RAPD APPROACH FOR VARIETAL IDENTIFICATION AMONG SESAME GENOTYPE

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Abstract

A study was conducted to entitle six "*Sesmum indicum*" varieties collected from different region of India. RAPD was performed to determine the polymorphism among the varieties. A total of 81 bands were amplified out of which 30 bands were polymorphic. Polymorphism calculated was found to be 37.03%. Dendrogram based on UPGMA analysis splitted into three clusters and each cluster occupied two genotypes. All the included genotypes clustered according to their geographical regions like Rajasthan genotypes (RT-46 & RT-54) were clustered into same group while Tamil Nadu genotypes (TMV-3 & TMV-5) and Orissa genotypes (N-8 & Rajeshwari) genotypes also clustered into their respective group. Rajasthan genotypes were clustered into the same group at 80 % genetic similarity while TMV-3 & TMV-5 at 95 % genetic similarity. The clustering indicates a strong resemblance among genotypes from the same geographical region. The observed similarity coefficient that was 0.81, showing a high degree of relationship between all sesame genotypes, indicating a common origin of all the accessions. The genotypes from Tamil Nadu were found most similar varieties at 95 % genetic similarity while cultivars from Rajasthan showed maximum (18 %) diversity. The maximum diversity was observed among TMV-5 and RT-46 (28 %) and can effectively be used for the crop improvement programme. The present study concluded that by using more numbers of genotype and primer pairs, RAPD markers can easily be used for the identification of genotypes from the same geographical regions.

Keywords: RAPD: *Sesame indicum*, Random Amplified Polymorphic DNA, UPGMA

INTRODUCTION

Sesame (*Sesamum indicum L.)* is one of the most important oil seed crops of pediliaceae family having excellent oil stability due to presence of antioxidants such as phenyl propanoid (Pathak et al., 2014a). The seeds are reported having various nutritional components which are beneficial for the health of human (Pathak et al., 2014b; Yosathai, 2014). It is used as active ingredient in antiseptics, bactericides, viricides, disinfectant and is a considerable source of calcium, tryptophan, methionine and many minerals.

The development of random amplified polymorphic DNA (RAPD) markers, generated by the polymerase chain reaction (PCR), allows the examination of genomic variation without prior knowledge of DNA sequences (Eissa et al., 2016; Williams et al., 1993; Hadrys, 1992). The number and the size of amplified fragments depend on length and sequence of short, single and arbitrary primers. Random amplified polymorphic DNA (RAPD) markers have been successfully used for cultivar analysis and species identification in most plants due to the technical simplicity and speed of RAPD methodology (Mukherjee et al., 2013; Biswas et al., 2010; Chiu, 2000). RAPD has also been used to study diversity in various crop cultivars of various origins (Mohammadi and Prasanna, 2003) However, material consisting of southern India and Rajasthan varieties including varieties derived from wide hybridization has not been studies. Looking on their wide applicapability RAPD techniques has been used in present research to estimate discriminatory power of primer for identification of genotypes. The

present study focuses to determine relative efficiency of RAPD in distinguishing and identifying Sesame *(Sesamum indicum L.)* accessions and to estimate diversity among genotypes using RAPD markers.

MATERIAL AND METHODS

Collection and maintenance of plant

For the present study, seedlings of sesame of six different varieties were collected from different states mainly northern part of India whichwasnamed as RT-46, RT-54, TMV-3, TMV-5, N-8 and Rajeshwari. The seedlings were grown in the premises of Kamla Nehru college, Nagpur. Approximately 3 seeds of each variety were grown in test tube on Paper Bridge.

DNA Isolation

For molecular analysis, DNA from different plants was isolatedusing modified CTAB method. About0.5g leaves obtained were homogenized in liquid nitrogen. The homogenized material was handled by Doyle and Doyle (1990) method.RNA was removed by treating the sample with DNase For molecular analysis, DNA from different plants was isolatedusing modified CTAB method. About0.5g leaves obtained were homogenized in liquid nitrogen. The homogenized material was handled by Doyle and Doyle (1990) method.RNA was removed by treating the sample with free RNase. It was further amplified and purified through RNAase treatment, quantified and separated on Agarose Gel to check the integrity of DNA that was extracted from the seedlings of collected seeds. The quality and integrity of DNA was judged through gel analysis on 0.8% agarose gel. The quantification of DNA was done by observing it at 260 nm and 280 nm wavelengths by using a spectrophotometer (NanoDrop ND-1000 version V3.1.1).

PCR amplification

PCR reaction was carried out in 50ulreactionin Bio-Rad Thermal Cycler. The reaction volume comprise of 1µl *Taq*polymerase (5U/µL), 5ul 10X *Taq* Buffer, 5µl each of the respective forward and reverse primers (equivalent to 10µmol/µl), 2.5µl of each dNTPs (equivalent to 20mM/ul), 2.5µl (50ng/µl) template DNA and remaining volume was adjusted with Mill Q water.The thermocycling profile for 18S rRNAprimerwas set as: initial denaturation at 95^{o}C for 3 min, followed by 35 cycles of denaturation at 95 ^{o} C for 30s, annealing at 58^{o}C for 20s, extension at 72^{o}C for 30s, with a final extension at 72^{o}C for 4min. The PCR products were visualized on 1.5 % agarose gel and purified using Axygen PCR Clean Up kit. The sequencing was done in the ABI 3730XL Sequencer through service provider.

RAPD analysis

For RAPD analysis 50 primers were selected based on literature and were obtained from local supplier. Out of them 15 primers were selected for the study and three primers were removed because of their monomorphic nature and poor reproducibility.

Discrimination Power

The discrimination (D) power of the RAPD primers for varietal identification was evaluated. This is an extension of the polymorphism information content (Anderson *et al*. 1993) available from the frequencies of different banding patterns generated by a primer in different genotypes.

RESULTS AND DISCUSSION

The absorbance was recorded as 260 nm (A260) and 280 nm (A280) for quantification and determination of any impurities if present in DNA. An absorbance value of one at 260 nm has been found to be equivalent to 50 mg of DNA/ml. The quality of plant DNA was determined as the ratio A260/ A280, which ranged from 1.8 to 1.9, which is indicative of good quality plant DNA.

S.No.	Sesame varieties	Ratio of A260/A280	Conc. of DNA (ng/µl)
1.	RT-46	1.97	2595.40
2.	RT-54	1.89	532.37
3.	TMV-3	1.8	5043.08
4.	TMV-5	1.8	815.30
5.	N-8	1.81	707.16
6.	RAJESHWARI	2.1	671.62

Table 1: DNA yield based on absorbance (260nm) obtained from various sesame varieties

- Intact high molecular weight plant nuclear DNA is essential for most of molecular studies. Though, RAPD analysis can be performed with little sheared DNA, a minimum of intact templates are still needed. DNA was isolated using CTAB method from sesame plant of different varieties

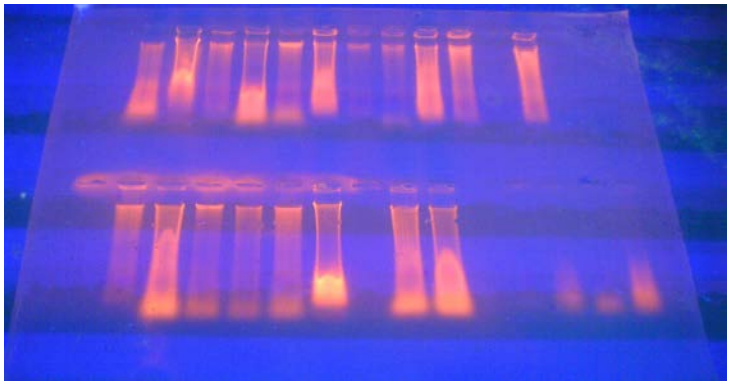
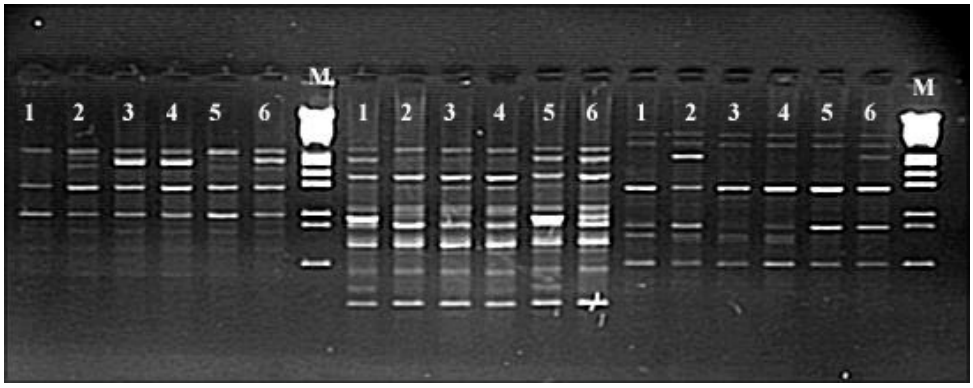
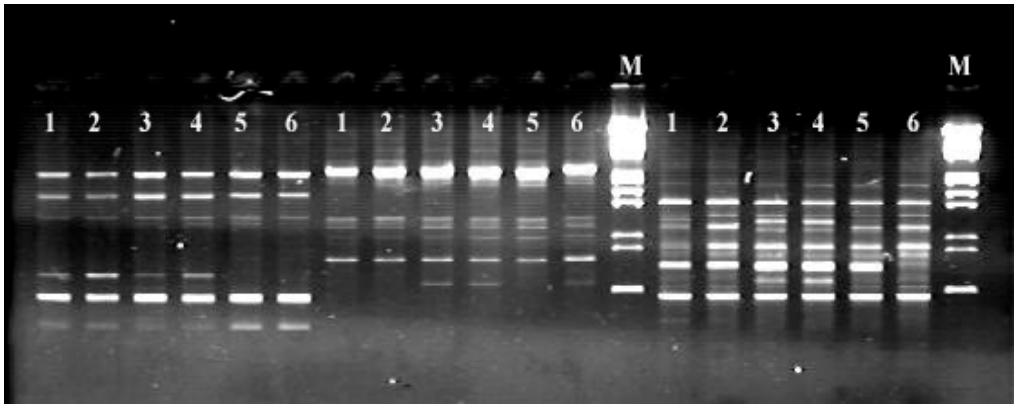


Fig 1: Genomic DNA isolated from Plant.

- The UBC random primers (decamer) procured from UBC (University of British Columbia) is most commonly used arbitrary primers in RAPD analysis of different plant species. Most of the primers produced amplicons below 2.0-kb range, though a few amplicons crossed 2.0-kb range. The number of polymorphic bands ranged from 2 to 6 with range of polymorphisms 22.22% (UBC-180) to 55.55% (UBC-186). Generally more the number of fragments generated higher could be number of polymorphic bands expected.



Primer no: 104,105 & 106



Primer no: 122,132 & 181

Fig 2: RAPD profile generated in six different Sesame Varieties

Genetic relationship among the genotypes and cluster analysis : The Jaccard;s similarity coefficient generated on the basis of RAPD profiling showed on average similarity of 81% among all the six varieties included in the study.

	RT-46	**RT-54**	**TMV-3**	**TMV-5**	**N-8**	**RAJESHWARI**
RT-46	1.00					
RT-54	0.82	1.00				
TMV-3	0.76	0.79	1.00			
TMV-5	0.72	0.78	0.95	1.00		
N-8	0.74	0.76	0.74	0.78	1.00	
RAJESHWARI	0.76	0.79	0.8	0.79	0.91	1.00

Table 2: Jaccard's similarity coefficient for ten varieties of sesame based on RAPD profiling.

Genetic similarity: Dendrogram based on UPGMA analysis splitted into three clusters and each cluster occupied two genotypes. All the included genotypes clustered according to their geographical regions like Rajasthan genotypes (RT-46 & RT-54) were clustered into same group while Tamil Nadu genotypes (TMV-3 & TMV-5) and Orissa genotypes (N-8 & Rajeshwari) genotypes also clustered into their respective group. Rajasthan genotypes were clustered into the same group at 80 % genetic similarity while TMV-3 & TMV-5 at 95 % genetic similarity. The clustering indicate a strong resemblance among genotypes from the same geographical region because all the included cultivars splitted into their respective groups.

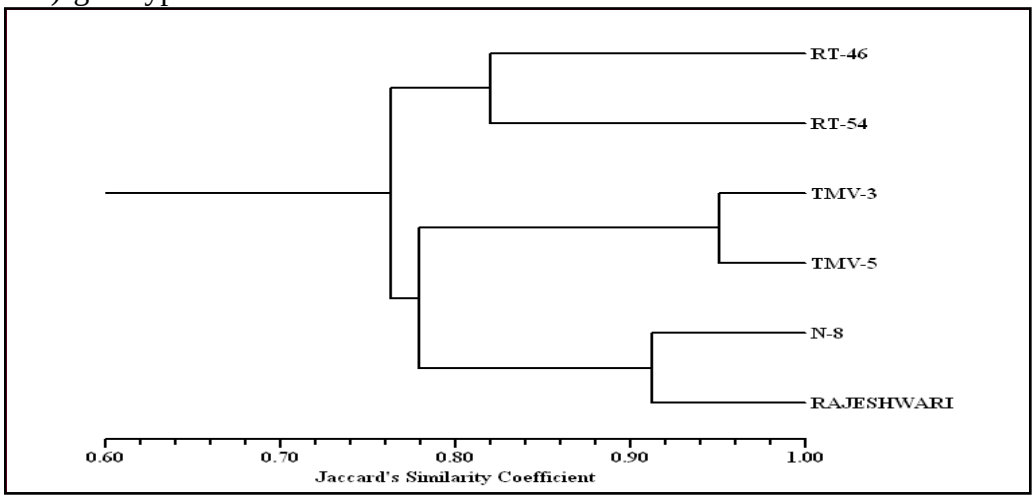


Fig 3: Dendrogram generated for six sesame cultivars using UPGMA cluster analysis based on Jaccards' similarity coefficient for RAPD data.

CONCLUSION:

1. RAPD can effectively distinguish genetic diversity in sesame.
2. Three group has been discovered according to the similarity-

i. RT-46 & RT-54(82%).
ii. TMV-3 & TMV-5(95%).
iii. N-8 & RAJESHWARI (91%).
iv.

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