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Full Length Research Paper

Study of genetic diversity in Sudanese sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers

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The random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity in *Sesame indicum* (L.). RAPD technique was carried out in a set of 10 sesame germplasm collected from different regions of Sudan. A total of 64 polymorphisms (6.4 polymorphic markers per primer) out of 75 reproducible products (7.5 fragments per primer) were obtained from the 10 primers used. The number of bands per primer ranged from 4 to 13, whereas the number of polymorphic bands ranged from 3 to 12, corresponding to 66.6% of the amplification products. Low level of genetic similarity was observed in the collected accessions. Unique bands were observed with the 10 primers. UPGMA clustering resulted in two major groups.

Key words: *Sesamum indicum*, population genetics, conservation, genetic diversity, RAPD.

INTRODUCTION

Sesame (*Sesamum indicum* L.), a member of the family Pedaliaceae, is widely grown in Sudan under rain fed conditions. It is one of the most important cash crops (Bedigian, 2003). Sudan, India, Myanmar and China are the most important sesame producers with 68% of the world production (Laurentin and Karlovsky, 2006). In spite of the economical importance of sesame for the Sudan economy big fluctuations in production and yield occurred. The average seed yield in Sudan is about 350 kg/ha (AOAD, 1998). This low yields are mainly due to absence of non-shattering cultivars suited for mechanical harvest, indeterminate growth, uneven ripening of capsules and biotic and abiotic stresses such as diseases, pests, drought etc. Moreover, Hamid et al. (2003) pointed out that low productivity was attributed partially to the traditional variety used.

In the Sudan the ultimate objective of the sesame breeding program since its inception in early 1950s has been the development of high yielding non shattering varieties for mechanized crop production (Khidir, 1969). Effective utilization of any sesame germplasm in a breeding program requires information on genetic variability, heritability and correlation among different characters in the germplasm. Knowledge of genetic diversity and relationships among sesame germplasm is essential for the long-term success of breeding programs since a wide range of genetic diversity among parents is essential for hybridization (Ganesh and Thangavelu, 1995).

Genetic diversity in crop species including sesame can be determined using morphological and agronomic characteristics, isozyme and DNA marker analysis (Liu, 1997; Koornneef, 1990; Reiter et al., 1993). However, morphological grouping may be based on characteristics which could be strongly affected by environmental factors (Liu, 1997). The morpho-agronomic markers may not reflect true genetic identities and diversities (Ferdinandez et al., 2001). Therefore, the use of DNA marker, such as random amplified polymorphic DNA (RAPD) (Williams et al., 1990) represents an alternative method in detection of polymorphism in sesame. Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms (Ko et al., 1998; Doldi et al., 1997).

RAPD markers are dominant markers and some loss of

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Table 1. List of sesame germplasm cultivars studied and their region and place of collection.

Genotype	Name	Collection area	Region
SUD1	Beladi	Elgadaref	Eastern Sudan
SUD2	Elgadaref-1	Elgadaref	Eastern Sudan
SUD3	Promo	Elgadaref	Eastern Sudan
SUD4	Elabassia	Kordofan -kamra	Southern western Sudan
SUD5	Aswad	Sinnar	Southern eastern Sudan
SUD6	Abusitta	Kazgill	Southern western Sudan
SUD7	Elobaied-1	Elobaied	Middle west Sudan
SUD8	Khidir	Elgadaref	Eastern Sudan
SUD9	Abusandoug	Abu karshulla	Southern western Sudan
SUD10	Ali Mahdi	Abu karshulla	Southern western Sudan

information may occur in comparison to the co-dominant markers. However, RAPD requires no previous knowledge of DNA sequences since arbitrary primers are used. In addition, storage of DNA extractions is not as critical with RAPDs, because DNA is relatively more stable than protein that is used for isozyme analysis. Bhat et al. (1999) used the RAPD technique to determine genetic diversity among Indian sesame germplasm. Parani et al. (1997) reported that RAPD markers are highly stable and reproducible under controlled experimental conditions. These studies showed that RAPD markers can be used successfully to determine genetic diversity. Despite the importance of sesame in Sudan, there has not been any investigation about the levels of genetic variation among sesame populations using molecular markers. In the present investigation, we report on genetic relationships and genetic variability within the population of sesame cultivars based on RAPD markers.

MATERIALS AND METHODS

Plant material

Ten genotypes of Sesame germplasm from different regions of Sudan were sampled (Table 1).

DNA extraction

Genomic DNA was extracted from fresh leaf tissue of ten individuals using modified CTAB method (Porebski et al., 1997). The modification made in intention to improve the quantity and the quality of the DNA. In this method the fine powdered plant materials were immediately transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water path at 65ºC with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamyl alcohol-chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform : isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol, and the ethanol was discarded after spinning with flash centrifugation. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at -20ºC for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then spectrophotometrically assessed following Sambrook et al. (1989) method.

RAPD analysis and primer selection

A total of twenty five primers were used for PCR amplification. The twenty five primers were initially tested using three cultivars from different regions. Ten primers that produced strongly amplified polymorphic bands with these test templates were selected for RAPD-PCR analysis (Table 2). The PCR reaction was conducted in 50 ml reaction volume 2 containing 1x PCR buffer, 1.5 mM MgCl , 0.2 mM of each dNTPs, 1 mM of forward and reverse primers, 1 U Taq DNA (promega) polymerase and 10 ng genomic DNA. Hot start and touchdown PCR temperature profile was used as follows: an initial denaturizing step at 94°C for 5 min, followed by 10 cycles of touchdown annealing temperature 60 to 50°C for 60 s in which the annealing temperature was decreased by 1°C every cycle. Another 30 cycles were starting, and then a final extension step at 72°C for 7 min was performed. The PCR product were mixed with 2.5 µl of 10 X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before loading. The PCR products and 1 kp DNA ladder were electrophoresed 2% agarose gel at 100 volts followed by staining with ethidium bromide and photographed on Polaroid 667 film under ultra-violet light.

Data analysis

For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were scored (1) for present, 0 for absent and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity (D) matrix among genotypes was estimated according to Nei and Lei (1979). Coefficient of similarity trees were produced by clustering the similarity data with the unweighted pair group method using statistical software package STATISTCA- SPSS (Stat soft Inc.). The similarity

Table 2. Polymorphism detected by the use of 10 random primers on 10 sesame local populations.

Name of primer	Sequence of primer	Total number of bands	Number of polymorphic bands	% of polymorphic
A2	TGCCGAGCTG	13	12	92.3
A4	AATCGGGCTG	6	5	83.3
A11	CAATCGCCGT	9	8	88.9
A15	TTCCGAACCC	9	8	88.9
A18	AGGTGACCGT	4	3	75
B1	GTTTCGCTCC	10	9	90
B5	TGCGCCCTTC	6	4	83.3
B7	GGTGACGCAG	7	6	85.7
B8	GTCCACACGG	6	6	100
B15	GGAGGGTGTT	5	3	60
Mean		**7.5**	**6.4**	**84.74**

Table 3. Matrix of RAPD dissimilarity among 10 Semseam indicum genotypes based on

Genotype	1	2	3	4	5	6	7	8	9	10
1	0.00									
2	0.20	0.00								
3	0.27	0.17	0.00							
4	0.17	0.16	0.19	0.00						
5	0.55	0.52	0.38	0.52	0.00					
6	0.31	0.21	0.22	0.27	0.35	0.00				
7	0.23	0.14	0.16	0.15	0.54	0.25	0.00			
8	0.33	0.24	0.25	0.25	0.48	0.25	0.21	0.00		
9	0.24	0.21	0.22	0.22	0.40	0.19	0.24	0.22	0.00	
10	0.26	0.19	0.17	0.18	0.47	0.15	0.18	0.22	0.21	0.00

coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA) according to Rohlf (1993).

RESULTS AND DISCUSSION

A total of twenty five primers were tested with the 3 genotypes of sesame (*S. indicum*). The result indicates that 10 primers (66.6%) showing at least 1 consistent polymorphic band. The ten informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships within and between all genotypes under study. A total of 75 amplified fragments were distinguished across the selected primers and the statistical analysis showed 64 polymorphic bands among the 10 genotypes with an average of 6.4 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer A2 (13) with 92.3% polymorphism while the minimum numbers of fragments were produced by the primer B15 (5) with 60% polymorphism. Pattern of RAPD fragments produced by the 10- mer primer A2, A4, A11, A15, A18, B1, B5, B7, B8, and B15 as shown in Table 2.

This study provides us with good knowledge about genetic variability of sesame which may allow more efficient and effective use of resources in plant improvement programs. In this study we utilized RAPD markers for better assessment of relationship of the accessions of sesame collected from different locations. The genetic dissimilarity values obtained with RAPD have been introduced for measuring genetic relationships in many plant species for easiness of the method, which only requires PCR technology. The low reproducibility of RAPD (Karp et al., 1997), introduces problem in their use for cultivar identification compared with other marker applications.

RAPD markers have been used in this study to evaluate the levels of genetic variation among Sudanese *S. indicum* populations. The choice of RAPD technique was motivated by the fact that no DNA sequence information is known about this species. Ten primers detected enough genetic variation among the 10 sesame cultivars to allow for complete differentiation. Li and Midmore (1999) reported that when the variation between genotypes is high, the use of a few primers will be sufficient. For instance in Millan et al. (1996), rose geno-

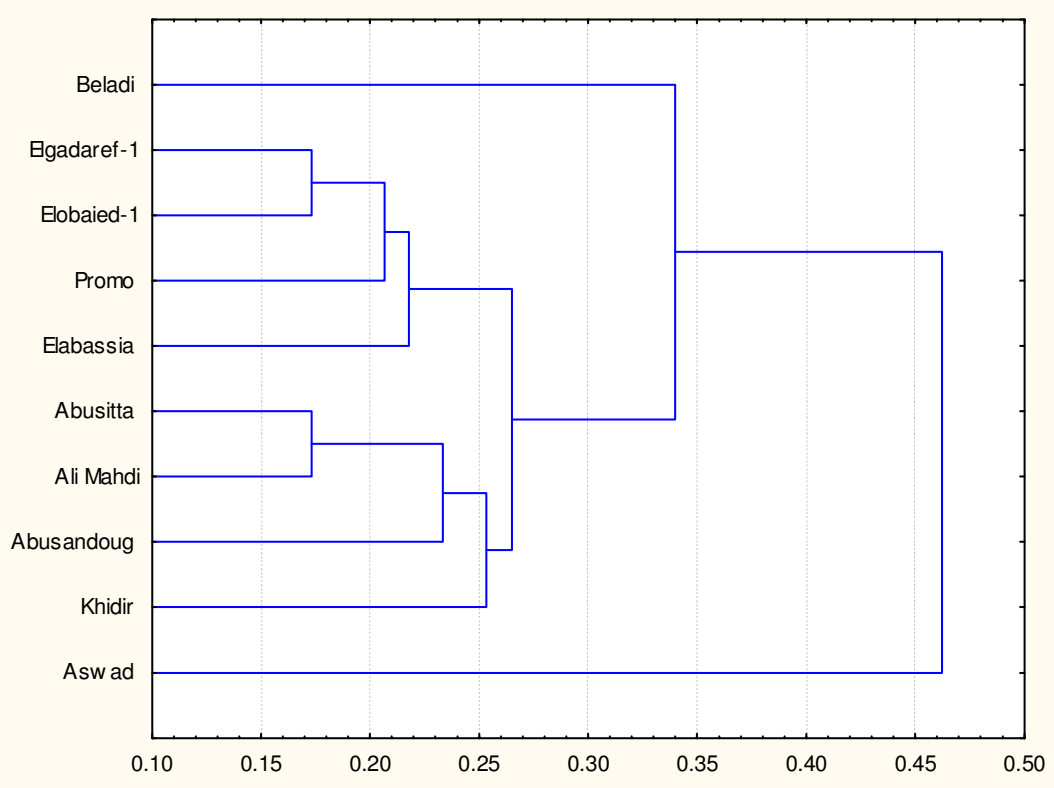

Figure 1. Dendrogram constructed for 10-sesame (*Sesasum indicum*) germplasm based on genetic distances using 10 RAPD primers

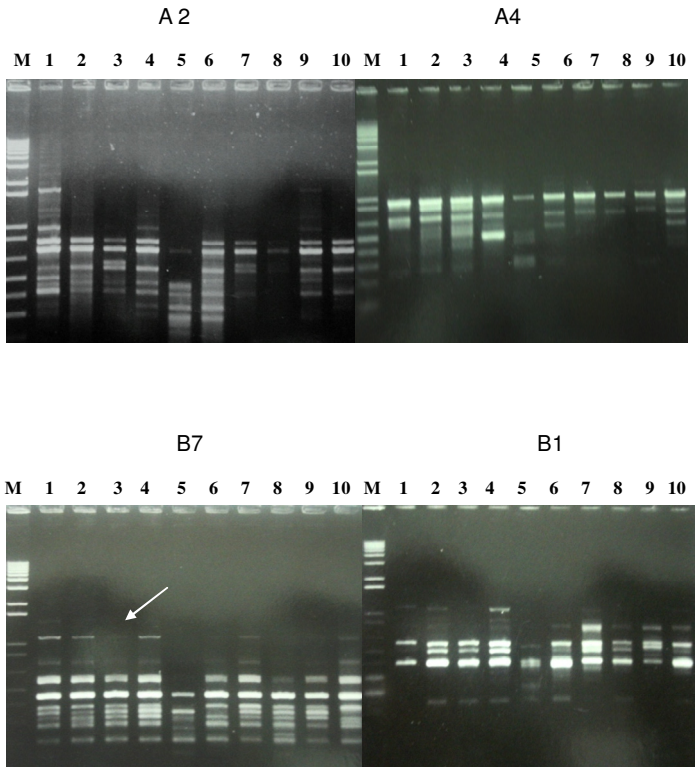

Figure 2. RAPD amplification patterns with primers A2, A4, B1, and B7. M, 1 kb ladder.

Table 4. Number of private bands detected across *Sesame* genotypes.

Sample name	Name of primer	Number of private bands
SUD1	A2	3
SUD5, SUD1	A11	2
SUD1	A15	1
SUD1	A18	1
SUD10, SUD8	B1	2
SUD5	B5	1
SUD5	B7	1
SUD8	B8	1

types were determined by only 10 primers while Schontz and Rether (1999) identified 37 lines of *Setaria italica* with 4 primers.

According to dendogram and cluster analysis (Figure 1), we found two main groups was originated by tree diagram, group one contained genotypes Elgadaref-1 and Elobaied-1. Group two contained genotypes Abusitta and Ali Mahdi; genotypes Aswad was placed in different group. The dissimilarity matrix obtained after multi variant analysis using Nei and Lei (1979) distance is presented in Table 3. RAPD fingerprint showed a total of 12 unique bands (Table 4); these could be useful for further study by designing special marker for discriminating the genotypes.

Molecular markers can be used to study the genetic diversity and genetic relationships among *S. indicum* accessions at the DNA level. Work is currently in progress to provide a large number of valid RAPDs as well as co-dominant molecular markers such as microsatellites (Khadari et al., 2003). In conclusion, RAPD analysis revealed high levels of genetic variability in *S. indicum* accessions, even with the use of limited set of primers. This high level of polymorphism among populations suggests that RAPD techniques can be useful for sesame systematic for the maintenance of germplasm banks and the efficient selection of parents in breeding.

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