. The most similarity with control parents was found in the HM2 and HM5 mutant populations, and the least similarity was related to the HM8

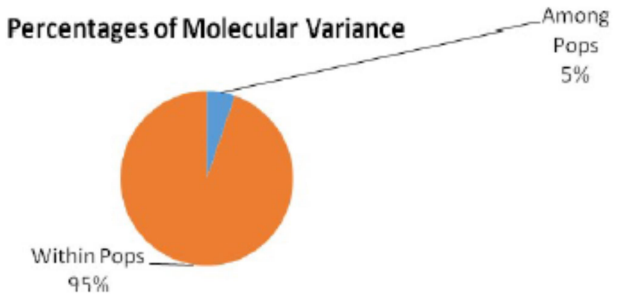

Figure 8. Analysis Of Molecular Variance (AMOVA) of 95 M_3 mutated genotypes with Hashemi parent cultivar (control), individual trees indicating seventeen populations based on ISSR analysis.

and HM9 mutant populations. Given the above dendrogram, cluster analysis using UPGMA illustrated that control plants were placed in a separate group from the selected mutated populations (Figure 6) (Sen and Alikamanoglu, 2014). Studying the molecular variation of the selected mutant populations using ISSR markers demonstrated that mutation treatment could have rather high polymorphism in 50% of them. Genetic variation in a population is a valuable source for magnifying a range of genetic materials in plant breeding, and molecular markers such as ISSRs are remarkable tools to determine genetic diversity (Nachimuthu *et al*., 2015; Thant *et al.,* 2021).

A model based on admixture was performed on *K* values varying from 1 to 10 with ten repetitions by all 95 mutant genotypes and 13 polymorphic markers for maximum probability and Delta *K (nK)* values (Figure 5). Δ*K* peak at *K*= 7 revealed that all the studied mutant populations (HM) could be categorized into seven clusters. The result was firmly in agreement with the obtained principal coordinates analysis and UPGMA tree. Population STRUCTURE analysis in various rice diversity panels has revealed the existence of two to eight sub-populations (Jin *et al.,* 2010; Das *et al*., 2013; Dang *et al*., 2015; Ming *et al*., 2015; Nachimuthu *et al*., 2015). Therefore, the results of this study representing the genetic diversity of the mutant genotypes can be employed to predict approaches such as analysis of mutant genotypes, classical mapping of population development; parental line selection in breeding programs and hybrid development for exploiting genetic variation existing in this population. Ninety-six mutant genotypes were mainly grouped into seven sub-populations and three distinctive groups by population STRUCTURE analysis, revealing the existence of moderate population structure within them. Thus, it is helpful to extend diversity in various strategies in crop improvement programs. The majority of the mutated genotypes were present in sub-population 6.

CONCLUSIONS

In fact, the mutagenesis modifies the normal biological combination of an organism and factual genetic changes create desirable characteristics. These derived mutant genotypes will experience observational yield trails to pick up the most superior genotypes of the Hashemi local variety with shorter maturity duration, plant height, and high yield. The overall evaluation of the newly developed genotypes revealed that the best genotype is em_3h280, followed equally by em_3h204. The study has shown that EMS mutagenesis was an efficient method for mutation induction in Hashemi local rice and the mutants were successfully identified through ISSR analysis. The existence of a notable amount of genetic variation in the studied populations can assist in the selection of suitable mutant lines for further breeding of the Hashemi local variety.

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REFERENCES

1. Aalami, A. and Karami, N. 2017. The Study of Genetic Diversity in Iranian Rice

Cultivars using ISSR, IRAP and REMAP Markers. *J. Crop Breed.,* **8(20)**: 51-41

2. Anupam, A., Imam, J., Quatadah, S. M., Siddaiah, A., Das, S. P., Variar, M. and Mandal, N. P. 2017. Genetic Diversity Analysis of Rice Germplasm in Tripura State of Northeast India Using Drought and Blast Linked Markers. *Rice Sci.*, **24:** 10–20.
3. Birla, D. S., Malik, K., Sainger, M., Chaudhary, D. and Jaiwal, P. K. 2017. Progress and Challenges in Improving the Nutritional Quality of Rice (*Oryza sativa* L.). *Crit. Rev. Food Sci. Nutr.,* **57:** 2455–2481.
4. Caldo, R. A., Sebastian, L. S. and Hernandez, J. E. 1996. Morphology-Based Genetic Diversity Analysis of Ancestral Lines of Philippine Rice Cultivars. *Phil. J. Crop Sci*., **21:** 86–92.
5. Chakhonkaen, S., Pitnjam, K., Saisuk, W., Ukoskit, K. and Muangprom, A. 2012. Genetic Structure of Thai Rice and Rice Accessions Obtained from the International Rice Research Institute. *Rice*, **5(1):** 19.
6. Choudhary, G., Ranjitkumar, N., Surapaneni, M., Deborah, D. A, Vip-parla, A., Anuradha, G., Siddiq, E. A. and Vemireddy, L.R. 2013. Molecular Genetic Diversity of Major Indian Rice Cultivars over Decadal Periods. *PLoS One*, **8(6):** 1-12.
7. Choudhury, D. R., Singh, N., Singh, A. K., Kumar, S., Srinivasan, K., Tyagi, R. K., Ahmad, A., Singh, N. K. and Singh, R. 2014. Analysis of Genetic Diversity and Population Structure of Rice Germplasm from North-Eastern Region of India and Development of a Core Germplasm Set. *PLoS One,* **9(11):** e113094.
8. Courtois, B., Frouin, J., Greco, R., Bruschi, G., Droc, G., Hamelin, C., Ruiz, M., Clement, G., Evrard, J. C., van Coppenole, S., Katsantonis, D., Oliveira, M., Negrao, S., Matos, C., Cavigiolo, S., Lupotto, E., Piffanelli, P. and Ahmadi, N. 2012. Genetic Diversity and Population Structure in an European Collection of Rice. *Crop Sci.*, **52:** 1663–1675.
9. Dang, X., Thi, T. G. T., Edzesi, W. M., Liang, L., Liu, Q., Liu, E., Wang, Y., Qiang, S., Liu, L. and Hong, D. 2015. Population Genetic Structure of *Oryza sativa* in East and Southeast Asia and the Discovery of Elite Alleles for Grain Traits. *Sci. Rep.*, **5:** 11254.
10. Das, B., Sengupta, S., Parida, S. K., Roy, B., Ghosh, M., Prasad, M. and Ghose, T. K. 2013. Genetic Diversity and Population Structure of Rice Landraces from Eastern and North Eastern States of India. *BMC Genet.*, **14:** 71-91.
11. Earl, D. A. and Von Holdt, B. M. 2012. Structure Harvester: A Website and Program for Visualizing Structure Output and Implementing the Evanno Method. *Conserv. Genet. Resour.,* **4(2):** 359–361.
12. Edzesi, W. M., Dang, X., Liang, L., Liu, E., Zaid, L. U. and Hong, D. 2016. Genetic Diversity and Elite Allele Mining for Grain Traits in Rice (*Oryza sativa* L.) by Association Mapping. *Front. Plant Sci.*, **7:** 1–13.
13. Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study. *Mol. Ecol.*, **14(8):** 2611–2620.
14. Fernandez, M., Figueiras, A. and Benito, C. 2002. The Use of ISSR and RAPD Markers for Detecting DNA Polymorphism, Genotype Identification and Genetic Diversity among Barley Cultivars with Known Origin. *Theor. Appl. Genet.*, **104(5):** 845-851.
15. Henry, I. M., Nagalakshmi, U., Lieberman, M. C., Ngo, K. J., Krasileva, K. V,. Vasquez-Gross, H., Akhunova, A., Akhunov, E., Dubcovsky, J., H. Tai, T. and Comai, L. 2014. Efficient Genome-Wide Detection and Cataloging of EMS-Induced Mutations Using Exome Capture and Next-Generation Sequencing. *Plant Cell*, **26:** 1382–1397.
16. Huang, R., Jiang, L., Zheng, J., Wang, T., Wang, H., Huang, Y. and Hong, Z. 2013. Genetic Bases of Rice Grain Shape: So Many Genes, so Little Known. *Trends Plant Sci.*, **18:** 218–226.
17. Islam, M. Z., Khalequzzaman, M., Bashar, M. K., Ivy, N. A., Mian, M. A. K., Pittendrigh, B. R., Haque, M. M. and Ali, M. P. 2017. Variability Assessment of

Aromatic Rice Germplasm by Pheno-Genomic Traits and Population Structure Analysis. *Sci. Rep.*, **8:** 9911.

18. Jabbarzadeh, Z., Khosh-khui, M., Salehi, H., Shahsavar, A. R. and Saberivand, A. 2013. Assessment of Genetic Relatedness in Roses by ISSR Markers. *World Appl. Sci. J.*, **28:** 2085-2090.
19. Jin, L., Lu, Y., Xiao, P., Sun, M., Corke, H. and Bao, J. S. 2010. Genetic Diversity and Population Structure of a Diverse Set of Rice Germplasm for Association Mapping. *Theor. Appl. Genet.*, **121:** 475–487.
20. Kanra, O. P. and Brunner, H. 1970. Chemical Mutagens. Mode of Action. In: "*Manual in Mutation Breeding*". *Tech. Rept. Ser.*, FAO/IAEA, **119:** 62–64.
21. Khaled, A. G. A., Motawea, M. H. and Said, A. A. 2015. Identification of ISSR and RAPD Markers Linked to Yield Traits in Bread Wheat under Normal and Drought Conditions. *J. Genet. Eng. Biot.*, **13:** 243–252.
22. Kshirsagar, S. S., Samal, K. C., Rabha, M., Bastia, D. N. and Rout, G. R. 2014. Identification of Variety Diagnostic Molecular Marker of High Yielding Rice Varieties. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.,* **84(2):** 389–396.
23. Lapitan, V. C., Brar, D. S., Abe, T. and Redofia, E. D. 2007. Assessment of Genetic Diversity of Philippine Rice Cultivars Carrying Good Quality Traits with SSR markers. *Breed. Sci.*, **57(4):** 263–270.
24. Lasalita-Zapico, F. C., Namocatcat, J. A. and Cariño-Turner, J. L. 2010. Genetic Diversity Analysis of Traditional Upland Rice Cultivars in Kihan, Malapatan, Sarangani Province, Philippines Using Morphometric Markers. *Phil. J. Crop. Sci.*, **139:** 177–180.
25. Liakat Ali, M., McClung, A. M., Jia, M. H., Kimball, J. A., McCouch, S. R. and Georgia, C. E. 2011. A Rice Diversity Panel Evaluated for Genetic and Agro-Morphological Diversity between Subpopulations and Its Geographic Distribution. *Crop Sci.,* **51(5):** 2021–2035.
26. Ming, H., Ya-hui, W. U., Xing-xing, T. A. O., Yong-zhu, L. I. U., Gui-li, Y. and Zhi-qiang, C. 2015. Genetic Diversity of Main Inbred Indica Rice Varieties Applied in Guangdong Province as Revealed by Molecular Marker. *Rice Sci.,* **22:** 1-8.
27. Maclean, J., Hardy, B. and Hettel, G. 2013. Rice Almanac: Source Book for One of the Most Important Economic Activities on Earth. 4th Edition, IRRI, Laguna.
28. Malekmohamadi, Z., Sabori, H., Biabani, A. and Hezarjaribi, E. 2017. Study of Genetic Diversity of Soybean (*Glycine max*) using ISSR Markers. *J. Crop Breed.*, **8(19):** 133-124.
29. Mohapatra, T., Robin, S., Sarla, N., Shehashayee, M., Singh, A. K., Singh, K., Singh, N. K., Amitha-Mithra, S. V. and Sharma, R. P., 2014. EMS Induced Mutants of Upland Rice Variety Nagina22: Generation and Characterization. *Proc. India Natl. Sci. Acad.*, **80:** 163-172.
30. Murray, M. G. and Thompson, W. F. 1980. Rapid Isolation of High Molecular Weight Plant DNA. *Nucl. Acids Res.*, **8:** 4321-4325.
31. Muthusamy, S., Kanagarajan, S. and Ponnusamy, S. H. 2008. Efficiency of RAPD and ISSR Markers System in Accessing Genetic Variation of Rice Bean (*Vigna umbellata*) Landraces. *Electr. J. Biotech.*, **11(3):** 1-18.
32. Nachimuthu, V. V., Muthurajan, R., Duraialaguraja, S., Sivakami, R., Pandian, B. A., Ponniah, G., Gunasekaran, K., Swaminathan, M. K. S. K. and Sabariappan, R. 2015. Analysis of Population Structure and Genetic Diversity in Rice Germplasm Using SSR Markers: An Initiative towards Association Mapping of Agronomic Traits in *Oryza sativa. Rice*, **8:** 2-24.
33. Nei, M. 1973. Analysis of Gene Diversity in Subdivided Populations. *Proc. Natl. Acad. Sci. USA*, 70: 3321-3323.
34. Peakall, R. and Smouse, P. E. 2006. GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research – An Update. *Mol. Ecol. Notes*, **6(1)**: 288-295.
35. Pervaiz, Z. H., Rabbani, M. A., Khaliq, I., Pearce, S. R. and Malik, S. A. 2010. Genetic Diversity Associated with Agronomic Traits Using Microsatellite

Markers in Pakistani Rice Landraces. *Electr. J. Biotech.*, **13(3):** 1–12.
36. Pritchard, J. K., Stephens. M. and Donnelly, P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetica*, **155:** 945–959.
37. Ab Razak, S., Nor Azman, N. H. E., Kamaruzaman, R., Saidon, S. A., Mohd Yusof, M. F., Ismail, S. N., Jaafar M. A. and Abdullah, N. 2020. Genetic Diversity of Released Malaysian Rice Varieties Based on Single Nucleotide Polymorphism Markers. *Czech J. Genet. Plant Breed.*, **56:** 62−70.
38. Reddy, C. S., Babu, A. P., Swamy, B. P. M., Kaladhar, K. and Sarla, N. 2009. ISSR Markers Based on GA and AG Repeats Reveal Genetic Relationship among Rice Varieties Tolerant to Drought, Flood, or Salinity. *J. Zhejiang Univ. Sci. B*, **10(2):** 133- 141.
39. Rohlf, F. J. 2002. Geometric Morphometrics and Phylogeny. In: "*Morphology, Shape and Phylogeny*". Systematics Association Special Volume, **64:** 175–193.
40. Sen, A. and Alikamanoglu, S. 2014. Biochemical Analysis of Drought Tolerant Sugar Beet Mutants Induced with Gamma Radiation. *J. Biotech*., **161:** 14–21.
41. Sharma, A. and Singh, S. K. 2013. Induced Mutation- A Tool for Creation of Genetic Variability in Rice (*Oryza sativa* L.). *J. Crop Weed*, **9:** 132–138.
42. Thant, A. A., Zaw, H., Kalousova, M., Singh, R. K. and Lojka, B. 2021. Genetic Diversity and Population Structure of Myanmar Rice (*Oryza sativa* L.) Varieties Using DArTseq-Based SNP and SilicoDArT Markers. *Plants,* **10(12):** 2564.
43. Thangjam, R., 2014. Inter-Simple Sequence Repeat (ISSR) Marker Analysis in Parkia timoriana (DC.) Merr. Populations from Northeast India. *Appl. Bioc. Biol.*, **172:** 1727–1734.
44. Tu Anh, T. T., Khanh, T. D., Dat, T. D. and Xuan, T. D. 2018. Identification of Phenotypic Variation and Genetic Diversity in Rice (*Oryza sativa* L.) mutants. *Agric.*, **8(2),** 30.
45. Viana, V. E., Pegoraro, C., Busanello, C. and Costa de Oliveira, A. 2019. Mutagenesis in Rice: The Basis for Breeding a New Super Plant. *Front. Plant Sci.*, 10:1326.
46. Waungh, R., Leader, D. J., Callum, M. C. and Caldwell, D. 2006. Harvesting the Potential of Induced Biological Diversity. *Trends Plant Sci.*, **11(2):** 71-79.
47. Wu, J. L., Wu, C., Lei, C. and Leung, H. 2005. Chemical- and Irradiation-Induced Mutants of Indica Rice IR64 for Forward and Reverse Genetics. *Plant Mol. Biol.*, **59:** 85–97.
48. Zhang, P., Li, J., Li, X., Liu, X., Zhao, X. and Lu, Y. 2011. Population Structure and Genetic Diversity in a Rice Core Collection (*Oryza sativa* L.) Investigated with SSR Markers. *PLoS One*, **6(12):** e27565.
49. Zhang, Y., He, Q., Zhou, X., Zheng, S., Wang, Y., Li, P. and Wang, Y. 2022. Genetic Diversity and Population Structure of 93 Rice Cultivars (lines) (*Oryza sativa* Xian Group) in Qinba in China by 3 Types of Genetic Markers. *BMC Genom.*, **23:** 550.
50. Yadav, S., Singh, A., Singh, M. R., Goel. N., Vinod, K. K., Mohapatra, T. and Singh, A. K. 2013. Assessment of Genetic Diversity in Indian Rice Germplasm (*Oryza sativa* L.): Use of Random versus Trait-Linked Microsatellite Markers. *J. Genet.*, **92(3):** 545–557.

تعیین تنوع ژنتیکی و ساختارجمعیت در موتانت‌های برنج هاشمی (*Oryza sativa* L.) بوسیله نشانگرهای مولکولی و مورفولوژیکی

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چکیده

جهش‌زایی بعنوان یکی از منابع مهم ایجاد تنوع ژنتیکی بوده و موتانت‌های گیاهی می‌توانند منابع مهم تنوع زیستی برای اصلاح گیاهان و مطالعه عملکرد ژن‌ها محسوب شوند. تنوع ژنتیکی و فنوتیپی ۹۵ ژنوتیپ موتانت انتخاب‌شده از ۱۷ جمعیت موتانت، حاصل از جمعیت جهش‌زای EMS روی رقم برنج هاشمی به صورت مولکولی در نسل M_3 با استفاده از سیزده آغازگر ISSR (تکثیر نواحی بین ریزماهواره‌ها) مورد مطالعه قرار گرفت. تنوع فنوتیپی جهش‌یافته‌ها نیز ارزیابی شد که نشان داد اجزای عملکرد دانه در بین جهش‌یافته‌های انتخابی نسبت به گیاهان شاهد متفاوت بودند. به طور موازی، تنوع ژنتیکی ارزیابی‌شده توسط نشانگرهای مولکولی نشان داد که تعداد کل قطعات تکثیر شده در هر آغازگر از ٤ (pr1-7) تا ۱۱ (ISSR-7، ISSR-11) متغیر است. به طور کلی، در مجموع ۹۹ قطعه بوسیله ۱۳ آغازگر بدست آمد که ۵۰ قطعه آن پلی مورفیک (۵۲/۹۲ درصد) بود. تنوع ژنتیکی ایجاد شده توسط نشانگرهای ISSR در میان ۱۷ جمعیت از ۱۱/۱۱٪ در HM9 تا ٤۵/٤۵٪ در HM2 متفاوت بود. میانگین مقدار پلی مورفیسم مولکولی ۰/۲۷ بود. از مجموع واریانس مولکولی، ۹۵ درصد از تفاوت‌ها به تنوع درون جمعیت و ۵ درصد تفاوت در میان جمعیت‌ها مربوط می‌شود. نمودار درختی روش جفت گروه بدون وزن با میانگین حسابی (UPGMA) نشان‌دهنده تنوع ISSR، جمعیت جهش‌یافته برنج را به هفت گروه طبقه‌بندی کرد، که با ارزیابی مدل مبتنی بر ساختار نیز تایید شد. به طور کلی، ژنوتیپ‌های موتانت مورد مطالعه، ویژگی‌های ژنتیکی مطلوبی را در جمعیت‌های ۱۳ و ۱۷ نشان دادند که ژنوتیپ‌های em_3h204 و em_3h280 واگراترین آنها بودند. بنابراین، این موتانت‌ها را می‌توان به عنوان ارقام جدید برنج مناسب برای محصول برنج و به‌عنوان والدین در برنامه به‌نژادی برای بهبود ارقام برتر در نسل‌های پیشرفته پیشنهاد کرد.