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Genetic Diversity and DNA Fingerprinting in Sesame (*Sesamum indicum* L.) Cultivars of ANGRAU

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ABSTRACT

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops and is widely cultivated in India and worldwide. India is rich in genetic variability of sesame. To determine the level of genetic diversity among 9 genotypes, 207 sesame-specific microsatellite markers were used in the present study. Of these, 46 markers were polymorphic. A dendrogram generated from the molecular profiles of these 46 polymorphic primers showed four clusters and one clade. Among these, the clade included 'Swetha', cluster I included 'Rajeswari', 'Madhavi', cluster II 'Chandana', 'YLM-11', and 'Hima', cluster III included 'YLM-17', and cluster IV included 'YLM-66' and 'Gouri'. The genetic similarity coefficient among the 9 genotypes ranged from 79 to 92% and PIC values ranged from 0.41 to 0.96. By DNA fingerprinting, all 9 genotypes could be easily distinguished with only 6 SSR primers. These results demonstrated that the cultivars developed at Acharya N G Ranga Agricultural University are from diverse origin exhibiting good variability.

Keywords: characterization, genotypes, microsatellite markers, polymorphism
Abbreviations: ANGRAU, Acharya N G Ranga Agricultural University; **NAIP**, National Agricultural Innovation Project; **NTSYS**, Numerical Taxonomy System; **SSR**, simple sequence repeat

INTRODUCTION

With the enforcement of Trade Related Intellectual Property Rights (TRIPS), one the three major agreements of World Trade Organization (WTO), member countries including India have amended either existing laws or enacted new laws related to Intellectual Property Rights (IPRs) to comply with TRIPS. Protection of plant varieties and plant breeders rights also come under the umbrella of IPRs. WTO facilitated for protection of plant varieties either under the existing patent law or by adopting a *sui generis* system or a combination of both. In India, Protection of Plant Varieties and Farmer's Rights Act (PPV&FRA) 2001 a *sui generis* kind provides a legal framework for registration of plant varieties and protection of rights of the concerned breeders. Novelty, Distinctness, Uniformity and Stability (NDUS) are the essential prerequisites for plant variety registration under PPV&FRA. Subsequent to the enforcement of the act and private sector becoming a major player in seed industry ownership related disputes are emerging. Therefore, it requires a precise identification system of varieties or hybrids and their pedigree in order to effectively implementation of the acts related to plant variety protection. DNA fingerprinting technique is a widely adopted method of providing unique identity across the disciplines. Genetic purity assessment by way of DNA fingerprinting technique during the process of protecting a plant variety offers a precise system of varietal identification barring all controversies or disputes related to ownership hitherto based on conventional morphological descriptors.

DNA fingerprinting technique is well standardized in crops like rice, maize, cotton etc., however a crop like sesame (*Sesamum indicum* L.) received very little importance. Sesame is one of the oldest oil seed crops and is widely cultivated in India and worldwide (Bhat *et al*. 1999). India is very rich in genetic variability of sesame. Today, India and China are the world's largest producers of sesame. The average productivity in India is 453 kg seed/ha, (Banerjee and Kole 2009). World production is estimated at 3.66 million tones, with Asia and Africa producing 2.55 and 0.95 million tons, respectively (Anonymous 2008). Sesame seeds contain 50-60% oil and 25% protein with lignan-type antioxidants such as sesamolin and sésamon. Seeds are used as an active ingredient in antiseptics, bactericides, viricides, and anti-tubercular agents (Bedigian *et al*. 1985). Sesame seeds also contain considerable source of calcium, tryptophan, methionine and many minerals (Johnson *et al*. 1979; Ali *et al*. 2007). It also has an antimicrobial effect on a Gram-negative bacterium, *Klebsiella* sp. which causes human urinary infections (Costa *et al*. 2007). Information on genetic diversity in sesame is limited (Laurentin and Karlovsky 2006).

Intervention of genomic tools in sesame improvement is sparse. Only a few reports are available in sesame on the use of molecular markers such as isozymes (Isshiki and Umezaki 1997), random amplified polymorphic DNA (Bhat *et al*. 1999; Ercan *et al*. 2004; Arriel *et al*. 2007; Abdellatef *et al*. 2008; Salazar *et al*. 2006), inter simple sequence repeats (Kim *et al*. 2002; Parsaeian *et al*. 2010; Anitha *et al*. 2010; Kumar and Sharma 2011), amplified fragment length polymorphism (Uzun *et al*. 2003) simple sequence repeats or SSRs (Dixit *et al*. 2005) and EST-SSRs (Suh *et al*. 2003; Wei *et al*. 2008; Ke *et al*. 2011). In India initiatives have been taken up in the recent past at national level to develop and use molecular tools in sesame (NAIP). In the process, ANGRAU has developed 274 number of SSRs by selective hybridization method comprising 25 (Jyothi 2009) and 249 (unpublished). 3328 EST sequences downloaded from NCBI were screened and identified 95 EST-SSRs (Jyothi 2009). SSRs are clusters of short tandemly repeated nucleotide bases and are a valuable polymerase chain reaction (PCR)-based DNA markers used in the genetic diversity analysis of many crop species. They are characterised by high variability, co-dominance, vast abundance and even

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Table 1 Description of origin, oil content and morphological characters 9 sesame accessions used in this study.

Accessions	Pedigree	Oil content (%)	Seed colour	Collection of samples	Plant height (cm)	No. of branches	No. of capsules/ plant	Capsule length (cm)	No. of seed/ capsule	1000-seed weight
Swetha	E-8 X IS-13	50	White seed	RARS-Jagitial, AP	136	6	154	2.4	68	2.05
Chandana	T-85X 5107		Light brown seed	RARS-Jagitial, AP	123	6	96	2.3	68	2.06
Rajeswari	Selection from 62-39 of Chhatarpur local (M.P)	50	White seed	RARS-Jagitial, AP	139	10	112	2.6	68	1.64
Hima	5039 X AT-1	-	White seed	RARS-Jagitial, AP	131	8	72	2.6	60	2.7
Madhavi (SP1181)	Selection from local A.P	44	Light brown seed	Visakhapatnam, AP	102	6	102	2	60	2.07
Gouri	Selection from Kokkirapalli local A.P	37	Brown seed	Visakhapatnam, AP	72	6	52	2.6	64	2.02
YLM-11 (IC305087)	Vinayak X Kanak	50	Brown seed	ARS-Yalamanchalli, AP	98	6	76	2.2	54	2.2
YLM-17 (IC527387)	Vinayak X Kanak	53	Light brown seed	ARS-Yalamanchalli, AP	98	6	64	2.2	54	3.3
YLM-66 (IC548002)	YLM 17 X P.S.201	51	Brown seed	ARS-Yalamanchalli, AP	112	6	98	2.2	60	2.2

distribution throughout genomes. For SSRs, each location in a chromosome that contains core repeats may have a different number of copies of the repeat. Polymorphism is based on the number of tandem repeats and, therefore, the length of PCR products (Dreisigacker *et al*. 2005; Blair *et al*. 2006; Milee *et al*. 2008; Gebremichael and Parzies 2011). Among the PCR based DNA marker, SSRs are the markers of choice for genetic diversity, seed genetic purity, germplasm characterization as they are more reliable due to their high fidelity profiles as a result of co-dominant nature and chromosome specificity (Nanda Kumar *et al.* 2004). SSRs have been extensively used in the molecular fingerprinting and assessment of pedigree in major crop species. For example, in rice there are several reports on use of SSRs for genetic profiling (Nandakumar *et al*. 2004; Sundaram *et al*. 2008; Aruna Kumari *et al*. 2011). However, with respect to sesame, it is still in the stage of building its genomic wealth in terms of developing molecular markers, tagging agronomically important genes and construction of linkage map. We utilized SSRs generated at our lab in an attempt to generate molecular IDs for sesame cultivars developed at Acharya N G Ranga Agricultural University (ANGRAU). The aim of the present research is to measure the diversity among 9 sesame varieties developed at ANGRAU using molecular markers (SSRs) to test the utility of sesame specific SSRs markers isolated in our lab.

MATERIALS AND METHODS

A selected set of 9 cultivars of sesame viz. 'Swetha', 'Rajeswari', 'Chandana', 'Madhavi', 'YLM-11', 'YLM-66', ' Hima', 'YLM-17' and 'Gouri', developed at various Regional Agricultural Research Stations (RARS) are used for DNA profiling in the present study. The selected set of 9 cultivars developed at ANGRAU represents varieties possessing high yield or resistance to various biotic and abiotic stresses and suitable to grow in different seasons (*kharif/rabi*). **Table 1** summarizes pedigree, seed colour, oil content and originating place, morphological characters, field data of nine sesame genotypes. Young leaves of sesame were collected from these genotypes grown in the fields of ANGRAU, Hyderabad, Andhra Pradesh, India during 2010. A total of 264 SSRs marker comprising of 95 ESTs (downloaded from NCBI) derived SSRs (Jyothi 2009), and 169-SSRs developed at Acharya N G Ranga Agricultural University by selective hybridization (Jyothi 2009 and unpublished) were used for generating molecular IDs of 9 sesame cultivars. DNA isolation was carried out using modified CTAB method (Murray and Thompson 1980; Jyothi 2009) followed by checking purity, yield and concentration using both UV-spectrophotometer and agarose gel electrophoresis. DNA amplifications were performed in 10 µl reaction volume containing approximately 50 ng template DNA, 5 pmol primer, 1 mM deoxyribonucleotide triphosphate (dNTPs), 1U *Taq* DNA polymerase (Himedia, Mumbai, India), 1X PCR Buffer with 15 mM $MgCl_2$ and volume was made with molecular grade water. PCR amplifications were carried out using Mastercycler Gradient PCR Machine (Eppendorf). The PCR schedules adopted were as : 94°C for 5' followed by 35 cycles of 94°C for 30 s, annealing temperature for 45 s, elongation at 72°C for 1'. After 35 cycles, the reaction was terminated with final extension of 10' at 72°C. The PCR products were run with gel electrophoresis system on a 3% agarose gel at 120 V constant power in 0.5 TAE buffer and stain with ethidium bromide. Both 100-bp and 50-bp molecular weight ladder (New England Biolabs, Massachusetts, USA) were used in the electrophoretic runs twice as size standards. PCR amplicon profiles were documented using UV trans-illuminator (Thermo Evolution 100 Dual Beam UV-VIS Spectrometer).For each primer, the number of polymorphic and monomorphic bands was recorded. The PCR amplifications were repeated twice for polymorphic primers. Bands clearly visible on agarose gel were scored as '1' for present, '0' for absent. The genetic distance between individuals was estimated by using the markers that produced the expected size (100-500 bp) of amplification product. Polymorphism information content (PIC) was calculated as described by Botstein *et al.* (1980) using the below formula:

$$PIC=1-[\sum_{i=1}^{n} P_i^2] - [\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2]$$

where p_i equals the frequency of the i^{th} allele and p_j the frequency of the $(I+1)^{th}$ allele. This computation was done using Genstat 7.10. For diversity analysis, only data from polymorphic SSR loci was used. Genetic diversity was estimated by computing the mean number of pairwise differences over each locus among SSR binary phenotypes using Genstat 7.10 software. Similarities between any 2 genotypes were estimated according to Nei and Li (1979) as;

$$S_{ij} = 2 N_{ij} (N_i + N_j),$$

where N_{ij} is the number of bands in common accessions i and j, N_i and N_j are the total number of bands in common between any 2 accessions and may range from 0 (no common bands) to 1 (identical band profile for the 2 accessions).

A dendrogram was constructed based on the S_{ij} values by adopting the sequential hierarchical agglomerative non-overlapping (SHAN) clustering technique of unweighted pair group method of arithmetic means (UPGMA), which is a variant of the average linkage clustering algorithm (Sneath and Sokal, 1973). The dendrogram was truncated at a similarity threshold value at which well-separated clusters, as indicated by MDS were obtained. These computations were performed using the statistical analysis package NTSYS-pc v2.10t (Rohlf 1994).

Table 2 Allele profiles of sesame.

A. Identity codes			**Allele code**			
Variety	**SM10-118**	**SM10-184**	**SM10185**	**SM10-188**	**SI-4**	**SEM-249**
Swetha	C	B	A	A	A	B
Rajeswari	B	C	A	A	A	A
Madhavi	B	C	A	A	A	B
Chandana	A	C	B	B	B	B
YLM-11	C	C	B	B	B	B
YLM-66	B	C	B	A	B	A
Hima	B	B	B	B	A	B
YLM-17	A	A	B	A	B	B
Gouri	B	A	B	A	B	B
B. Code key			**Allele size in base pairs**			
Allele code	**SM10-118**	**SM10-184**	**SM10-185**	**SM10-188**	**SI-4**	**SEM-249**
A	190	150	235	188	200	290
B	200	170	250	200	210	300
C	210	185				

RESULTS AND DISCUSSION

The 9 sesame cultivars subjected to DNA fingerprinting analysis in the present study are developed at various regional centres of ANGRAU, Andhra Pradesh, India. Of the total 264 SSRs screened, only 207 showed amplification, of which 46 were polymorphic. The size of the polymorphic alleles ranged from 140 to 280 bp. The PIC values ranged from 0.41 to 0.96 with an average of 0.68. However, no sequence information related to these SSRs has been published and is not available in the databases. The SSR primer information available is for a set of 10 microsatellite sequences isolated from an enriched library of sesame (Dixit *et al*. 2005). After screening 50 microsatellite sequences isolated from an enriched library of sesame, 10 polymorphic primers were used to determine their usefulness in diversity analysis among 16 sesame accessions. The number of alleles ranged from three to six per locus with an average of 4.6 alleles. The fragment size varied from 150 to 307 bp. Expected heterozygosites (*He*) and Polymorphic Information Contents (PICs) ranged from 0.437 to 0.858 and 0.34 to 0.80, respectively, which indicated the highly informative nature of the microsatellites.

In the present study, the dendrogram generated of 9 genotypes grouped them into four clusters and one clade, with similarity coefficient ranging from 0.76 to 0.92 (**Fig. 1**). Cluster I included 2 cultivars 'Rajeswari' and 'Madhavi'. Cluster II included 3 cultivars 'Chandana', 'YLM-11', and 'Hima' Cluster III included a single genotype 'YLM-17. Cluster IV included 2 cultivars 'YLM-66' and 'Gouri'. Interestingly, cultivar 'Swetha' separated as a clade. 'Rajeswari' and 'Madhavi' in cluster 1 were shown to have 90% genetic similarity, though both have a diverse origin. 'Rajeswari' is white colored seed from RARS, Jagitial, and 'Madhavi' is light brown colored seed from Visakhapatnam. 'Chandana', 'YLM-11', and 'Hima', of second cluster exhibited highest similarity coefficient of 0.92, despite variation in their origin and seed colour. 'Hima' is a white colored seed and 'Chandana' is a light brown colored seed both developed at RARS, Jagitial and 'YLM-11' is a brown colored seed, developed at ARS, Yalamanchalli. 'YLM-66' and 'Gouri' in the fourth cluster have a similarity coefficient of 0.86. Both these cultivars are brown seeded and were released from ARS Yalamanchalli and Visakhapatnam, respectively. 'Swetha' separated as a clade. It is a high yielding variety in the state diverged from the other genotypes with a similarity coefficient of 0.76. Zhang *et al*. (2007) studied variability and genetic divergence in a fairly large sample of 192 accessions 134 of Chinese origin, 41 alien germplasm and 17 improved cultivars using 270 SRAP markers and 25 EST-SSR markers by UPGMA analysis. The analysis revealed the 192 lines to group into three robust clusters. Cluster I had six subgroups and there was no association between genotype groups and geographical origin.

In addition, the data was subjected to the 2D and 3D

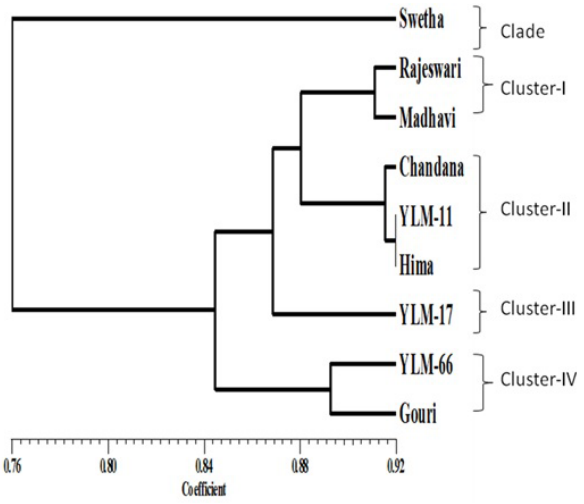

Fig. 1 Dendrogram showing phylogenetic relationships between 9 sesame cultivars.

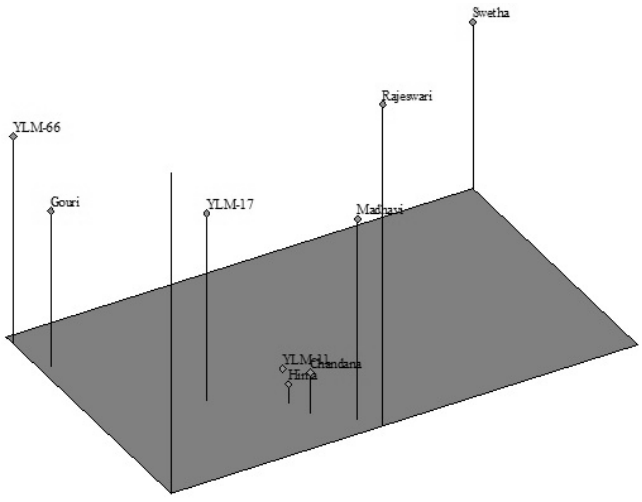

Fig. 2 3D plot from principle component analysis of 9 sesame cultivars from ANGRAU.

principal component analysis. The results from PCA plots of the 9 sesame varieties with 207 microsatellite markers reveal a clear distinction in the varieties as shown in **Fig. 2**. The PCA plot analysis was in congruence with UPGMA cluster analysis. As the molecular data were correlated with variation at the agro morphological level in the crop plants and therefore provide good guidance on the distribution of the useful variation as well as on the existence. These results demonstrated that these SSR markers can be used to

assess the genetic variability and cultivars identification. The values measured for diversity parameters based on allelic information exhibited the differences in sesame varieties. The current study demonstrated that there exists a considerable genetic variability in the sesame germplasm, and also the potentiality of the microsatellite markers in exploring the genetic relatedness among the genotypes. The six primers viz., SM 10118, SM 10184, SM 10185, SM10188, SI-4 and SEM 249 can differentiate sesame accessions through varietal specific genotype profiles. This profile can be used to identify variety, however further efforts should be made to assess the stability of DNA profiles generated in the present study. **Table 2** summarizes the table of Identity codes and allele codes (A) allele codes and Allele size in base pairs (B). This preliminary study was successful in understanding the extent of genetic variability in sesame cultivars of Andhra Pradesh.

In the beginning of the molecular marker era, RAPDs were used in sesame (Bhat *et al*. 1999; Ercan *et al*. 2004; Arriel *et al*. 2007; Abdellatef *et al*. 2008; Salazar *et al*. 2006) in assessing the extent of genetic diversity at molecular level indicating low genetic variation. Similarly ISSR markers were used (Kim *et al*. 2002) in studying the genetic relatedness among 75 *S. indicum* accessions, and grouped them into two major clusters. The dendrogram did not indicate any clear diversity among the accessions based on their geographical origin. However, comparative analysis of RAPD and ISSR markers for characterization of sesame genotypes made by Sharma *et al*. 2009 revealed the marker types to show maximum discrimination power and produce putative variety specific bands. Conversion of putative variety specific RAPD and ISSR markers into co-dominant sequence characterized amplified region/ sequence tagged site (SCAR/STS) markers has been suggested for development of robust variety specific markers. Reliability of AFLP marker technique for determining association between geographical origin and genetic diversity has been studied in sesame (Laurentin and Karlovsky 2006; Ali *et al*. 2007).

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