



An insight into morphological and molecular diversity in Indian sesame cultivars

E. Ramprasad¹, S. Senthilvel^{*}, Jawahar Lal Jatoth, K. N. Yamini¹, Kuldeep Singh Dangi¹, A. R. G. Ranganatha² and K. S. Varaprasad

ICAR-Indian Institute of Oilseeds Research, Hyderabad 500 030; ¹Professor Jayashankar Telangana State Agricultural University, Hyderabad 500 030; ²All India Coordinated Research Project on Sesame and Niger, JNKVV Campus, Jabalpur 482 004

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Abstract

Extent of genetic diversity in a collection of 41 sesame genotypes comprising advanced breeding lines and varieties was assessed based on phenotypic (19 morphological and quantitative traits) and genotypic (68 SSR loci) data. Wide variation was observed for quantitative traits, namely, plant height (86-137 cm). No. of capsules/plant (42-116), days to maturity (81-103) and seed yield/plant (10-23g)., SSR markers showed 29% polymorphism with an average of 2.8 alleles/locus and 0.409 PIC value indicating a trend of moderate level of diversity at molecular level in the collection. The results reinforce the need for using both phenotypic and molecular data for genetic diversity assessments and selecting diverse lines for breeding programmes.

Key words: *Sesamum indicum*, working germplasm, cultivars, genetic diversity, SSR markers

Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop and often referred as 'Queen of oilseeds' due to its high oil content and nutritive and therapeutic value. It is a predominantly self-pollinated crop widely cultivated in many parts of the world, primarily in tropical and subtropical areas and recently in semi-arid regions (Elleuch et al. 2007). Sesame seeds are highly nutritive and contain 50 to 58 per cent oil along with proteins, vitamins, niacin, minerals and lignans. Sesame oil is highly stable due to the presence of antioxidants such as sesamin and sesamol. It is used in the preparation of number of food products and manufacturing of perfumes, soaps, paints and insecticides (Bedigian and Harlan 1986). Refined sesame oil is used in

pharmaceutical and cosmetic products.

India, Myanmar and China are the top three sesame producing countries in the world. But, the productivity of sesame in India is low (377 kg/ha) as compared to China (1,642 kg/ha) and Myanmar (617 kg/ha) (FAOSTAT 2013). There is a great scope to improve productivity through improved varieties and hybrids. The success of any crop improvement programme depends on the nature and magnitude of variability available in the crop species. India, being one of the centres of origin of sesame, possesses rich genetic diversity (Vavilov 1951; Nayar and Mehra 1970; Bedigian 2003). However, the extent of diversity introduced into the breeding materials across sesame breeding centres in India is largely unknown. Understanding the extent of genetic diversity in the working collection helps in orienting the future breeding programmes.

Morphological characteristics, being the important determinants of the agronomic value and taxonomic classification are widely used in assessing the genetic diversity. Growth habit, seed coat color, leaf shape, number of capsules, days to maturity and seed yield are some of the agro-morphological traits, which are used to estimate the genetic diversity in sesame. Compared with other means of studying genetic diversity, the morphological evaluation is direct, inexpensive and easy approach but has limitations. Phenotypic trait measurements are more subjective due to the influence of environment, which could be misleading. Alternatively, DNA-based

*Corresponding author's e-mail: senthilvel.senapathy@icar.gov.in

molecular markers allow a more precise way of determining genetic diversity as the genotypic data are not influenced by the environment.

DNA markers have been extensively used to decipher genetic diversity across crop species. In sesame, various kinds of DNA markers such as, random amplified polymorphic DNA [RAPD] (Bhat et al. 1999), inter-simple sequence repeat [ISSR] (Kim et al. 2002), amplified fragment length polymorphism [AFLP] (Laurentin and Karlovsky, 2006), sequence-related amplified polymorphisms [SRAP] (Zhang et al. 2010) and simple sequence repeat [SSR] (Zhang et al. 2010; Cho et al. 2011) have been used to analyze genetic diversity. Among DNA markers, SSRs are considered more ideal to study genetic diversity and relationships among closely related breeding lines due to its abundance, hyper-variability, multi-allelic and codominant nature (Powell et al, 1996). Thus the use of molecular markers to accrue information on genetic diversity has been a great potential for broadening the genetic base in several crops (Durgesh et al. 2015; Saxena et al. 2016; Singh et al. 2016) including sesame. This study was undertaken to assess the genetic diversity present in a set of advanced breeding lines and varieties, which represents the breeders' working collection at major sesame breeding centres in India, using morphological traits and microsatellite markers.

Materials and methods

Plant material and field experimentation

A set of 41 advanced breeding lines and varieties developed at 14 sesame breeding centers in India were used in this study. The list of genotypes and their source are given in Table 1. Phenotypic evaluation was carried out during *kharif* 2013 (rainy season) at the experimental fields of Indian Institute of Oilseeds Research (IIOR), Hyderabad, India. The lines were sown on August, 2013 in the field in a randomized complete block design with two replications. Each line was grown in a row of 2m length with a row spacing of 30 cm and plant to plant spacing of 10-15 cm. The crop was provided with recommended dose of nutrients (40:20:20 kg N: P: K per ha). Half of the of nitrogen (20 kg/ha) and the entire quantity of phosphorus and potash were applied during sowing. Remaining quantity of nitrogen was applied 30 days after sowing as top dressing. Three hand weeding were carried out during the crop growth period. Proper soil moisture was maintained throughout the crop growth period through supplementary irrigations. Necessary plant protection measures were taken to control pests and diseases.

Table 1. A list of advanced breeding lines and varieties of sesame used in the study

S. No.	Name of the genotype	S. No.	Name of the genotype
	A.R.S. Amreli	23	Hawari
1	Patan-64	24	JLS-403-33
2	Nesadi Selection	25	IsAgi-95-10
3	Nana Bhamodra-5	26	JLT-408
4	AT-213		C.S. Azad Univ. of Agric. & Tech., Kanpur
5	G.Til-1	27	CST-2001-1
6	G.Til-2	28	CST-2008-2
7	G.Til-10		P.A.U., Ludhiana
	Orissa Agric. Univ. & Tech. Bhubneswar	29	LT-8
8	Nirmala		A.R.S. Mandore
9	Prachi	30	RT-125
	M.A.R.S., Dharwad	31	RT-346
10	DS-1	32	RT-356
11	DS-5	33	RT-358
12	DSS-9		Crop Research Farm
13	DS-10	34	MT-10-81
14	DS-30		Cotton Res. S., Srivilliputhur
	Regional A.R.S., Jagtial	35	SVPR-1
15	JCS-1020		College of Agric. Tikamgarh
16	Madhavi	36	TKG-22
17	Rajeswari	37	TKG-87
18	Swetha		Oilseed Res. S.Tindivanam
19	Hima	38	TMV-3
	Oilseed R.S., Jalgaon		R.R.S., Vridhachalam
20	JL-Sel-05-3	39	VS-07-023
21	JLS-9707-2	40	VRI-2
22	JLS-408-2		Agril. Res. S., Yelamanchili
		41	YLM-17

Trait evaluation

Morphological characters were recorded on five plants per genotype in each replication according to the descriptors prescribed by Bioversity International (formerly IPGRI), Rome. A total of 19 traits (10 quantitative and 9 qualitative) namely, plant height (cm), diameter of stem (cm), number of branches/plant, first capsule height (cm), capsule length (cm), capsules/plant, number of seeds/capsule, days to maturity, 1000-seed weight (g), seed yield/plant (g), leaf hairiness, stem hairiness, stem branching, leaf arrangement, stem shape in cross section, leaf shape (middle and top), number of flowers/leaf axil, number of carpels/capsule and seed coat color were recorded.

SSR genotyping

Genomic DNA was isolated from the leaf samples

collected from 20 day-old seedlings according to the plant genomic DNA extraction procedure described by Doyle and Doyle (1990). The quality and concentration of DNA was assessed through 0.8% agarose gel electrophoresis. The DNA samples were diluted to uniform concentration of 10ng/ μ l using sterile distilled water and stored at -20°C. A set of 75 sesame SSR primer pairs were used for genotyping. PCR amplification was done in 10 μ l reaction volume containing 1X PCR buffer (Merck Millipore) with 1.5mM MgCl₂, 0.2mM each of dNTPs 0.4 μ M each of forward and reverse primer, 0.5U Taq polymerase (Merck Millipore) and 10ng genomic DNA as template using a thermal cycler (Applied Biosystems, USA). The initial denaturation cycle of 94°C for 5 min was followed by 35 cycles comprising 30 s at 94°C, 30 s at 50-55°C and 30 s at 72°C. An additional step of 7min at 72°C was used for final extension. The PCR products were separated on 6% polyacrylamide gels using Dual Triple-wide Mini-Vertical Electrophoresis system. Ethidium bromide stained gel was placed on UV transilluminator of gel documentation system (Syngene, India) and it was exposed to UV light for about 20 s or until the bands appeared clearly. Then, the gel image was captured and saved.

Data analysis

Genetic diversity parameters namely major allele frequency (M_{AF}), number of alleles (N_A), observed heterozygosity (H_o), gene diversity (H_e) and polymorphic information content (PIC) were calculated using the software programme PowerMarker version 3.25 (Liu and Muse, 2005). Three kinds of pairwise dissimilarity coefficients: 'Manhattan on standardized variables' for quantitative (continuous) data, 'Rogers-Tanimoto' for qualitative data (modalities) and 'simple

matching' for SSR (allelic) data were calculated using the software, Darwin 5.0 (Perrier and Jacquemoud-Collet 2006) with 1,000 bootstraps. These dissimilarity coefficients were used for constructing the dendrogram based on the weighted Neighbor-Joining method (Saitou and Nei 1987) as implemented in Darwin 5.0. The correspondence between the similarity matrices derived from quantitative, qualitative and marker data was tested using the Mantel test (Mantel and Valand 1970).

Results and discussion

In this study, the extent of genetic diversity prevailing in a collection of Indian sesame varieties and advanced breeding lines was assessed based on 10 quantitative traits, 9 qualitative traits and genotypic data from 75 sesame SSR markers.

Morphological diversity

The mean and range for 10 quantitative traits scored across 41 sesame genotypes are given in Table 2. Wide variation was observed for quantitative traits. More than two fold differences were noted for plant height, number of branches, capsules per plant and seed yield. The physiological maturity showed about three week differences from the early to late maturing genotypes. The extent of variation for different quantitative traits in the breeding lines and varieties covered a reasonable spectrum of diversity reported in the Indian germplasm collection consisting of 3,129 accessions representing all eco-geographical regions of the country (Bisht et al. 1998). The range for different quantitative traits in the varieties were within the range reported in germplasm accessions studied by Bandila et al. (2011).

Table 2. Mean and range of quantitative traits studied across the genotypes

Trait	Mean	Range	C.V. (%)	S.E.±
Plant height (cm)	86.17	59.09 -137.09	6.05	3.68
Number of branches	4.74	3.30 - 7.80	7.13	0.24
First capsule height (cm)	18.60	13.34 - 25.59	10.89	1.43
Capsule length (cm)	3.15	2.61 - 3.88	4.77	0.11
Capsules/plant	79.34	42.05 -115.50	1.78	1.00
Diameter of stem (cm)	1.44	1.08 - 1.95	10.22	0.10
Number of seeds/capsule	63.70	54.85 - 79.20	6.65	3.00
Days to maturity	92.00	81.00 -103.00	4.47	2.90
1000-seed weight (g)	3.08	2.70 - 3.85	7.38	0.16
Seed yield/plant (g)	15.16	10.35 - 22.65	19.67	2.11

The pairwise dissimilarity coefficients based on 10 quantitative traits (Manhattan on standardized variables) ranged from 0.303 (RT-358 and JLT-408) to 2.486 (Swetha and JLS-9707-2) with a mean of 1.122. The NJ tree based dendrogram showed mainly three clusters (Fig. 1). However the bootstrap support was

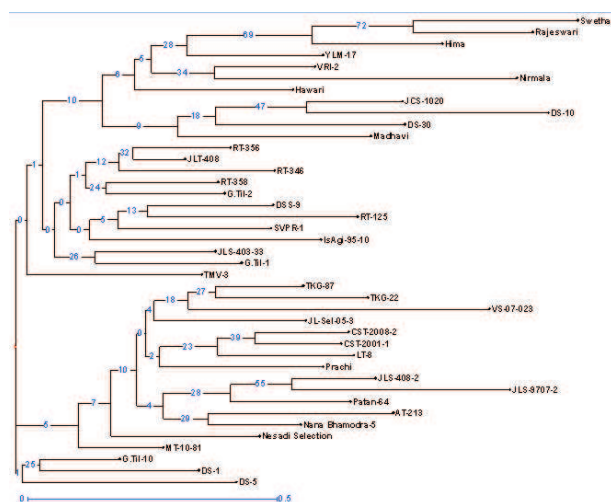


Fig. 1. Weighted Neighbor-Joining tree showing distribution of 41 sesame genotypes based on quantitative traits. Bootstrap values supporting nodes are shown. Distances were obtained using Manhattan dissimilarity coefficient

very low indicating a very fragile grouping. The cluster 1 consisted of 23 genotypes with several sub-clusters within itself. The second cluster consisted of 15 genotypes and the third contained only three genotypes. All four lines developed at Jagtial were placed in close proximity in cluster 1 because of high morphological similarity among the lines. Similarly, all four lines developed at Mandore were placed together. Sesame lines from other breeding centers were scattered across clusters indicating substantial genetic variability among lines that are developed at each center.

Genotypes differed widely for leaf shape and seed coat colour. Seven different seed coat colour were observed among the genotypes with predominance of white seed colour. However, only a little variability was observed among the lines for other qualitative traits. Germplasm collection also tends to possess limited variability with respect to qualitative traits (Bisht et al. 1998; Bandila et al. 2011). All genotypes except Prachi from Bhubaneshwar are of glabrous type in terms of leaf hairiness. Prachi has sparse hairs on the leaves. Stem hairiness among the genotypes ranged from

'absence' to 'medium'. None of the genotype used in this study had more than one flower per axil. Bandila et al. (2011) have found two out of 60 germplasm accessions having more flowers per axil, when they studied germplasm collection from seven states of India. However, about 32 per cent of 3,129 germplasm accessions studied by Bisht et al. (1998) showed more than one capsule per leaf axil.

The data on eight qualitative traits except carpels per capsule was used to calculate pair wise dissimilarity indices (Rogers-Tanimoto dissimilarity coefficient). The dissimilarity index ranged from 0.00 to 0.94 (DSS-9/Prachi; G.Til-2/Prachi and Prachi/JLS-9707-2) with mean of 0.55. Six genotype pairs namely Nirmala/TKG-22, RT-125/LT-8, JLS-403-33/CST-2008-2, TKG-87/YLM-17, Swetha/DS-10 and IsAgi-95-10/Rajeshwari showed no variability for all qualitative traits studied.

Molecular diversity

A set of 75 sesame SSR primer pairs (47 EST-SSRs and 28 genomic SSRs) were initially used to assess the molecular diversity among the breeding lines and varieties. Out of 75 SSR primer pairs, seven did not amplify properly, 48 were monomorphic and 20 were polymorphic (29.4% polymorphism). The level of polymorphism was higher in this study compared to earlier report by Yepuri et al. (2013), who found only 12 % of 156 primer pairs polymorphic in a set of 49 sesame accessions consisting of germplasm and improved lines including one accession of wild relative (*S. mulayanum*). The low level of polymorphism might be due to the use of EST-SSR markers. In present study also, out of 47 EST-SSR markers used, only two were polymorphic. This phenomenon of genomic SSRs being more polymorphic than EST-SSR had been observed in many plant species. Since the EST-SSRs are developed from the transcribed region of the genome, the level of polymorphism shown by this class of SSRs is low (Varshney et al. 2005).

The numbers of alleles detected for each of the 20 polymorphic SSR loci were slightly variable and ranged from 2 to 4 per locus with a mean of 2.80 alleles per locus. Thus, the SSR allelic diversity appears to be very less in sesame but comparable to other oilseed crops such as sunflower, 4.95 alleles/locus (Filippi et al. 2015), safflower, 3.6 alleles/locus (Kiran et al. 2015), groundnut, 3.14 alleles/locus (Jiang et al. 2007) and Brassica, 3.52 alleles/locus (Praval et al. 2015). The polymorphism information content (PIC) value varied from 0.126 to 0.676 with mean of 0.409 (Table 3). The SRR markers, zm_11, SM10184,

Table 3. Diversity parameters of polymorphic SSR loci

Marker	Major allele frequency	No. of alleles	Gene diversity	Heterozygosity	PIC
GBssr-sa-8	0.863	2	0.237	0.175	0.209
GBssr-sa-72	0.585	2	0.485	0.146	0.368
ZM_38	0.720	2	0.404	0.171	0.322
SM10192	0.784	3	0.362	0.216	0.333
SM10-110	0.878	2	0.214	0.000	0.191
SM10189	0.927	2	0.136	0.000	0.126
SM10-171	0.625	3	0.501	0.000	0.416
SM10184	0.390	4	0.723	0.000	0.675
SM10188	0.474	4	0.669	0.026	0.615
ZM_6	0.342	3	0.667	0.122	0.593
ZM_11	0.313	4	0.728	0.025	0.676
Si43	0.925	2	0.139	0.000	0.129
Si24	0.781	3	0.364	0.000	0.331
Si28	0.561	3	0.565	0.024	0.486
SM10194	0.675	4	0.508	0.025	0.474
SEM83	0.744	3	0.413	0.000	0.375
SEM445	0.526	2	0.499	0.000	0.374
SEM-12-178	0.512	2	0.500	0.000	0.375
SM10-116	0.450	3	0.645	0.000	0.572
SM10-118	0.439	3	0.626	0.000	0.547
Mean	0.626	2.8	0.469	0.047	0.409

SM10188, ZM_6, SM10-116 and SM10-118 recorded high PIC values (>0.5). SSR markers used in the study were robust, informative and polymorphic as evident from its PIC value. These markers could be potentially used for molecular characterization of sesame germplasm from various sources.

The major allele frequency for the 20 SSR loci ranged from 0.3125 (ZM_11) to 0.9268 (SM10189) with the mean of 0.6256. Sesame being a self-pollinated crop, a narrow range of heterozygosity was detected in the investigated material, from 0.00 to 0.2162, with mean of 0.0465. Out of 20 polymorphic SSR loci, 11 loci detected no heterozygosity while the remaining SSR loci showed a narrow range of heterozygosity. However, the gene diversity ranged from 0.1356 to 0.7278 with mean of 0.4692. The same level of gene diversity was observed when 130 Chinese sesame accessions including 82 cultivars, 44 landraces and 4 wild accessions were assessed using Insertion-Deletion (InDel) and SSR markers (Wu et al. 2014).

SSR allelic data was used to work out the pairwise dissimilarity among the genotypes. The simple

matching dissimilarity index ranged from 0.100 (RT-125 and LT-8) to 0.775 (DS-5 and RT-356) with a mean of 0.478. This indicated that there were very closely related as well as highly diverse pairs of genotypes available in the study materials. Some of the closely related pairs were LT-8/RT-125, Swetha/G.Til-10 and IsAgi-95-10/JL-408. Some of the distantly related genotype pairs include DS-5/RT-356, G.Til-10/AT-213, IsAgi-95-10/G.Til-2, IsAgi-95-10/ Madhavi, RT-358/DS-5, JLS-403-33/DS-5, RT-356/DSS-9 and RT-125/LT-8. Crosses between these genotypes are expected to yield heterotic materials.

The simple matching dissimilarity coefficient matrix was used to construct the dendrogram based on weighted Neighbor-Joining method. The 41 sesame genotypes assembled from different breeding centers in India were broadly divided into three clusters (Fig. 2). The lines developed from Jagtial and Mandore tend

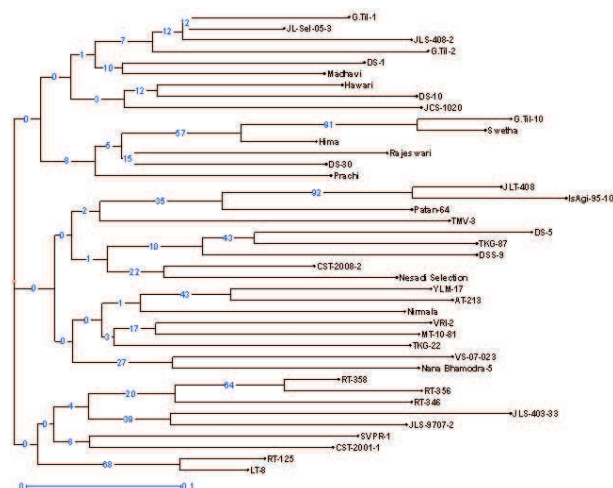


Fig. 2. Weighted Neighbor-Joining tree showing distribution of 41 sesame genotypes based on SSR allelic data. Bootstrap values supporting nodes are shown. Distances were obtained using simple matching dissimilarity coefficient

to group together indicating a possibility of narrow genetic bases in this material. The low level of genetic diversity among the lines developed at Jagtial was profound in the phenotypic diversity analysis too.

Moderate level of diversity revealed by SSR markers in this study is only indicative and not conclusive because of small number of loci analyzed. Conclusions on levels of genetic diversity would be risky when studies involve inadequate sampling of genotypes and low genome coverage of marker loci (Fu 2015). However, genetic relationships among

breeding lines from across the sesame breeding centres have been fairly revealed in this study.

The congruence among the genetic distance calculated from phenotypic and genotypic data was assessed based on Mantel's Test. The correlation coefficients among the distance matrices of quantitative data, qualitative data and marker data were not significant. Zhang et al. (2012) have also noted significant inconsistency in terms of diversity as revealed by phenotypic and molecular marker data while assessing the genetic diversity of sesame core collection in China. Similar observations were made in other crops also (Johnson et al. 2007; Reed and Frankham 2001). The mismatch between levels of genetic diversity detected by phenotypic traits and DNA markers raises a question that which is more informative or superior. In our view, both ways of looking at genetic diversity is important considering that they are of mutually exclusive and complementary in nature. The phenotypes mostly reveal functional diversity and are perhaps confounded by environmental influences. The DNA markers mostly reveal unknown diversity (cryptic), which include both neutral and functional diversity. Furthermore, DNA marker based diversity information has been highly valuable for deciphering genetic relationships and pedigree, which are very difficult to accomplish with phenotypes alone. It is also our view that diversity estimates from phenotypes and DNA markers cannot be compared due to their very different nature and utility for different purposes. It is not uncommon that highly diverse genotypes at phenotype level are genetically very similar and vice versa. Therefore, judicious handling of tools/methods is important for characterization of plant genetic diversity.

Authors' contribution

Conceptualization of research (SS, KSV); Designing of the experiments (SS, KSD); Contribution of experimental materials (LJL, ARGR, KNY); Execution of field/lab experiments and data collection (ER, LJL, KNY); Analysis of data and interpretation (ER, SS, ARGR); Preparation of manuscript (ER, SS).

Declaration

The authors declare no conflict of interest.

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