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GENETIC CHARACTERIZATION OF SESAME VARIETIES THROUGH RAPD FINGERPRINTING TECHNIQUE IN BANGLADESH

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ABSTRACT

DNA fingerprinting helps to document plant genetic resources of a particular species through banding patterns and plays an important role in genetic conservation and breeding program. Four varieties of sesame had been fingerprinted by three decamers of randomely amplified polymorphic DNA (RAPD) markers. A total of 21 PCR amplification products were found by using 3 decamer primers (OPA-09, OPC-05 and OPL-07) among which 14 bands were polymorphic. The size of the amplification products ranged from 347bp to 1224bp for OPA-09, 317bp to 1592bp for OPC-05 and 423bp to 1024bp for OPL-07 primer. Within the individuals of a single variety no genetic variation was observed. UPGMA dendogram based on Nei's (1972) genetic distance indicated segregation of 4 varieties of sesame into two main clusters: T 6 with BARI Til-2 grouped in cluster 1 and BARI Til-3 with BINA Til 1 in cluster 2. Molecular characterization of the varieties will provide support for broadening the genetic basis of selective breeding programs.

Keywords: sesame, genetic characterization, RAPD fingerprinting, Bangladesh

INTRODUCTION

Sesame (*Sesamum indicum* L.), family Pedaliaceae, is one of the most ancient oilseed crop known to mankind. It is grown in tropical and subtropical areas (Ashri, 1998) on 6.5 million hectares worldwide, producing more than three million tons of seed (FAO, 2005). India, Sudan, Myanmar, China and Bangladesh, are the most important sesame producers with 68 % of the world production. However, Asia is rich in diversity of cultivated sesame. It is an important source of edible oil and is widely used as a one of the ingredients in food products especially in bakery foods and animal feed. Sesame seed contains 50-60% oil and 25% protein with natural antioxidants such as sesamolin, sesamin and considerable source of calcium, tryptophan, methionine and many minerals (Johnson *et al.*, 1979) and has been used as active ingredients in antiseptics, bactericides, viricides, disinfectants, moth repellants, anti-tubercular agents (Bedigian *et al.*, 1986, Bedigian, 2003). Composition of fatty acid in sesame oil is variable between different cultivars (Brar, 1982). These important characteristics have lead researchers' interest in identifying the sesame cultivars using advanced molecular technologies.

Information on the genetic diversity in sesame is limited. However, more recently, a high level of variability of morphological characters within different sesame collections was reported (Bedigian *et al.*, 1986 and Bisht *et al.*, 1998). Molecular markers are more reliable for genetic studies than morphological characteristics because the environment does not affect them. Genetic variability in sesame has also been studied by molecular techniques, including isozymes (Isshiki and Umezake, 1997), RAPD (Ercan *et al.*, 2004), ISSR (Kim *et al.*, 2002) and AFLP markers (Ali *et al.*, 2007). In Bangladesh, four cultivars of sesame have been registered and released for cultivation (Rahman *et al.*, 2007). It is necessary to study cultivars at the molecular level to distinguish them for their special characters and to differentiate varieties available in Bangladesh. In this context, the aim of the present study is to decipher the DNA fingerprinting of the sesame cultivars in Bangladesh and to find out the phylogenetic relationships among the cultivars using Randomly Amplified DNA (RAPD) markers.

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MATERIALS AND METHODS

Genomic DNA isolation

Seeds of four sesame varieties were obtained from Bangladesh Agricultural Research Institute, Gazipur, Bangladesh and grown in the experimental field of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Genomic DNA was isolated following protocol described by Saghai-Maroof *et al.* (1984) with some modifications. Juvenile leaves (unfolded) of 30 days old plants were used in genomic DNA isolation. Leaf tissues were cut into small pieces, homogenized and digested with extraction buffer (pH= 8.0): 50 mM Tris-HCl, 25 mM EDTA (Ethylene-diaminetetraacetic acid), 300 mM NaCl and TEN buffer + 5% SDS (Sodium Dodecyl Sulfate) +10% PVP (Poly Vinyl Pyrolideone) +20% CTAB (Cetyl Trimethyl Ammonium Bromide). After incubation for 20 minutes at 65°C with intermittent swirling, the mixture was emulsified with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v). DNA was precipitated using two volume of absolute alcohol in presence of 0.3 M sodium acetate and pelleted by centrifugation. The pellets were then washed with 70% ethanol, air dried and resuspended in an appropriate volume of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH=8.0) and finally treated with 2 µl of RNAse. Quality and quantity of DNA were controlled via gel electrophoresis and spectrophotometer, repectively.

Primers identification

A set of five Random primers were chosen to estimate the potential of these marker for variety identification. Finally three primers, OPA-09, OPC-05 and OPL-07 were selected based on their performance for RAPD data analysis.

Polymerase Chain Reaction

Polymerase Chain Reactions were done in a volume of 10 µl containing 10 x PCR Buffer, 0.25 mM each of the dNTPs, 25 pmol of primer, 1 unit ampli *Taq* DNA polymerase, 100 ng template DNA and a suitable amount of sterile deionized water. Amplification were carried out in a oil free thermal cycler (Thermal cycler gradient, Eppendorf) with the following thermal profile: initial denaturation step at 94°C for 4 min followed by 45 cycles at 94 °C for I min, 34-36°C for 1 min, and 72 °C for 2 min and a final cycle at 72 °C for 7 min. PCR was the confirmed by electrophoresis on 2% agarose gel.

RAPD data analysis

Following electrophoresis, the sizes of the PCR products were estimated by comparisons of distance travelled by each fragment with distance travelled by known size fragments of the DNA molecular weight markers (100 bp DNA ladder, Genei, India). All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to size and scored visually on the basis of their presence (1) or absence (0), separately for each variety for each primer. The scores obtained using all primers in the RAPD analysis were then pooled to create a single data matrix. This was used to compare the frequencies of all polymorphic RAPD markers among populations with 1000 simulated samples using POPGENE (version 1.31) (Yeh *et al.*, 1999) computer program. The size of the RAPD markers were estimated by using the software DNAfrag, version 3.03 (Nash, 1991). Nei (1972) genetic distance values were computed from frequencies of polymorphic markers using the POPGENE (Version 1.31) computer package (Yeh *et al.*, 1999). The dendrogram was constructed using POPGENE (Version 1.31) (Yeh *et al.*, 1999).

RESULTS AND DISCUSSION

A total of 21 PCR amplified products were detected with 3 decamer primers (OPA-09, OPC-05 and OPL-07) among which 14 bands were polymorphic. The size of the amplified DNA fragments ranged from 347bp to 1224bp for OPA-09, 317bp to 1592bp for OPC-05 and 423bp to 1024bp for OPL-07 primer (Table 1). Detailed RAPD fingerprinting profiles of studied varieties across 3 primers are shown in Figure 1. In the DNA fingerprinting profile, three individuals for each variety were tested as replications. Although three individuals of a single variety contained genomic DNA of their own, the

primers did not show any polymorphic bands among them. This illustrates that the seeds of sesame varieties were maintained with proper management and no mixture of genetic resources was found. The highest number of bands was generated by the primer OPC05 whereas the least number of bands were found by the primer OPL07 (Table 1).

Table 1. RAPD primers with corresponding bands scored and their size ranges across 4 sesame (*Sesamum indicum*) varieties

Primer Codes	Sequences (5'-3')	Total no. of bands scored	Size ranges (bp)	Sizes of observed bands (bp)	Number of polymorphic bands
OPA09	GGGTAACGCC	6	347-1224	347, 402, 477, 630, 809, 1224	4
OPC05	GATGACCGCC	10	317-1592	317, 485, 519, 602, 673, 857, 908, 1116, 1392, 1592	6
OPL07	AGGCGGGAAC	5	423-1024	423, 462, 573, 744, 1024	4

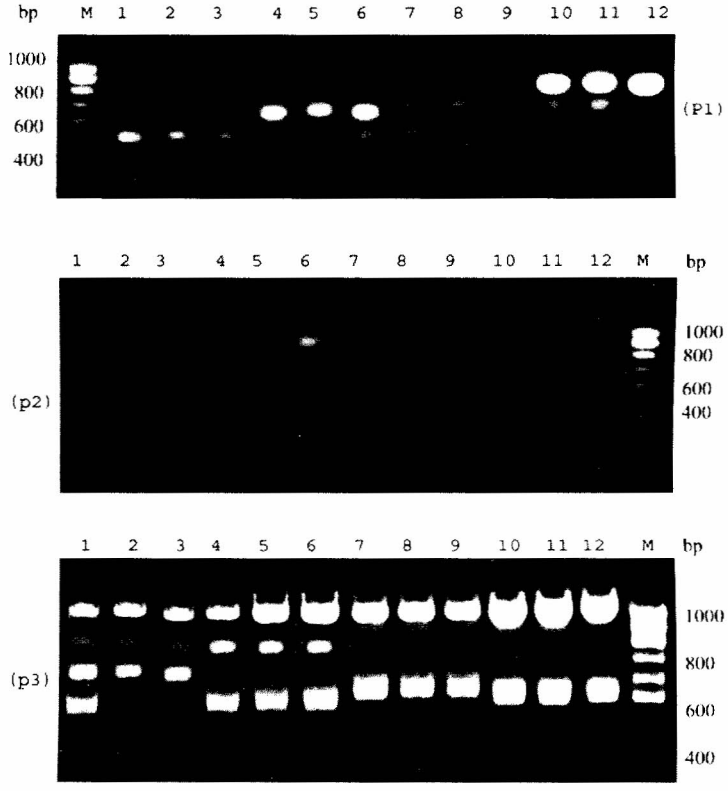

Fig. 1. RAPD profiles of four sesame varieties across 3 primers, P1 (OPA-09), P2 (OPC-05), P3 (OPL-07). Lanes, 1-3= T6, 4-6= BARI Til 2, 7-9= BARI Til 3 and 10-12= BINA Til 1. M= Molecular weight marker (100 bp).

Across all the three RAPD primers, the highest average inter-varietal similarity indices (70.83%) was found between BARI Til 2 and T6 varieties and the lowest highest inter-varietal similarity indices (31.85%) was found between BARI Til 3 and BINA Til 1 varieties (Table 2).

Table 2. Inter-variety similarity indices (S_{ij}) between individuals of four sesame varieties

Between individuals	Band sharing percentages			Average S_{ij} (%)
	OPA09 (%)	OPC05 (%)	OPL07 (%)	
T6 vs BARI Til 2	75.00	62.50	75.00	70.83
T6 vs BARI Til 3	75.00	62.50	20.00	52.50
T6 vs BINA Til 1	50.00	37.50	50.00	45.83
BARI Til 2 vs BARI Til 3	100.00	50.00	25.00	58.33
BARI Til 2 vs BINA Til 1	40.00	37.50	66.67	48.05
BARI Til 3 vs BINA Til 1	40.00	22.22	33.33	31.85
Average S_{ij} (%)	63.33	45.37	45.00	**51.23**

UPGMA dendogram based on Nei's (1972) genetic distance indicated segregation of 4 cultivars of sesame into two main clusters: T 6 with BARI Til-2 grouped in cluster 1 and BARI Til-3 with BINA Til 1 in cluster 2 (Fig. 2). Ali *et al.* (2007) noticed that due to genetic difference, major genotype clusters were related to main geographic origin while analyzing genetic diversity in sesame using AFLP markers. However, small clusters were also formed based on some known characteristics, pedigree relations or belonging to close area of cultivation within main group. The highest genetic distance (1.064) was found between T 6 and BINA Til 1 varieties and lowest (0.322) between T 6 and BARI Til 2. The gene flow between the BARI Til 2 and T6 varieties was the highest (0.724) and the lowest gene flow (0.345) was observed between the T6 and BINA Til1 varieties (Table 3).

Table 3. Gene flow, N_m (above diagonal) and genetic distance (below diagonal) observed in 4 sesame (*Sesamum indicum*) varieties across all RAPD primers

Cultivars	T 6	BARI Til 2	BARI Til 3	BINA Til 1
T 6	-	0.724	0.446	0.345
BARI Til 2	0.322	-	0.656	0.345
BARI Til 3	0.807	0.422	-	0.638
BINA Til 1	1.064	1.064	0.450	-

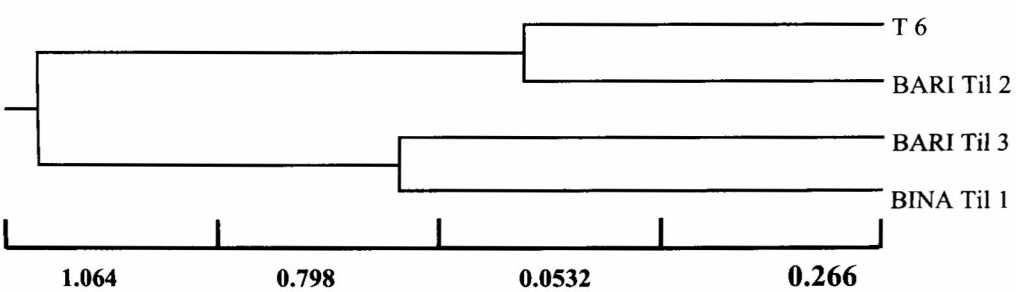

Fig. 2. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation between four Sesame varieties according to RAPD data analysis.

Sesamum indicum L. has a large genetic variability, which should be taken into account during planning of conservation strategies or when sesame variability is used in breeding programs. Isozyme studies concluded that cultivated sesame has a narrow genetic base (Isshiki and Umezake, 1997). A RAPD-based study on sesame carried out on 36 Indian accessions and 22 accessions from other countries (Bhat *et al.*, 1999) and a study on a Turkish sesame collection (Ercan *et al.*, 2004) concluded that sesame has a high level of genetic variability.

The identification of genetic relationship among the cultivars based on biochemical and molecular analysis will be used in further genetic improvement. It will also provide support for selection of

crossing combinations from bulk parental genotypes and for broadening the genetic basis of breeding programs.

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