

GENETIC DIVERSITY OF SESAME (*SESAMUM INDICUM* L.) GERMPLASM FROM PAKISTAN USING RAPD MARKERS

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Abstract

Genetic diversity among 20 sesame (*Sesamum indicum* L.) accessions was examined at DNA level by means of random amplified polymorphic DNA (RAPD) analysis. Ten primers used produced a total of 93 RAPD fragments, of which 70 (75%) were polymorphic. Each primer generated 5 to 17 amplified fragments with an average of 9.3 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 accessions. Pair-wise similarity indices varied from 0.65 to 0.91. A UPGMA cluster analysis based on these genetic similarities located most of the accessions far apart from one another, showing a high level of polymorphism. Genetically, all the genotypes were classified into two major groups and six subgroups or clusters. A single accession (22243) was relatively distinct from rest of the accessions and created independent cluster. In conclusion, even with the use of a limited set of primers, RAPD technique revealed a high level of genetic variation among sesame accessions collected from diverse ecologies of Pakistan. This high level of genetic diversity among the genotypes suggested that RAPD technique is valuable for sesame systematic, and can be helpful for the upholding of germplasm banks and the 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 & 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 & 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 & Karlovsky, 2006).

Sesame is grown over 50 countries in the world. According to FAO (Anon., 2003), over 7 million of hectares were harvested worldwide in 2005, producing almost three million of 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. The country like Pakistan that spends a vast amount of foreign exchange worth USD\$1354 million on edible oil trade annually (Anon., 2008) is facing a sensitive shortage of irrigation water; at the present sesame is the best choice as an edible oil crop for agriculture. Its yield (219 kg/ha) is near to the

ground as compared to other chief sesame producing countries of the world such as China, Egypt and Honduras which are generating 1185, 1143 and 1133 kg seed yield per hectare, respectively (Anon., 2000). This low yield (219 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 & Umezaki, 1997), RAPD (Bhat *et al.*, 1999), ISSR (Kim *et al.*, 2002), AFLP (Uzun *et al.*, 2003) and SSR (Dixit *et al.*, 2005). In Pakistan 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 & 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 Pakistani sesame germplasm.

Pakistan is wealthy of sesame variation, over 172 sesame accessions are present in our country (Masood *et al.*, 2003). 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 Pakistani 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 accessions collected from different geographical areas of Pakistan through RAPD technique.

Materials and Methods

Plant material: Plant material for the study consisted of 20 sesame genotypes, collected from diverse ecologies of Pakistan (Table 1). These accessions were selected on the basis of their agronomic presentation in field conditions. Around 15~25 seeds of each sesame accession were grown in the greenhouse. After 20 days of seed sowing, fresh leaflets were collected from 10 plants of each accession, bulked together and stored at -80°C till the leaves were subjected for the extraction of total genomic DNA.

Table 1. List of sesame germplasm from Pakistan used for RAPD analysis.

Sr. No.	Accession Number	Collection locale	Province of collection
1.	19533	Lahore	Punjab
2.	19583	Multan	Punjab
3.	21334	Jhang	Punjab
4.	21376	Muzaffargarh	Punjab
5.	21406	Attock	Punjab
6.	21478	Toba Tek Singh	Punjab
7.	21530	Dera Ghazi Khan	Punjab
8.	21674	Narowal	Punjab
9.	21880	Faisalabad	Punjab
10.	22180	Ghotki	Sindh
11.	22193	Nawabshah	Sindh
12.	22209	Dera Ismail Khan	Khyber Pakhtunkhwa
13.	22217	Dera Ismail Khan	Khyber Pakhtunkhwa
14.	22243	Bhakkar	Punjab
15.	22247	Mianwali	Punjab
16.	22256	Bahawalpur	Punjab
17.	22272	Leyyah	Punjab
18.	SG62	Islamabad	Federal area
19.	SG119	Islamabad	Federal area
20.	21885	Faisalabad	Punjab

DNA extraction and PCR analysis: Total genomic DNA was extracted from the leaflet tissues of each sesame genotype using micro-prep DNA extraction protocol reported by Fulton *et al.*, (1995) 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 20ng/μl with TE buffer. Altogether, 40 decamer oligonucleotides from Operon Technologies Inc. (Alameda, California, USA) were experienced for identifying polymorphisms. After a

preliminary screen, 10 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 20μl. The reaction mixture contained 1x PCR buffer [10mM Tris-HCl (pH 8.3), 50mM KCl], 2mM MgCl₂, 0.25mM dNTP, and 0.4mM of 10-mer primer (Operon Technologies Inc., Alameda, CA), 1 unit Taq DNA polymerase and approximately 20ng of genomic DNA. For the prevention of reaction mixtures evaporation a one drop of mineral oil was added. DNA 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 94°C for first strand separation, pursued by 45 cycles of 1 min at 94°C for denaturation, 1 min at 36°C for annealing the DNA double strand and 2 min at 72°C for primer extension. At last, 1 cycle of 7 min at 72°C was employed for concluding extension, followed by drenched at 4°C. The reproducibility of the amplification products was confirmed twice for each RAPD primer. After amplification, 10µl of amplification products plus loading dye were loaded in 1.3% 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, gels were carefully stained with Ethidium bromide for 40 minutes and photographed under UV light by means of black and white film # 667 (Polaroid, Cambridge, Mass., USA).

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 accessions based on absence or presence of unique and shared DNA bands were utilized to make similarity coefficients. Estimates of genetic similarity (F) were calculated between all pairs of sesame accessions through Dice algorithm. The Dice algorithm is the same to that of Nei & Li (1979) as follows:

$$\text{Similarity (F)} = 2N_{xy} / (N_x + N_y)$$

Where N_x = the total number of fragments detected in individual 'x',

N_y = the total number of fragments shown by individual 'y' and

N_{xy} = the number of fragments shared by individuals 'x' and 'y'

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

The genetic diversity and the relationships among 20 sesame genotypes were evaluated by RAPD markers using 10 primers. Fig. 1 shows the pattern of amplified

products across sesame accessions generated with the primers OPA-04, OPA-09 and OPB-03, respectively. In most of the cases, sesame germplasm collections exhibited different banding patterns. Some of the accessions shared relatively lower number of bands with other germplasm accessions, showing their distant relationship to them. The accession '22243' demonstrated unique bands in contrast with all other sesame accessions. Interestingly, many primers showed characteristic bands in this accession which were not produced in any of the other collections. A total of 40 decamer primers were primarily screened for their potential to generate polymorphic patterns by means of four sesame accessions. Ten primers that exhibited reliable and consistent banding patterns were chosen for the evaluation of variability across all the accessions. Remaining thirty primers performed poorly and produced weak bands or did not amplify the DNA in some of the accessions utilized, were removed from the analysis. Each of the chosen 10 primers varied greatly in their ability to determine variability among the accessions. A total of 93 amplification products were produced by 10 primers across 20 accessions, out of which 70 (75%) fragments were polymorphic (Table 2). The number of amplification products generated by each primer ranged from 5 (OPA-03) to 17 (OPA-09) with an average of 9.3 fragments per primer. The size of the amplified fragments ranged from 800 (OPA-04) to 5000bp (OPD-08).

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 accessions. Pair-wise estimates of similarity for 20 accessions ranged from 0.65 to 0.91. Two pairs of accessions, 'SG62' and 'SG119', and 'SG62' and '21885' were the closest genotypes with the highest similarity index of 91%, while '21376' and '22209' were the least similar accessions. No accession was exactly similar to any other accession. Based on analysis carried out on Nei and Li's similarity matrix via UPGMA, 20 accessions were grouped together into main clusters (Fig. 2). First cluster (A) consisted of eleven genotypes, whereas second cluster (B) comprised of nine accessions. Each of the two clusters may be further sub-divided into three sub-groups. As expected from the similarity estimates, cluster analysis positioned most of the sesame accessions far apart from each other showing a high level of genetic diversity. However, some of the accessions of the same locality were grouped together in the same cluster revealed a nearer genetic relationship (e.g. cluster B2). One accession '22243' did not fall into any of sub-groups and formed a separate cluster which was distant from all the other sesame accessions evaluated.

Discussion

RAPD markers have been used in this study to assess the genetic diversity among the Pakistani sesame genotypes. 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).

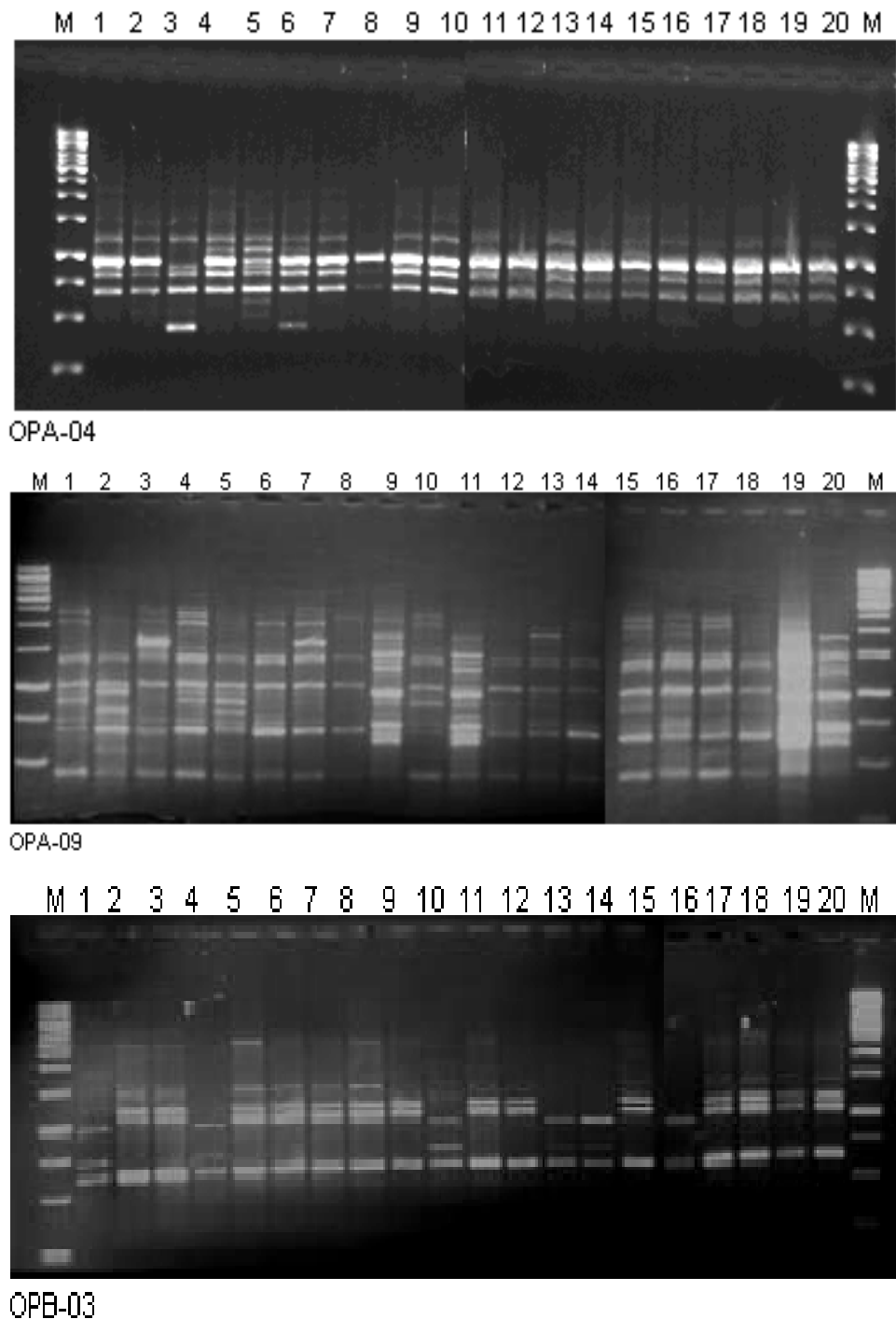


Fig. 1. Amplification of sesame accessions by RAPD markers OPA-04, OPA-09 and OPB-03. M = 1kb DNA ladder, 1 = 19533, 2 = 19583, 3 = 21334, 4 = 21376, 5 = 21406, 6 = 21478, 7 = 21530, 8 = 21674, 9 = 21880, 10 = 22180, 11 = 22193, 12 = 22209, 13 = 22217, 14 = 22243, 15 = 22247, 16 = 22256, 17 = 22272, 18 = SG62, 19 = SG119, 20 = 21885.

A high level of genetic variation was observed among the 20 Pakistani sesame genotypes. 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 & Godt, 1989). Hence, some cross-pollination could clarify the high level of genetic diversity examined in the same sesame accessions. 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 10 RAPD primers noticed sufficient genetic diversity among the 20 sesame genotypes 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 & 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 & 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).

Table 2. Primers used for generating RAPDs in Pakistani sesame genotypes.

Sr. No.	Primer names	Sequence (5' - 3')	Amplified fragments (a)	Polymorphic fragments (b)	Percentage polymorphism (bx100/a)	Fragment size range (bp)
1.	OPA-02	TGCCGAGCTG	11	8	72%	1500-3300
2.	OPA-03	AGTCAGCCAC	5	3	60%	900-2500
3.	OPA-04	AATCGGGCTG	11	9	82%	800-2500
4.	OPA-09	GGGTAACGCC	17	15	88%	1150-3900
5.	OPA-10	GTGATCGCAG	12	11	92%	900-4000
6.	OPA-16	AGCCAGCGAA	6	4	67%	1300-3300
7.	OPA-18	AGGTGACCGT	11	6	55%	1200-3250
8.	OPB-03	CATCCCCCTG	7	6	86%	900-2500
9.	OPD-08	GTGTGCCCA	7	4	57%	1400-5000
10.	OPK-04	CCGCCCAAAC	7	5	71%	1400-2000
Total		-	93	71	75%	-

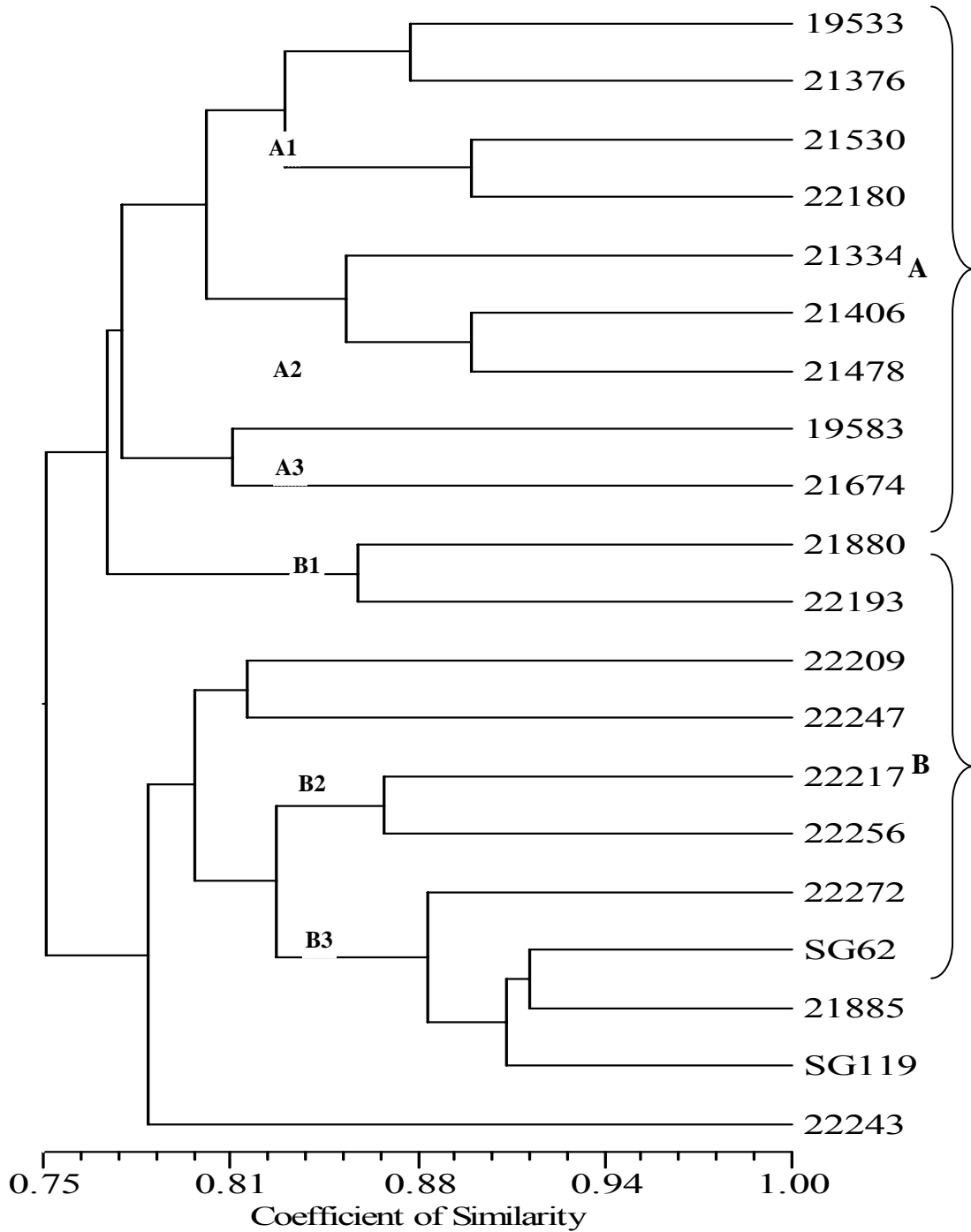


Fig. 2. UPGMA cluster analysis showing the relationship and diversity among 20 Pakistani sesame genotypes based on 93 RAPD fragments generated by 10 random primers.

Table 3. Genetic similarity coefficients among 20 sesame genotypes collected from diverse ecologies of Pakistan.

No.	Accession	19533	19583	21334	21376	21406	21478	21530	21674	21880	22180	22193	22209	22217	22243	22247	22256	22272	SG62	SG119	21885	
1	19533	1.00																				
2	19583	0.75	1.00																			
3	21334	0.79	0.79	1.00																		
4	21376	0.87	0.79	0.82	1.00																	
5	21406	0.77	0.80	0.83	0.82	1.00																
6	21478	0.82	0.79	0.88	0.83	0.89	1.00															
7	21530	0.83	0.76	0.85	0.81	0.78	0.85	1.00														
8	21674	0.76	0.81	0.78	0.78	0.75	0.80	0.82	1.00													
9	21880	0.87	0.77	0.78	0.81	0.80	0.83	0.85	0.78	1.00												
10	22180	0.86	0.73	0.76	0.82	0.75	0.80	0.89	0.76	0.85	1.00											
11	22193	0.75	0.77	0.69	0.74	0.72	0.72	0.70	0.74	0.85	0.71	1.00										
12	22209	0.72	0.70	0.69	0.65	0.71	0.73	0.79	0.75	0.74	0.74	0.72	1.00									
13	22217	0.78	0.72	0.76	0.76	0.76	0.78	0.79	0.75	0.78	0.80	0.76	0.82	1.00								
14	22243	0.78	0.70	0.69	0.75	0.66	0.69	0.79	0.80	0.71	0.73	0.69	0.81	0.81	1.00							
15	22247	0.78	0.74	0.78	0.73	0.75	0.80	0.77	0.78	0.78	0.74	0.78	0.82	0.82	0.77	1.00						
16	22256	0.82	0.68	0.72	0.76	0.69	0.73	0.81	0.73	0.80	0.80	0.76	0.77	0.86	0.84	0.80	1.00					
17	22272	0.79	0.74	0.75	0.76	0.70	0.75	0.81	0.76	0.80	0.79	0.80	0.82	0.80	0.82	0.82	0.80	1.00				
18	SG62	0.75	0.73	0.78	0.72	0.77	0.76	0.82	0.72	0.78	0.78	0.78	0.80	0.88	0.77	0.84	0.82	0.90	1.00			
19	SG119	0.78	0.73	0.76	0.74	0.72	0.74	0.79	0.70	0.81	0.78	0.80	0.76	0.83	0.73	0.80	0.82	0.89	0.91	1.00		
20	21885	0.70	0.71	0.74	0.68	0.73	0.74	0.77	0.67	0.77	0.76	0.78	0.79	0.85	0.72	0.77	0.81	0.85	0.91	0.90	1.00	

It should be noted that RAPD molecular markers could give high level of genetic diversity as compared to other molecular markers for example Isshiki & Umezaki (1997) identified a low level of genetic diversity in sixty eight sesame germplasms applying isozymes. Similarly Laurentin & 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 accessions by means of RAPD molecular markers.

In our study a high level of polymorphism was detected among sesame genotypes from diverse geographical regions of Pakistan. 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 (75%) 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 diverse sesame genotypes collected from various geographical regions of Pakistan, it was found that some accessions present geographically far apart clustered together in the identical group such as accession '22180' (from Sindh) and '21530' (from Punjab) grouped together in sub-group A1 (Fig. 2). This could be a result of large movement of Pakistani farmers to various regions (in Pakistan) moving sesame seeds for farming into their new geographical locations. According to Stankiewicz *et al.*, (2001) the human factor has earlier been revealed to be accountable for the lack of association between genetic and geographical detachment in some cases. The accession '22243' from Punjab region was found to be an extremely distinct genotype as it did not group with any other sesame accession. It seemed to be dissimilar species as it displayed a low level of similarity with all the other germplasm collections. This accession revealed certain unique bands which were not produced in any of the sesame collections evaluated. This result was further supported by its characteristic pattern of total seed proteins (data not shown) and important phenotypic features from the other genotypes. Some of the accessions evaluated appeared to be linked with their geographical sites and these genotypes from the similar geographical location were found to have a near genetic association. For instant sub-group B2 included accessions '21885', 'SG62' and 'SG119', from Federal region Islamabad. It also included accessions '22272' from Leyyah (Punjab). A close relation of this accession may be a result of free traffic between Leyyah and federal area farmer community. In conclusion, RAPD analysis showed a considerable level of genetic diversity among sesame accessions collected from different areas of Pakistan, even using as few as 10 RAPD primers. This high level of genetic variability among the sesame accessions proposed that the RAPD technique can be fruitful for the sesame systematics and selection of parents for breeding programs.

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