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Assessment of genetic diversity of sesame (*Sesamum indicum* L.) genotypes using morphological and RAPD markers

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Abstract

A comparison was made between phenotypic and molecular markers (RAPD) to assess the genetic diversity of twenty sesame genotypes representing, different agro-ecological zones of India. A higher range of genetic similarity (0.88-0.99) was observed on the basis of 20 phenotypic markers whereas, it was less (0.78-0.95) on the basis of RAPD markers, indicating better distinction between and within population of sesame when RAPD markers were used. Genotypes in RAPD based dendrogram were clustered into their respective geographical group while, a random grouping was observed in dendrogram based on the morphological markers. The genotypic classification agreed closely with the grouping observed in RAPD based principal coordinate analysis (PCA). The mean coefficient of genetic differentiation (Gst 0.619) indicated a high level of genetic diversity among population (Ht 0.169) and low level of genetic diversity within population (Hs 0.048). A negative correlation was found among discrimination power (Dj) and polymorphism information content (PIC) but the former was found effective in distinguishing all the populations as well as genotypes individually using specific band positions for them.

Key words: Sesame, genetic diversity, morphological markers, RAPD marker

Introduction

Sesame is one of the most important oilseeds crops. It is grown in tropical and subtropical areas on 6.5 million hectare worldwide producing more than three million tones of seeds. It belongs to the pedaliaceae family and is an annual, self pollinated oilseeds crop. Sesame oil comprising 50 % of the dry seed weight has been preferentially consumed in oriental food because of its distinctive flavor and the stabilizing antioxidant properties. It is used as active ingredient in antiseptics, viricides and disinfectants, and is a considerable source of calcium, tryptophan, methionine and many minerals. However, in spite of its importance sesame has been mentioned as an 'orphan crop' because it is not mandated to any of the CGIAR institutes which could also be one of the reasons for lack of research efforts [1].

Hence, the first and foremost need is the Identification or cataloguing of sesame genotypes along with the assessment of genetic diversity prevalent in different geographical regions in India. The genetic diversity analysis in sesame has been done using either morphological characteristics or molecular markers such as isozyme [2], RAPD [3, 4], ISSR [5] and AFLP [6]. But there are no reports on the identification and characterization of sesame germplasm using both the morphological and molecular markers so as to compare the two systems. However, the use of qualitative and quantitative morphological characteristics has only been accepted by ISTA as the uniqueness of the genotypes for grant of protection. But these characteristics are often affected due to gene x environment interaction, hence not much dependable.

Grouping of genotypes to their respective geographical locations and their individual identification with diversity analysis using both morphological markers as well as molecular markers (RAPD) are the main emphasis of the study. Among a large category of molecular markers, random amplified polymorphic DNA (RAPD) is useful for the assessment of genetic diversity [7] owing to their simplicity, speed and relatively low cost [8]. Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms [9, 10]. The resolving power of this tool is several folds higher than morphological or isozyme

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markers and is much simpler and technically less demanding than RFLP and other new generation markers. RAPD markers have proved their importance for diversity analysis in several crops and horticultural plants like neem [11], common bean [12], strawberry [13] and particularly in sesame [3, 4]. Since molecular based characterization of genotypes is independent of G x E interaction it may be an efficient and effective tool to understand and explain the genotype variability between and within geographical regions and ultimately in granting protection and crop improvement program.

The purpose of this study was i) to identify molecular markers in genotypic variability assessment of sesame so as to replace and/or supplement the morphological characteristics for grant of intellectual property rights and ii) to determine the genetic variability among and within the geographical regions to make use of it in crop improvement program of sesame.

Materials and methods

The genetically pure seed material of 20 sesame genotypes representing different geographical locations in India were collected from the Project Coordinator (sesame & niger), Indian Council of Agricultural Research, India. (Table 1).

Morphological marker analysis

All the 20 accessions for phenotypic characteristics analysis were planted in the experiment field of the Department of Seed Technology & Research, Agricultural Research Station, Durgapura, Jaipur (Rajasthan) in a plot size of 3.0 x 4.0 m (10 rows & 30 cm R x R spacing), replicated in randomized complete block design for three consecutive years (2005–2008). Twenty quantitative (8) and qualitative (12) characteristics were recorded according to UPOV, NBPGR, IBPGR & ISTA to analyze genotypic variability (Table 2). However, two characteristics, locule number per capsule and capsule number per leaf axil were not considered because of monomorphic nature and only 18 morphological characteristics were scored for all the genotypes.

Data analysis: morphological markers based Manhattan dis-similarity coefficients were calculated to find out the genotypic relationship using NTSYSpc 2.02e software. Dendrogram was constructed using Euclidean distance coefficients and Pearson correlation coefficients were calculated using the 18 variables to know the degree of relationship among them. The PCA was also carried out to display the distribution of the included genotypes. All these statistical calculations were carried out using NCSS 2007 version 07.1.14.

RAPD analysis

DNA Extraction / PCR amplification and electrophoresis: Total genomic DNA was extracted using the method of Doyle and Doyle, 1990 [14] from five days old seedlings. The quality of extracted DNA treated with RNase was assessed on 0.8% agarose gel and was finally quantified using Nano Drop spectrophotometer (ND-1000, Version 3.1.1, USA). For RAPD analysis, 200 random decamer primers from set # 1 & 2 obtained from the University of British Columbia, Vancouver, Canada were screened, of which 50 were amplified. Out of the 50 amplified primers, twenty were removed because of their monomorphic nature and poor reproducibility. The PCR reactions were performed in a 25 µl reaction mixture containing 1X *Taq* assay buffer, 0.5 units of *Taq* DNA polymerase, 200 µM of each dNTPs (Bangalore Genei Pvt. Ltd., India), 0.2 µM primers and 50 ng of template DNA. The PCR reactions were carried out in DNA thermal cycler (Model-CGI-96, Corbett Research, Australia). The PCR reactions were repeated thrice for each primer to ensure the reproducibility of RAPD results. The PCR amplification conditions for RAPD consisted of an initial extended step of denaturation at 94°C for 4 min followed by 44 cycles of denaturation at 94°C for 1 min, primer annealing at 37 °C for 1 min and elongation at 72 °C for 2 min followed by a final step of extension at 72 °C for 4 min. The PCR reaction products were fractionated on 1.2 % agarose gel containing 0.5 µg/µl ethidium bromide. After separation, gels were documented using Biovis Image Plus software (Expert Vision Pvt. Ltd. Mumbai).

Scoring and Data analysis: RAPD data were scored for the presence (1) or absence (0) and bands with same molecular weight and mobility were considered as a single locus. The total numbers of alleles, polymorphic alleles, average number of alleles per primer, polymorphism percentage, polymorphism information content (PIC) and discrimination power (Dj) [15] were calculated. Under discriminatory tool, total number of unique bands (NUB), different fingerprints (NF) per primer and the number of genotypes with unique fingerprints per primer (NGUF) were recorded. Similarity matrix for RAPD primers was constructed using the Jaccard's similarity coefficient values to find out genotypic relationship. These data were then subjected to UPGMA (unweighted pair-group method with arithmetic averages) analysis to generate dendrograms using NTSYSpc-version 2.02e (16).

Table 1. List of cultivars and their geographical location used in study

S. N.	Genotypes	Geographical region	S. N.	Genotypes	Geographical region
1.	RT-46	Mandor (RJ)	11.	T-12	Kanpur (UP)
2.	RT-54	Mandor (RJ)	12.	T-13	Kanpur (UP)
3.	RT-103	Mandor (RJ)	13.	T-78	Kanpur (UP)
4.	RT-125	Mandor (RJ)	14.	Usha (OMT-11-6-5)	Bhubaneshwar (Orissa)
5.	RT-127	Mandor (RJ)	15.	Uma (OMT-11-6-3)	Bhubaneshwar (Orissa)
6.	TMV-3	Vridhachalam (TN)	16.	Nirmala (OS-Sel-164)	Bhubaneshwar (Orissa)
7.	TMV-4	Vridhachalam (TN)	17.	Rajeshwari	Jagtial (AP)
8.	TMV-5	Vridhachalam (TN)	18.	Chandana (JCS-94)	Jagtial (AP)
9.	TMV-6	Vridhachalam (TN)	19.	TKG-21 (JT-21)	Tikamgarh (MP)
10.	T-4	Kanpur (UP)	20.	TKG-22 (JT-22)	Tikamgarh (MP)

RJ: Rajasthan, TN: Tamil Nadu. UP: Uttar Pradesh, AP: Andhra Pradesh, MP: Madhya Pradesh

Bootstrapping was done to test the robustness of clustering pattern using 1000 re-samplings with Free Tree software (version 0.9.1.50). To study the genetic structure of *S. indicum* species, the accessions were grouped in five sets according to their geographical distribution. The 0/1 matrix for this purpose was treated as a binary diploid data. Number of polymorphic loci, observed number of alleles (na), effective number of alleles (ne), Nei's gene diversity and Shannon information index (I) were calculated to find out the level of genetic diversity. We also calculated the total genotypic diversity among population (Ht), within population (Hst) and mean coefficient of genetic differentiation (Gst). All the calculations were done using POPGENE version 1.32 [17]. Principal coordinate analysis (PCA) was also carried out to display the location of the 20 genotypes in three dimensions using GenAlEx version 6.2 [18].

Results and discussions

Genetic variability as revealed by morphological markers

On an average 96 % genetic similarity was observed among 20 genotypes on the basis of Manhattan dissimilarity coefficients. TMV-4 from Tamil Nadu showed maximum dissimilarity (0.12) from two genotypes of Rajasthan RT-125 and RT-127. The maximum group diversity (0.08) was found between Rajasthan and Tamil Nadu. The maximum within group similarity (98 %) was found among genotypes from Uttar Pradesh followed by 97 % among Tamil Nadu (Table 5). The dendrogram based on the Euclidean distance coefficient clustered all the genotypes broadly into four groups. Genotypes from Tamil Nadu and Andhra Pradesh were grouped into same cluster while two genotypes from Andhra Pradesh, Rajeshwari and Chandana were splitted as single entries from rest of the genotypes of Tamil Nadu. However, geographically genotypes from Tamil Nadu were most distinct from others. Genotypes from Uttar Pradesh were grouped into a single cluster except, T-78 that splitted as a single entry from rest of the genotypes (Fig. 2A). Mixed clustering was also observed in case of Rajasthan genotypes though, three genotypes RT-125, RT-127 & RT-103 appeared in one cluster but RT-46 and RT-54 splitted with other populations. In PCA analysis, total 18 components are required for 100 % variation while first three coordinates axis accounted for 72.19 % of the total variation and the results were consistent with that revealed by Euclidean distance coefficient (Fig. 3A).

Polymorphism as revealed by RAPD

Twenty sesame accessions (Table 1) were analyzed by using 30 polymorphic and reproducible RAPD primers. Each primer-template yielded distinct, easily detectable bands of variable intensities. Indistinct bands produced by nonspecific amplification were ignored. The bands used for fingerprinting were those reproducible over repeated runs, with sufficient intensity to detect the presence or absence with confidence (Fig. 1). Considering all the primers and accessions, a total of 309 scorable amplification products were obtained, of which 43.68 % were polymorphic (Table 2). The number of amplification products produced per primer varied from 5 to 19, with a mean of 10.3 bands per primer. The size of scored bands ranged from 139 to 4346 bp. Although some bands were monomorphic, most cultivars produced unique amplification profiles sufficient to distinguish from each other confirming the efficiency of RAPD markers for the identification of sesame

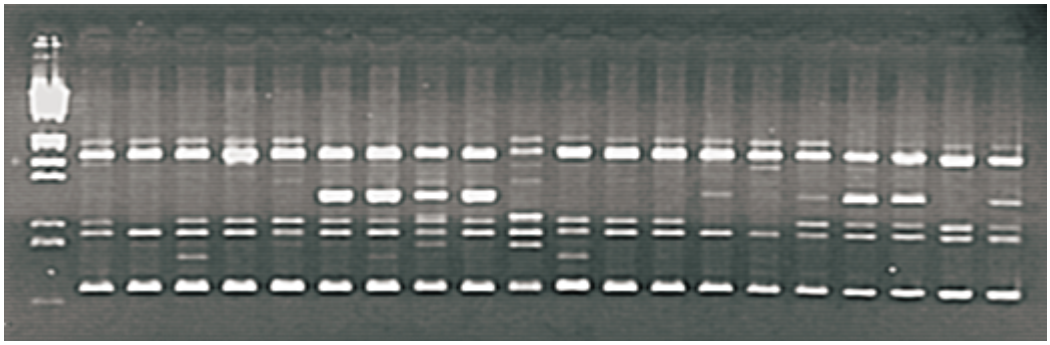

Fig. 1. PCR amplification profile of 20 sesame genotypes generated using RAPD primer no. 71; Lane1: RT-46; Lane 2: RT-54; Lane 3: RT-103; Lane 4: RT-125; Lane 5: RT-127; Lane 6: TMV-3; Lane 7: TMV-4; Lane 8: TMV-5; Lane 9: TMV-6; Lane 10: T-4; Lane 11: T-12; Lane 12: T-13; Lane 13: T-78; Lane 14: Usha; Lane 15: Uma; Lane 16: Nirmala; Lane 17: Rajeshwari; Lane 18: Chandana; Lane 19: TKG-21; Lane 20: TKG-22; M: ë DNA *Eco* RI/*Hind* III double digest

Table 2. List of morphological chcracterstics and their stage of observation employed for the study.

S. N.	Characteristics	Range of variable characteristics	Stage of observation	Type of assessment
1.	Initiation of flowering (days)	Early, medium, late	Flowering	VG
2.	Time of 50% flowering (days) Early <40, Medium 41-49, Late >50	Early, medium, late	Flowering	VG
3.	Flower : petal colour	White, light purple, dark purple	Flowering	VG
4.	Flower : petal hairiness	Absent, sparse, dense	Flowering	VS
5.	Plant : height of main stem (cm)	Short (<75 cm), medium (75-125 cm), long (>125 cm)	Completion of flowering	MS
6.	Plant : branching	Absent, few (1-2), many (>4)	Completion of flowering	VS
7.	Plant : branching pattern	Basal, top	Completion of flowering	VS
8.	Stem : hairiness	Absent, sparse, dense	Completion of flowering	VS
9.	Leaf : lobes	Slightly lobed, deeply lobed	Completion of flowering	VG
10.	Leaf : size	Small, medium ,large	Completion of flowering	VG
11.	Leaf : serration of margin	Week, strong	Completion of flowering	VG
12.	Capsule : hairiness	Absent, sparse, dense	Green capsule	VS
13.	Capsule : locule number per capsule	4, 6, 8	Green capsule	VS
14.	Capsule : shape	Tapered, narrow oblong, broad oblong, square	Harvest maturity	VG
15.	Capsule : number per leaf axil	One, more than one	Harvest maturity	VS
16.	Capsule : arrangement	Alternate, opposite	Harvest maturity	VG
17.	Capsule : length	Short (<1.6 cm), medium (1.6-2.5 cm), long (>2.5 cm)	Harvest maturity	MS
18.	Time of maturity	Early (<76 days), medium (76-85 days), late (>85 days)	Maturity	VG
19.	Seed : coat colour	White, light brown, dark brown, bleck	Post harvest	VG
20.	Seed : 1000 seed weight	Low (<2.6 g), medium (2.6-3.0 g), high (>3.0 g)	Post harvest	M

MG : Measurement by a single observation of a group of plants or parts of plants; MS : Measurement of a number of individual plants or parts of plants; VG : Visual assessment by a single observation of a group of plants or parts of plants; VS : Visual assessment by observation of individual plants or parts of plants

genotypes used in the study. In the present study 24 genotype specific band positions for 14 genotypes and 11 geographical specific band positions were obtained. Among the obtained band positions maximum were specific for Rajasthan and Tamil Nadu (Table 4).

Maximum percent polymorphism was obtained using RAPD primer No. 71 (83.71) followed by 189 (81.81). Maximum discriminating power was obtained with primer No. 189 (0.97) followed by primer No. 98 (0.96) (Table 3). There was no correlation between percent polymorphism and discriminating power as RAPD primer no. 71 showed maximum percent polymorphism (89.7) while its Dj value was 0.86. In another example, primer no. 98 showed 0.96 Dj value with 72.72 % polymorphic bands. Jaccard's similarity coefficients among the all pair-wise combinations of accessions ranged from 0.78 to 0.95 with a mean genetic similarity of 0.85. Maximum similarity (0.95) was found among the genotypes from the same geographical region as Usha and Uma from Orissa and TMV-4 and TMV-6 from Tamil Nadu (Table 5). Dendrogram based on UPGMA analysis separated nearly all the genotypes clearly into their respective groups. Genotypes from Rajasthan (except RT-54), and UP were clustered into a single group which further separated into two sub-groups according to their state of origin. Genotypes from Orissa, Andhra Pradesh and Madhya Pradesh were clustered into same group which sub-clustered into their respective groups except Nirmala that was clustered with genotypes from Madhya Pradesh (Fig. 2B). The first three coordinate axes accounted for 71.63 % of the total variation using PCA analysis (Fig. 3B). Data on number of alleles (na), effective number of alleles (ne), Nei's genetic diversity and Shannon's information index, for all the populations were analyzed and their values were found as 1.440±0.497, 1.2165±0.3249, 0.1048 and 0.2012±0.2599, respectively.

Comparison on genetic variability as revealed by morphological and RAPD markers

A weak correlation between Euclidean based genetic distance matrices indicated the discrepancy between the morphological and RAPD markers based analysis which is also supported by the greater genetic similarities (0.88-0.99) based on morphological characteristics as compared to that of RAPD based (0.78-0.95) analysis (Table 5). The greater similarity among phenotypic data might be either due to lack of genetic variability and/or due to G x E interaction resulted in different and variable patterns. The two methods were not found comparable in distinguishing all the genotypes individually which was evident by the dendrogram patterns and PCA analysis (Fig. 2 & 3). Several other comparisons between morphological and molecular marker based study also indicated similar results [19, 20, 21]. So, phenotypic markers are required to be assessed very carefully and timely. Highest value for similarity coefficient (0.86) was observed between capsule shape and petal hairiness followed by leaf size and 50 % flowering (0.85) while it was negative (-0.82) between plant branching and time of maturity followed by plant branching and leaf size (-0.80). All quantitative characteristics were positively correlated except capsule length which was negatively correlated with rest of the variables. Similar observation was also observed by Sarwar *et al*. [22]. A positive correlation (0.73) was observed between stem hairiness and capsule hairiness, which was in accordance with the previous study of Mahajan *et al*. [23].

In case of sesame, seed coat color was found most stable character followed by stem hairiness while petal color, leaf size and serration of leaf margin were most fluctuating in a given environmental condition that was probably the reason why mixed grouping was observed in the dendrogram based on phenotypic markers (Fig. 2A) whereas, RAPD marker based dendrogram clustered all the genotypes corresponding to their geographical origin (Fig. 2B). Genotypes from Rajasthan and Uttar Pradesh were grouped into same cluster but subdivided into their respective geographical groups. One of the genotype, RT-54 from Rajasthan was clustered outside the group indicating more novel band positions of this genotype. The distinctness of RT-54 was reported by Sharma *et al*. [24] and as indicated earlier it is also morphologically different, early maturing and dwarf genotype.

Level of genetic diversity between and within populations

Genotypes from Tamil Nadu were clustered into a different group indicating a strong resemblance among genotypes showing 94 % genetic similarity while they were genetically far distant from other genotypes at 0.144 Nei's genetic distance. A high level of genetic diversity among populations and low genetic diversity within populations were detected based on the total genotypic diversity among populations (Ht – 0.169) and within population (Hs – 0.0483) and also on the basis of mean coefficient of gene differentiation (Gst – 0.619) indicating 61.9 % genetic variability among populations and 38.1 % within populations. Low level of genetic diversity within populations and significant differentiation

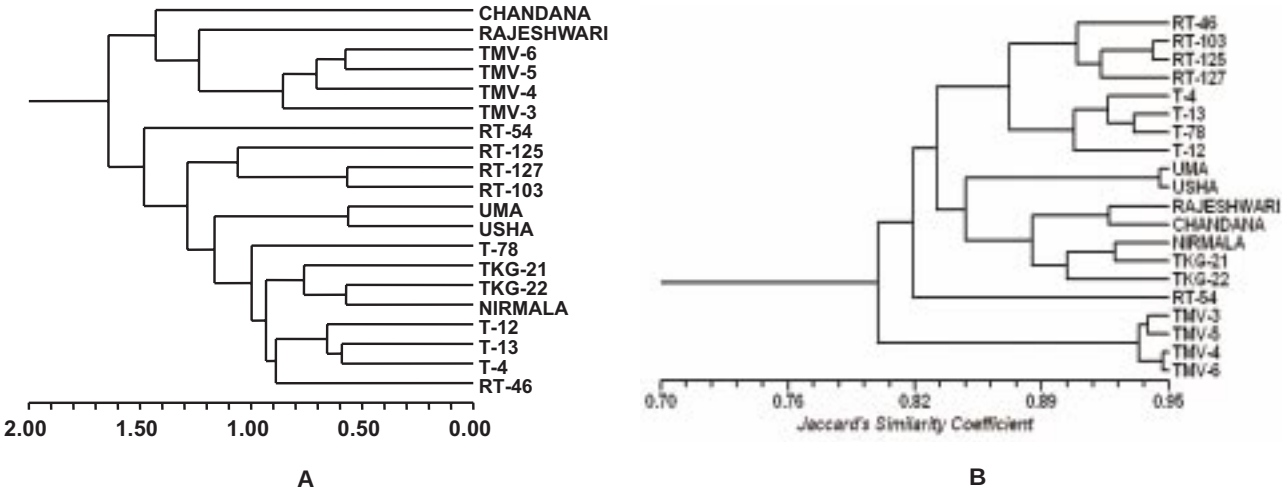

Fig. 2. Dendrogram derived from UPGMA cluster analysis using A) Euclidean distance coefficient of morphological markers and B) Jaccard's similarity coefficient of RAPD based markers

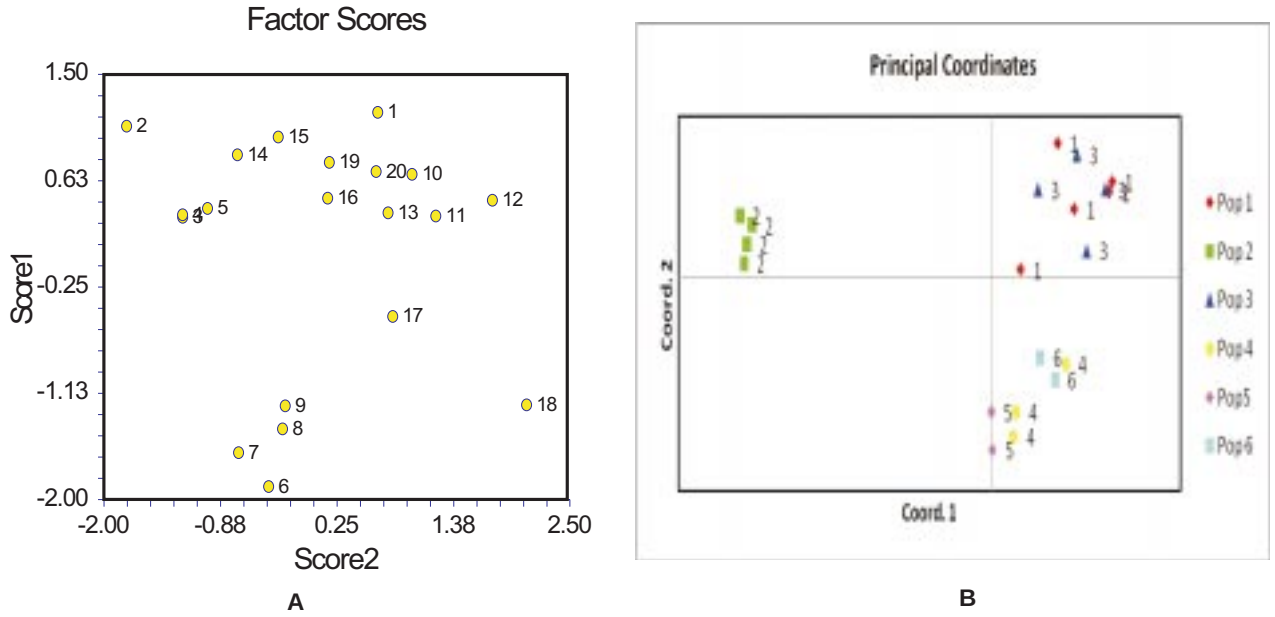

Fig. 3. Two dimensional scaling of 20 sesame genotypes by principal co-ordinate analysis (PCA) using the pooled genetic distance matrix from A) morphological markers B) RAPD markers

among populations might be due to the localized breeding system in which gene flow across the geographical regions and inbreeding depression and/or selection within the population played a significant role. The average genotypic variability (0.01-0.12) of morphological marker analysis was much less than that of RAPD marker analysis (0.09-0.25). The reason was the RAPD markers scattered throughout the genome which revealed the diversity of the entire genome while morphological markers either the target gene are less and/or modified by the G x E interaction, resulted in poor differentiation under field conditions.

Uniqueness of genotypes

The Dj and PIC values were calculated to find out the efficiency of primers in distinguishing individual genotypes. A negative correlation (-0.05) was found between the two discriminatory tools i.e. Dj & PIC, indicating different aspects of the two, as the Dj depends on the unique banding patterns while the later on the

Table 3. Primer sequence, total bands, polymorphic bands, banding patterns and other calculated parameters of polymorphism using RAPD primers.

Primer	Sequence (5'-3')	TB	PB	% P	TBP	UBP	Dj	PIC
6	CCTGGGCCTA	14	07	50.00	11	07	0.91	0.13
12	CCTGGGTCCA	15	04	26.26	07	01	0.93	0.11
23	CCCGCCTTCC	08	05	62.50	09	03	0.85	0.20
34	CCGGCCCCAA	07	02	28.57	03	01	0.42	0.67
61	TTCCCCGACC	06	02	33.33	03	01	0.56	0.10
71	GAGGGCGAGG	07	06	85.71	10	07	0.86	0.23
72	GAGCACGGGA	07	03	42.85	04	01	0.71	0.14
77	GAGCACCAGG	05	01	20.00	02	00	0.44	0.08
82	GGGCCCGAGG	05	02	40.00	03	02	0.19	0.20
83	GGGCTCGTGG	08	05	62.50	06	01	0.17	0.16
95	GCGGGGTTGG	14	07	50.00	12	05	0.70	0.16
96	GGCGGCATGG	19	08	50.00	10	00	0.91	0.14
98	ATCCTGCCAG	11	08	72.72	15	07	0.96	0.21
101	GCGGCTGGAG	17	03	17.64	06	02	0.81	0.06
103	GTGACGCCGC	13	06	46.15	09	04	0.90	0.16
104	GGGCAATGAT	13	06	46.15	10	05	0.89	0.18
105	CTCGGGTGGG	13	04	30.76	13	09	0.95	0.10
106	CGTCTGCCCG	15	07	46.66	05	01	0.68	0.17
122	GTAGACGAGC	8	03	37.50	04	02	0.62	0.08
125	GCGGTTGAGG	15	04	26.66	09	04	0.90	0.09
127	ATCTGGCAGC	10	04	40.00	05	02	0.68	0.08
132	AGGGATCTCC	14	04	28.57	07	03	0.82	0.09
134	AACACACGAG	07	01	14.28	02	01	0.10	0.01
147	GTGCGTCCTC	07	04	57.14	03	00	0.63	0.18
154	TCCATGCCGT	07	04	57.14	06	03	0.72	0.15
156	GCCTGGTTGC	10	06	60.00	10	06	0.89	0.17
157	CGTGGGCAGG	06	04	66.66	05	02	0.75	0.21
180	GGGCCACGCT	07	03	42.85	04	02	0.62	0.09
186	GTGCGTCGCT	10	03	30.00	07	03	0.70	0.10
189	TGCTAGCCTC	11	09	81.81	15	11	0.97	0.25

polymorphic band positions hence, Dj was found better in distinguishing genotypes. RAPD primers showing more than 0.80 Dj value were found effective and could be employed for the identification of the genotypes because of their unique fingerprints. The importance of the Dj value was proved earlier by Tissier *et al*. [15] for horticultural plant (*Vitis vinifera*) and particularly for sesame by Sharma *et al*. [24]. Genotype specific as well as population specific band positions made this technique highly applicable and effective for the genotyping of sesame collections. The band positions specific for a genotype or population were only included after confirmation of their reproducibility, the only few RAPD primers were identified with clear differentiation. The consistent clustering of most breeding entries close to each other in the present study apparently substantiates that the RAPD marker system have a high potential in quantifying the level of similarity and relationship among sesame genotypes. Furthermore, the results showed that by using the RAPD technique, a large set of informative data could be generated in less time than with morphological characteristics which

Table 4. Unique band positions for individual genotypes and geographical location produced by RAPD primers.

S.No.	Genotype	RAPD primer	Marker zize (bp)	A/B	RAPD primer	MW	Location	A/B
1.	RT-46	134	502	B	103	3406 0963	Rajasthan Tamil Nadu	 B
2.	RT-54	82 127	1014 1850	B B	147	4346 1214	Tamil Nadu Tamil Nadu	B A
3.	RT-103	125	1840	A	189	2871 1357	Rajasthan Rajasthan	B B
4.	RT-125	157	1766	A	072	1375	Tamil Nadu	A
5.	RT-127	95	1161	A	096	0599	Tamil Nadu	A
6.	TMV-3	06 98 156	3530 633 927	B A	095 B	0764	Uttar Pradesh	A
7.	TMV-4	189	1135	B	127	2401	Andhra Pradesh	B
8.	TMV-6	61 95	284 1289	A A	101	564	Orissa	B
9.	T-4	154 189	1268 1531	B A				
10.	T-12	06 71	820 1352	A B				
11.	T-78	127	807	A				
12.	Usha	34 71	724 1014	B B				
13.	Uma	83	1648	A				
14.	TKG-22	72 96 189	552 1537 1255	A A A				

A: Present; b: Absent

took more time and resulted in mixed grouping of genotypes.

It can be concluded from the present investigation that the RAPD marker based analysis is an effective and reproducible technique for the characterization of sesame genotypes as grouping was found good and in accordance with their geographical regions. The obtained genotype/population specific band positions could be converted into sequence characterized amplified regions (SCARs) that could replace and/or supplement the morphological characteristics for the identification of these genotypes. The identification of sesame genotypes using RAPD primers is a cost and time effective and robust technique making it suitable for the DUS characterization and might eliminate the need to grow the genotypes for their morphological marker analysis. The reduced genetic diversity within populations is alarming to the crop improvement program of sesame.

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Table 5. Jaccard's similarity coefficients (below diagonal) and Manhattan dis-similarity coefficients (above diagonal) based on RAPD and morphological markers for 20 sesame genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
RT-46	-	0.04	0.03	0.02	0.02	0.10	0.11	0.09	0.06	0.03	0.03	0.02	0.05	0.03	0.04	0.03	0.08	0.05	0.02	0.04
RT-54	0.85	-	0.04	0.05	0.04	0.08	0.10	0.07	0.05	0.03	0.03	0.04	0.05	0.01	0.02	0.04	0.06	0.05	0.04	0.05
RT-103	0.91	0.83	-	0.03	0.03	0.08	0.09	0.06	0.04	0.02	0.03	0.02	0.03	0.03	0.03	0.02	0.06	0.03	0.03	0.03
RT-125	0.91	0.85	0.94	-	0.02	0.11	0.12	0.09	0.07	0.04	0.04	0.03	0.05	0.04	0.05	0.04	0.09	0.05	0.02	0.04
RT-127	0.89	0.85	0.92	0.91	-	0.10	0.12	0.09	0.07	0.04	0.04	0.03	0.05	0.04	0.04	0.04	0.08	0.05	0.02	0.04
TMV-3	0.82	0.80	0.79	0.82	0.80	-	0.03	0.02	0.04	0.08	0.07	0.08	0.07	0.08	0.06	0.07	0.03	0.07	0.09	0.07
TMV-4	0.81	0.79	0.80	0.82	0.82	0.94	-	0.03	0.06	0.09	0.09	0.10	0.09	0.09	0.08	0.09	0.05	0.08	0.11	0.08
TMV-5	0.83	0.79	0.79	0.81	0.81	0.94	0.94	-	0.03	0.06	0.06	0.07	0.07	0.06	0.05	0.06	0.02	0.05	0.08	0.06
TMV-6	0.79	0.78	0.78	0.81	0.81	0.93	0.95	0.93	-	0.04	0.04	0.05	0.05	0.04	0.03	0.04	0.03	0.04	0.06	0.04
T-4	0.86	0.79	0.87	0.87	0.86	0.78	0.81	0.81	0.80	-	0.01	0.01	0.03	0.02	0.02	0.02	0.05	0.04	0.02	0.03
T-12	0.87	0.81	0.89	0.87	0.85	0.81	0.81	0.81	0.79	0.88	-	0.02	0.03	0.02	0.02	0.02	0.05	0.04	0.03	0.03
T-13	0.86	0.81	0.86	0.87	0.88	0.83	0.85	0.84	0.85	0.92	0.92	-	0.03	0.03	0.03	0.02	0.07	0.03	0.02	0.02
T-78	0.87	0.83	0.89	0.90	0.87	0.80	0.82	0.82	0.81	0.92	0.91	0.93	-	0.04	0.04	0.04	0.06	0.04	0.04	0.03
Usha	0.78	0.84	0.81	0.83	0.81	0.78	0.79	0.79	0.80	0.82	0.81	0.84	0.86	-	0.02	0.03	0.06	0.05	0.04	0.04
Uma	0.79	0.83	0.81	0.84	0.81	0.78	0.80	0.79	0.80	0.83	0.80	0.82	0.86	0.95	-	0.03	0.04	0.05	0.04	0.04
Nirmala	0.84	0.80	0.87	0.87	0.86	0.80	0.82	0.81	0.80	0.86	0.86	0.86	0.90	0.85	0.86	-	0.05	0.03	0.03	0.02
Rajeshwari	0.79	0.80	0.81	0.83	0.83	0.81	0.83	0.81	0.80	0.83	0.81	0.85	0.84	0.83	0.83	0.89	-	0.05	0.07	0.06
Chandana	0.79	0.82	0.80	0.84	0.81	0.81	0.82	0.83	0.81	0.82	0.80	0.83	0.84	0.86	0.86	0.90	0.92	-	0.04	0.02
TKG-21	0.86	0.82	0.87	0.88	0.87	0.80	0.83	0.82	0.81	0.87	0.85	0.87	0.89	0.84	0.85	0.92	0.88	0.90	-	0.03
TKG-22	0.83	0.82	0.85	0.86	0.84	0.79	0.80	0.80	0.79	0.82	0.82	0.83	0.85	0.85	0.86	0.90	0.85	0.88	0.90	-

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