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Characterization and analysis of genetic diversity in Indian Sesame (*Sesamum Indicum* L.) genotypes

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Abstract

Limited information is available on the extent of genetic variability in sesame. In this study, twenty phenotypic (qualitative and quantitative) traits and 200 RAPD markers were used to determine the extent of genetic diversity among 60 sesame varieties grown in different geographical regions of India. Fourteen RAPD markers were found useful in assessing the diversity. A high level of genetic variability among population (H_T = 0.1991) and less variability within population (H_S = 0.0749) was observed. Mean coefficient of gene differentiation (G_{ST} = 0.6238) was 62.38 % among sesame population and 37.62% within population. The study indicated that the Indian sesame lines are genetically diverse, which should be utilized in the improvement of sesame crop.

Key words: Genotype characterization, genetic diversity, phenotype; RAPD markers, *Sesamum indicum* L.

Introduction

Sesame belonging to Pedaliaceae family is one of the most important ancient oilseed crops. It was cultivated and domesticated in the Indian subcontinent during Harappan and Anatolian eras [1, 2] but now it is grown in tropical and subtropical areas in many part of the world. Sesame oil is highly nutritive (50% oil and 25% protein), used as oriental food for its distinctive quality due to presence of natural antioxidants such as sesamin and sesamol [3]. Though, India is the largest sesame growing country with 1.8 to 1.9 mha, accounting for about 25% of the global sesame cultivated area, the sesame productivity is 386.53 Kgha^{-1} (Sesame & Niger, ICAR unit, http:// www.jnkvv.nic.in) still lower than the world productivity (442.73 Kgha^{-1}). Genetic diversity is the key to successful crop improvement program. Sesame improvement program in India has been lagging behind as no systematic efforts have been made to characterize and document the extent of genetic variability available among Indian sesame varieties released for cultivation in different parts of the country [4].

Morpho-physiological descriptors are conventionally used for establishing the uniqueness of a variety and also for genetic diversity analysis [5]. In recent years, molecular markers have been employed to determine the distinctness of crop varieties [6, 7]. Molecular markers define differences in nucleotide sequences which remain unaffected by growth stage, season, location and agronomic practice [8, 9] as against morphological markers which are controlled by polygenes and are influenced by environment [10, 11]. Among a large category of molecular markers, random amplified polymorphic DNA (RAPD) markers are useful for the assessment of genetic diversity [12, 13] owing to their simplicity, speed and relatively low cost. The usefulness of RAPD, ISSR and SSR markers have been demonstrated in genetic diversity assessment for selected sesame varieties and molecular markers were found superior than the phenotypic traits in genotypic characterization [9, 14-16]. The present study was conducted with the objectives (a) whether RAPDs, in comparison to morphological descriptors, may be used to assess

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the genetic diversity in sesame varieties released for cultivation in India and (b) to determine the extent of genetic variability among and within twelve diversity centers in India so that the information on variability of desirable traits be exploited in the sesame improvement program.

Materials and methods

The genetically pure seed materials of 60 sesame varieties, representing 12 different geographical locations of India, were collected from the Project Coordinator (Sesame & Niger), Indian Council of Agricultural Research, New Delhi, India (Table 1). Each geographical region represents a combination of soil type, latitude and longitude and all the varieties grown in a given geographical region are regarded as one sub-population (Subp). The present study was carried out on 12 sub-populations.

Morphological marker analysis

Seeds of 60 varieties were planted in the experimental field of the department of Seed Science and Technology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur in a plot size of 3.0 x 4.0 m (10 rows with 30 cm row x row spacing), replicated in randomized complete block design for three consecutive years. Twenty phenotypic traits including 6 quantitative and 14 qualitative traits were recorded as per the guidelines of Union for the Protection of New Varieties of Plants (UPOV) [17], National Bureau of Plant Genetic Research (NBPGR) and International Plant Genetic Resources Institute (IPGRI) [18] and International Seed Testing Association (ISTA) [5] to analyze phenotypic variability. One characteristic, locule number per capsule was not included in the analysis because only single state was observed in all the varieties.

Phenotype data analysis

The qualitative data were arranged as a nominal variable whereas quantitative data were used as discrete variable. All the modified morphological variables were standardized by subtracting the mean and dividing by maximum values. The Manhattan dissimilarity coefficients were calculated using the standardized morphological data to find out the genotypic relationship among sesame varieties through cluster analysis (NTSYSpc 2.02e software; [19]). The Principal Coordinate Analysis (PCA) was also carried out to display the distribution of the varieties in three-dimensional space, using NCSS 2007 version 07.1.14.

DNA extraction

Total genomic DNA was extracted using the method of Doyle and Doyle [20], from five days old seedlings. The quality of extracted DNA after RNase treatment was assessed on 0.8% agarose gel and was finally quantified using Nano-Drop Spectrophotometer (ND-1000, Version 3.1.1, USA).

RAPD primer selection and PCR amplification

Sixty RAPD primers were amplified from a set of 200 random decamer primers of set number 1 and 2, obtained from the University of British Columbia, Vancouver, Canada. Out of 60 RAPD primers amplified only 14 were included because of their 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), 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/ml 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 the bands with same molecular weight and mobility were considered as a single locus. These data were then subjected to UPGMA (Unweighted Pair-Group Method with Arithmetic Average) analysis to generate dendrograms using NTSYSpc-version 2.02e [19]. A dendrogram demonstrating the relationship among the 12 sub-populations based on Nei's genetic distance was established according to the UPGMA. Bootstrapping was done to test the robustness of clustering pattern using 1000 re-samplings with Free Tree software (version 0.9.1.50). Principal component analysis (PCA) was also carried out to depict the relationship among 60 cultivated sesame varieties in three dimensions using GenAlEx version 6.2 [21]. Correlations between molecular and morphological distance matrices was performed by Mantel test using

Table 1. List of sesame genotypes with their parentage and the location of their cultivation used in the study

Cultivar	Parentage	Location	Cultivar	Parentage	Location
RT-46	T-12 x Punjab Til-1	Mandor (Raj.)	Sweta Til	E-8 x IS-13	Jagtial (A.P.)
RT-54	A6-3 x ES 6-1	"	YLM-11 (Varaha)	Vinyak x Kanak	"
RT-103	C-7 x A6-5	"	YLM-17 (Gautama)	Vinayak x Kanak	"
RT-125	Type-13 x RT-1	"	Hima	No. 5039xAT-1	"
RT-127	S I 3500 x Patan- 64	"	Usha (OMT-11-6-5)	Mutant of Kanak	Bhubaneshwar (Orissa)
Kanchan (JT-7)	NP 6 x Punjab Til and SH-446	Tikamgarh (M.P.)	Uma (OMT-11-6-3)	Mutant of Kanak	"
TKG-21 (JT-21)	Punjab Til-1 x TC-25	"	Nirmala (OS-Sel-164)	Mutant of B-67	"
TKG-22 (JT-22)	HT-6 x JLT-3	"	Vinayak	Sel. from Matnasani local	"
TKG-55 (JT-55)	TC-25 x TNAU-10	"	Kanak	Vinayak x T-4	"
TKG-306(JT-306)	CST-785 x TKG-22	"	Kalika (B-3-7)	Mutant of Vinayak	"
PKDS-11(Venktesh)	Selection from Bichhua Local-3	Powerkheda (M.P.)	Prachi (ORM-17)	Mutant of B-67	"
TMV-3	Local x Malabar wild	Vridhachalam (T. N.)	Kayam Kulum-1	Sel.from Kayamkulam local	Kayamkulum (Kerala)
TMV-4	Selection from Sattur local	"	Thilarani	Thilak x Kayamkulam-1	"
TMV-5	Selection	"	Thilothama	PT-58-35 x Kayamkulam-1	"
TMV-6	Sel. from local material of AP	"	Thilthara	CST-785 x B-14	"
SVPR-1	Selection of Western Ghat (TN)	"	Phule Til-1	D-7-11-1x N-58-2	Jalgaon (MS)
CO-1	TMV-3 x SI-1878	Coimbatore (T.N.)	JLT-7	N-58-2 x C-50	"
Paiyur-1	Six white 2511 x SI-2314	Paiyur (T. N.)	JLT-8	Padma x Yuzhi-8	"
VRI-I	Selection from local material	Tamil Nadu	JLT-26	Phule Til-1x N-32.	"
VRI-II	VS-9003 x TMV-6	Tamil Nadu	AKT-64	N-128 x C-50	Nagpur (MS)
T-4	T-10 x T-3	Kanpur (U.P.)	AKT-101	N-62-10 x R-19	"
T-12	Selection	"	N-32	Sel.from Chattarpur district	"
T-13	Selection	"	GT-1	M.T- 67-52	Amreli (Gujarat)
T-78	NP-6 x T-4	"	GT-2	Gujarat Til-1x TC-25	"
Pragati (MT-75)	JLT-26 x RT-127	Mauranipur (U.P.)	GT-10	Selection from TNAU-17	"
Shekhar	T-4 x T-12	Kanpur (U.P.)	Kapli	Local genotype of Gujarat	Local Variety (Gujarat)
Local Chaupala	Sel. from local collection of UP	Local Variety (U.P.)	DS-1	Gulbarga local x JT-58-135	Dharwad (KK)
Rajeshwari	N-62-39 x I-5001	Jagtial (A.P.)	E-8	Sel. from Northern KK	"
Chandana (JCS-94)	T-85 x L-5107	"	Brijeshwari	Sel.from Kangra Local	Kangra (HP)
Madhvi (SP-1181)	Sel.from local germplasm	"	Tilottama	Sel. from Jinardi Ducca-2	Berhampura (WB)

Sel. = Selection; Raj= Rajasthan; U.P.= Uttar Pradesh; M.P.= Madhya Pradesh; T.N.= Tamil nadu; A.P.= Andhra Pradesh; MS= Maharashtra; KK= Karnataka; H.P.= Himachal Pradesh

Passage software 1.1 [22, 23] which assumes that the two matrices were obtained independently. Genetic diversity was measured by the percentage of polymorphic bands, Shannon information index [24] and Nei's gene diversity [25]. The sub-population differentiation (G_{ST}) was calculated using POPGENE [26].

Results and discussion

Phenotypic analysis of sesame varieties

Due to mismatch of quantitative scores observed during consecutive years of experimentation, the average score of three years was considered to analyze the data for distinctness and the relationship among varieties. Manhattan dissimilarity coefficient clustered 60 varieties into four distinct groups at an average cut off value of 0.17. However, the clustering of varieties was not found according to their geographical locations (Fig. 1a). When morphological markers were used for diversity assessment, similar results and conclusions were drawn in other crops earlier [27-29]. The first three axis of principal component analysis accounted 58.04% of the total variation, it was 25.92 % for the first axis while 20.02 % and 12.11 % was observed for the second and third axis, respectively. When axis first was plotted against axis second, no distinct group was outlined in three dimensional plot of PCA (Fig. 2a). Quantitative traits such as leaf size, seed weight, plant height, days to maturity were found inconsistent in subsequent years of experimentation. Similarly, some qualitative traits *viz*., leaf lobe, petal color and leaf serration were not found stable in a given environmental conditions that might be the reason why mixed grouping was observed in dendogram based on phenotypic markers. The seed coat color was found most consistent throughout the study followed by stem/ capsule/petal hairiness. The capsule/leaf axil was an exclusive characteristic of GT-1, GT-2 and Local Chopala.

Polymorphism as revealed by RAPD

Out of 200, only 14 RAPD primers were found reproducible and amplified distinct, easily detectable bands of variable intensities. Considering all the primers and varieties, a total of 148 amplicons were obtained, of which 69.59 % were polymorphic (Table 2). Maximum polymorphic amplicons were observed in varieties from Tamil Nadu (45.95 %) followed by Orissa (35.81 %). The number of amplification products per primer varied from 6 to 18, with a mean of 10.6 bands per primer. The size of scored bands ranged from 300 to 3000 bp. Although some bands were monomorphic but most of the cultivars produced unique amplification profiles sufficient to distinguish from each other, confirming the efficiency of RAPD

Table 2. List of RAPD markers employed in the study and their statistics

Primer	Sequence (5'-3')	TB	PB	% P	TBP	UBP	Dj
06	CCTGGGCCTA	14	13	92.85	20	09	0.91
23	CCCGCCTTCC	18	16	88.88	37	28	0.97
95	GCGGGGTTGG	12	09	75.00	32	19	0.96
96	GGCGGCATGG	15	07	46.66	19	07	0.91
101	GCGGCTGGAG	10	06	60.00	22	12	0.90
103	GTGACGCCGC	08	05	62.50	16	05	0.90
104	GGGCAATGAT	06	01	16.66	02	00	0.43
125	GCGGTTGAGG	10	07	70.00	10	03	0.70
127	ATCTGGCAGC	07	03	42,85	05	00	0.67
132	AGGGATCCTCC	13	07	53.84	26	15	0.94
134	AACACACGAG	07	07	100	12	06	0.79
147	GTGCGTCCTC	09	08	88.88	17	09	0.80
156	GCCTGGTTGC	08	06	75.00	09	04	0.39
157	CGTGGGCAGG	11	08	72.72	13	04	0.87
Total		148	103	69.59			

TB = total number of bands, PB = polymorphic bands, %P = % polymorphism, TBP = total number of banding patterns, UBP = unique banding pattern, Dj = discrimination power

markers for the identification of individual sesame variety and also for grouping of varieties in to their respective geographical region (sub- population). UPGMA based dendrogram clustered all the varieties according to their geographical regions at an average cut off value of 0.77 (Fig. 1b). First three axis of principal component analysis (PCA) accounted 64.57% of the total Eigen value or variation (Fig. 2b) and the results were in accordance with the UPGMA based dendrogram. According to the polymorphic bands, the amount of genetic variation among the twelve subpopulations was of the order of subp-11 < subp-10 < subp-12 < subp-9 < subp-8 < subp-1 < subp-7 < subp-4 < subp-2 < subp-5 < subp-3 < subp-6. Almost similar order of genetic variation was obtained on the basis of Shannon information index, subp-11 > subp-10 < subp-12 < subp-9 < subp-1 < subp-8 < subp-7 < subp-4 < subp-2 < subp-5 < subp-3 < subp-6 indicating greater genetic distance between the subp-6 and subp-11. The subp-6 and subp-11 are located far away from

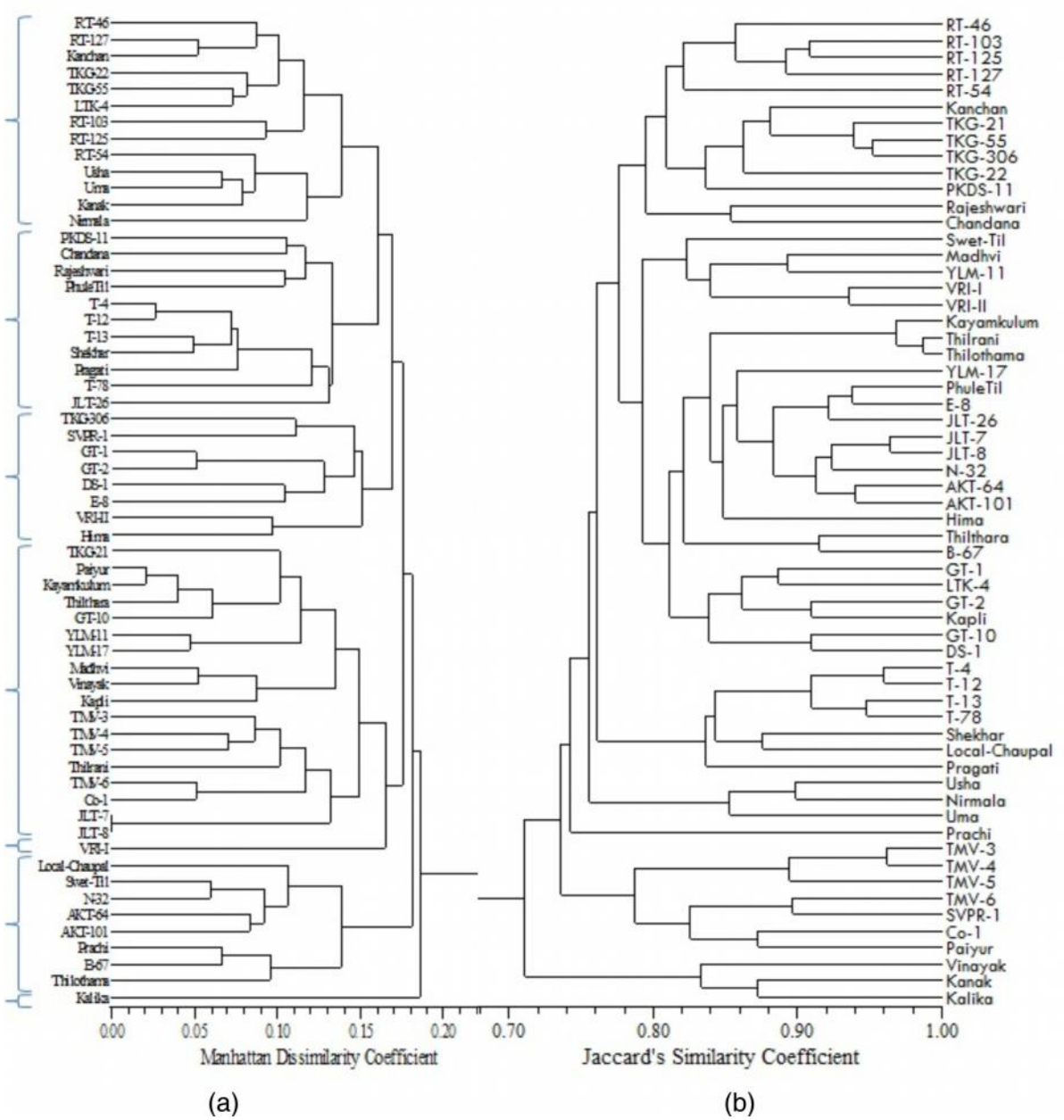

Fig. 1. Dendrogram derived from UPGMA cluster analysis using (a) Euclidean distance coefficient of morphological markers and (b) Jaccard's similarity coefficient of RAPD based markers

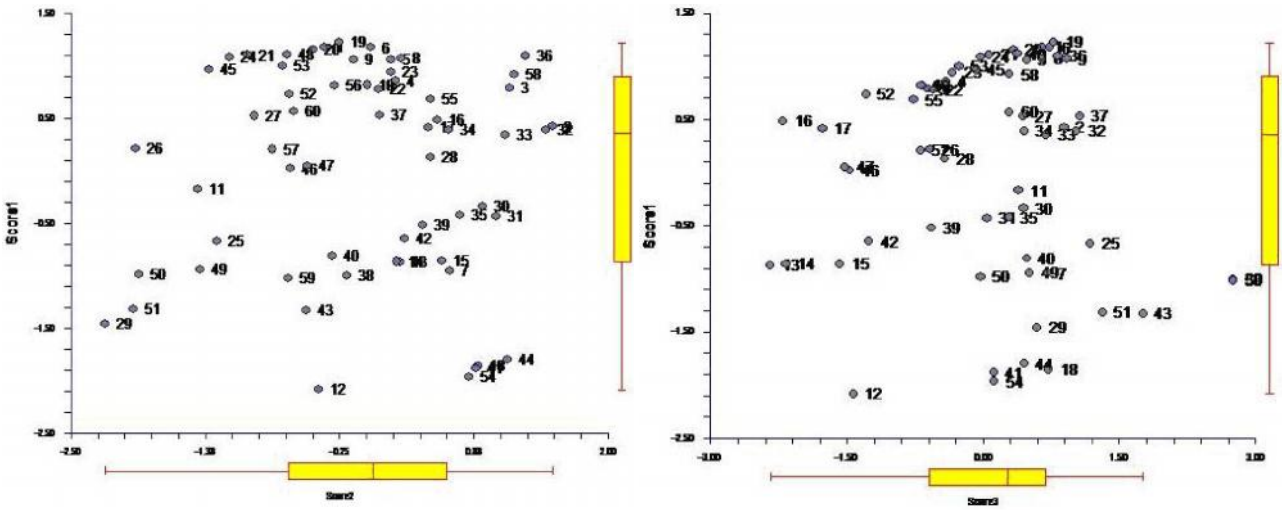

Fig. 2a. Two dimensional scaling of 60 sesame varieties by principal coordinate analysis (PCA) using the pooled genetic distance matrix from morphological descriptors. Because of the variability constraints both combinations of three coordinates are shown (I) PCA axis I and PCA axis II and (II) PCA axis I and PCA axis III

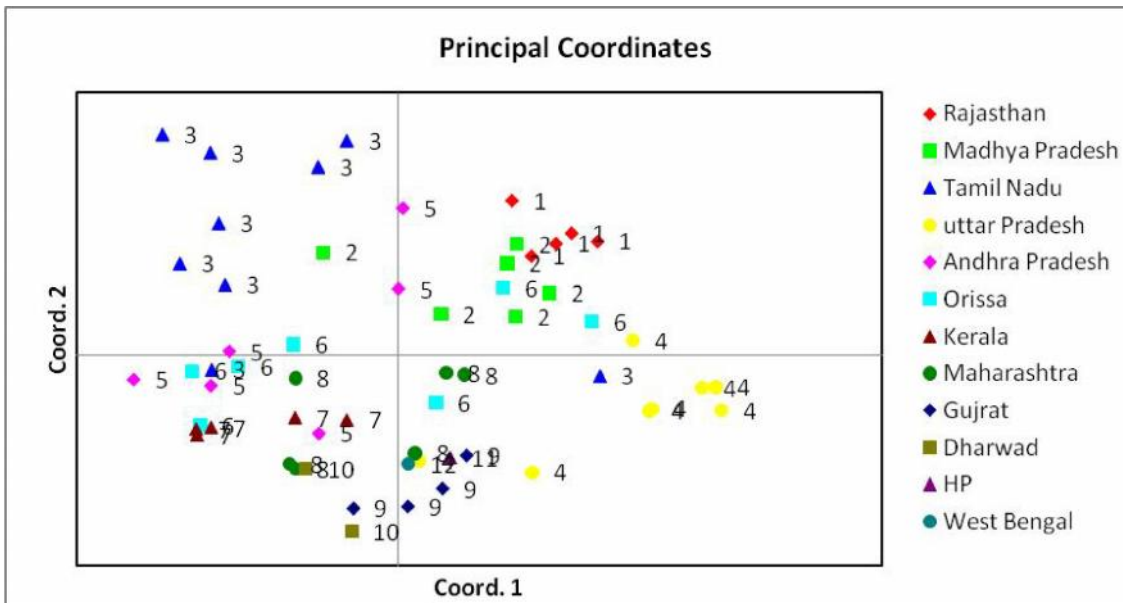

Fig. 2b. Two dimensional scaling of 60 sesame varieties by principal co-ordinate analysis (PCA) using the pooled genetic distance matrix from RAPD data

each other, subp-6 (Orissa) is a representative of coastal region in eastern part of India whereas subp-11 (HP) is a representative of hilly region in the northern part of the country. The genetic distance was minimum (0.0578) between subp-8 and subp-9 and it was maximum (0.4523) between subp-7 and subp-11 which is in accordance to their geographical locations. The analysis of genetic identity among the sub-populations of *Sesamum indicum* indicates that the subp-8 and subp-9 from the adjoining locations, are most (0.9438) identical, whereas subp-7 and subp-11 from far located areas exhibit least (0.6362) identity. Shannon information index and Nei's gene diversity index were used to partition the diversity within and between sub-populations. 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-0.1991) and within

populations (Hs-0.0749) and also on the basis of mean coefficient of gene differentiation (Gst-0.6238) indicating 62.38% genetic variability among populations and 37.62% within populations (Table 3). Low level of genetic diversity within populations and significant differentiation among populations might be due to the localized breeding system in which gene flow across the geographical region and/or selection within populations played a significant role.

Genetic variability as revealed by morphological traits and RAPD markers

In the present study, a week correlation (r = 0.098, P = 0.001) was found between the phenotype based clustering matrix and RAPD-based clustering matrix in terms of relationship between and within sub populations. RAPD data segregated varieties into their respective geographical region (sub-population) at a broad range of genetic diversity (0.03-0.38) whereas, no region-specific grouping of varieties was seen on the basis of phenotypes with a narrow range of genetic diversity (0.00-0.27) (Fig. 1ab). The reason was probably that RAPDs revealed the diversity of the entire genome to a greater extent, while for phenotypes either the target genes are less and/or are modified by the G X E interactions which resulted in poor differentiation and low variability under field conditions. Several other comparisons between morphological and molecular based studies also indicated that the two methods were different and highly variable [11, 27]. Also probably it implies the differences between molecular markers and morphological traits in the degree of genomic coverage [30- 33]. Phenotypes are therefore, not much dependable and should be supplemented with molecular analysis for the characterization of individual genotype and also for claiming the intellectual property right. A significant relationship between molecular markers and morphology could perhaps be obtained if the markers were linked to morphological traits under study [34, 35]. Phenotype traits could also effectively be used to document varieties which were developed using parents with wide genotypic variability. For example, one variety, Pragati of subp-4 segregated in a separate group with more dissimilarity coefficient (0.07) than the remaining varieties of this region (T4, T12, T13, T78 etc).

Table 3. Genetic diversity analysis of the twelve sub-populations (*Subp) of *Sesamum indicum*

Geographical region (Sub-population)	Shannon information index (mean value)	Nei's gene diversity index (mean value)
Rajasthan (Subp1)	0.1133	0.0771
Madhya Pradesh (Subp2)	0.1377	0.0927
Tamil Nadu (Subp3)	0.1772	0.1178
Uttar Pradesh (Subp4)	0.1180	0.0797
Andhra Pradesh (Subp5)	0.1664	0.1129
Orissa (Subp6)	0.1987	0.1339
Kerala (Subp7)	0.1141	0.0766
Maharashtra (Subp8)	0.1135	0.0784
Gujrat (Subp9)	0.0884	0.0594
Karnataka (Subp10)	0.0409	0.0280
Himachal Pradesh (Subp11)	0.0000	0.0000
West Bengal (Subp12)	0.0613	0.0420
Gene diversity index among population (H_T)		0.1991
Gene diversity index within sub-population (H_S)		0.0749
Gene diversity within sub-population (H_S/H_T)		0.3762
Sub-population differentiation ($G_{ST} = 1-H_S/H_T$)		0.6238

This is the first molecular marker based documentation and its comparison with conventionally used phenotypes on the genetic variability in 60 released sesame varieties, representing all the sesame growing areas in the country. RAPD markers with high discriminatory power proved an effective technique for the characterization of individual sesame variety and also for determining the extent of genetic variability in the population of *Sesamum indicum*. The sesame breeding program would benefit from using RAPD genome profiling technique to maximize genetic polymorphism for important traits. It is concluded that sesame lines under study are genetically variable which need to be exploited for desirable traits in sesame improvement program.

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