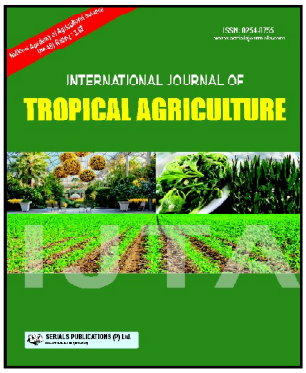

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Assessment of Genetic Diversity Using RAPD Marker Among Different Varieties of Rice (*Oryza sativa*)

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Abstract: Assessment of the extent and distribution of genetic variation in a crop species and its relatives is essential in understanding pattern of diversity and is highly significant for the improvement of many crop species including rice. The genetic divergence among 12 Indian varieties of rice (*Oryza sativa* L.) was assessed employing random amplified polymorphic DNA markers. A total of 136 clear score able bands were generated, out of which 87.25% were polymorphic. The total number of amplicon varied from 7 to 10 with a mean of 8 amplicon per primer while in case of polymorphic amplicon it varied from 5 to 9 with a mean of 7 polymorphic amplicon per primer. The amplified product size ranged from 0.3 to 1.8 kb. The Jaccard's similarity coefficient values ranged 0.56 to 0.90 with a mean of 0.70. A dendrogram constructed based on the UPGMA clustering method revealed two major clusters CL1 and CL2. The dendrogram has put all the genotypes in two clusters.CL1 comprises of three varieties with 85 per cent similarity coefficient whereas remaining 9 varieties clustered together in CL2, which is further classified into two sub clusters with 65.50 per cent similarity. Current study highlights genetic diversity among various varieties and attributed to recent human selection pertinent to few genes. The study revealed rich genetic diversity among rice varieties.

Key words: Rice, genetic diversity, PCR, RAPD, dendrogram.

INTRODUCTION

Rice (*Oryza sativa* L.) is principal cereal crops belonging to the family *Graminae*. It is one of the leading crop of the world, adapted as staple food for half of the world's population (Parsons *et al*., 1997, Aggarwal *et al*, 2002) which is source of predominant dietary major calories (Sasaki 2000). It has also become a model organism for genome analysis, having a diploid chromosome number of 24 and the smallest genome size of all major crop plants of 430Mb (Arumuganathan and Earle, 1995). Assessment of genetic diversity becomes important in establishing relationships among different cultivars (Sivaranjani *et al*, 2010., Kibria, 2009).

DNA is master molecule occurs in all living organism; hence it is very important for the studies of molecular biology. So, reliable methods must be applied that can correctly identify cultivars to assess the genetic diversity in the genotypes. Indeed, advances in molecular techniques have enabled the study of genetic variability at the DNA level, which has significantly increased the accuracy in assessing the genetic diversity and identifying cultivars. Molecular markers have become important tools in studies of genetic diversity (Bered *et al*, 2005), due to the high resolution and reliability in the identification of variety. RAPD (Random Amplified Polymorphic DNA) markers have the advantage of detecting polymorphism work sequence independent, simply, quickly, cost-effective, and requires small amount of DNA (Haque *et al*, 2007, Demeke *et al*, 1996). Thus, identification of genotypes and their inter-relationships is important. Development of new biotechnological techniques provides increased support to evaluate genetic variation in both phenotypic and genotypic levels and the results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic basis of commercially grown varieties. The purpose of this study was to evaluate the genetic diversity in 12 rice varieties that are being made available to rice growers in Maharashtra, and to develop a molecular profile using RAPD markers.

MATERIALS AND METHODS

Materials Plant Material

Seed samples of selected for work were collected from Dry land rice research centre, Tuljapur and Agriculture Government College, Kolhapur, Maharashtra.

Table 1
List of selected rice varieties

Sr. No.	*Name of variety*	*Source*
1.	Anjali 12108	*Varieties Collected from Dry land rice research centre, Tuljapur*
2.	IVT-12113	
3.	TJP-48	
4.	Terna	
5.	MAULS-11	
6.	TJP-61	
7.	IVT-12110	
8.	MAULS-21	
9.	Ratnagiri 24	*Agriculture Government college Kolhapur*
10.	Bhogawati	
11.	Mohini	
12.	Pusa Basmati	

METHODOLOGY

Population Raisings

Seeds of sown in pot containing soil, sand and proportional amount of organic manure in laboratory and general precautions were taken for germination of like water logged condition provide to seed sample by using pots. Also each and every bag was tagged by different labels for identification of varieties.

DNA Isolation

The genomic DNA was isolated from rice leaflets by protocol developed and standardized by Chuan *et al*., 2010. The extracted DNA was resolved on 0.8

% Agarose gel. The quantification was done by spectrophotometer and stored at –20°C until further use.

$$\text{DNA } (\mu g/ml) = \frac{A260 \times 50 \times \text{dilution factor}}{1000}$$

Quantification of DNA

DNA was quantified spectrophotometrically 5 ml of DNA was diluted to 3 ml and absorbance was measured at 260 nm and 280 nm using quartz cuvette. The amount of DNA was calculated by using the formula,

Where, A260- absorbance at 260 ηm and 50- a conversion factor used to convert optical density (OD) concentration in (µg/µl)

Dilution of DNA Samples

A part of DNA sample was diluted with appropriate quantity of sterilized distilled water to yield a working concentration of 30 ng/ml and stored at 4°C until PCR amplification.

Agarose Gel Electrophoresis

The agarose gel electrophoresis unit was cleaned properly before use. 0.8% agarose gel was prepared by dissolving 0.8 g of agarose in 100 ml 1X TAE buffer and heated in microwave oven and ethidium bromide was added to it after cooling down to 50°C. The gel was poured in mini casting tray in which comb was inserted previously and kept for 1 hour. After solidification the comb was removed. 5 ml of DNA was mixed with 1 ml 6X × gel loading dye and loaded on the gel. The electrophoresis was carried out at 100 V for 45 minutes using 1X TAE buffer. The result was then visualized under U.V transilluminator.

RAPD Analysis

PCR Reaction

The PCR reaction of 25µl volume was set for each strain by using an individual RAPD primer. All PCR reactions were carried out in a final volume containing 1 µl (10 pmol) of primer, 0.3 µl Taq DNA polymerase(1.5 U/µl), 2.5 µl PCR buffer, 1.5 µl 1.5 mM Mg Cl2, 0.5 µl 0.2 mM dNTPs (for each), 1 µl of template DNA (approximately25 ng/ µl) and 18.2 µl sterile distilled H_2O. In present study, 16 RAPD primers were screened for polymorphism survey in pooled DNA accessions of rice (*Oriyza sativa*), sequencesof the RAPD primers used in this study are shown in table 2. PCR reaction mixture was prepared with the above said components and equally distributed (24µl) into 5 PCR tubes. Genomics DNA (1µl) derived from 12 different variety of Rice were added. PCR tubes were placed in thermal cycler (Eppendrop) for amplification of the genomic DNA as per the standardized protocol. 94°C for 5 min as initial denaturation and 40 cycles of 94°C for 0.5 min, 36°C for 1 min and 72°C for 1.5 min. This was followed by a 10 min final extension at 72°C.

Table 2
List of primers along with their sequences used for RAPD analysis

No.	*Primer Name*	*Primer Sequence*	*No. of Bases*	*GC content %*
1.	OPA-08	GTGACGTAGG	10	60
2.	OPA-15	TTCCGAACCC	10	60
3.	OPA-17	GACCGCTTGT	10	60
4.	OPA-19	CAAACGTCGG	10	60
5.	OPE-02	GGTGCGGGAA	10	70
6.	OPK-02	GTCTCCGCAA	10	60
7.	OPL-02	TGGGCGTCAA	10	60
8.	OPN-06	CCACGGGAAG	10	70
9.	OPB-06	TGCTCTGCCC	10	70
10.	OPB-07	GGTGACGCAG	10	70
11.	OPB-08	GTCCACACGG	10	70
12.	OPB-11	GTAGACCCGT	10	60
13.	OPB-12	CCTTGACGCA	10	60
14.	OPC-07	GTCCCGACGA	10	70
15.	OPD-08	GTGTGCCCCA3	10	70
16.	OPF-06	GGGAATTCGG	10	60

Resolution of amplified product

The amplified products were resolved on 1.5% agarose gel at 100 V for 2 hours. The gel was stained with ethidium bromide (0.5ml/1ml). After electrophoresis, the gel was carefully taken from the casting tray and photograph was taken on a gel documentation system (Bio Era Gel documentation unit II).

Data Scoring and Analysis

Amplification products in the gel images were scored for presence (1) or absence (0) missing. Homology of bands based on the distance of migration of amplified DNA fragments according to their molecular weights in the gel was determined. Molecular weights of the bands were estimated using 100 bp to 1000 bp DNA ladder (GeNei.) as standard.

Polymorphism Per centage

The polymorphic per centage of the obtained bands was calculated by using following formula,

Polymorphic % = (No. of polymorphic bands/ Total bands) × 100.

RESULT AND DISCUSSION

Molecular diversity in rice by using molecular marker tool was also documented earlier by many workers (Ravi *et al.*, 2003, Mahmoud *et al.*, 2005, Rahman *et al.,* 2007, Rabbani *et al.*, 2008, Pangal *et al.*, 2010, Upadhyay *et al.*, 2011). In this study, 12 varieties of rice were subjected to amplification by RAPD primers in PCR master cycler. The banding pattern thus obtained by RAPD markers clearly distinguished varieties into different clusters showing genetic diversity. Diversity estimates provide useful information to understand the genetic structure of rice by using RAPD markers. In present study, 16 RAPD primers were screened for polymorphism survey in pooled DNA accessions of Rice (*Oriyza sativa*) and out of that, four RAPD primers showed positive results. These positive primers were then screened in all rice varieties under present study and polymorphic banding pattern was observed in all the DNA sample of rice.

In present study, RAPD markers have been successfully amplified for cultivar identification and genetic diversity analysis in a large number of crops, and these RAPD marker gas given a good polymorphic data and hence they can be used for genetic diversity analysis. These finding were found similar as given by (Raghunathachari *et al.*2000; Ravi *et al.* 2003; Nazari and Pakniyat 2008; Kibria *et al.* 2008, Hossain *et al.* 2012, Chakraborty *et al.* 2013). The PCR analysis of 12 rice varieties taken in this study, with 4 polymorphic random markers generated 136 scorable bands. Among RAPD markers, OPA-08 produced maximum number of bands (42 in all varieties) (Figure 1) followed by OPA-19 (38) (Figure 2) and OPK-02 (34) (Figure 4). While RAPD marker marker OPE-02 generated minimum number of bands (22) (Figure 3) in the genomic pool.

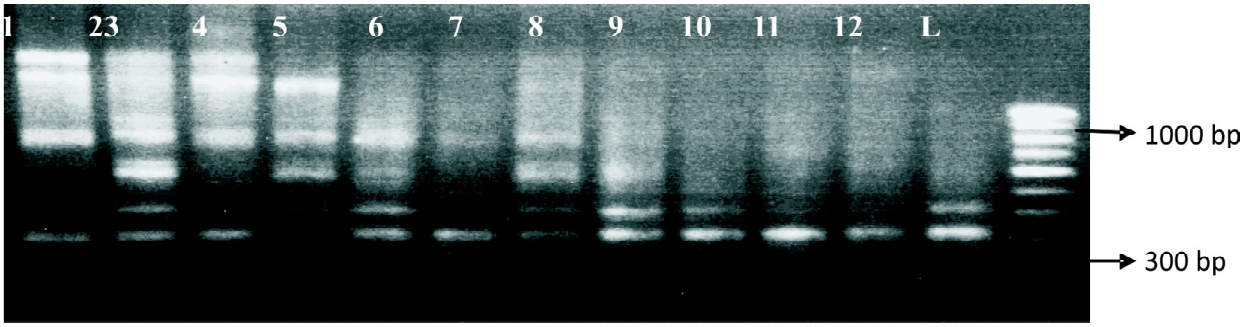

Figure 1: RAPD profile of *Oryza sativa* with primer OPA-08

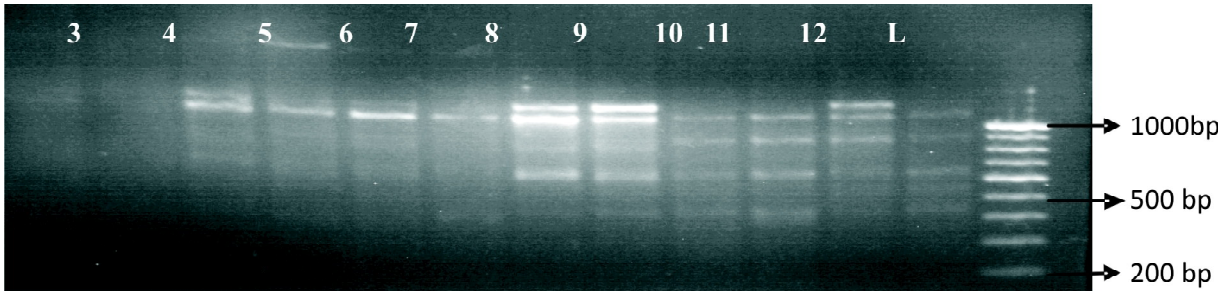

Figure 2: RAPD profile of *Oryza sativa* with primer OPA-19

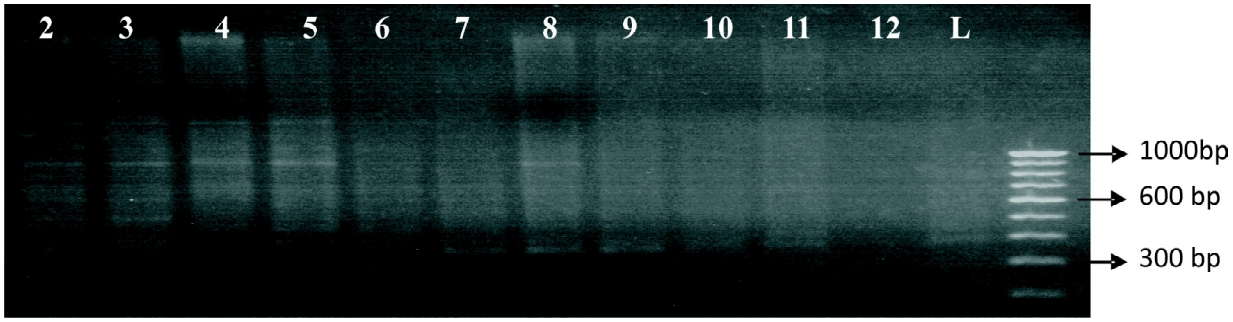

Figure 3: RAPD profile of *Oryza sativa* with primer OPE-02

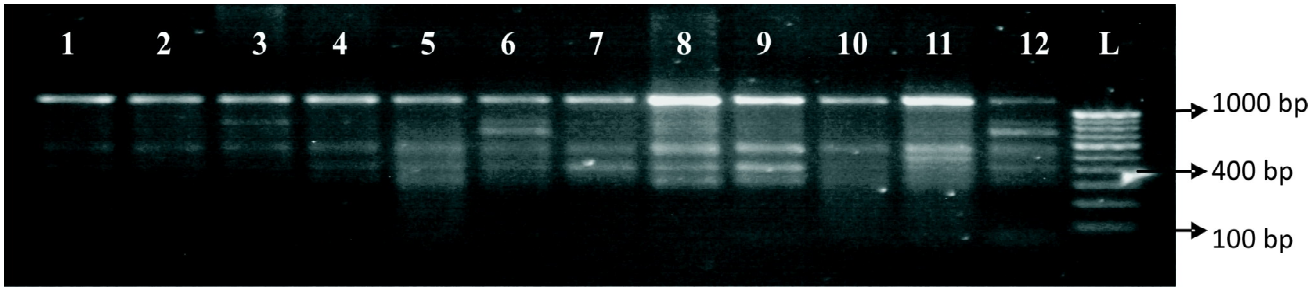

Figure 4: RAPD profile of *Oryza sativa* with primer OPK-02

The observed amplicon size ranged from 300 bp to 1800 bp. Total 4 RAPD primer *viz.,* OPA-08, OPA-19, OPK-02 and OPE-02 were screened successfully. The total primers were able to generate total 32 amplicons with an average 8 amplicons per primer (Table 3) out of total 28 ampilcons were found polymorphic. They show 87.25% polymorphism and the average number of the polymorphic per primer were 7 (Table 3). Different primer produced different level of polymorphism among the different genotypes. The number of DNA amplified amplicon per primer ranged from 6 to 10 (OPA-08, OPA-19, OPK-02 and OPE-02). (Table 3). The primer OPE-02 generated 22 bands but this primer showed 100 per cent polymorphism out of 6 all 6 amplicons

Table 3
List of RAPD primers and polymorphic amplicons generated

Sr. No.	*Primer code*	*Total no. of amplicons*	*No. of polymorphic amplicons*	*Per cent polymorphism*
1.	OPA-08	10	09	90
2.	OPA-19	09	08	88
3.	OPE-02	06	06	100
4.	OPK-02	07	05	71
	Total	32	28	–
	Average	8	7	87.25

are polymorphic. The OPE-02 primer was the most informative primer. The primer OPK-02 stood least informative primer by generating only 5 polymorphic amplicon showed 71 per cent. The average sizes of amplicons were between 300 bp to 1800 bp. Ravi *et al.* (2003) carried out genetic diversity analysis of 40 rice genotypes and reported 90 per cent polymorphism. Raghunathachari *et al.* (2000) evaluated analysis of genetic variability in Indian scented rice using 18 varieties which showed 95.1 per cent polymorphism. Whereas low level of polymorphism (50 per cent) was reported by Beverley *et al.* (1997) in his study in which he evaluated the genetic diversity in rice with RAPD markers. Kanawapee *et al.* (2011) also reported (68.94 per cent) low level of genetic diversity among four important rice varieties. The data obtained by RAPD markers was analyzed by NTSYS-PC version 2.02*i* and dendrogram was constructed by using Jaccard's similarity coefficient value to estimate the genetic similarity of the rice cultivars. A similarity matrix (Table 4) is obtained by Jaccard's similarity coefficient value.

Table 4
Similarity matrix

Varieties	*Mohini*	*TJP-48*	*Ratnagiri-24*	*Terna*	*Pusa basmati*	*Bhogawati*	*MAULS-21*	*IVT-12113*	*MAULS11*	*Anjali-12108*	*TJP-61*	*IVT 12110*
Mohini	1.000000											
TJP-48	0.843750	1.0000000										
Ratnagiri-24	0.8437500	0.6875000	1.0000000									
Tenar	0.6562500	0.6875000	0.6250000	1.0000000								
Pusa basmati	0.6562500	0.6250000	0.5625000	0.7500000	1.0000000							
Bhogawati	0.6875000	0.5312500	0.6562500	0.5312500	0.7187500	1.0000000						
MAULS-21	0.6250000	0.6562500	0.5312500	0.7187500	0.7812500	0.6875000	1.0000000					
IVT-12113	0.6250000	0.5312500	0.5312500	0.5937500	0.7812500	0.7500000	0.8750000	1.0000000				
MAULS-11	0.6250000	0.5312500	0.5312500	0.5312500	0.7187500	0.8125000	0.7500000	0.8750000	1.0000000			
Anjali-12108	0.6562500	0.5000000	0.5625000	0.5000000	0.6250000	0.7812500	0.6562500	0.7812500	0.9062500	1.0000000		
TJP-61	0.6562500	0.5000000	0.6250000	0.5000000	0.5625000	0.7187500	0.5937500	0.7187500	0.8437500	0.8750000	1.0000000	
IVT12110	0.5937500	0.5000000	0.5000000	0.5000000	0.5625000	0.7187500	0.5937500	0.7187500	0.8437500	0.8750000	0.8125000	1.0000000

Cluster Analysis Using RAPD Markers

A dendogram was generated using UPGMA cluster analysis based on Jaccard's similarity coefficient value revealed that average similarity per centage was 70. Diversity analysis of *Oryza sativa* using RAPD markers through clustering (Figure 5) showed that two major cluster were observed in rice (*Oryza sativa* L) varieties with 59 per cent similarity*viz.* CL1 and CL2 cluster CL1 included two subcluster consisted of three varieties *i.e.* Mohoni and TJP-48 with 85 per cent similarity coefficient and remaining one verity *i.e.* Ratnagiri-24 with 77 per cent similarity second subcluster. Remaining 9 varieties consisted under CL2 cluster with 65.50 per cent similarity divided in to again two subclusters. In which one cluster covered Terna 69 per cent dissimilar to another cluster consisted of 3 varieties *viz.,* Pusa basmati, MAULS-21 and IVT-12113. Pusa basmati

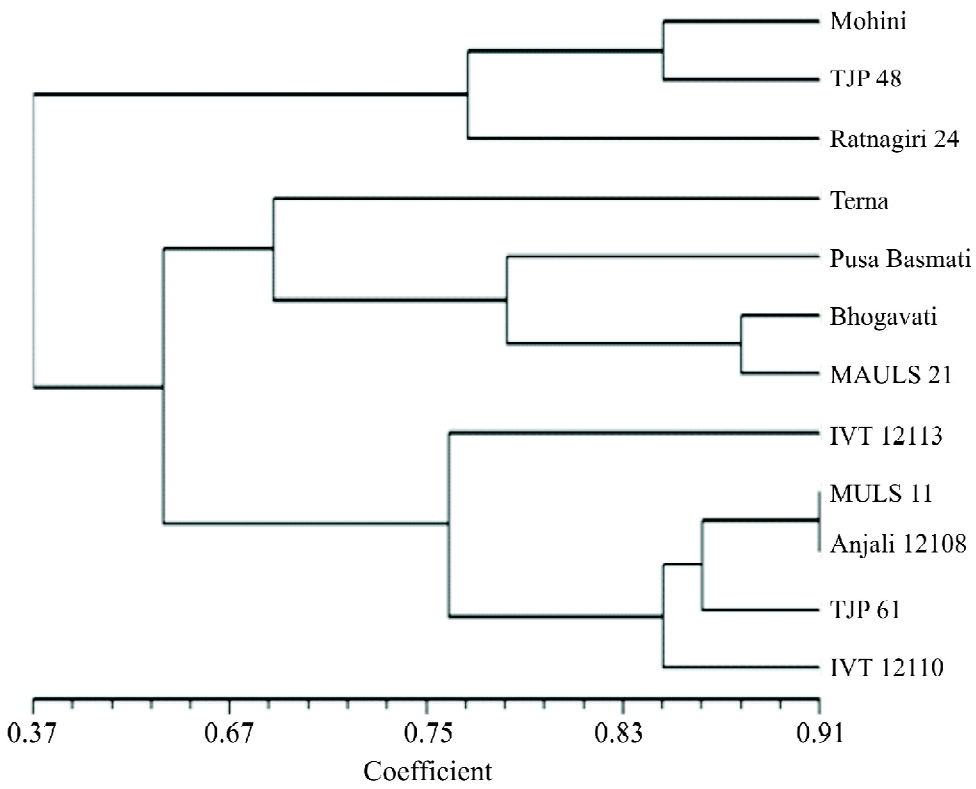

Figure 5: Dendogram showing genetic diversity for RAPD marker in rice (*Oryza sativa*)

was 79 per cent similarity. MAULS-21 and IVT-12113 were 89 per cent similar. Second subcluster covered 5 varieties. Bhogawati 76 per cent similar to MAULS-11, Anjali-12108, TJP-61 and IVT-12110. IVT-1210 was 85 per cent similar to three varieties. TJP-61 was 87 per cent similar to two varieties. MAULS-11 and Anjali12108 was 91 per cent similarity and which is highest similarity among all other verities.

CONCLUSION

In conclusion, the result of this study indicates that the efficiency and ease of using RAPD markers for investigating genetic relationship and identification of varieties is good tool.The present study revealed moderate level of polymorphism among 12 rice varieties and it stands intermediate. For the screening of selected 12 rice vsariety OPE-02 primer is best shows 100 per cent polymorphism and for further study it will be used. These medium to high polymorphic results indicated a wide genetic base in rice and genetic diversity may be due to their characteristics, wide distribution, amplification protocol used or selection of suitable primers as reported by the earlier workers. These diverse genotypes can be used in hybridization program as they show less similarity with the other genotypes.

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