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Use of RAPD Marker for the Assessment of Genetic Diversity of Sesame (*Sesamum indicum* L.) Varieties

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A B S T R A C T

Genetic diversity among 28 sesame (*Sesamum indicum* L.) varieties was examined at DNA level by means of random amplified polymorphic DNA (RAPD) analysis. Fifteen primers used produced a total of 132 RAPD fragments. Each primer generated 3 to 26 amplified fragments with an average of 8.8 bands per primer. Based on pair-wise comparisons of RAPD amplification products, Nei and Li's similarity coefficients were computed to assess the associations among the varieties. Pair-wise similarity indices varied from 0.35 to 0.84. A UPGMA cluster analysis based on these genetic similarities located most of the varieties far apart from one another, showing a high level of polymorphism. Genetically, all the genotypes were classified into two major groups at similarity coefficient 0.35 and these major groups are further divided into small clusters as the similarity coefficient level increases. Accession Swetha Til and AKT-101 was show maximum distance from each other. In conclusion, even with the use of a limited set of primers, RAPD technique revealed a high level of genetic variation among sesame varieties. This high level of genetic diversity among the genotypes suggested that RAPD technique is valuable for sesame systematic, and can be helpful for competent choice of parents in breeding programs.

Introduction

Sesame (*Sesamum indicum* L.) of the family Pedaliaceae, is one of the oldest oil crops being cultivated in Asia for more than 5000 years. The genus Sesamum contains more than 30 species of which *S. indicum* is the commonly cultivated (Nayar and Mehra, 1970; Kobayashi *et al.*, 1990). The exact natural origin of the sesame is mysterious. India and Africa are the two expected places of its origin. Ashri (1998) felt that settling the dispute on the origin of sesame will involve more detailed cytogenetic and fitting DNA comparisons. Sesame seeds are in high demand because of its significance in the confectionary industry universally. Sesame contains about 50–60% odorless and colorless oil (Uzun *et al.*, 2003), which is of superior class with antioxidants, almost matching olive oil. Sesame oil is used as a cooking medium mainly in the Indian subcontinent and African countries. Small uses of sesame oil consist of pharmaceutical and skin care products and are synergic for insecticides (Hatam and Abbasi, 1994). Sesame oil also contains a high level of polyunsaturated fatty acids (Wood, 1999). It has a reducing consequence on plasma

cholesterol and it also lowers the blood pressure (Sankar *et al.,* 2005). Potential reimbursement of sesame on human health has freshly rehabilitated the attention in this ancient crop (Laurentin and Karlovsky, 2006). Sesame is grown over 50 countries in the world. According to Agriculture statistic division, Directorate of economics and statistics, New Delhi 2nd advance estimation,2015-16 over 7.53 lakh hectares were harvested in India, producing almost 8.32 lakh tons. India, Sudan, Myanmar, Uganda and China are the supreme sesame producers, covering 75% of world production. Sesame has many returns as it needs a little water as compared to other crops such as cotton (half of sesame crop), etc Its yield (426 kg/ha) is near to the ground as compared to other chief sesame producing countries of the world such as China, Egypt and Hondrous which are generating 1185, 1143 and 1133 kg seed yield per hectare, respectively (Anon., 2000). This low yield (426 kg/ha) can be straightforwardly increased up to 2000 kg/ha. In spite of being the first oilseed crop known to man, its extended history and importance sesame is a naturally neglected crop. Sesame has been mentioned as an 'orphan crop' because it is not commanded to any of the CGIAR institutes which could also be one of the reasons for lack of research works (Ashri, 1995). As a natural result of this condition, the use of molecular techniques for the enhancement of sesame is very restricted. Only a few reports are accessible on the use of molecular markers such as isozyme (Isshiki and Umezaki, 1997), RAPD (Bhat *et al.,* 1999), ISSR (Kim *et al.,* 2002), AFLP (Uzun *et al.,* 2003) and SSR (Dixit *et al.,* 2005). In India as well as in other countries, the average seed yield of sesame is quite low owing to lack of enhanced cultivars, making the plants vulnerability to diseases, pest and environmental stresses. Furthermore, properties such as undefined growth habit and asynchronous capsule ripening highlight seed shattering and there is a lack of inputs in the cultivation of sesame (Ashri, 1998). Lower productivity of the sesame has also been attributed to some extent to the use of conventional varieties (Hamid *et al.,* 2003). Comparatively, low seed yield is one of the most important reasons that sesame wants breeding to provide more yields (Furat and Uzun, 2010). Selection for good yield types should be very functional and donate to breeding programs in our country. Hence, the first and prime need is the detection or cataloguing of sesame genotypes along with the assessment of genetic diversity widespread India sesame germplasm. India is wealthy of sesame variation. Such local and simple sesame varieties offer raw material for improved agricultural products (Ali *et al.,* 2009). Although genetic variation subsists for agronomically important characters, but the inadequate genetic information regarding Indian sesame populations is warning the access to helpful traits present among adapted landraces of sesame all over the country. This deficient genetic information about the sesame populations is the key factor for limited cultivation of modern varieties and small yield (Baydar *et al.,* 1997) because efficient utilization of any sesame germplasm in a breeding program need information on genetic variability, heritability and correlation among diverse characters in the germplasm. Among a large group of molecular markers, random amplified polymorphic DNA (RAPD) is suitable for the estimation of genetic diversity (Williams *et al.,* 1990) due to its simplicity, speed and relatively low cost. Being a quick and sensitive method, RAPD can be quickly and effectively applied to distinguish useful polymorphisms (Ko *et al.,* 1998). The resolving influence of this tool is numerous folds superior than morphological or biochemical markers and is much simpler and technically less demanding than RFLP and other new generation markers. RAPD markers have proved their significance for

assortment analysis in a number of field crops such as rice (Rabbani *et al.,* 2008; Pervaiz *et al.,* 2010), and horticultural plants like strawberry, common bean, neem, turmeric (Jan *et al.,* 2011) and particularly in sesame (Ercan *et al.,* 2004). Since molecular based characterization of genotypes is independent of G x E interaction it may be a successful and competent tool to understand and validate the genotype variability between and within geographical regions and ultimately in conceding protection and crop development program. In the present study, we report on the genetic diversity and genetic associations within the sesame varieties through RAPD technique.

Materials and Methods

Plant material

Twenty eight genotypes of Sesame germplasm from Project coordinating Unit (Sesame and Niger) were sampled (Table 1). Leaves were collected at 20-25 days after sowing from three plants of each of 28 genotypes and DNA was isolated to study the genotypic diversity based on RAPD markers.

DNA extraction and PCR analysis

Total genomic DNA was extracted from the leaf tissues of each sesame genotype using CTAB DNA extraction protocol reported by Saghai-Maroof (1984) with few alterations. Concentration of DNA was checked by visual evaluation of band intensity in contrast with lambda DNA molecular standards of recognized concentrations with 0.8% agarose gel. For PCR analysis all the extracted DNA samples were diluted to a running concentration of 40ng/µl with TE buffer. After a preliminary screen, 15 primers which proved obvious and reliable banding patterns and amplification were eventually selected to amplify the DNA of each sesame accession. Amplification reactions were carried out in a volume of 25µl. The reaction mixture contained10X optimized DyNAzymesTM Buffer (Thermo scientific), Primer (10p moles/ µl), dNTPs (2.5mM each) (Fermentas, USA), DyNAzymes TM-II DNA polymerase (2U/µl), Template DNA (40ng/ µl), Nuclease free water (Merck,USA). Thermocycler - 480 (Perkins Elmer Cetus, Norwalk, USA) was used for the DNA amplification. The thermal cycler was planned to 1 cycle of 5 min at 94o C for first strand separation, pursued by 45 cycles of 45 second at 94o C for denaturation, 1 min at 36o C for annealing the DNA double strand and 2 min at 72o C for primer extension. At last, 1 cycle of 10 min at 72o C was employed for concluding extension, followed by drenched at 4o C. After amplification, 10µl of amplification products plus loading dye were loaded in 2% agarose gels for electrophoresis in 1xTBE (10mM Tris-Borate, 1mM EDTA) buffer to analyze the PCR products. A 1kb DNA ladder was used as a molecular size marker. After the complete run of electrophoresis, the separated bands were visualized under UV transilluminator and photographed using Syngene Gel Documentation system.

Data analysis

All RAPD product amplified by given primers were measured as a single locus and data were scored as the absence (0) or presence (1) of a DNA band for each of the primer-accession combination. The intensity of the DNA fragments was not taken into consideration and the bands with the same mobility were considered to be the same bands. Only main DNA fragments constantly amplified were scored and weak bands were not measured for analysis. The molecular size of the DNA fragments was deliberated from a standard curve based on known size of DNA fragments of a 1kb marker. Pair-wise comparisons of all the sesame varieties based

on absence or presence of unique and shared DNA bands were utilized to make similarity coefficients. The resulting similarity coefficients were employed to assess the relationship among sesame genotypes with a cluster analysis by means of unweighted pair-group method with arithmetic averages (UPGMA) and then designed in the form of dendrogram. We selected this way of calculation over other general similarity indices because of the enlarged weighting of band matches versus that of non-matches. All calculations were carried out using NTSYS-pc, Version 2.1 package (Rohlf, 2000).

Results and Discussion

The genetic diversity and the relationships among 28 sesame genotypes were evaluated by RAPD markers using 15 primers. Fig. 1 shows the pattern of amplified products across sesame varieties generated with the primer OPF-10. In most of the cases, sesame germplasm collections exhibited different banding patterns. Some of the varieties shared relatively lower number of bands with other germplasm varieties, showing their distant relationship to them. 15 primers that exhibited reliable and consistent banding patterns were chosen for the evaluation of variability across all the varieties. Each of the chosen 15 primers varied greatly in their ability to determine variability among the varieties. A total of 123 amplification products were produced by 15 primers across 28 varieties (Table 2). The number of amplification products generated by each primer ranged from 3 (OPC- 13) to 26 (OPA-03) with an average of 8.8 fragments per primer. A similarity matrix based on the proportion of shared RAPD fragments was utilized to set up the level of relatedness between the diverse sesame germplasm varieties. Pair-wise estimates of similarity for 28 varieties ranged from 0.35 to 0.84. Two varieties, 'PKDS-11' and 'HT-2' were the closest genotypes with the highest similarity index of 83%, while 'AKT-101' and 'SWETHA TIL' were the least similar varieties. No accession was exactly similar to any other accession. Based on analysis carried out on Nei and Li's similarity matrix via UPGMA, 28 varieties were grouped together into main clusters (Fig. 2).

Table.1 List of sesame germplasm cultivars studied

Swetha Til	NT-32
Nirmala	GT-1
PKV NT-11	GT-10
Hima	GT-4
TKG-21	Prachi
TKG-22	RT-135
TKG-306	GT-2
TKG-308	MT-75
TKG55	PKDS-8
PT-1	PKDS-11
JLT-408	HT-2
RT-54	Shekhar
RT-351	AKT 101
RT-346	DSS-9

Table.2 List of 15 Primers were used in present study from Operon series

		Sequence
1.	OPA-12	GTGATCGCAG
2.	OPA-3	CAATCGCCGT
3.	OPA-18	CAGCACCCAC
4.	OPA-20	AAAGCTGCGG
5.	OPB-11	TGTCATCCCC
6.	OPC-13	AGCGAGCAAG
7.	OPC-15	GAACACTGGG
8.	OPF-09	CCCTACCGAC
9.	OPF-10	GTGCAACGTG
10.	OPAG-03	GGTTGTACCC
11.	OPK-09	CCCGCTACAC
12.	OPK-18	CCCAGCTGTG
13.	OPL-14	CCTAGTCGAG
14.	OPN-04	CACAGGCGGA
15.	OPV-15	CAGCCCAGAG

Fig.2 UPGMA cluster analysis showing the relationship and diversity among 28 sesame varieties based on 123 RAPD fragments generated by 15 random primers

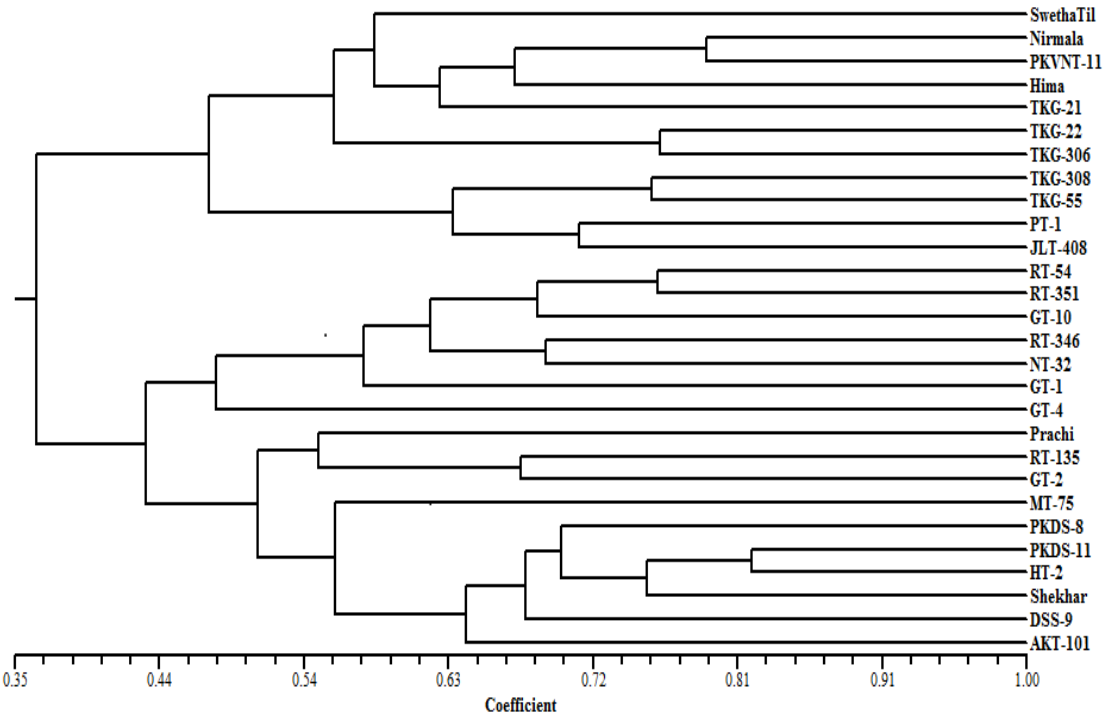
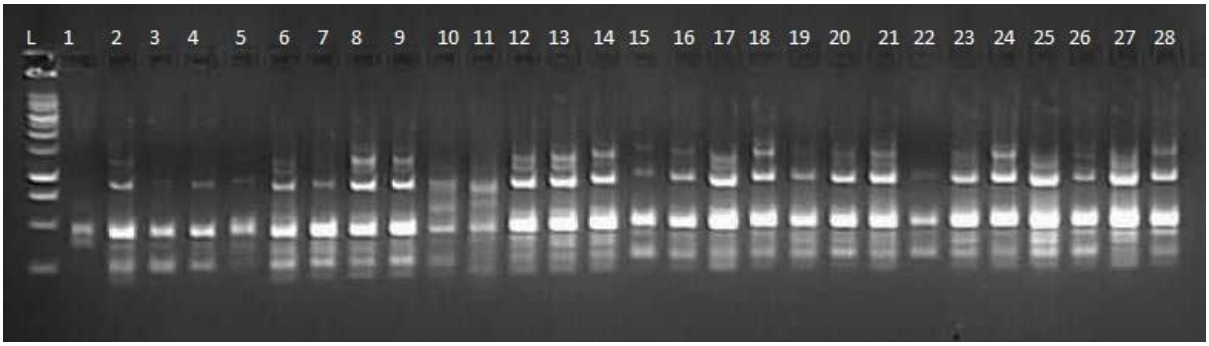


Fig.1 Amplification of sesame varieties by RAPD markers OPF-10, L = 1kb DNA ladder



First cluster (A) consisted of eleven genotypes, whereas second cluster (B) comprised of seventeen varieties. Each of the two clusters may be further sub-divided into other sub-groups. As expected from the similarity estimates, cluster analysis positioned most of the sesame varieties far apart from each other showing a high level of genetic diversity. However, some of the varieties of the same locality were grouped together in the same cluster revealed a nearer genetic relationship.

RAPD markers have been used in this study to assess the genetic diversity among the sesame varieties. The selection of the RAPD technique was inspired by the fact that no DNA sequence knowledge is known about sesame crop and RAPD technique does not need any prior information of DNA sequencing. In addition it is simple to use for the evaluation of genetic diversity in sesame (Bhat *et al.*, 1999; Ercan *et al.*, 2004; Salazar *et al.*, 2006). A high level of genetic variation was observed among the 28 sesame varieties.

Though sesame is generally a self-pollinated crop but cross-pollination from 5 to 60% has been reported in it (Yermamos, 1980). About 10 to 20% of the genetic diversity among populations is due to cross-pollinations (Hamrick and Godt, 1989). Hence, some cross-pollination could clarify the high level of genetic diversity examined in the same sesame varieties. Our results are in agreement to other studies based on RAPD markers which have reported high level of genetic variations in sesame genotypes (Ashri, 1998; Bhat *et al.*, 1999; Ercan *et al.*, 2004; Salazar *et al.*, 2006). The 15 RAPD primers noticed sufficient genetic diversity among the 28 sesame varieties to allow for full separation. A number of other investigations have reported on the use of the same number of RAPD primers for evaluating genetic variation. Li and Midmore (1999) detected a high level of genetic diversity among germplasm of Chinese water chestnut with 14 RAPD primers. Similarly, Millan *et al.*, (1996) reported a high level of genetic variation in rose germplasm using merely 10 RAPD primers. Rether and Schontz (1999) identified 37 lines of Foxtail millet (*Setaria italica* L.) using just four RAPD primers, whereas genetic diversity of rice landraces and cultivars from Pakistan has successfully been assessed by RAPD markers (Rabbani *et al.*, 2008; Pervaiz *et al.*, 2010) and SSR markers (Rabbani *et al.*, 2010). It should be noted that RAPD molecular markers could give high level of genetic diversity as compared to other molecular markers for example Isshiki and Umezaki (1997) identified a low level of genetic diversity in sixty eight sesame germplasms applying isozymes. Similarly Laurentin and Karlovsky (2006) noticed a very low genetic variation in thirty two sesame genotypes using AFLP molecular markers. Even Kim *et al.*, (2002) reported a low level of genetic diversity in sesame germplasm collected from Korea and some other countries using microsatellite ISSR molecular markers. While Bhat *et al.*, (1999) and Ercan *et al.*, (2004) identified a very high level of genetic diversity among sesame varieties by means of RAPD

molecular markers. In our study a high level of polymorphism was detected among sesame varieties. This was also supported by earlier RAPD marker results from other sesame investigations by applying the OPM-06 primer (100% polymorphism). In our study a high level of polymorphism observed is analogous to the 78% polymorphism noticed in the evaluation of genetic diversity in Turkish sesame (Ercan *et al.*, 2004). Bhat *et al.*, (1999) also observed 86.75% polymorphism in a study of genetic diversity in Indian and exotic sesame germplasm. Although a considerable level of genetic diversity was present among sesame varieties. In conclusion, RAPD analysis showed a considerable level of genetic diversity among sesame varieties, even using as few as 15 RAPD primers. This high level of genetic variability among the sesame varieties proposed that the RAPD technique can be fruitful for the sesame systematics and selection of parents for breeding programs.

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