Analysis of Genotypic Diversity in Sesame (*Sesamum indicum* L.) Based on Some Physiological Characters

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Abstract: Genetic diversity among 30 sesame genotypes, collected from different parts of India, was studied using measurements of leaf area index (LAI) obtained 30, 45, 60 and 75 days after sowing (DAS), crop growth rates (CGR) estimated between the above leaf area measurements (i.e. 30 to 45 DAS, 45 to 60 DAS and 60 to 75 DAS), days to peak flowering, duration of flowering, duration from peak flowering to maturity and oil yield per plant. The normalised Euclidean distance was calculated from the data, and, independently, the Mahalanobis D^2 statistics was calculated after dimensionality was reduced by pivotal condensation. The clustering pattern obtained by D^2 analysis agreed closely with the dendrogram constructed from the Euclidean distance matrix. In general, the distribution pattern of genotypes in different clusters indicated that genetic divergence was not related to geographical differentiation. However, it was evident that a certain degree of genotypic divergence resulted from the geographic origin of the cultivars. Duration from peak flowering to maturity contributed most to the observed diversity, followed by days to peak flowering, duration of flowering, LAI at 30 DAS and 75 DAS, oil yield per plant and LAI at 60 DAS. Therefore, a greater emphasis should be laid on these characters in the selection of parents for further breeding programmes.

Keywords: D^2 analysis; Euclidean distance; genotypic diversity; physiological traits; *Sesamum indicum* L.

Sesame (*Sesamum indicum* L.) is one of the most important ancient oil seed crops (Bedigian & Harlan 1986). It is grown in tropical and subtropical areas (Ashri 1998) of the world. Sesame contains about 50–60% seed oil (Uzun *et al.* 2002; Arslan *et al.* 2007), which is of superior quality, nearly matching olive oil. Sesame oil is highly stable compared to other edible oils, mainly due to the presence of antioxidants like sesamin, sesaminol, sesamol, sesamolinol and squalene (Mohamed & Awatif 1998). Sesame oil also contains a high level of polyunsaturated fatty acids (Wood 1999). It has a reducing effect on plasma cholesterol and it also lowers the blood pressure (Sankar *et al.* 2005). Potential benefits of sesame on human health have recently renewed the interest in this ancient crop (Laurentin & Karlovsky 2006). Sudan, India, Myanmar and China are the most important sesame producers with 68% of the total world production (Laurentin & Karlovsky 2006). Despite the nutritional value and oil quality, the research on sesame has been scarce (Bedigian 2003). Average productivity of this important oil seed crop in India is 453 kg of seed/ha, which is far below the average productivity in China (1127 kg/ha) and in Egypt (1211 kg/ha) (Banerjee & Kole 2009).

There is an ample scope for improving the productivity of this important oil seed crop through varietal improvement and hybrid cultivar development. Genotypic diversity is very important in selecting the parents for hybridization programmes for identifying heterotic crosses and obtaining desirable recombinants in the segregating gen-

erations. Ramanujam *et al.* (1974) reported that hybrids between genetically diverse parents were more heterotic than those between more closely related parents. Information on genetic diversity in sesame is limited (Laurentin & Karlovsky 2006). A high level of variability in morphological characters within different sesame collections was reported by Bishit *et al.* (1998) and Banerjee & Kole (2006a). But information on genetic diversity based on physiological traits is very limited in sesame. Studies on morphological differences among genotypes provided little understanding of the underlying physiological-genetic controls over accumulating the yield (Banerjee & Kole 2009). Loomis (1993) suggested that crop yield could be raised by optimizing crop structure and physiology. Mean rates of biomass accumulation, days to maturity and assimilate partitioning are the three major physiological genetic components of the process of accumulating crop yield (Wallace & Yan 1998). Crop growth rate (CGR) and leaf area index (LAI), although it requires sequential samplings of the aerial biomass and leaf area at different crop growth stages, are estimated from simple measurements. Flowering and maturity characters are easily determined by simple observations under field conditions. Banerjee & Kole (2006b) observed a significant contribution of LAI, CGR and days from flowering to maturity to sesame oil yield. A strong influence of leaf area index on yield was reported by Liu *et al.* (2005) and Yoshida *et al.* (2007) in soybean and rice, respectively.

Several methods are available to measure such diversity quantitatively. D^2 statistics of Mahalanobis (1936) is one of the widely used methods to effectively measure such genetic diversity among the genotypes in a number of crop plants (Murty & Arunachalam 1986). Estimation of genetic dissimilarity based on 'Euclidean distance' is also widely used to effectively estimate the genotypic dissimilarity in crop research.

Genetic variability among Indian sesame accessions is very high as shown both for the morphological (Bisht *et al.* 1998; Banerjee & Kole 2006a) and molecular markers (Bhat *et al.* 1999; Laurentin & Karlovsky 2006). As mentioned earlier, divergence studies on important physiological parameters in sesame are scarce. In the present experiment an attempt was made to assess the magnitude of this unexplored physiological genetic divergence by common and widely used methods. The aim of this study was to bring results that could help sesame breeders to select suitable parental material for crossing and increase the efficiency of selection in conjugation with other available information on morphological and molecular diversity.

MATERIALS AND METHODS

Thirty sesame genotypes, collected from major sesame growing regions of India, were grown in a randomized complete block design with three replications during summer 2002 and 2003 (March to June) on an Agricultural Farm, Institute of Agriculture, Sriniketan, Visva-Bharati University (23°19'N latitude and 87°42'E longitude and at an elevation of 58.9 m a.s.l.), located in the sub-humid, sub-tropical lateritic belt of West Bengal, India. The soil of the experimental plot was sandy loam in texture (61% sand, 10.7% silt and 28.3% clay) with medium to low fertility status (N: 235.4 kg/ha, P: 20.4 kg/ha, K: 172.3 kg/ha, and organic C: 0.5%) and acidic (pH: 5.2) in nature. Each genotype was grown in 10 row plots of 4 m long, with a spacing of 30 cm between rows and 15 cm between plants. Standard agronomic package and practices were followed to raise a healthy crop. The analysis is based on the determination of the following 11 physiological parameters: leaf area indices (LAI) determined at 30, 45, 60 and 75 days after sowing (DAS) (denominated as LAI 1,LAI 2, LAI 3 and LAI 4, respectively), crop growth rates (CGR) estimated for the period between 30 and 45 DAS (CGR 1), 45 and 60 DAS (CGR 2), and 60 and 75 DAS (CGR 3), days to peak flowering (DPF), duration of flowering (DF), duration from peak flowering to maturity (DFM) and oil yield per plant (OY). For the computation of different growth parameters, destructive sampling was done by uprooting plants from one-meter row length, kept in oven at a temperature 75°C for drying and dry weight of leaves and stems was taken separately when constant weight was obtained. Leaf samples were also collected during each destructive sampling from randomly selected plants from each treatment in each replication. Considerable care was taken while collecting leaf samples so as to represent the leaves from the lower, middle and upper portion of the plant. After recording the leaf area with a leaf area meter, leaf samples were oven dried and dry weight of each leaf sample was recorded. Leaf area and dry weight of such sampled leaves and the dry

weight of all leaves under destructive samplings from each genotype in each replication were used for the estimation of leaf area. LAI and CGR were estimated according to the method of Hunt (1978). Data on all the flowering characters, i.e. DPF, DF (duration in days from initiation of flowering to completion of flowering) and DFM (duration in days from peak flowering to maturity) were recorded on the basis of observations of the whole plot for each treatment. Oil content (%) was measured in 2 ml of dry, clean and healthy seeds from the bulked seeds of harvested plants, taken three times separately, for each treatment from each replication using the non-destructive method of low resolution nuclear magnetic resonance (NMR) with a Newport Analyzer Mark III instrument (Newport Oxford Instruments, Ltd., Newport Pagnell, UK). OY (g) was estimated by multiplying the oil content by the seed yield per plant for each treatment.

Statistical Analysis

Genetic divergence among 30 genotypes was estimated using the Mahalanobis D^2 statistics. Character means were transformed into sets of uncorrelated variables using the pivotal condensation of common dispersion matrix according to Rao (1952). Although the D^2 statistics can handle a multidimensional situation, higher order interactions do not contribute very much in any experiment. Therefore, some interaction effects were sacrificed while reducing dimensionality using the pivotal condensation method. In all the D^2 combinations, the characters were ranked 1 to 11 on the basis of their contribution to D^2. Then the rank of each character was summed up over all the D^2 combinations to get rank total and rank average. Grouping of genotypes into different clusters was done according to Tocher's method (Rao 1952).

Hierarchical clustering using Euclidean distance matrix and average linkage method

Euclidean distance is a multivariate generalization of the Pythagorean Theorem. Trait means were normalized (mean zero and standard deviation one) by using R-statistical software. Standardization of data is essential to reduce the effect of the scale difference within different variables and as a consequence the 'normalized Euclidean Distance' becomes scale independent. Once the distance matrix was found, a dendrogram was constructed according to the average linkage method. Hierarchical clustering was performed by R-statistical software using the 'agricolae' package.

RESULTS AND DISCUSSION

The analysis of variance revealed statistically significant differences among the 30 sesame genotypes for all 11 characters studied (Table 1). Significant G × E interactions for CGRs, LAI 1 and LAI 2 and flowering and maturity traits indicated differential responses of the genotypes over seasons. A wide range of variation for physiological parameters in sesame was reported by Banerjee & Kole (2006b). Most of the D^2 values (data not presented) were statistically significant. In the pooled analysis, D^2 values exhibited a high range,

Table 1. Analyses of variance (mean squares) for 11 physiological characters

Factors	df	CGR 1	CGR 2	CGR 3	LAI 1	LAI 2	LAI 3	LAI 4	DPF	DF	DFM	OY
Season (S)	1	15.66**	5.50	15.85	0.03**	0.19**	0.07	0.43*	15.63	7.19	166.28**	184.50
Replication (R)	2	0.17	7.04	2.73	0.01	0.02	0.16 *	0.04	15.52	9.31	15.25	2770.75*
R × S	2	0.22	1.99	23.36	0.01	0.01	0.03	0.16	1.17	28.56	2.70	776.50
Genotypes (G)	29	5.19**	57.47**	155.49**	0.10**	0.25**	1.16**	8.46**	605.21**	554.00**	324.04**	13910.49**
G × S	29	0.97**	6.54*	33.43*	0.01**	0.05**	0.07	0.07	22.48**	57.87**	45.49**	12.35.43
Error	116	0.47	3.91	19.15	0.01	0.01	0.05	0.08	6.57	13.61	11.15	816.46

*, **Significant at $P = 0.05$ and 0.01, respectively; df – degrees of freedom; CGR – crop growth rates at 3 phases; LAI – leaf area index at 4 terms; DPF – days to peak flowering; DF – duration of flowering; DFM – duration from peak flowering to maturity; OY – oil yield per plant

indicating less divergence among some genotypes, while others were quite diverse. It was possible to group the examined sesame genotypes into eight different clusters (Table 2). Cluster I (nine accessions) contained genotypes from Gujarat, Maharastra, Madhya Pradesh, Uttar Pradesh and Orissa. Cluster II (four accessions) contained accessions coming from Assam, Orissa, Rajasthan and Gujarat. Cluster III (nine accessions) was constituted by the genotypes from West Bengal, Orissa, Maharastra, Uttar Pradesh and Rajasthan. Cluster IV (three accessions) contained genotypes from Uttar Pradesh and Rajasthan and cluster V (two accessions) was represented by genotypes coming from Orissa and Madhya Pradesh. Clusters VI, VII and VIII consisted of only one genotype originating from West Bengal, Rajasthan and Andhra Pradesh, respectively. Average intra- and inter-cluster distances for eight different clusters in respect of 11 physiological characters are

Table 2. Place of origin (constituent province in India), geographical position and clustering pattern of sesame genotypes based on the Mahalanobis D^2 statistic and hierarchical clustering

Genotype	Place of origin	Region	Latitude	Longitude	Cluster (D^2)	Cluster (hierarchical)
MT75	Uttar Pradesh	north	27°30'N	80°12'E	I	II–I
Sekhar	Uttar Pradesh	north	27°30'N	80°12'E	I	II–I
CST2001	Uttar Pradesh	north	27°30'N	80°12'E	I	II–IV
TKG22	Madhya Pradesh	central	31°22'N	79°30'E	I	II–III
JTS111	Madhya Pradesh	central	31°22'N	79°30'E	I	II–I
AT91	Gujarat	west	22°48'N	71°48'E	I	III–I
AT93	Gujarat	west	22°48'N	71°48'E	I	II–I
OS-Sel-164	Orissa	south-east	20°30'N	84°36'E	I	II–III
Ajit130	Maharastra	central	19°48'N	76°00'E	I	II–III
OS-Sel-2	Orissa	south-east	20°30'N	84°36'E	II	I
AAUDT9304-14-4	Assam	north-east	26°12'N	93°18'E	II	I
AT88	Gujarat	west	22°48'N	71°48'E	II	II–III
RT333	Rajasthan	north-west	27°00'N	74°00'E	II	II–III
CST2002	Uttar Pradesh	north	27°30'N	80°12'E	III	II–II
MT34	Uttar Pradesh	north	27°30'N	80°12'E	III	II–II
MT101	Uttar Pradesh	north	27°30'N	80°12'E	III	II–II
Rama	West Bengal	east	23°30'N	87°42'E	III	I
OS-Sel-24	Orissa	south-east	20°30'N	84°36'E	III	III
OS-Sel-253	Orissa	south-east	20°30'N	84°36'E	III	II–II
OS-Sel-185	Orissa	south-east	20°30'N	84°36'E	III	II–IV
RT53	Rajasthan	north-west	27°00'N	74°00'E	III	II–IV
Aji 131	Maharastra	central	19°48'N	76°00'E	III	II–I
MT24	Uttar Pradesh	north	27°30'N	80°12'E	IV	II–IV
MT32	Uttar Pradesh	north	27°30'N	80°12'E	IV	II–I
RT 334	Rajasthan	north-west	27°00'N	74°00'E	IV	II–I
OS-Sel 6-1	Orissa	south-east	20°30'N	84°36'E	V	II–III
JTS103	Madhya Pradesh	central	31°22'N	79°30'E	V	II–IV
B67	West Bengal	east	23°30'N	87°42'E	VI	I
RT331	Rajasthan	north-west	27°00'N	74°00'E	VII	I
JCS9489	Andhra Pradesh	south	16°00'N	79°00'E	VIII	IV

Table 3. Average intra- and inter-cluster distance based on D^2 analysis among eight clusters

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**54.53**	85.22	184.58	113.15	116.08	103.18	144.35	694.22
II		**50.49**	242.54	124.98	113.72	81.34	96.98	742.20
III			**64.05**	409.39	406.17	132.14	312.17	1303.98
IV				**63.51**	74.17	248.78	146.05	545.48
V					**79.84**	211.57	195.22	587.85
VI						**0**	185.20	1079.27
VII							**0**	523.54
VIII								**0**

Diagonal values (bold face) are average intra-cluster distances and off-diagonals are average inter-cluster distances

presented in Table 3. Barring solitary clusters, cluster II had the lowest intra-cluster divergence (D^2 = 50.49), followed by cluster I (D^2 = 54.53), cluster IV (D^2 = 63.51), cluster III (D^2 = 64.05) and cluster V (D^2 = 79.84). It was evident that cluster VIII was highly diverse from all the other clusters. High inter-cluster divergence was also observed between cluster III and clusters V (406.17) and IV (409.39). However, lower inter-cluster divergence was noticed between clusters IV and V (74.17) and also clusters II and VI (81.34), clusters I and II (85.22) and clusters II and VII (96.98). Laurentin & Karlovsky (2006) also reported the high genetic variability among sesame accessions from different parts of India. Sesame seems to be domesticated in India (Bedigian 2003), which could explain this high genetic divergence among different Indian accessions. A high heterosis effect and superior pure lines can be obtained especially after crossing genotypes belonging to cluster III with cultivars of clusters IV and V. Cluster VIII, represented by JCS 9489, was highly diverse from the others, but exhibited poor performance *per se* in these environmental conditions.

The high diversity of JCS 9489 was also evident from the Euclidean distance matrix (data not presented here). The highest dissimilarity was observed between JCS 9489 and Sekhar. OS-Sel-24 also appeared to be diverse from most of the other genotypes. A low level of physiological divergence was observed between Sekhar and JTS 111; TKG 22 and OS-Sel-164; and MT34 and CST2002. Based on the Euclidean distance matrix derived from the standardized data and average linkage method of clustering, the 30 genotypes were grouped into four major clusters (Figure 1). Cluster II appeared as the largest cluster (containing 13 genotypes), which can be subdivided into four sub-clusters again.

An interesting similarity was observed among the clusters derived from both D^2 analysis and hierarchical clustering. Accessions of hierarchical cluster I were the elements of clusters II, III, VI and VII of D^2 analysis. It is evident from Table 3 that the inter-cluster distance between these clusters was quite low, except clusters III and VII, in which it was medium. In the case of members of four sub-clusters of hierarchical cluster II, the distribution pattern of these genotypes in different D^2 clusters also revealed low inter-cluster divergence. The cultivar JCS9489 appeared as highly diverse from the rest of the genotypes and this was evident from both types of divergence study. JCS9489 had non-branching habit, late flowering and maturity, high degree of indeterminacy and poor yield.

In general, the clustering patterns based on both D^2 analysis and hierarchical clustering indicated no strict and narrow relationship between the observed genetic diversity and geographical differentiation. Anuradha and Reddy (2004) and Laurentin and Karlovsky (2006) concluded that genetic diversity among the different sesame accessions was not associated with geographical diversity. However, all the accessions of hierarchical cluster I, except RT331, represented the north-eastern regions of India. JCS9489 coming from the southern parts of India (Andhra Pradesh) was highly diverse from all other accessions that originated from the northern, central, western and eastern parts of India. Navale *et al.* (2001) reported a link between the geographic diversity and genetic divergence.

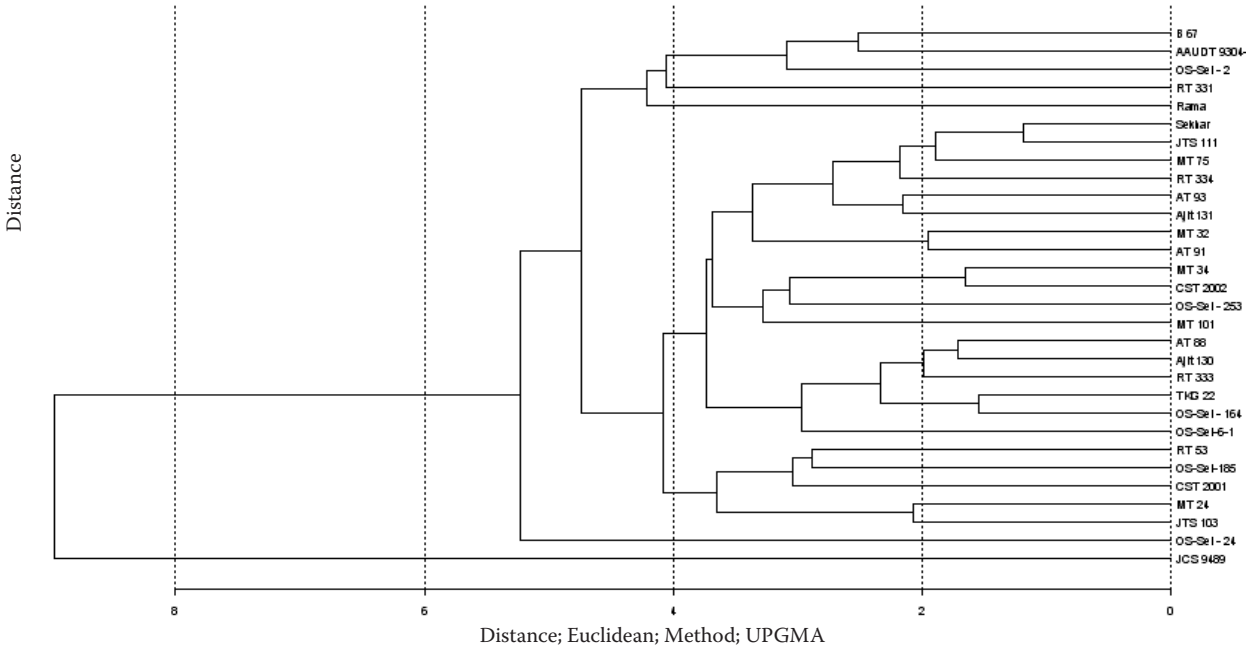

Figure 1. Grouping of 30 sesame accessions based on the Euclidean distance matrix and average linkage method of clustering

On the other hand, the results revealed that many genotypes of close geographic proximity fell into different clusters and *vice-versa*. Laurentin and Karlovsky (2006) also reported that the regional isolation was not the only factor contributing to diversity in a natural population. Clustering of genotypes from different eco-geographic locations into one cluster could be attributed to the possibility of free exchange of breeding materials among widely separated locations. However, unidirectional selection for a particular trait or a group of linked traits in several places may result in obtaining similar phenotypes, which can fall into one cluster irrespective of their geographic region. The formation of different clusters among the genotypes of common geographic origin may be due to their parentage, developmental traits, past history of selection and different outcrossing rates (Bhat *et al*. 1999).

D^2 analysis revealed that out of the examined physiological traits DFM contributed most (14.00%) to the observed diversity, followed by DPF (11.64%) and DF (10.17%). The traits, LAI-1 (9.67%), LAI-4 (9.29%), OY (8.74%) and LAI-3 (8.41%) contributed moderately to the diversity and CGR-2 (7.65%), LAI-2 (7.55%), CGR-1 (6.50%) and CGR-3 (6.38%) contributed relatively little. Therefore, the flowering and the maturity traits had the highest impact on genetic diversity among the sesame populations studied. Among the plant growth characters, LAIs contributed more to the observed diversity than CGRs.

When considering data on genotypic divergence derived with the use of both methods, as well as relative importance of characters in determining the oil yield in this particular population and performance *per se* of the genotypes, together with the cluster means, the crosses between the genotypes B67, AAUDT9304-14-4, OS-Sel-2, Rama, AT91, CST2002, AT88 and JTS103 are most likely to express a considerable amount of heterosis in F_1 generation and to provide a wide spectrum of recombinants in segregating generations.

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