Scientific Research Publishing

American Journal of Plant Sciences, 2016, 7, 2399-2411
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Agromorphological and Molecular Characterization of *Sesamum indicum* L.—An Oil Seed Crop

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How to cite this paper: Hota, T., Pradh, C. and Rout, G.R. (2016) Agromorphological and Molecular Characterization of *Sesamum indicum* L.—An Oil Seed Crop. *American Journal of Plant Sciences*, **7**, 2399-2411.
http://dx.doi.org/10.4236/ajps.2016.717210

Received: October 10, 2016
Accepted: December 5, 2016
Published: December 8, 2016

Abstract

Sesamum indicum L. (family: Pedaliaceae) is an economically important oil seed crop grown in tropical and sub-tropical countries. It is widely used in food, nutraceutical, pharmaceutical industries. *Sesamum* is widely distributed in all the climatic stages and great diversity. The exploration of genetic diversity is a pre-requisite for genome organization in the landraces and the related domesticated ones. Agromorphological and molecular markers were used to assess the identification of 33 *Sesamum* genotypes and determination of the genetic relationships among these genotypes. Out of 30 Inter-Simple Sequence Repeat (ISSR) primers tested, 18 primers produced 114 detectable fragments, of which 97 (85.08%) were polymorphic across the varieties. Molecular profiling could be solely used for their identification of genotypes. Genetic relationships among these genotypes were evaluated by generating a similarity matrix based on the Jacard's coefficient and the Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendogram. The results showed a clear cut separation of the 33 genotypes and were in broad agreement with the morphology. Both molecular and morphological markers will be useful for preservation of the germplasm as well as breeding program.

Keywords

Sesamum, PCR Based Marker, Phylogeny, Molecular Profiling, Agro-Mophological Traits

1. Introduction

Sesame (*Sesamum indicum* L.) family Pedaliaceae, is one of the popular high-value,

multipurpose oil seed crop grown widely in tropical and subtropical regions. Production of Sesame in India is encouraging, as more than 30% of world production is contributed by India alone and identified as the first rank in total production. The average yield of sesame on global scale is 5.1 quintals/ha while, current world production is estimated at about 4.04 million tons annually. India placed the second in the world after Myanmar with 18.20 lakh ha and 6.10 lakh tons production respectively. The average yield of sesame on global scale is 5.11 q/ha, while in India, it is 3.30 q/ha which is very low. It is widely used against various diseases including cancer, cold, colic etc. [1]. *Sesame* oil contains a unique compound known as lignans. Lignans comprise sesamin, sesamolin, and a small amount of sesamol [2]. Lignans are also phytoestorgens and their conversion to enterolactone is very important in preventing hormone-dependent cancers like breast and prostate and cardiovascular diseases. *Sesamum* has also a unique anti-oxidant compound "sesamol" and poly-unsaturated fatty acid [3] [4] [5]. Even, *Sesamum* has great economic importance but the farmers do not attracted much for cultivation as the low productivity and low economic return. Even India is self-sufficient in production, the productivity is decline due to unavailability of high yielding varieties [6] [7] [8]. *Sesamum* species are represented by a large number of genotypes/accessions which are facing genetic erosion. Genetic diversity analysis is utmost important for breeding program. Morphological, physiological, agronomic traits and genetic analysis through molecular marker are the prerequisite to know the genome organization of wild land races and related cultivated ones. Recently, molecular marker technology helps the identification of plant genetic resources. Moreover, the precise number of cultivars/accessions is still unverified since problems of mislabelling are very often detected. Therefore, it is essential to establish the strategies for the preservation of local genotypes. Till now, very scanty systematic work has been made to identify the indigenous as well as exotic collections along with the local germplasms to study the genetic diversity [9]. Different PCR and non-PCR based molecular markers have been utilized to identify the varieties/accessions [9]-[19]. The information of genetic variation between indigenous and exotic collection of sesame will provide better management strategy for crop improvement programme under adverse climatic conditions [20] [21]. The combination of both agro-morphological and molecular investigation would be more appropriate to analyse the germplasms of sesame. The present investigation was to identify the phylogenetic analysis of 33 cultivars of *Sesamum* representing through agro-morphological and molecular markers.

2. Materials and Methods

2.1. Plant Materials

The genetically pure seed material of 33 *Sesamum* varieties representing different geographical locations in India was collected from the germplasm centre of AICRP in *Sesamum* at Orissa University of Agriculture & Technology, Bhubaneswar, India (**Table 1**). The seeds were sown in the pots and grown in green house with 30˚C ± 2˚C with 75% relative humidity to study the morphological, biochemical and molecular analysis.

The experimental layout was a randomized block design with three replications. The Soil was mixed with cow-dung at a ratio of 3:1. The plants were irrigated every three days interval. The morphological data were taken every 7 days interval.

2.2. DNA Preparation

DNA was isolated using CTAB method following the protocol of Doyle and Doyle [22], with minor modifications. 1.0 - 1.5 g of young non-senescent leaves were ground in liquid nitrogen. Then they were incubated in CTAB buffer (3% w/v CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4M NaCl, 2% v/v β-mercaptoethanol, 2% w/v polyvinyl pyrrolidine, pH 8.0) for 2 h at 65˚C. The homogenate was then extracted with an equal volume mixture of chloroform:isoamylalcohol (24:1) and centrifuged at 9000 rpm for 10 min. The upper aqueous layer was recovered and precipitated with pre-chilled isopropanol. The pallet was suspended with Tris-EDTA buffer (pH 8.0). The crude DNA was treated with RNase and incubated for 30 min at 37˚C and again extracted with 1 volume phenol and subsequently with 1 volume of chloroform: isoamylalcohol (24:1). The supernatant were collected and precipitated with 3 M sodium acetate and pre-chilled ethanol. The DNA pellet was washed with 70% ethanol, dried, and re-suspended in TE buffer. The high molecular weight DNA was checked for quality and quantity electrophoretically using 0.8% agarose gel against a known amount of DNA taken as standard.

2.3. Primer Screening

Thirty synthesized ISSR primers (M/S Merck Bioscience, India) were initially screened using six genotypes to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved, and polymorphic amplified products within the genotypes. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded. A few well-amplified fragments that were not reproducible across 2 replicates of DNA extraction were also discarded from analysis.

2.4. PCR Amplifications and Electrophoresis of PCR Products

For ISSR experiment, the initial optimization of PCR was conducted including concentration of template DNA, primer, $MgCl_2$, number of PCR cycles, and annealing temperature. The PCR reaction had a total volume of 25 µl containing 20 ng templates DNA, 100 mM each dNTPs, 20 ng of oligonucleotides synthesized primer (M/S Emerck Bioscience, India), 2.5 mM $MgCl_2$, 1x Taq buffer (10 mM Tris-HCl) pH 9.0, 50 mM KCl, 0.01% gelatin], and 0.5 U Taq DNA polymerase (M/S Emerck Bioscience, India). DNA amplification was performed in a thermal cycler (Peqlab, Germany) programmed for a preliminary 5 min denaturation step at 94˚C, followed by 40 cycles of denaturation at 94˚C for 20 s, annealing temperature depending on the primer (50˚C - 56˚C) for 30 s and extension at 72˚C for 45 s, and finally at 72˚C for 5 min. Amplification products were separated alongside a low range molecular weight marker (M/S Emerck Bios-

cience, India) on a 2% (w/v) agarose gel electrophoresis in 1x TAE (Tris Acetate-EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (UVITECH, UK) and the amplification product sizes were evaluated using the software Quantity one (BioRad, California, USA).

2.5. Analysis of Amplification Profile

Clearly defined ISSR bands that behave as dominant markers were scored for the presence (1) or absence (0) for all the species/cultivars and entered into a data matrix. The genetic relationships among the species/cultivar were determined by calculating the Dice coefficient, estimated as S = 2NAB/NA + NB. Where NAB is the number of amplified product common to both A and B. NA and NB corresponds to number of amplified product in A and B, respectively. Diversity patterns were represented in the form of a dendogram that was generated by subjecting the genetic similarity matrix to Unweighted Pair-group Method Arithmetic average (UPGMA) cluster analysis with software NTSYS-pc, Version 2.0 [23].

3. Results and Discussion

All the varieties grew and developed well. The day of emergence after sowing was ranged from 3 - 5 days and percentage of germination varied from 50% - 100%. The data relating to plant height, stem length to the first capsule, days to maturity, number of fruiting branches and number of capsules/plant were presented in **Table 1**. The present study also illustrates to provide additional and rapid molecular marker suitable for identification of genetic resources and also to assess phylogenetic relationship among the *Sesamum* varieties occurring in India. Thirty ISSR primers were used to assess the 33 genotypes of *Sesamum* is presented in **Table 2**. Out of 30 primers, 18 primers generated clear multiplex banding profiles (good/good with less bands), among which ten primers produced the best ISSR profiles. On modification of annealing temperature, $MgCl_2$, primer concentration and template concentration did not improve their patterns. In addition, the results also showed that the most of the primers based on GA/AG and CA/GT dinucleotides core repeats generated good banding profiles. The amplification of ISSR profiles was consistent across three replicate DNA extractions from three samples, over with 98% of scorable fragments reproducible. This was in concordance with previous studies an oilseed where the reliability of the protocol was notified between PCRs and DNA extraction [24]. A higher concentration of $MgCl_2$ (2.5 mM) showed good results. This may be due to non-specific amplification because of reduced enzyme activity [25]. The concentration of magnesium chloride affects the specificity and yield of reaction by increasing the stringency of primer annealing or has a direct effect on Taq DNA polymerase [26]. The selected primers showed highly polymorphic banding profile. ISSR amplification for all samples resulted in multiple band fingerprint profile for the selected ISSR primers (**Figure 1**). The average number of identified fragments per primer was 6.33, with a range from 4 to 8, while the average

Table 1. Agromorphological characteristics of 33 genotypes of *Sesamum indicum* L.

Sr. No.	Genotypes	Days of Emergence after sowing	Germination percentage	Plant Height (cm)	Stem height to the first capsule (cm)	Days to maturity	Days to 50% flowering	Number of fruiting branches	Number of capsule per plant	Seed colour	Number of seeds per capsule	1000 seeds weight in (gm)	Number of seeds per plant
1	S1-2138-2	3	60	163.7 ± 10.2	55.37 ± 1.4	92	42	3	62	Black	50	2.43	3100
2	ES-120-1-84-13	5	60	160.4 ± 11.4	42.67 ± 1.8	90	40	2	52	Greyish white	35	2.18	1820
3	S-0140	3	75	150.9 ± 12.6	33.78 ± 1.7	89	41	3	53	Greyish black	47	3.18	2491
4	NIC-8463	4	75	167.5 ± 10.7	51.56 ± 1.5	85	42	3	56	Greyish white	33	2.71	1848
5	Amrit	3	80	143.3 ± 10.3	35.81 ± 1.3	78	37	4	67	Brownish white	36	2.82	2412
6	Nirmala	4	80	155.7 ± 11.4	33.52 ± 1.5	80	38	3	54	Brownish white	51	2.23	2754
7	Usha	6	60	112.5 ± 12.8	41.65 ± 1.7	81	40	3	62	Brownish white	40	2.36	2480
8	Prachi	3	90	160.4 ± 10.7	43.68 ± 1.4	85	39	3	63	Black	48	3.42	3024
9	Kalika	4	75	158.8 ± 10.2	46.48 ± 1.8	86	40	2	54	Brownish grey	51	2.46	2754
10	Kanaka	3	95	160.5 ± 11.4	41.40 ± 1.5	81	38	2	63	White	42	3.03	2646
11	S1-44	5	55	129.8 ± 12.8	25.40 ± 1.7	89	41	3	44	Brownish White	47	3.05	2068
12	S1-199-2-8 4	4	70	129.4 ± 10.5	25.40 ± 1.5	87	37	3	57	White	37	2.74	2109
13	RJS-44	4	60	135.3 ± 11.4	30.48 ± 1.9	91	37	4	43	White	45	3.34	1935
14	S1-1025	3	50	153.2 ± 12.6	55.88 ± 1.3	96	39	4	58	White	46	3.52	2668
15	S1-1671	5	70	164.7 ± 10.3	63.50 ± 1.2	84	40	8	60	White	39	3.67	2340
16	S1-85	5	70	140.5 ± 10.6	33.02 ± 1.5	92	38	4	61	White	38	3.60	2318
17	1S-684	5	85	150.4 ± 11.2	60.96 ± 1.7	89	43	3	58	White	36	3.48	2088
18	S1-339	6	60	145.3 ± 12.3	55.88 ± 1.4	91	40	3	57	Brownish black	40	3.14	2280
19	NIC-17508	4	75	148.7 ± 11.3	60.96 ± 1.6	88	41	4	59	White	38	2.71	2242
20	S1-1585	5	80	149.9 ± 10.5	22.86 ± 1.3	87	34	6	62	White	41	3.95	2542
21	S1-205	4	90	151.7 ± 11.2	25.04 ± 1.8	93	38	5	61	White	39	3.23	2379
22	KMR-11	4	90	142.4 ± 13.2	35.56 ± 1.5	86	41	6	55	White	40	3.13	2200
23	IC-1634-3	6	50	153.3 ± 11.4	40.64 ± 1.7	90	37	7	56	White	41	3.56	2296
24	KM-13	6	80	150.5 ± 10.4	60.96 ± 1.2	85	38	3	58	White	45	3.29	2610
25	S1-205	4	80	163.7 ± 11.4	68.58 ± 1.5	91	39	6	63	White	44	2.68	2772
26	S-0337	4	100	160.1 ± 12.5	63.50 ± 1.6	86	37	5	61	Black	44	2.72	2684
27	IS-607-1-84	4	50	160.3 ± 11.2	68.58 ± 1.2	86	42	4	45	Brownish black	41	3.16	1845

Continued

28	SI-1926	4	80	168.9 ± 10.8	78.74 ± 1.7	93	41	12	47	Brownish black	42	2.56	1974
29	NIC-13598	5	50	167.5 ± 10.5	84.32 ± 1.4	89	43	11	57	Brownish black	48	2.66	2736
30	ENT-78-30 1	3	80	158.7 ± 11.3	62.32 ± 1.5	86	42	4	45	Brownish black	47	2.50	2115
31	ES-71-A	5	60	154.6 ± 13.2	33.78 ± 1.3	88	40	4	45	Black	44	2.63	1980
32	S-336	4	100	165.4 ± 11.5	67.31 ± 1.5	95	38	12	46	Black	57	2.90	2622
33	KIS-398	4	90	165.5 ± 12.6	26.67 ± 1.4	85	40	4	48	Brownish black	53	2.36	2544

Table 2. Polymorphism detected by using the eighteen selected ISSR primers.

Name of the Primer	Sequence 5' - 3'	Total number of fragments	No. of polymorphic fragments	Percentage of polymorrphism (%)	Band range (bp)
UBC-818	(CA)8G	08	07	87.5	280 - 900
UBC-825	(AC)8T	06	02	33.3	350 - 1000
UBC-890	(ACG) 2ACTGTG (TGT) 2GTT	05	05	100	200 - 800
UBC-855	(AC) 8CTT	06	06	100	350 - 1200
USB-807	(AG) 8T	08	08	100	550 - 3500
USB-808	(AG) 8C	05	04	80.0	600 - 1200
USB-810	(GA) 8T	07	06	85.7	200 - 800
USB-811	(GA) 8C	09	07	77.8	300 - 1000
USB-835	(AG) 8AC	04	02	50.0	270 - 850
USB-838	(GA) 8AT	07	07	100	300 - 2000
USB-839	(GA) 8GC	03	02	66.7	450 - 1700
USB-840	(CT) 8T	06	06	100	400 - 1500
USB-843	(GA) 8GT	05	04	80.0	300 - 850
USB-844	(GA) 8AG	06	04	66.7	200 - 800
AM1	(GGC) 5AT	08	07	87.5	350 -1500
AM2	(AAG) 5GC	06	06	100	350 - 1200
AM4	(AAG) 5CC	08	07	87.5	200 - 1250
AM6	(AGC) 5GG	07	07	100	350 - 1200
		114	97	85.08	

number of polymorphic fragments per primer was 5.38 with a range from 2 to 8. Out of the 114 scorable fragments, 97 were polymorphic revealing 85.08% polymorphism across the 33 varieties studied (Table 2). In this study primers based on GA/AG and GT/TG dinucleotides core repeats generated good profiles, which seem to indicate that the more frequent microsatellite in *Sesamae* contains the repeated dinucleotides

(AG/GA) n and (GT/TG) n. Our results are in accordance with the SSR analysis reported earlier [18]. Baydar [27] reported that the sesame varieties with non-branching character having higher yield advantages for mechanized cultivation, because of their uniform maturity in a short growing period. The varieties were ranked with low to high seed yield as reported [28]. Sharma *et al.* [29] reported that RAPD and ISSR markers may be converted to co-dominant sequence characterized amplified region/sequence tagged site (SCAR/STS) markers to develop variety specific markers. The present study indicates that there was a distant variation in DNA amplification of 33 varieties of *Sesamum* collected from Eastern India. The matrix calculated for all possible pair wise comparisons between varieties showed that the index value varied from a minimum of 0.45 between "SI-2138-2" and "S-0140" to a maximum of 0.87 between "Usha and KIS-398" and "Kanaka" and "KIS-398" (Table 3). All the others ones have displayed

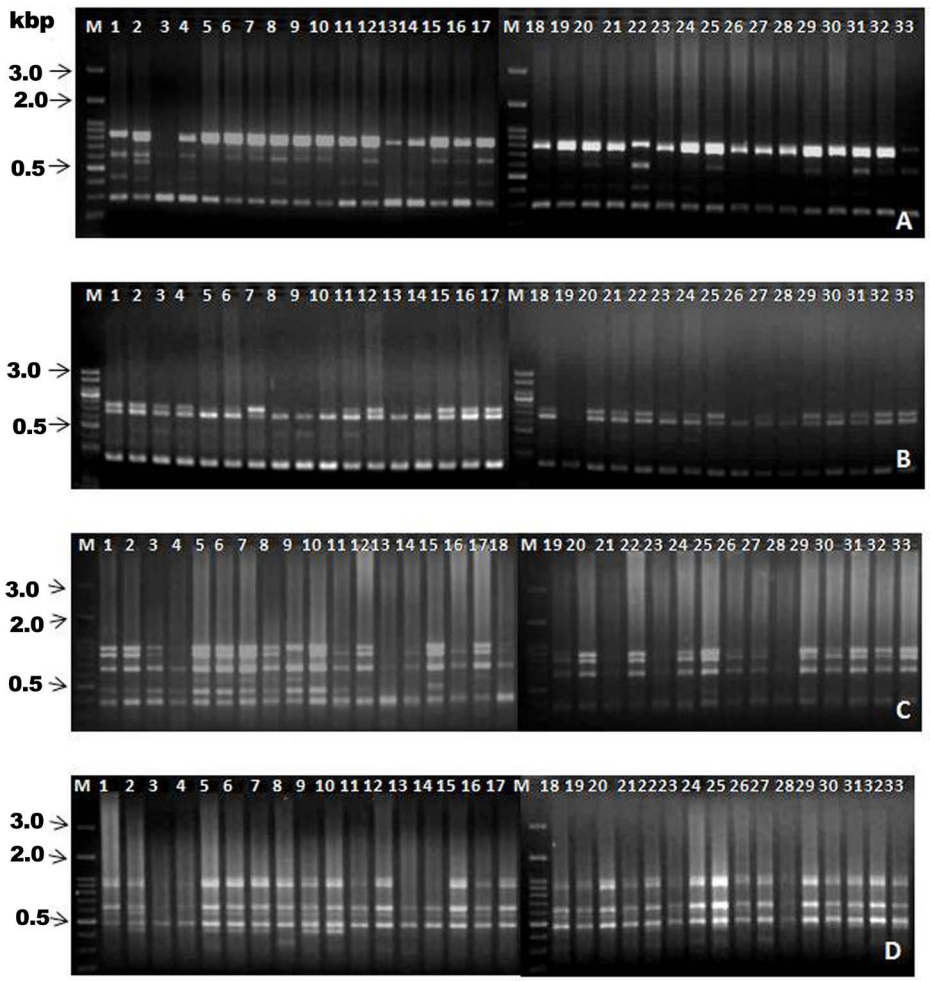

Figure 1. ISSR banding pattern of 33 varieties of Sesamum using synthesis primer: USB 843 (A), USB-835 (B), UBC-818 (C) and UBC-825 (D). M-low range DNA marker. No. 1 - 33 is the varieties of Sesamum as in Table 1.

Table 3. Similarity coefficient among the 33 varieties of *Sesamum indicum* obtained from ISSR markers. (V1-V33 is the varieties as indicated in **Table 1**).

	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31	v32	v33
v1	1																																
v2	0.63	1																															
v3	**0.45**	0.53	1																														
v4	0.6	0.64	0.56	1																													
v5	0.64	0.71	0.51	0.64	1																												
v6	0.69	0.69	0.46	0.59	0.77	1																											
v7	0.68	0.68	0.43	0.62	0.83	0.81	1																										
v8	0.63	0.67	0.5	0.64	0.64	0.72	0.71	1																									
v9	0.64	0.74	0.48	0.68	0.83	0.89	0.83	0.74	1																								
v10	0.71	0.71	0.45	0.64	0.83	0.84	0.91	0.78	0.87	1																							
v11	0.57	0.74	0.58	0.68	0.71	0.73	0.72	0.74	0.75	0.71	1																						
v12	0.73	0.72	0.52	0.74	0.73	0.74	0.81	0.84	0.77	0.84	0.8	1																					
v13	0.33	0.39	0.43	0.47	0.4	0.41	0.38	0.42	0.4	0.43	0.47	0.41	1																				
v14	0.45	0.53	0.57	0.61	0.48	0.4	0.46	0.47	0.42	0.45	0.58	0.56	0.57	1																			
v115	0.66	0.66	0.46	0.59	0.67	0.65	0.74	0.66	0.7	0.74	0.6	0.76	0.31	0.49	1																		
v16	0.62	0.66	0.48	0.71	0.6	0.61	0.67	0.7	0.63	0.7	0.7	0.72	0.5	0.59	0.65	1																	
v17	0.67	0.63	0.53	0.67	0.71	0.65	0.71	0.74	0.71	0.71	0.67	0.8	0.39	0.57	0.77	0.74	1																
v18	0.49	0.5	0.41	0.49	0.48	0.56	0.56	0.53	0.48	0.55	0.55	0.56	0.57	0.53	0.49	0.64	0.5	1															
v19	0.64	0.68	0.53	0.53	0.65	0.63	0.59	0.68	0.61	0.65	0.61	0.67	0.43	0.53	0.63	0.63	0.64	0.57	1														
v20	0.62	0.77	0.49	0.63	0.74	0.76	0.82	0.73	0.82	0.78	0.78	0.76	0.4	0.49	0.73	0.69	0.7	0.6	0.68	1													
v21	0.59	0.59	0.51	0.68	0.64	0.54	0.61	0.59	0.64	0.6	0.71	0.69	0.46	0.56	0.58	0.66	0.63	0.47	0.64	0.66	1												
v22	0.76	0.6	0.5	0.57	0.65	0.66	0.72	0.67	0.68	0.72	0.65	0.73	0.29	0.47	0.67	0.59	0.71	0.4	0.61	0.67	0.6	1											
v23	0.52	0.57	0.53	0.61	0.58	0.49	0.55	0.5	0.54	0.51	0.58	0.52	0.47	0.53	0.46	0.51	0.47	0.49	0.57	0.6	0.64	0.5	1										
v24	0.7	0.7	0.47	0.6	0.78	0.76	0.87	0.7	0.78	0.86	0.74	0.8	0.42	0.5	0.73	0.7	0.7	0.57	0.68	0.77	0.67	0.75	0.6	1									
v25	0.66	0.7	0.49	0.56	0.67	0.61	0.71	0.66	0.67	0.7	0.67	0.68	0.37	0.49	0.73	0.65	0.7	0.49	0.72	0.77	0.7	0.74	0.6	0.77	1								
v26	0.56	0.57	0.53	0.65	0.65	0.63	0.62	0.64	0.65	0.65	0.61	0.63	0.56	0.53	0.52	0.59	0.57	0.62	0.66	0.68	0.64	0.57	0.71	0.68	0.63	1							
v27	0.62	0.7	0.6	0.67	0.74	0.68	0.71	0.66	0.74	0.7	0.74	0.68	0.4	0.52	0.69	0.65	0.66	0.49	0.63	0.73	0.7	0.7	0.56	0.77	0.73	0.68	1						
v28	0.47	0.49	0.43	0.65	0.53	0.44	0.51	0.49	0.53	0.5	0.57	0.55	0.41	0.52	0.48	0.58	0.52	0.43	0.51	0.55	0.74	0.49	0.61	0.56	0.55	0.61	0.59	1					
v29	0.72	0.68	0.55	0.65	0.76	0.74	0.8	0.68	0.8	0.8	0.69	0.74	0.36	0.44	0.75	0.64	0.72	0.48	0.62	0.75	0.64	0.81	0.58	0.84	0.79	0.66	0.83	0.54	1				
v30	0.76	0.61	0.51	0.61	0.76	0.67	0.77	0.65	0.72	0.76	0.65	0.74	0.36	0.44	0.75	0.6	0.76	0.48	0.62	0.71	0.68	0.73	0.62	0.8	0.75	0.66	0.75	0.57	0.86	1			
v31	0.74	0.6	0.5	0.64	0.71	0.76	0.83	0.74	0.74	0.82	0.71	0.8	0.42	0.47	0.73	0.66	0.74	0.53	0.6	0.73	0.67	0.79	0.57	0.82	0.77	0.68	0.73	0.56	0.84	0.84	1		
v32	0.58	0.66	0.59	0.67	0.7	0.64	0.67	0.7	0.7	0.67	0.74	0.68	0.42	0.47	0.61	0.65	0.66	0.55	0.68	0.78	0.7	0.63	0.59	0.7	0.73	0.72	0.78	0.63	0.76	0.71	0.74	1	
v33	0.75	0.67	0.51	0.64	0.79	0.77	0.87	0.74	0.79	0.87	0.71	0.84	0.37	0.48	0.74	0.67	0.78	0.48	0.65	0.74	0.71	0.8	0.54	0.82	0.78	0.61	0.74	0.57	0.84	0.84	0.86	0.7	1

various intermediate levels of similarity. Cluster analysis showing a dendrogram using Jaccard's coefficient under UPGMA method divided into two major clusters at 0.45 similarity level: A1 and A2 (Figure 2). Cluster A1 had 2 varieties and cluster A2 had 31 varieties. Cluster A1 had two varieties "RJS-44" and "SI-339". Similarly, A2 major cluster was divided into two minor groups *i.e.* B1 and B2 with 56% similarity with each other. The minor cluster B1 had only two varieties *i.e.* "S-0140" and "SI-1025". Another minor cluster B2 was further divided into two sub-minor clusters *i.e.* C1 and C2 having 65% similarity with each other. The minor sub cluster C1 having four varieties ("SI-205", "IC-1634-3", "S-0337" and "SI-1926") with 70% similarity with each other... However, "SI-205" and "SI-1926" have 76% similarity among themselves. The minor sub cluster C2 was further divided into two clusters *i.e.* D1 and D2 with 67% similarity. In this study, it was observed that the var. Usha and Kanaka have highest similarity (87%) among themselves and similar days of maturity and day to 50% flowering. But, morphologically they were very close similarity among themselves. The varieties "Nirmala" and "Kalika" have 82% similarity with each other and 80% similarity with "Amit". All these three varieties were morphologically alike with high germination ability and 1000 seed weight ranged from 2.46 to 2.82 g. Our results indicate that the genetic relationship among the *Sesamae* varieties, inferred by ISSR markers, were in accordance with their morphological characters. The principal component analysis (PCA) and 2D analysis were reflected in Figure 3 & Figure 4. The molecular markers consistently show that pollinator wasp species are deeply and consistently well resolved phylogenetically, both within and among the varieties that are associated with different sesamae germplasms as reported [21]. The *Sesamae* is a short day plant and is normally

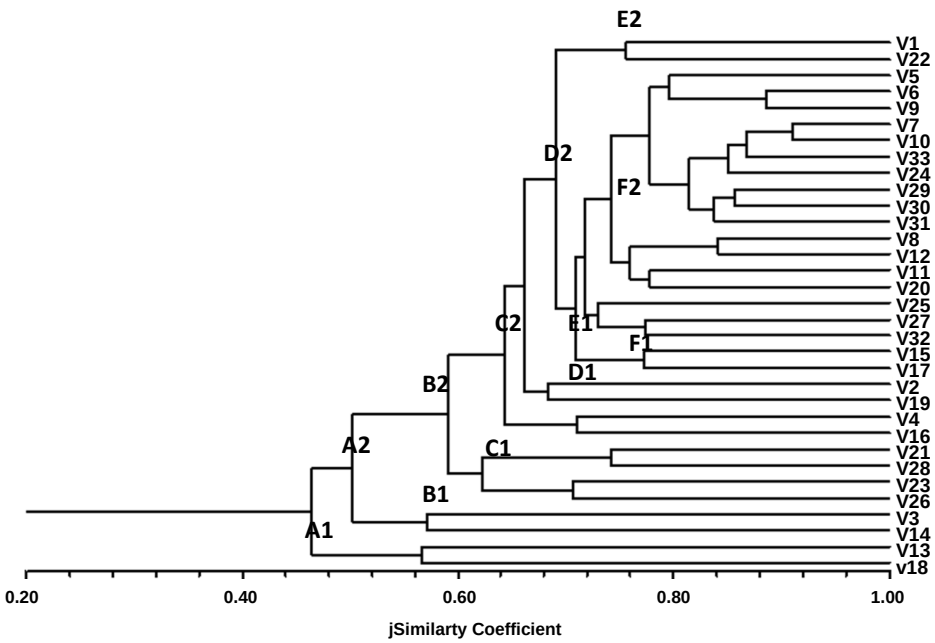

Figure 2. UPGMA dendrogram based on the similarity coefficient, showing the clustering pattern among the 33 varieties of Sesamum. V1 - V33 is the varieties of Sesamum as in Table 1.

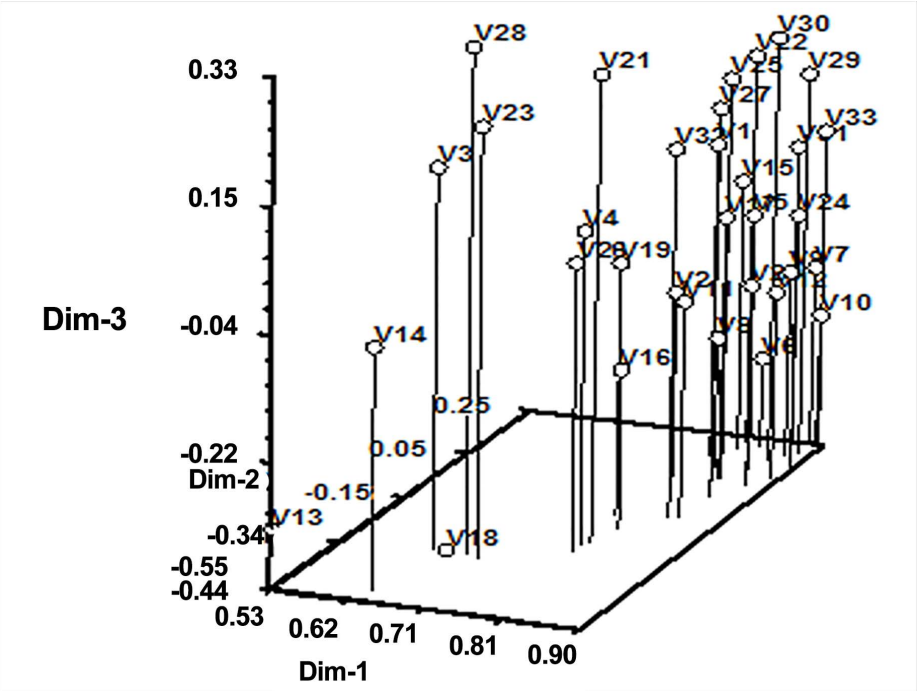

Figure 3. Principal Component analysis based on the similarity coefficient, showing the distribution pattern among the 33 varieties of Sesamum. V1 - V33 is the 33 varieties of Sesamum as in Table 1.

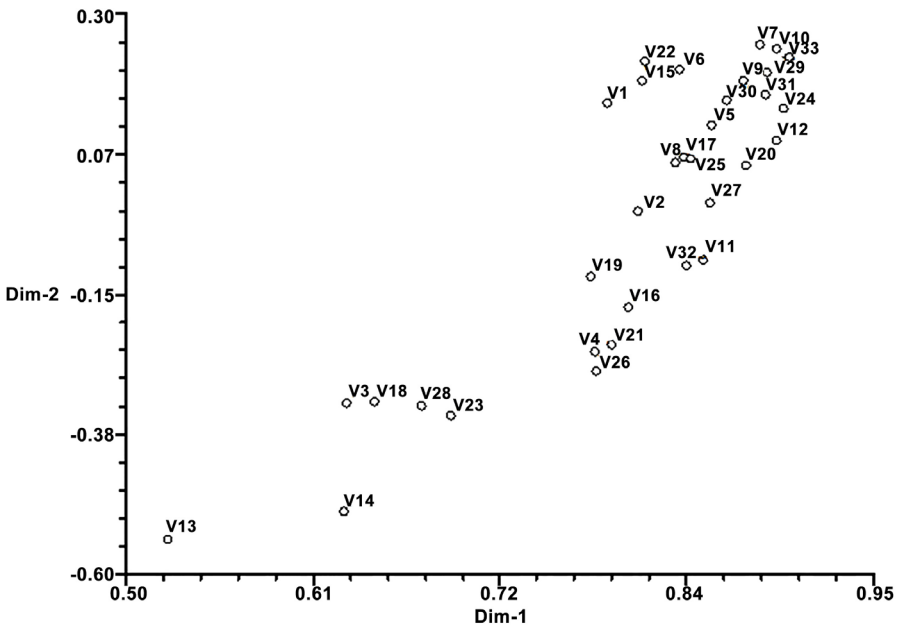

Figure 4. 2D-analysis based on the similarity coefficient, showing the distribution pattern among the 33 varieties of Sesamum. V1 - V33 is the 33 varieties of Sesamum as in Table 1.

self pollinated, although cross pollinated ranging from 5 to over 50% occurs. Most of

the varieties show an indeterminate growth habit.

4. Conclusion

This study indicates that the information for variety identification and the presence of genotypes in the distribution of parental species for *Sesamae* have been confirmed with both agro-mophological and ISSR markers. This analysis is quick and reproducible, and can generate sufficient polymorphism to identify the varieties, although most ISSR alleles are dominant rather than co-dominant. This investigation could be refined to include more markers and individual variety analysis for detailed characterization of *Sesamae* taxa that would be essential for future breeding and crop improvement program.

Acknowledgements

The authors also acknowledge to Officer-in-Charge, AICRP Sesamum, OUAT, Bhubaneswar for providing seed materials for the experiment.

References

[1] FAO (2000) The State of Food and Agriculture, 2000. www.fao.org

[2] Morris, J.B. (2002) Food, Industrial, Nutraceutical, and Pharmaceutical Uses of Sesame Genetic Resources. In: Janick, J. and Whipkey, A., Eds., *Trends in New Crops and New Uses*, ASHS Press, Alexandria, 153-156.

[3] Ashri, A. (1989) Sesame. In: Robbelen, G., Downey, R.K. and Ashri, A., Eds., *Sesame-Oil Crops of the World*, McGraw-Hill Publishing Company, New York, 375-387.

[4] Fukuda, Y., Nagata, M., Osawa, T. and Namiki, M. (1986) Contribution of Lignin Analogues to Antioxidative Activity of Refined Unroasted Sesame Seed Oil. *Journal of the American Oil Chemists' Society,* **63**, 1027-1031. https://doi.org/10.1007/BF02673792

[5] Bedigian, D., Seigler, D.S. and Harlan, J.R. (1985) Sesamin, Sesamolin and the Origin of Sesame. *Biochemical Systematics and Ecology*, **13**, 133-139. https://doi.org/10.1016/0305-1978(85)90071-7

[6] Ram, S.G., Sundaravelpandian, K., Kumar, M., Vinod, K.K., Bapu, J.K. and Raveendran, T.S. (2006) Pollen-Pistil Interaction in the Inter-Specific Crosses of *Sesamum* sp. *Euphytica,* **152**, 379-385. https://doi.org/10.1007/s10681-006-9225-1

[7] Anthony, O., Ogunshakin, R., Vaghela, S. and Patel, B. (2015) Towards Sustainable Intensification of Sesame-Based Cropping Systems Diversification in North Western India. *Journal of Food Security*, **3**, 1-5.

[8] FAOSTAT (2015) Food and Agricultural Organization of the United Nations. Statistical Database.

[9] Kim, D.H., Zur, G., Danin-Poleg, Y., Lee, S., Shim, K., Kang, C. and Kashi, Y. (2002) Genetic Relationships of Sesame Germplasm Collection as Revealed by Inter-Simple Sequence Repeats. *Plant Breeding*, **121**, 259-262. https://doi.org/10.1046/j.1439-0523.2002.00700.x

[10] Bhat, K.V., Babrekar, P.P. and Lakhanpaul, S. (1999) Study of Genetic Diversity in Indian and Exotic Sesame (*Sesamum indicum* L.) Germplasm Using Random Amplified Polymorphic DNA (RAPD) Markers. *Euphytica*, **110**, 21-34. https://doi.org/10.1023/A:1003724732323

[11] Ercan, A.G., Taskin, M. and Turgut, K. (2004) Analysis of Genetic Diversity in Turkish Sesame (*Sesamum indicum* L.) Populations Using RAPD Markers. *Genetic Resources and*

Crop Evolution, **51**, 599-607.

[12] Hawkes, J. (1983) The Diversity of Crop Plants. Harvard University Press, Cambridge. https://doi.org/10.4159/harvard.9780674183551

[13] Dixit, A., Jin, M.H., Chung, J.W., Yu, J.W., Chung, H.K., Ma, K.H., Park, Y.J. and Cho, E.G. (2005) Development of Polymorphic Microsatellite Markers in Sesame (*Sesamum indicum* L.). *Molecular Ecology Notes*, **5**, 736-738. https://doi.org/10.1111/j.1471-8286.2005.01048.x

[14] Laurentin, H.E. and Karlovsky, P. (2006) Genetic Relationship and Diversity in a Sesame (*Sesamum indicum* L.) Germplasm Collection Using Amplified Fragment Length Polymorphism (AFLP). *BMC Genetics*, **7**, 10-18. https://doi.org/10.1186/1471-2156-7-10

[15] Laurentin, H.E. and Karlovsky, P. (2007) AFLP Fingerprinting of Sesame (*Sesamum indicum* L.) Cultivars Identification, Genetic Relationship and Comparison of AFLP Informativeness Parameters. *Genetic Resources Crop Evolution*, **54**, 1437-1446. https://doi.org/10.1007/s10722-006-9128-y

[16] Ali, G.M., Yasumoto, S. and Seki-Katsuta, M. (2007) Assessment of Genetic Diversity in Sesame (*Sesamum indicum* L.) Detected by Amplified Fragment Length Polymorphism Markers. *Electronic Journal of Biotechnology*, **10**, 12-23. https://doi.org/10.2225/vol10-issue1-fulltext-16

[17] Wu, K., Yang, M., Liu, H., Tao, Y., Mei, J. and Zhao, Y. (2014) Genetic Analysis and Molecular Characterization of Chinese Sesame (*Sesamum indicum* L.) Cultivars Using Insertion-Deletion and Simple Sequence Repeat (SSR) Markers. *BMC Genetics*, **15**, 35-45. https://doi.org/10.1186/1471-2156-15-35

[18] Zhang, P., Zhang, H.Y., Guo, W.Z., Zheng, Y.Z., Wei, L.B. and Zhang, T.Z. (2007) Genetic Diversity Analysis of *Sesamum indicum* L. Germplasms Using SRAP and EST-SSR Markers. *Genes and Genomics*, **32**, 207-215. https://doi.org/10.1007/s13258-009-0888-6

[19] Pandey, S.K., Das, A., Rai1, P. and Dasgupta, T. (2015) Morphological and Genetic Diversity Assessment of Sesame (*Sesamum indicum* L.) Accessions Differing in Origin. *Physiology and Molecular Biology of Plants*, **21**, 519-529. https://doi.org/10.1007/s12298-015-0322-2

[20] Banerjee, P.P. and Kole, P.C. (2009) Analysis of Genetic Architecture for Some Physiological Characters in Sesame (*Sesamum indicum* L.). *Euphytica*, **168**, 11-22. https://doi.org/10.1007/s10681-008-9871-6

[21] Tabatabaei, I., Pazouki, L., Bihamta, M.R., Mansoori, S., Javaran, M.J. and Niinemets, U. (2011) Genetic Variation among Iranian Sesame (*Sesamum indicum* L.) Accessions vis-à-vis Exotic Genotypes on the Basis of Morpho-Physiological Traits and RAPD Markers. *Australian Journal of Crop Science*, **5**, 1396-1407.

[22] Doyle, J.J. and Doyle, J.L. (1990) Isolation of Plant DNA from Fresh Tissue. *Focus*, **12**, 13-15.

[23] Rohlf, F.J. (2000) NTSYS-PC Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics, Exerter Publishing Ltd., New York.

[24] Charters, Y.M., Robertson, A. and Wilkinson, M.J. (1996) PCR Analysis of Oil Seed Rape Cultivars (*Brassica napus* L. ssp. *Ollifera*) Using 5'-Anchored Simple Sequence Repeat (SSR) Primers. *Theoretical and Applied Genetics*, **92**, 442-447. https://doi.org/10.1007/BF00223691

[25] Hopkins, K.L. and Hilton, A.C. (2001) Optimization of Random Amplification of Polymorphic DNA Analysis for Molecular Sub Typing of *Escherichia coli*. *Letters in Applied Microbiology*, **32**, 126-130. https://doi.org/10.1046/j.1472-765x.2001.00871.x

[26] Saiki, R.K. (1989) The Design and Optimization of the PCR, In: Erlich, H.A., Ed., *PCR*

Technology: *Principles and Applications for DNA Amplification*, Palgrave Macmillan, New York, 7-16. https://doi.org/10.1007/978-1-349-20235-5_1

[27] Baydar, H. (2005) Breeding for the Improvement of the Ideal Plant Type of Sesame. *Plant Breeding*, **24**, 263-267. https://doi.org/10.1111/j.1439-0523.2005.01080.x

[28] DuePham, T., ThiNguyen, T.D., Carlsson, A.S. and Buui, T.M. (2010) Morphological Evaluation of Sesame (*Sesamum indicum* L.) Varieties from Different Region. *Australian Journal of Crop Science*, **4**, 498-504.

[29] Sharma, S.N., Kumar, V. and Mathur, S. (2009) Comparative Analysis of RAPD and ISSR Markers for Characterization of Sesame (*S. indicum* L.) Genotypes. *Journal of Plant Biochemistry and Biotechnology*, **18**, 37-43. https://doi.org/10.1007/BF03263293