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Genetic Diversity of Mutant Lines of Rice Using RAPD Markers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Present study was conducted to characterized mutant lines of rice variety Rajendra Mahsuri-1. Genetic diversity in rice mutant lines (M_3) was characterised by using RAPD markers among them at the molecular level. Altogether 78 alleles were amplified, of which 53 were polymorphic. Polymorphic information content (PIC) ranged from 0.082 for OPA2 to 0.424 for OPA3. Among the primers used, OPA3, OPA7, OPA8, OPA13 and OPB6 had a remarkably higher number of variants with greater gene diversity and rice mutant lines discrimination ability. Major allele frequency was found higher in case of OPA2, OPA4, OPA9 and OPB7. Using a similarity coefficient in the numerical taxonomic approach of classification, the mutant lines were differentiated and classified into different groups. Principal coordinate analysis based two-dimensional plotting of genetic profiles completely supported the results obtained from the hierarchical classification of mutant lines were distinctly discriminated from the remaining lines.

Keywords: Rice; Rajendra Mahsuri-1; RAPD; PCA.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most economically important crops because it is a nutritional source of more than one-third of the world population. Rice is the important staple food crop for more than half of the world's population. About 90% of the world's rice is grown and consumed in Asia, whereas 50% of the population depends on rice for food [1]. In India, rice accounts for more than 43% of food grain production. It is cultivating in 44.8 million

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hectares under four main ecosystems viz. irrigated, rainfed lowland, rainfed upland and flood-prone with an average annual production of 100 million tons [2]. Also, it is an ideal model monocot species for the study of crop genomics due to relatively small genome size (approximately 430 Mb), diploid origin (2n = 24), and close relationship to other important cereal crops Rabbani et al. 2003. Food security is becoming more urgent and important due to the increasing global population, declining arable land and dramatic climate change. Mutation research was continued in rice with the following aims:(a) To enhance the frequency and spectrum of mutations in Rajendra Mahshuri1 and Rajendra Kasturi rice varieties; (b) To change the grain quality of' the Rajendra Mahshuri1 and Rajendra Kasturi variety, (c) To improve the grain quality of the Rajendra Mahshuri1 and Rajendra Kasturi; (d) To increase the recombination frequency in japonica-indica hybrids. Fast neutrons gave a high mutation frequency. Rajendra Mahshuri1 and Rajendra Kasturi variety were more sensitive to all mutagens than the indica types. Chemical mutagens had no particular advantage over ionising radiations with reference to either mutation frequency or spectrum. Mutants with Rajendra Mahshuri1 and Rajendra Kasturi type of grain occurred readily in all treatments. Such mutants had a larger grain length/ width ratio and were more resistant to abiotic stress. Fine grain types with better cooking quality occurred in the M3 populations of Rajendra Mahshuri1 and Rajendra Kasturi.

2. MATERIALS AND METHODS

Experimental materials comprised twenty mutant lines of Rajendra Mahsuri-1 and two varieties namely, RM-1 and Sabour Surbhit (Table 1). Seeds of each of these entries were planted in Petri plate with germination paper. The immature leaves from two to three weeks old seedlings were collected from each genotype and used for DNA extraction in the Molecular Biology Laboratory of Department of Molecular Biology and Genetic Engineering Bihar Agricultural University, Sabour, Bhagalpur-813210.

2.1 Extraction of Genomic DNA

Following CTAB method based protocol [3] with slight modification, total genomic DNA was isolated. Leaf tissues (150–200 mg) were ground to a fine powder and used for DNA extraction. The extraction buffer was prepared by using 100 mM Tris-HCl (pH 8.0), 20 mM Na-EDTA salt pH 8.0). 1.4 M NaCl, 2% (w/v) CTAB and 20% β-Mercaptoethanol. The purification of the extracted DNA sample was performed by RNase A treatment at the rate of 0.1mg/ml and the mixture was incubated at 37°C to 39°C for 45 minutes. The aqueous phase was separated in a fresh tube and chloroform: Isoamyl alcohol was added in the ratio of 24: 1. The mixture was again centrifuged for ten minutes. The separated supernatant was collected after centrifugation and mixed with $1/10^{th}$ of its volume of 7.5 M ammonium acetate and DNA was precipitated by adding double volume of chilled isopropanol. After this step, DNA was pelleted by centrifugation of mixture at 5,000 rpm for 3 minutes and then dried and dissolved in TE buffer.

Table 1. List of Mutant lines used in the study

Sl. No.	Mutant lines	Designation
1	Rajendra Mahshuri-1	RM-1
2	RM450-68	M_3-1
3	RM450-70	M_3-2
4	RM450-5	M_3-3
5	RM450-11	M_3-4
6	RM450-84	M_3-5
7	RM450-118	M_3-6
8	RM450-42	M_3-7
9	RM450-133	M_3-8
10	RM450-134	M3-9
11	RM450-74	M3-10
12	RM450-75	M3-11
13	RM450-59	M3-12
14	RM450-92	M3-13
15	RM450-33	M3-14
16	RM450-38	M3-15
17	RM450-21	M3-16
18	RM450-24	M3-17
19	RM450-29	M3-18
20	RM450-201	M3-19
21	RM450-54	M3-20
22	Sabour Surbhit	SS

2.2 Amplification of Genomic DNA

Using standard protocol of polymerase chain reaction adjusted to laboratory conditions, extracted genomic DNA samples were subjected to amplification with known ten RAPD primers. The amplification was carried out by using 10 µl reaction mixture prepared by a combination of 3.0 µl water (Protease and Nuclease-free), 1.0 µl 5X PCR buffer, 1.0 µl 10 mM $MgCl_2$, 1.5 µl 1 mM dNTPs mixture, 0.5 µl Primer, 0.5 µl Taq Polymerase (1 unit) and 1.0 µl DNA sample. A panel of ten primers flanking sequences were used for the amplification of specific regions existing in the genome of rice (Table 1).

Table 2. Programme of polymerase chain reaction

Sl. No.	Reaction condition	Time and Temperature
1.	Initial denaturation	4 min. at 94°C
2.	35 cycles of	
(a)	Denaturation	1 min. at 94°C
(b)	Annealing	1 min. at 35-40°C
(c)	Extension	2 min. at 72°C
3.	Final extension	10 min. at 72°C

Polymerase chain reaction based amplification of specific regions was achieved by optimising the reaction condition in a thermal cycler and following a standardised programme (Table 2). After amplification, the amplified product was subjected to 1.5% agarose gel electrophoresis at 90 V for 1 hour and then visualised and documented in gel documentation system (England gene). The size of the amplified fragment was estimated with the help of 1kb ladder (Gbioscience).

2.3 Analysis of Amplified Products

Using the ladder, the size of amplified products was determined in relation to the size of markers in the ladder. The position of the bands on the gel corresponded to the location of the bands along Y-axis (ranging from 0 to 1). The Rf value for each band was also determined to assume the location of well as initial position (Rf=0) and the position of migrated dye as final position (Rf=1) as a frame of reference. The different bands produced by each one of the ten primers were compared and classified into the two different categories of monomorphic and polymorphic bands.

2.4 Analysis of Markers Based Polymorphism

Computational analysis for determining the major allele frequency, gene diversity, polymorphism information content and heterozygosity was performed using the software Power Marker (Liu and Muse, 2005). Allelic diversity at each marker locus was assessed by comparison of the polymorphism information content (PIC) of the primers Pankaj et al. 2018.

***Heterozygosity*:** Proportion of heterozygous genotypes in the population at a single locus in question was estimated Pankaj et al. 2018,

***Gene diversity*:** The probability that two randomly chosen alleles from the population are different at the locus was estimated Pankaj et al. 2018.

2.5 Analysis of Markers Based Divergence

All the mutant lines were scored for the presence and absence of the bands and the data were entered into the binary matrix as discrete

Table 3. List of ten primers utilised for amplification of rice genomic DNA extracted from twenty-two mutant lines along with two non-mutated used in the present study

SL.no.	Primer (Operon code)	Total no. of amplified fragment	Monomorphic bands	Polymorphic bands	Polymorphism (%)
1.	OPA-02	06	2	4	66.67
2.	OPA-08	12	3	9	75.00
3.	OPA-09	09	6	3	33.33
4.	OPB-06	07	0	7	100.00
5.	OPB-17	10	4	6	60.00
6.	OPA-04	06	5	1	16.67
7.	OPA-03	06	1	5	83.33
8.	OPB-07	07	2	5	71.43
9.	OPA-07	08	0	8	100.00
10.	OPA-13	07	2	5	71.43

variables. Genetic similarities among genotypes were calculated on the basis of presence and absence of common bands. The genetic associations among genotypes were analyzed by calculating the similarity coefficient [4] for pair-wise comparisons based on the proportions of shared bands as follows:

Similarity coefficient = 2a/(2a+b+c)

Where,

a= Number of shared bands between J^{th} and K^{th} genotypes
b= Number of bands present in J^{th} genotype but absent in K^{th} genotype
c= Number of bands absent in j^{th} genotype but present in K^{th} genotype

Cluster analysis was performed using the data on similarity coefficients. The method used for tree building in the cluster analysis involved sequential agglomerative hierarchical non-overlapping clustering based on similarity coefficients. The dendrogram based on similarity indices was obtained by un-weighted pair-group method using arithmetic mean. The analysis was performed with the help of NTSYS-pc software [5]. The nature of diversity between mutant lines under evaluation in the present investigation was assessed by identifying the clusters at appropriate phenon levels.

Principal coordinate analysis was conducted to obtain a two-dimensional ordination of RAPD primers dependent genetic profiles of the mutant lines under evaluation. The binary data matrix was also subjected to further analysis and genetic similarities among entries were calculated on the basis of presence and absence of common bands. Genetic association among entries was analyzed by calculating the similarity coefficient [4] for pair-wise comparisons based on the proportions of shared bands produced by the primers using the software NTSYS-pc (Rohlf, 2000).

3. RESULTS AND DISCUSSION

The amplification of genomic region characterised by the RAPD primers was achieved by using the primers. The PCR reaction condition was optimized by adopting a method designed to reveal the effects and interactions of specific reaction components simultaneously using few reactions. This approach resulted in the optimization of reaction condition for targeted amplification. Finally, the amplification reaction was set up with the ten primers keeping suitable annealing temperature in order to amplify.

Altogether 78 alleles including 53 polymorphic and 25 monomorphic bands were detected using ten primers (Table 3).

3.1 Polymorphism per Cent (PP)

The polymorphism per cent revealed in the form of a percentage of the unique allele was recorded to be the maximum in the case of OPB-06 and (100.00%) and the minimum in the case of OPA-04 (16.67%) (Table 3).

3.2 Polymorphic Information Content (PIC)

The level of polymorphism exhibited amongst the entries under evaluation in the present study, as assessed by calculating polymorphism information content (PIC) of each of the primers, which revealed allele diversity and frequency among the entries, varied from 0.033 (OPA-09) to 0.424 (OPA-08) with an average of 0.185 across the primers (Table 4).

Table 4. Analysis of primers used for the amplification of genomic regions in the mutant lines and control

Sl. no.	Primer	Major allele frquency	Gene diversity	PIC
1	OPA2	0.951	0.088	0.082
2	OPA3	0.792	0.301	0.424
3	OPA4	0.979	0.037	0.033
4	OPA7	0.623	0.410	0.315
5	OPA8	0.758	0.268	0.211
6	OPA9	0.907	0.121	0.097
7	OPA13	0.816	0.225	0.180
8	OPB6	0.756	0.342	0.275
9	OPB7	0.946	0.096	0.087
10	OPB17	0.850	0.188	0.151

Among the primers used, OPA3, OPA7, OPA8, OPA13 and OPB6 had a remarkably higher number of variants with greater gene diversity and rice mutant lines discrimination ability. Major allele frequency was found higher in case of OPA2, OPA4, OPA9 and OPB7 (Table-4). The results obtained in the present study are in accordance with earlier reports [6,7,8,9,10].

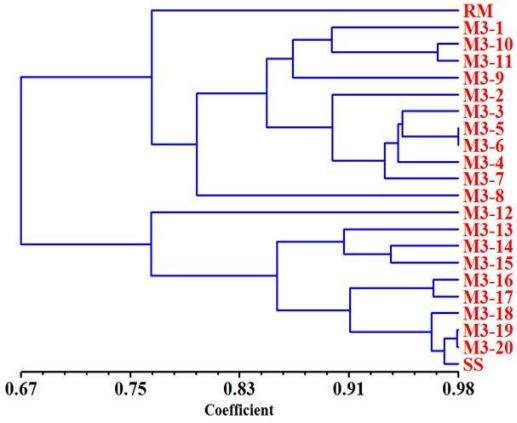

Fig. 1. Dendrogram of mutant lines of rice

3.3 Clustering of Entries Based on RAPD Markers

Using a similarity coefficient as a measure of similarity, an assessment of the nature and extent of differentiation and divergence was made amongst the entries under evaluation. The method used for the tree building in the analysis involved sequential agglomerative hierarchical nested clustering based on similarity matrix and a dendrogram was generated following un-weighted pair group method using the arithmetic mean. Clusters were identified at an appropriate phenon level to deduce the nature of relationships among the entries.

Considering broad classification of entries, as indicated by dendrogram, basically, the entries were divided into five groups (Fig.1), which were further divided into clusters, sub-clusters and sub-sub clusters.

The first mono-genotypic group consisted of one entry, namely RM whereas the second multi-genotypic group consisted of ten entries, namely M3-1, M3-10, M3-11, M3-9, M3-2, M3-3, M3-5, M3-6, M3-4 and M3-7. Similarly, the third mono-genotypic group consisted of one entry, namely M3-8 and the fourth mono- genotypic group consisted of one entry, namely, M3-12 whereas the fifth multi-genotypic group consisted of nine entries, namely M3-13, M3-14, M3-15, M3-16, M3-17, M3-18, M3-19, M3-20 and SS.

Principal coordinate analysis (Fig.2) based two-dimensional plots of 10 RAPD primers dependent genetic profiles revealed the spatial distribution pattern of entries along the two principal axes. Although some of the entries were placed far away from the centroid of the clusters and remaining entries were placed more or less around the centroid, it was evident that entries were distinctly divided into four genotypic groups. Therefore, the inferences derived from the similarity coefficients based hierarchical classification were completely supported by the principal coordinate analysis. The results obtained in the present study are in accordance with earlier reports [6,7,8,9,10].

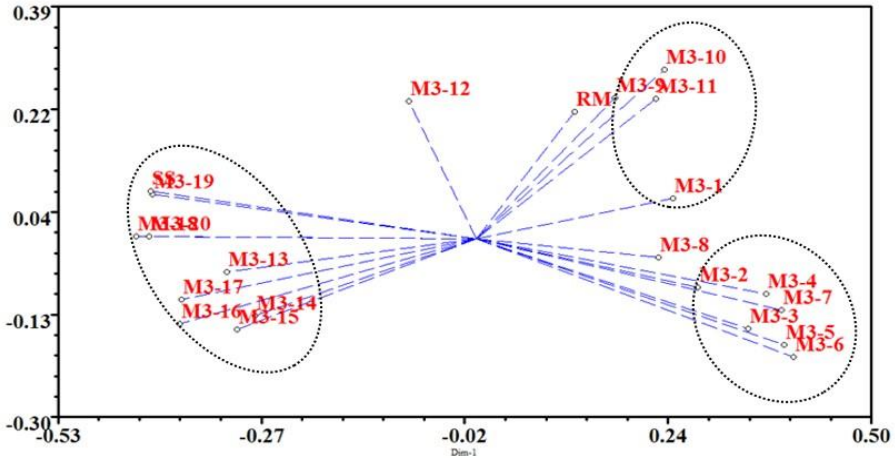

Fig. 2. Principal coordinate analysis of mutant lines

4. CONCLUSION

The present study was conducted to characterized mutant lines of rice variety Rajendra Mahsuri-1. The PCR reaction condition was optimized by adopting a method designed to reveal the effects and interactions of specific reaction components simultaneously using few reactions. Genetic diversity in rice mutant lines (M_3) was characterised by using RAPD markers among them at the molecular level. Principal coordinate analysis based two-dimensional plotting of genetic profiles completely supported the results obtained from the hierarchical classification of mutant lines were distinctly discriminated from the remaining lines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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