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RAPD-ANALYSIS OF GENETIC VARIATION OF FOUR IMPORTANT RICE VARIETIES USING TWO OPR PRIMERS

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ABSTRACT

To assess the genetic diversity in four *Oryza sativa* genotypes using randomly amplified polymorphic DNA (RAPD) markers. Genomic DNA from *O. sativa* leaves was isolated, quantified by UV visible spectrophotometer and amplified using different primers OPR1 and OPR2. OPR1 showed four rice non-polymorphic genotypes, whereas OPR2 showed polymorphic amplified fragments in 2% agarose gel. Above described data phylogenic tree was constructed according to Nei's genetic distance using the free tree software program with help of tree view. Genetic variation of four rice varieties that ADT 38 and ASD 16 followed by IR 20 and PONNI has the highest genetic diversity determined by RAPD. Which are crossed resulting hybrid with maximum heterosis and better characteristic such as high yield is possible.

Keywords: rice varieties, genetic diversity, genotypes, RAPD analysis, phylogenetic tree, primers, OPR1, OPR2.

INTRODUCTION

Rice is one of the important crops in the world, providing more calories for the human beings who are consuming all over the world. Currently, 90% of the rice has been produced and consumed in Asian countries (Paranthaman *et al.*, 2009). In India during 2003-2004, 87 million tonnes of rice was produced from an area of 42.41 million per hectare (James Martin, 2007). It was estimated that demand for rice in 2010 will be 100 million tonnes and in 2025, it will be 140 million tonnes (Singh, 2004). Rice has smallest genome of all cereals: 430 million nucleotides and can serve as a model genome of flowering plants. ADT 38 is suitable rice variety adaptable to aerobic irrigation (James Martin, 2007), resistant to blast, green leaf hopper, gall midge and the power yield is 58 quintals per hectare. ASD 16 is tolerant to salinity and moderately resistant to brown plant hopper, sheath rot and its yield is 56 quintals per hectare. As well as rice the varieties such as PONNI and IR 20 are resistant to the bacterial leaf blight, rice tungro virus, stem borer and the yield is more than 45 quintals per hectare.

Now-a-days, the PCR-based RAPD is technically simple and cheaper compared to the RFLP (Williams *et al.*, 1990). Advantages of RAPD are simplicity, rapidity requirement for small quantity of DNA, and ability to regenerate polymorphisms (Yu and Nguyen, 1994). It has universal primers, which are commercially available probe isolation, filter preparation and nucleotide sequence. In many instance, the small number of primers are necessary for polymorphism identification within species. Hence, the present study was RAPD analysis of PONNI, ASD 16, ADT 38 and IR 20 with the help of two essential primers.

MATERIALS AND METHODS

Plant material

Four different varieties of *Oryza sativa* seeds namely PONNI, ADT38, IR20 and ASD 16 were collected from seed bank of Agricultural University, India. Collected seeds were grown in pots in the laboratory conditions.

Genomic DNA isolation

Isolation of genomic DNA method was followed by Sambrook *et al.*, (1989) with slight modification. Plant leaves sample (0.5 g/kg) was taken and grinded with 1ml of homogenization buffer. The grinded samples 1ml of lysis was added and then centrifuged at 8000 rpm for 10 min at 4°C. Supernatant was transferred into a new 1.5 ml tube and mixed with equal volume of phenol, chloroform isoamylalcohol and centrifuge at 10,000 rpm for 10 min at 4°C. Aqueous layer was separated and equal volume of ice cold ethanol was added and incubated (80°C, 30 min). The DNA was pelleted by centrifugation (10,000 rpm, 12 min) and allowed to air-dry before being re-suspended in 50 µl of TE buffer.

Gel electrophoresis

Isolated DNA (15 µl) was mixed with 3 µl loading dye (Genei, Bangalore) and loaded on 0.8% agarose gel (Himedia). Electrophoresis was conducted at 75 volts for 1 hour for genomic DNA and 55 volts for 1 to 2 hours for PCR amplifications. The gels were stained with ethidium bromide and visualized under UV-Transilluminator.

Quantification of DNA by UV-spectrophotometer

The isolated DNA samples were quantified by UV visible spectrophotometer absorbance at 260-280 nm and DNA concentration was determined.

DNA concentration = OD260 × 50 µg/ml× dilution factor.

Primers

Random primers were synthesized in Genei Bangalore. The length of each primer is 10 bp and their sequences are listed below.

Primer	Sequence
OPR1	GCACCGATCT
OPR2	GATTCCGCGG

RAPD-PCR reaction

Amplification reaction was performed according to the method described by Saker *et al.* (2005) with slight modification, which contains a template (1.5 µl), primer (1 µl), enzyme master mix (12.5 µl) and Milli Q water (10 µl). The amplification was carried out in DNA thermal cycler with PCR profile: pre denaturation at 94°C for 5 min, 36 cycles at 94°C for 30 sec, 36°C for 30 sec, and 72°C for 1 min, with final extension at 72°C for 5 min, finally amplified product was hold at 4°C. The 15 µl of amplified products were resolved in 2% agarose gel with ethidium bromide. The electrophoresis gel was document under UV light.

Data analysis

Individual RAPD patterns were compared with samples. The reproducible well-marked amplified fragments were scored for each genotype represented graph data. The pair wise comparison of banding patterns was evaluated by Nei's (1978) genetic distance using the Free-Tree-Free ware programme (Pavlicek *et al.*, 1999). The Phylogenetic tree was constructed with help of tree view software.

RESULTS AND DISCUSSIONS

The present investigation, isolated genomic DNA from high yield rice varieties were resolved in electrophoresis and distinct band for the presence of genomic DNA of *Oryza sativa* (Figure-1). In addition, the concentrations were presented in different nanograms such as ADT38- 89 ng, ASD16-100 ng, PONNI-88.3 ng and IR20-93 ng.

In RAPD-PCR reactions, the four rice genotypes have been carried out using with 2 random primers. Genomic DNA was isolated in 30 days young leaves for RAPD analysis and total 27 scorable bands were determined in four species of rice varieties ranging from 1600 bp to 300 bp. The amplified product of primer OPR1 showed the identical band patterns in four rice varieties genotypes (Figure-2).

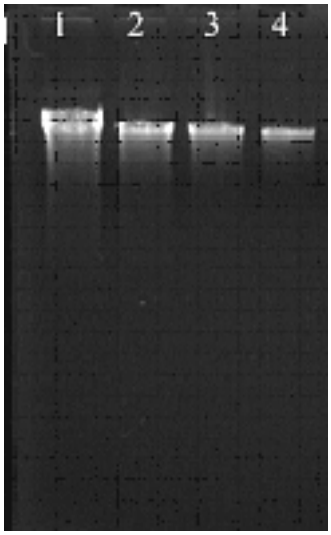

Figure-1. Genomic DNA of *Oryza sativa*.

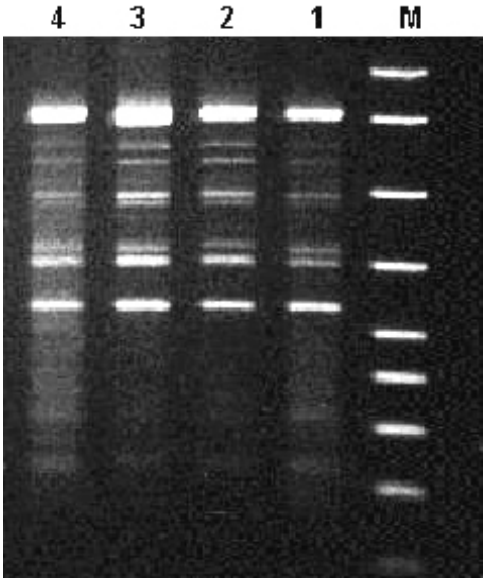

Figure-2. Amplified RAPD patterns of OPR1 Genomic DNA of *Oryza Sativa*.

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M	-	1 Kb DNA Ladder
1	-	ADT38
2	-	ASD16
3	-	IR20
4	-	PONNI

While, Figure-3 depicts different band patterns with primer OPR2 are used genetic diversity of rice genotypes as well as this primer was more informative than OPR1. The primer OPR1 showed no differences between four rice varieties genotypes and appear the sequence of GCACCGATCT to the genomic DNA, where this sequence appears. The priming regions binding in genomic DNA, it replicates and amplifies the polymerase chain reaction and the amplified products are resolved on the agarose gel showed with identical band patterns

indicates the primer with non polymorphic form of rice genotypes. Primer OPR2 showed the different band patterns in four varieties of sequences GATTCCGCGG with OPR2 used for distinguish rice varieties through RAPD analyze with genetic relationship. Although none primers were individually informative as to differentiate all the genotype, highly polymorphic profiles were obtained with different primers.

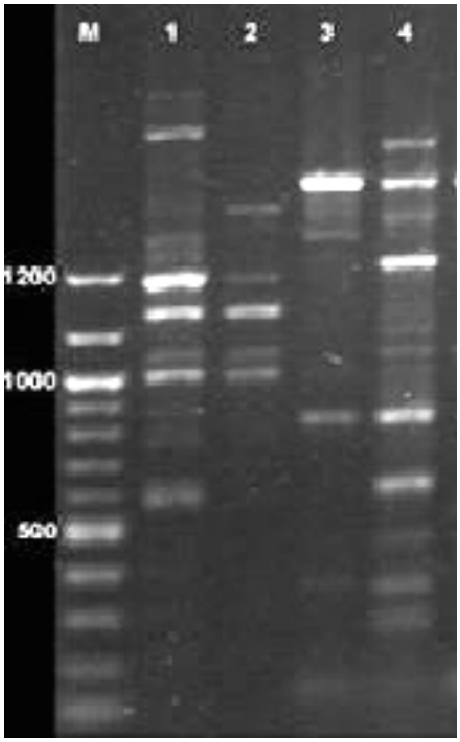

Figure-3. Amplified RAPD patterns of OPR2.

M	-	100 bp DNA Ladder
1	-	ADT38
2	-	ASD16
3	-	IR20
4	-	PONNI

Whereas, RAPD data analysis the fragments were recorded in presence and absence of fragments on gel photographs (Figure-4). Above two methods showed the clusters were described; first cluster was divided into ADT 38 and ASD 16; second cluster, which contains IR 20 and PONNI. Highest genetic diversity revealed in RAPD analysis was determined between IR 20 and ASD 16, whereas ADT 38 and PONNI as shown in normal genetic diversity (Table-1). Moreover, our data indicated that the intra species disparities of four species rice genome in ranges 0.39138 to 1.95601. The intra species disparities were the biggest between ASD 16 and IR 20 of 1.95601 followed by ADT 38 and PONNI of 1.77767. While the relationship between PONNI and IR 20, ADT 38 and ASD 16 are closer. Ren *et al.* (2003) reported that dendogram constructed using UPGMA from a genetically similar matrix based on RAPD data supported for clustering of distinct five groups with few exceptions. In general RAPD finger printing has number of potential applications, including the determination of cultivator's purity, efficient and management of genetic resources. Mackill (1995) classified the Indica and Japonica cultivars into separate groups by cluster analysis. The present study gives evidence that support the RAPD analysis in determining variability with in a population and method of finger printing is relatively easy to obtain the valuable data. Furthermore, it can be model for other studies relating to genetic diversity and highly polymorphic genotypes were transformed the species in order to produce more hybrid varieties.

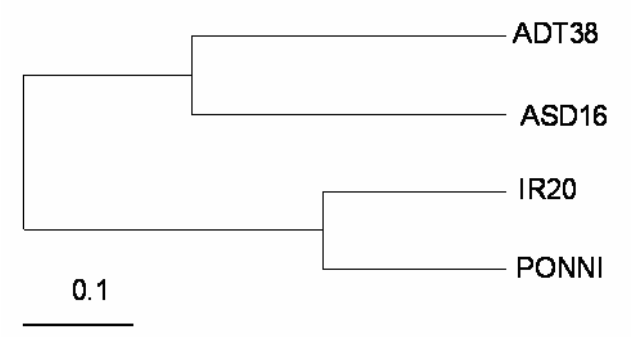

Figure-4. UPGMA dandogram based on Nei's (1978) original measure of genetic distance, summarizing the data on differentiation between four samples of *O. sativa* genotypes according to RAPD analysis.

Table-1. Genetic distance between *O. sativa* populations of four different rice varieties based on Nei's 1978 measures of genetic distance.

	PONNI	**IR20**	**ADT38**	**ASD16**
PONNI		0.39138	1.77767	1.02564
IR20	0.39138		1.60944	1.95601
ADT38	1.77767	1.60944		0.8574
ASD16	1.02564	1.95601	0.8574	

In conclusion, our current investigation revealed that the highest genetic diversity of important rice varieties ADT 38, ASD16, IR 20 and PONNI were used for RAPD analysis. Present study reveals that PCR based fingerprinting technique, RAPD were informative for estimating the extent genetic diversity as well as to determine the genetic relationship between different species of *Oryza sativa*. However, the data polymorphism level was sufficiently established and informative by finger prints. In such a phenomenon our research works on important rice varieties in genetic diversity revealed through two primers. In future our research work is maybe useful for cheap and best RAPD analysis of new genetic species diversity.

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