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Variation in seed oil content and fatty acid composition in sesame (*Sesamum indicum*)

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Sesame (*Sesamum indicum* L.) is one of the worlds oldest spice and oilseed crop grown mainly for its seeds that contain approximately 50% oil and 25% protein (Burden 2005). Sesame contains high levels of antioxidants such as sesamol, sesamin, sesamolin and sesaminol. Therefore, sesame oil is called the queen of the vegetable oils because of its antioxidants (ASGA 2011).

The presences of antioxidants (sesamum, sesamolin and sesamol) make sesame oil to be one of the most stable vegetable oils in the world. Among oil crops, sesame is one of the highest in oil content. Generally, the oil content in sesame ranges from 34 to 63% (Uzun *et al.* 2002, Were *et al.* 2006). Genetic and environmental factors influence the oil content and fatty acid compositions in sesame (Carlsson *et al.* 2008). Several workers have studied the genetic diversity of seed oil content and fatty acid composition in cultivated species *S. indicum* (Hiremath *et al.* 2007) and also in other edible oilseed crops like sunflower (Mandal *et al.* 2006), Brassica (Meena and Sachan 2009), soybean (Anita Rani *et al.* 2006), linseed (Sivaraj *et al.* 2011), and non edible oilseed crop like *Jatropha* (Dhyani *et al.* 2011). Electrophoresis analysis is also used to study molecular systematic for identification of genotypes based on proteins and this technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins (Ullah *et al*. 2010).

The present study was undertaken to assess the genetic diversity in seed oil content and fatty acid composition in sixty indigenous species of *Sesamum indicum.* The purpose of this study was to characterize various sesame accessions to gain information that could help design strategies for future breeding program and conservation of this crop. The germplasm of 60 accessions of *Sesamum indicum* collected from eight different states of India were procured from National Bureau of Plant Genetic Resources (NBPGR), Hyderabad (Table 1). The experiment was carried out in October 2010.

Lipid analysis was done with sesame seeds employing Nuclear Magnetic Resonance (NMR) spectroscopy technique. Seed oil was estimated by conventional Soxhlet method using petroleum ether as extraction solvent (SER 148 Solvent extractor). Oil content in the twenty grams of dried seed sample was fed into the NMR machine and the oil quantity was recorded as percentage.

The fatty acid content of ten selected accessions of which (three accessions from low and three accessions from high range and four accessions from medium range of oil content) was analysed by gas chromatography.

Seeds of each accession were cleaned and ground. One hundred mg of ground sample was taken in 1.5µl eppendorf tube and defatted with 1 ml of hexane for 30 min and the hexane was decanted. The defatting was repeated twice. The defatted cake was dried under vacuum and used for protein extraction by mixing with 1 ml buffer (Tris-HCl pH-7.5, 50 mM) for 60 min in an automatic mixer. The samples were centrifuged for 5 min at 13 000 rpm in a centrifuge (Eppendorf, Germany), the supernatant was taken and the protein was estimated by the method of Lowry *et al.* (1951)

Equal amount of protein from different samples were made up to 25µl of sample and 25µl of buffer (1:1) containing ß-mercaptoethanol was kept in a waterbath at 95^0 for 5 min, and centrifuged for 5 min and the supernatant was used for loading, sample prepared from each genotype was loaded into wells of stacking gel by layering them under electrode buffer using micropipette. The upper and lower reservoirs of

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Table 1 Details of the experimental material included for the study

Accession No.	State	Accession No.	State
IC751		IC14329	
IC16225		IC21705	
IC16236		IC23233	
IC16238	Maharastra(13)	IC23271	
IC16243		IC23321	
IC16248		IC23325	
IC16249		IC23327	
IC16250		IC23332	Madhya Pradesh(24)
IC41906		IC23335	
IC41910		IC23341	
IC41911		IC23346	
IC41912		IC41932	
IC41978		IC41948	
IC14080		IC41953	
IC14106		IC41962	
IC14135	Rajasthan(8)	IC41964	
IC14155		IC41966	
IC14174		IC42200	
IC26303		IC52585	
IC42965		IC52586	
IC42987		IC52592	
IC14163		IC52593	
IC43169		IC52599	
IC43171	Gujarat(8)	IC52600	
IC43177		IC96098	
IC43179		IC96109	Uttar Pradesh(3)
IC43181		IC96113	
IC43185		IC16832	
IC43217		IC31379	Punjab(2)
IC20156	Nagaland(1)	IC96079	Himachal Pradesh(1)

electrophoretic unit were filled with Tris glycine (0.5x) buffer. The gel having thirteen loaded wells was placed in the electrophoretic unit and fixed. Then the unit was adjusted so as to pass a current of 15 mA, for overnight. After the gel dye front almost reached to the bottom of the gel plate the unit was switched off and the gel carefully removed and placed in staining solution. The gels were later stained in 0.04% Coomassie Brilliant Blue in methanol, acetic acid and distilled water (45:10:45 v/v) for 8–10 followed by destaining in the same solution without Coomassie Brilliant Blue with occasional shaking till the gels became clear.

The banding patterns obtained were observed to detect the differences among the genotypes in terms of presence and absence of a particular Coomassie blue stained band.

Fifty out of 60 accessions were chosen for oil content based on variation in phenotypic characters and the popularity of the accession. The oil content of *S. indicum* accessions estimated by Nuclear Magnetic Resonance (NMR) also showed large variations among the accessions.

The oil content of *S. indicum* accessions estimated by Nuclear Magnetic Resonance (NMR) also showed large variations among the accessions (Table 2).

The oil content ranged from 43.32 % (IC 16225) to 51.74 % (IC 20156). Fourteen genotypes of the experimental material recorded more oil content in when comparison to grand mean (47.58 ±1.47 %).

The range of seed oil content in this work is similar to those reported by Tinay *et al*. (1976) (42.2 to 52.2%) and lower (40.4 to 59.8%) than reported by Yermanos *et al*. (1972).

Principal fatty acids in sesame seed oil are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids as reported by Hiremath *et al* (2007).

Ten *S. indicum* accessions for fatty acid composition are selected based on oil content with three accessions (IC 2332, IC52600, IC2016) with high range of oil content, i.e. from 49 to 51% and three accessions (IC 16225, IC16238, IC16248) with low oil content with range 43 to 45 % and four accessions (IC 16250, IC 14329, IC 23327, IC 43177) with medium range oil content from 46 to 48% was estimated by Gas

Table 2 Percentage of oil content in 50 *S. indicum* accessions

Accession No.	Oil content (%)	Accession No.	Oil content (%)
IC 16225	43.32	IC52585	47.00
IC 16236	47.02	IC52586	46.59
IC 16238	44.33	IC52599	49.00
IC 16243	46.83	IC52600	49.64
IC 16248	45.05	IC14106	49.13
IC 16250	46.88	IC14135	48.43
IC 41906	47.45	IC14155	45.35
IC 41910	48.55	IC14174	47.65
IC 41911	48.22	IC26303	46.04
IC 41978	47.12	IC42965	48.64
IC 14329	47.61	IC42987	46.98
IC 21705	49.37	IC14163	47.03
IC 23233	48.47	IC43169	48.99
IC 23271	46.63	IC43171	48.16
IC 23321	48.16	IC43177	48.52
IC 23325	49.38	IC43179	48.49
IC 23327	48.67	IC43181	47.34
IC 23346	45.36	IC43185	48.33
IC 41932	46.66	IC43217	47.66
IC 41948	47.91	IC96098	48.67
IC 41953	47.91	IC96109	44.60
IC 41962	47.49	IC96113	48.52
IC 41964	46.51	IC16832	47.54
IC 41966	47.46	IC31379	48.41
IC 42200	48.59	IC20156	51.74

Range : 43.32 % to 51.74 % , Grand mean: 47.58 ±1.47 %.

Table 3 Biochemical characteristics of 10 accessions of *S. indicum* including fatty acid composition, oleic/linoleic acids ratio

Accession No.	Fatty acid composition (%)				O/L ratio*
	Palmitic	Stearic	Oleic	Linoleic	
Standard (retn time)	8.806	11.66	11.92	12.47	
IC16225	8.94	5.80	39.03	44.80	0.87
IC16238	9.35	5.40	38.99	45.00	0.86
IC16248	9.66	5.67	39.25	43.90	0.89
IC16250	9.18	5.75	40.57	43.28	0.93
IC14329	9.20	6.04	40.36	42.40	0.95
IC23325	9.35	5.63	41.72	41.95	0.99
IC23327	9.64	5.95	42.42	40.61	1.04
IC52600	8.95	5.79	39.59	44.31	0.89
IC43177	9.24	5.77	40.83	42.80	0.95
IC20156	9.34	5.27	38.57	45.47	0.84

* Oleic/Linoleic

chromatography which showed large variations. Based on the retention time of the standard fatty acids the composition of the unknown sesame samples were identified (Table 3). The area under the peak was used to calculate the percentage fatty acid based on the total oil content present in the sample.

The soluble seed proteins of sesame were prepared and separated by PAGE and Cluster analysis was carried out for the protein profile among the sesamum accessions obtained on PAGE based on Euclidian genetic identity. Two major clusters were observed with only twenty-one accessions in Cluster I and the remaining thirty-nine accessions in Cluster II at 45 % similarity level. Cluster I was again divided into two sub-clusters in which eight accessions were placed in sub-cluster Ia and remaining thirteen in sub-cluster Ib at 55% similarity level.Cluster II was again divided into two subclusters of which eleven accessions are present in Cluster IIa and twenty-eight in Cluster IIb. The cluster analysis did not separate the germplasm based on their geographical origins. This result was in agreement with findings of Dixit and Swain (2000) and Gupta *et al*. (2001). This might be migration of the sesame materials from one region to another in collection sites through farmer to farmer exchange of seeds.

Among the saturated fatty acids the highest value (9.64%) was for palmitic acid (16:0) which was present in IC 23327 and the lowest (8.94%) was in the accession IC16225. Stearic acid (18:0) was highest (6.04%) in IC 14329 and lowest (5.27%) in IC 20156. Arachidic acid (20:0) was the highest (0.65%) in IC 16250, whereas the lowest (0.57%) in IC 20156.. Among the unsaturated fatty acids, the highest value (42.42%) was for oleic acid (18:1) that was present in IC 23327 and the lowest (38.57%) in IC 20156. The highest value (45.47%) for linoleic acid (18:2) was observed in IC 20156 and the lowest in IC 23327 (40.61%). And linolenic acid (18:3) was highest (0.71 %) in IC 23327, whereas the lowest (0.36%) in IC 16250. Among the accessions the highest O/L ratio was found to be 1.04 in IC 23327 and the lowest was 0.84 in IC 20156.

Linoleic acid and its derivative fatty acids are essential fatty acids and human being cannot synthesize these but must obtain it from dietary sources. High level of linoleic acids in the oil reduces the blood cholesterol level and plays an important role in preventing atherosclerosis (Ghafoorunissa 1994). Thus, edible oil with high linoleic acid content is premium oil.

SUMMARY

Sesame is a widely cultivated crop in tropics and sub-tropics. It is widely cultivated crop in India which mostly used for oil. The commercial importance of sesame as oil with the large-scale cultivation in both tropics and sub-tropics has resulted in establishing breeding programmes for its improvement. For the study of inter accession diversity among the sesame accessions biochemical analysis (oil content by NMR; fatty acid composition by GC) was used. Oil content was estimated using NMR which revealed that IC20156 accession had high oil content of 51.7%. The oil content among the accessions was observed between 43.3-51.7%. Fatty acid composition was estimated using GC which revealed that linoleic acid was high compared to all other major fatty acids (PA, SA, OA). Accessions from different regions were sometimes closely related and accessions from the same region had different genetic background. The work has shown that significant diversity exists for oil content and fatty acid composition in the sesame germplasm evaluated. Sesame accessions with potential use as parental lines in future breeding for improvement of many of the qualitative and quantitative traits have been identified.

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