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Analysis of genetic diversity in sesame (*Sesamum indicum* L.) germplasm using RAPD markers and disease resistance screening through RGA markers

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Abstract

Sesame (*Sesamum indicum* L.) belongs to an angiosperm family Pedaliaceae. It is an ancient oilseed crop known to mankind and it is a diploid species. Its oil is highly prized as it consists of natural antioxidants. *S. indicum* has a large number of varieties and are susceptible to many pathogens. There is a huge yield loss due to an insect pathogen *Antigastra catalaunalis*, viral, bacterial and mycoplasma. In the present study, molecular characterization of 40 sesame germplasm from different geographical regions of the world and Indian accessions were included. The study was under taken to determine the extent of genetic diversity between the varieties at molecular level. OPA, OPB and OPM series of RAPD decamers were screened and a total of 150 amplified fragments were scored, out of which 122 fragments are polymorphic with an average of 7.62 polymorphic fragments per primer. The average percent polymorphism was 80.5 among the accessions studied. The similarity indices were ranging from 0.41 to 0.98. Cluster analysis was done by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based dendrogram. The results indicates that, with the use of a limited number of decamers, RAPD technique revealed a high level of polymorphism among the accessions and are reliable markers to assess the genetic diversity within sesame germplasm effectively for breeding programme. Screening for disease resistant varieties was carried out using RGA markers and two primers showed positive test for presence of '*R*' gene in the sesame accessions and diploid wild species.

Key words : Sesame; NTSYS; RAPD; Pedaliaceae; RGA

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an ancient oil yielding crop and known as "Queen of Oilseeds" belongs to family Pedaliaceae. Oilseeds constitute major agricultural crop and rank next only to the cereals. Seed oil or vegetable oils are consumed as food and several of them have industrial uses. Commercially, only about 30 species have been exploited as source of vegetable oil. About 90% of total world production of vegetable oil comes from only 12 species [1]. Vegetable oil accounts for about 70% of the world's edible oil source and remainder of 30% comes from animal fat. Most important edible oil seed crops are *Arachis hypogea, Glycine max, Brassica* species*, Heliantus annuus, Carthamus tinctorius* and *Guizotia abyssinica.* Among, edible oilseed crops *Sesamum indicum,* is an important crop yielding high quality seed oil. *Sesamum* oil is a premium commodity due to its stability against prolonged storage and heating due to its natural antioxidants, sesamin and sesamolin. No other cultivated edible oil seed contain these antioxidants. Sesame oil has large proportion of unsaturated fatty acids i.e. oleic (41%) and linoleic acid (45%) and do not contain anti-nutritional or toxic compounds [2, 3, 4] and it also contains up to 25% proteins with an exceptionally high amount of methionine. This is a favourable feature as an additional food element for human diet. Sesame generally requires a hot climate (23-28 °C) for its growth and development. Sesame is reasonably drought resistant but the crop grows well in the area with 500-600 mm annual rainfall. It is usually grown on rain fed areas and its drought tolerant qualities are its chief advantages. It can grow in a wide variety of soils, provided they are well drained but it is very difficult to harvest the crop because of indeterminate growth and dehiscent capsules [5].

Sesame is attacked by many viral, fungal and bacterial pathogens and severe insect pests also cause significant yield loss. Among viral pathogens, phyllody and leaf curl diseases are most prominent and many leads to severe yield loss. Most serious fungal diseases are leaf spots caused by *Cercospora sesami* Zimm., *Alternaria* species, *Fusarium oxysporum* var. *sesami*, stem and root rot caused by *Phytophthora* and *Rhizoctonia* species. Bacterial leaf spot disease due to *Pseudomonas sesami* also causes considerable yield loss. Among the insect pests, leaf roller and capsule borer *Antigastra catalaunalis* and hairy caterpillar *Diacrisia obliqua* causes acute damage to sesame crop [3, 6]. Therefore, it is important to improve this neglected oil seed crop. For the selection of better genotypes required in plant breeding programme, molecular techniques for assessing molecular diversity and screening for disease resistant genotypes is very essential.

The principal sesame growing countries in the world are China, India, Burma, Pakistan, Thailand, Ceylon, Turkey, Iraq, Syria, Sudan, Uganda, Nigeria, Tanzania, Congo, Egypt, Somaliland, Ethiopia, Greece, Mexico, Costa-Rica, El-Salvador, Columbia and Venuzuela [6].

During 2007, India was the world's largest producer followed by Myanmar (Burma), China, and Thailand. China and India have alternate ranking as highest producer of sesame for several decades [7, 8]. Sesame is cultivated in 7.8 million hectare area with a production of 3.83 million tons worldwide [9]. During 2008, the total world production was about 3.5 million tons that was grown on 7 million hectares. India covers about 45% of the world's area under sesame cultivation and the total production was nearly 52 thousand tons. India ranks first, both in the total area under cultivation and production of this oilseed crop in the world. However, the productivity is quite low (368 kg/ha) as compared to worlds average (489 kg/ha) (www.fao.org).

In India, it is mainly grown in the states of Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh, Tamil Nadu, Orissa, Karnataka and Gujarat.

Genetic markers are the powerful tools for evolutionary and population studies, constructing genetic maps and designing applied breeding programs. When the genetic marker data is interpreted correctly, these are invaluable for examining genetic variation among and within populations, assessing level of out crossing and inbreeding and genetic identification or fingerprinting of varieties or pedigrees. Genetic marker data can also be used for assisting with early selection of better genotypes [10].

Use of PCR based RAPD markers has profoundly increased the potentiality to detect genetic polymorphism among organisms, particularly for those in which information about genome relations are unknown [11, 12]. In recent years, there has been increased interest in the use of DNA based molecular markers for a variety of applications in population genetics, evolutionary genetics, conservation, crop improvement and hybrid seed production.

Only limited reports are available on genetic diversity studies of this oilseed crop with the use of PCR based molecular markers viz., RAPD [13, 14, 15, 16], comparative studies with RAPD and morphological traits [17, 18, 19], AFLP [20, 21, 22, 23, 24, 25], ISSR [26, 27, 28], SSR [29, 30, 31, 32], isozymes [33], morphological and agronomic characters [34].

Li and Midmore [35] reported that when the variation between genotypes is high, the use of a few primers will be sufficient. Many researchers have been reported their findings on genetic variability among various crop species with the use of limited RAPD primers. Ercan *et al.* [15] have studied the genetic diversity among thirty eight accessions of sesame germplasm from different regions of Turkey using twelve RAPD markers. Similarly, Sandigwad and Patil [36] have studied the genetic diversity in different genotypes of *Cinnamomum zeylanicum* Blume (Lauraceae) collected from south India using eleven RAPD primers, Maheshwari and Patil [37] analyzed the genetic divergence among *Capsicum annuum* genotypes using sixteen RAPD markers and Sankannavar and Patil [38] were undertaken RAPD marker system to study the genetic relatedness between *Phyllanthus* species, whereas Tahira *et al.* [39] have used twelve RAPD decamers to evaluate the genetic divergence in thirty lines of *Brassica juncea*.

Similarly, efforts are being made to identify the markers tightly linked to the genes responsible for disease resistance which will be useful for marker assisted breeding. Zhang *et al.* [29] found an AFLP marker linked to Powdery mildew resistance in cucumber. Prashanthi *et al.* [40] screened fifteen lines of mungbean and they identified three resistant sources with specific amplification generated by OPT16 primer at 900 bp. Liu *et al.* [41] studied the relationship between blast resistance phenotypes and resistant gene analogue profiles in rice. A total of twenty one rice varieties were assayed based on RGA-PCR using six pairs of RGA primers and evaluated for leaf blast resistance. Suvendu *et al.* [42] have studied the differential response to rust and leaf spot disease in groundnut using ISSR markers. Claire *et al.* [43] while working on isolation and functional characterization of a cluster of TIR-NBS-LRR genes linked to powdery mildew resistance in grapevine found that the North American grapevine *Muscadinia rotundifolia* is completely resistant to powdery mildew due to the action of a single, dominant locus designated *Run1*. In tomato, RAPD markers for *Fusarium* crown and root rot resistant gene (*Frl*) [44], *Pto* locus conferring resistance to *Pseudomonas syringae* were identified using near isogenic lines [45]. In apples, RAPD markers have been identified as linked to resistance gene *Vfl* [46].

Table 1: List of sesame germplasm and their place of collection

S No.	Accession Number	Place of Collection
1	IC 132680	Maharashtra
2	EC 361722	Sri Lanka
3	IC 132692	Rajasthan
4	IC 31843	Madhya Pradesh
5	IC 201123	Madhya Pradesh
6	EC 334963	Bangladesh
7	EC 361725	Sri Lanka
8	IC 132392	Punjab
9	IC 413234	Andhra Pradesh
10	EC 358989	Nepal
11	IC 132702	Madhya Pradesh
12	IC 132362	Maharashtra
13	IC 96221	Andhra Pradesh
14	IC 141169	Rajasthan
15	EC 361724	Sri Lanka
16	IC 96234	Karnataka
17	EC 350653	Japan
18	EC 359900	Mayanmar
19	IC 413262	Andhra Pradesh
20	EC 359013	Nepal
21	EC 334956	Bangladesh
22	IC 413239	Andhra Pradesh
23	IC 132692	Rajasthan
24	IC 201123	Karnataka
25	IC 132692	Rajasthan
26	EC 357019	Mayanmar
27	IC 132659	Maharashtra
28	IC 132174	Tamil Nadu
29	IC 132671	Maharashtra
30	IC 41257	Gujarat
31	LC Shekar	Karnataka
32	LC MT16	Karnataka
33	LC SSD21	Karnataka
34	LC SVPR1	Karnataka
35	LC SSD22	Karnataka
36	LC RT351	Karnataka
37	LC DS05	Karnataka
38	LC JCS1020	Karnataka
39	LC MT10	Karnataka
40	LC DS01	Karnataka

IC - Indian Collection; EC - Exotic Collection; LC - Local Collection

Hence, present investigation is carried out with the objectives: (1) to assess genetic polymorphism among local, national and international sesame accessions using RAPD markers and (2) to screen the accessions for disease resistant gene through RGA markers. This investigation may pave the way to identify the sesame genotype with disease resistant genes and the possibility of its transfer to the local/cultivated varieties of sesame through classical plant breeding methods or through transformation method.

MATERIALS AND METHOD

Plant material

Present investigation includes 40 sesame genotypes consisting of international, national and local accessions

representing different geographical areas. The details of sesame accessions with their place of collection were given in Table 1. Out of fourty sesame accessions, ten exotic accessions are from Bangladesh, Nepal, Sri Lanka, Mayanmar and Japan, and thirty Indian accessions from different states namely Maharashtra, Rajasthan, Andhra Pradesh, Madhya Pradesh, Punjab, Tamil Nadu, Gujarat including ten local accessions of Karnataka state.

DNA extraction

All the sesame accessions were grown and maintained in the Botany experimental garden. Five to six young leaves were taken from each accession for DNA extraction using modified CTAB method [47].

Leaves were ground into fine powder in liquid nitrogen, transferred to 50 ml centrifuge tube containing 10 ml preheated (65° C) CTAB extraction buffer. The tubes were kept in water bath for 10-15 minutes at 65° C with periodical shaking at an interval of five minutes. Later, the tubes were incubated at room temperature for 15 minutes. 10 ml of chloroform:Isoamyl alcohol (CIA) mixture (24:1) was added and the contents were mixed by shaking gently. Then the tubes were centrifuged for 10 minutes at 15,000 rpm at room temperature. The supernatant was carefully transferred to fresh centrifuge tubes and 10 ml of chilled Isopropanol was added to each tube and mixed by inverting and stored overnight at -20° C. The contents were centrifuged for 10 minutes at 15,000 rpm at 4° C. DNA pellet obtained was washed with 70 per cent ethanol/wash buffer and the tubes were inverted on blotting paper to dry the pellet. DNA was dissolved in 200 µl $T_{10}E_1$ buffer and stored at -20° C until it's use.

PCR amplification

A total of twenty eight RAPD decamer primers belonging to OPA, OPB and OPM series (Operon Technologies Inc., Almeida, USA) and twenty RGA markers were used in the present work. The PCR reaction was carried out to amplify the genomic DNA by using master cycler gradient 5331 eppendorf, Germany and the results were analyzed as described by Williams *et al* [11]. Each 25 µl of the PCR reaction mixture consists of 50 ng genomic DNA, 10X *Taq* buffer with 15 mM $MgCl_2$, 10 mM each dNTP's, 5 pM primer, 0.5 units of *Taq* DNA polymerase (GeNei™, Bangalore, India). 10 pM of each forward and reverse primers were used in the RGA-PCR reactions, while all other conditions were the same. The standardized PCR reaction programme was with initial denaturation temperature of 94 ºC for 4 min, 40 cycles (35 cycles for RGA) of 1 min denaturation at 94 ºC, 1 min annealing at 38 ºC (43º- 49ºC for RGA), 2 min for extension at 72 ºC, followed by final extension of 7 min at 72 ºC to ensure the completeness of the primer extension and cooling at 4°C.

Agarose gel electrophoresis

Amplified RAPD-PCR products were resolved on 1.2% (w/v) agarose gel with ethidium bromide (5µg/ml) in 1X TAE, while RGA-PCR products were separated on high-resolution 3% (w/v) Metaphor agarose gel together with a 100 bp ladder. The agarose gel electrophoresis was carried out for 50 V for the first 15 minutes and it is raised to 100 V for rest of the period. Gels were visualized, documented and photographed in Gel-documentation unit (UVtech, Cambridge). The size of PCR amplicon was estimated by comparing it with a 1 kb and 100 bp molecular weight DNA ladder (GeNei™, Bangalore, India). All the reactions were repeated thrice and only the consistently reproducible bands were taken into consideration for data analysis.

Statistical analysis

RAPD profile of all the sesame accessions were scored manually for the presence (1) or absence (0) of a band and were recorded as a binary matrix. Faint bands were not considered for the analysis. Similarity indices were calculated between all the accessions according to Jaccard's similarity matrix [48]. The resulting similarity matrix was used to evaluate the relationships among accessions with cluster analysis using an Unweighted pair group with arithmetic mean (UPGMA). All computations were carried out using the phylogenetic software NTSYS-pc version 2.1 [49]. For RGA, presence of a band was marked as "+" and

Table 2: RAPD Primers and their sequences, Total Number of Bands, Number of Polymorphic Bands, Percentage Polymorphism and Molecular weight of the product.

S. No	RAPD primer	Sequence	Total Number of Bands	Polymorphic Bands	% Polymorphism	Mol. Wt. of the Product (bp)
1	OPA01	CAGGCCCTTC	08	05	62.50	250 – 3000
2	OPA02	TGCCGAGCTG	08	07	87.50	700 – 2600
3	OPA03	AGTCAGCCAC	10	08	80.00	600 – 2200
4	OPA04	AATCGGGCTG	11	10	90.90	700 – 2300
5	OPA05	AGGGGTCTTG	11	08	72.72	500 – 3000
6	OPA07	GAAACGGGTG	08	05	62.50	300 – 2200
7	OPA09	GGGTAACGCC	13	12	92.30	450 – 3200
8	OPA10	GTGATCGCAG	12	10	83.33	500 – 2500
9	OPA20	GTTGCGATCC	10	09	90.00	800 – 2800
10	OPB07	GGTGACGCAG	09	06	66.66	500 - 2800
11	OPB08	GTCCACACGG	07	04	57.14	400 – 2500
12	OPM01	GTTGGTGGCT	13	11	84.61	600 – 2200
13	OPM02	ACAACGCCTC	06	05	83.33	350 – 2000
14	OPM03	GGGGGATGAG	09	08	88.88	300 – 2500
15	OPM04	GGCGGTTGTC	08	08	100.00	600 – 2800
16	OPM05	GGGAACGTGT	07	06	85.71	500 - 2500

absence of band was marked as "-". The allele sizes were determined by comparing with the 100 bp marker.

RESULTS

RAPD analysis

Of the twenty eight RAPD decamers used for the initial screening of accessions for polymorphism, sixteen primers were proved to be genetically reliable and reproducible. Each of the sixteen primers varied greatly in their ability to resolve variability among the accessions. A total of 150 distinct major RAPD bands were recorded, in which 122 bands were polymorphic, which were constantly generated from sixteen primers across all the sesame accessions. Each primer generated 4 to 12 polymorphic bands with an average 7.62 bands per primer (Table 2; Fig. 1). The size of the amplicon ranged from 250 - 3200 bp. The highest number of bands (13) was obtained with primer OPA09, while lowest number (7) was obtained with primers OPB08 and OPM05. A high level of variability was observed within all the accessions with an average genetic polymorphism 80.50%.

The dendrogram based on UPGMA analysis grouped the fourty sesame accessions into five close knit clusters (Fig. 3) comprising 33 out of 40 accessions studied. Cluster I includes 6,

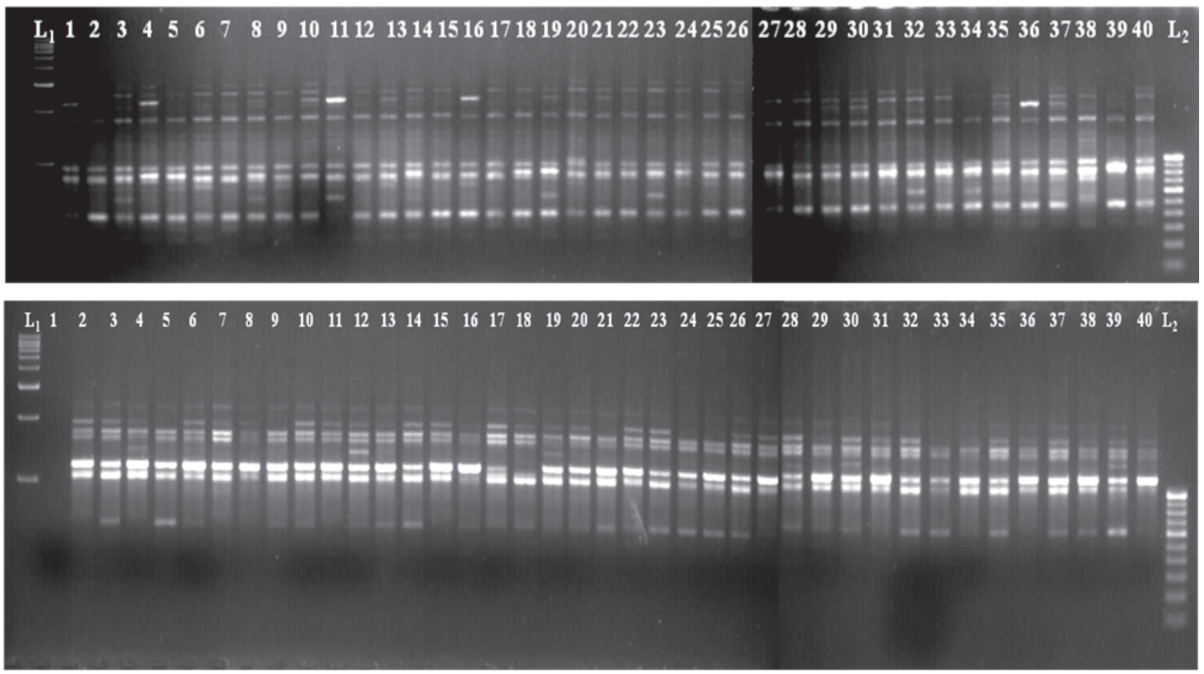

Fig. 1: RAPD profile of 40 sesame accessions with OPA05 (top) and OPM04 (bottom). The lanes 1 40 correspond to the accessions as listed in Table 1. The lanes L_1 and L_2 correspond to 1kb and 100bp standard molecular weight markers respectively.

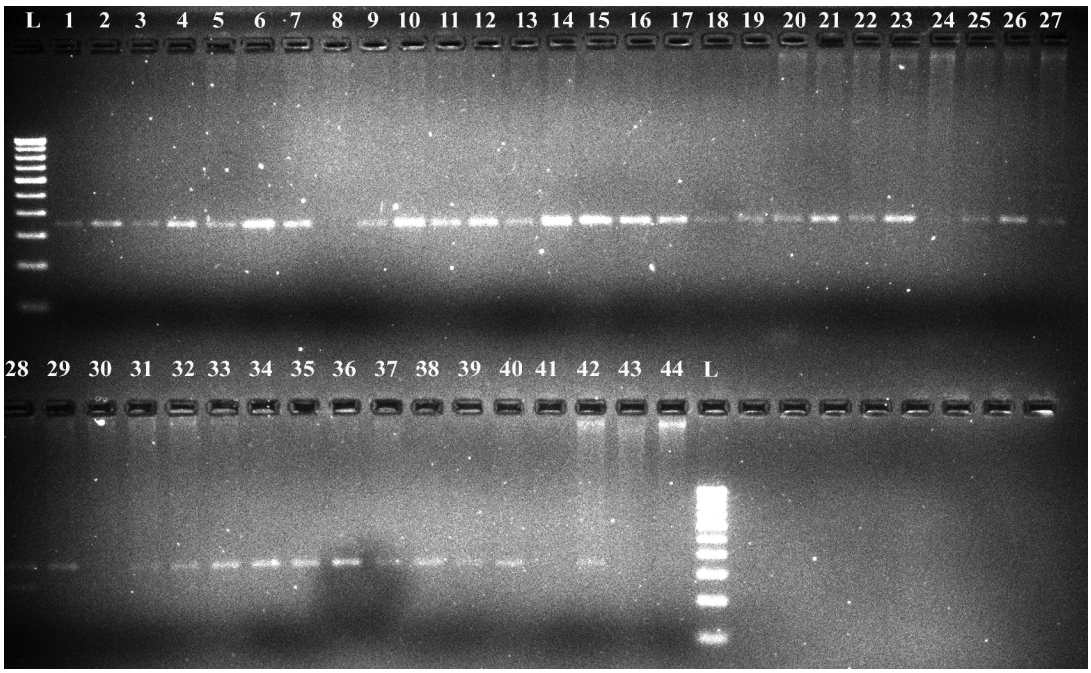

Fig. 2: PCR amplification of genomic DNA from 40 sesame genotypes and its diploid wild taxa with primer *Pto-kin1IN*.

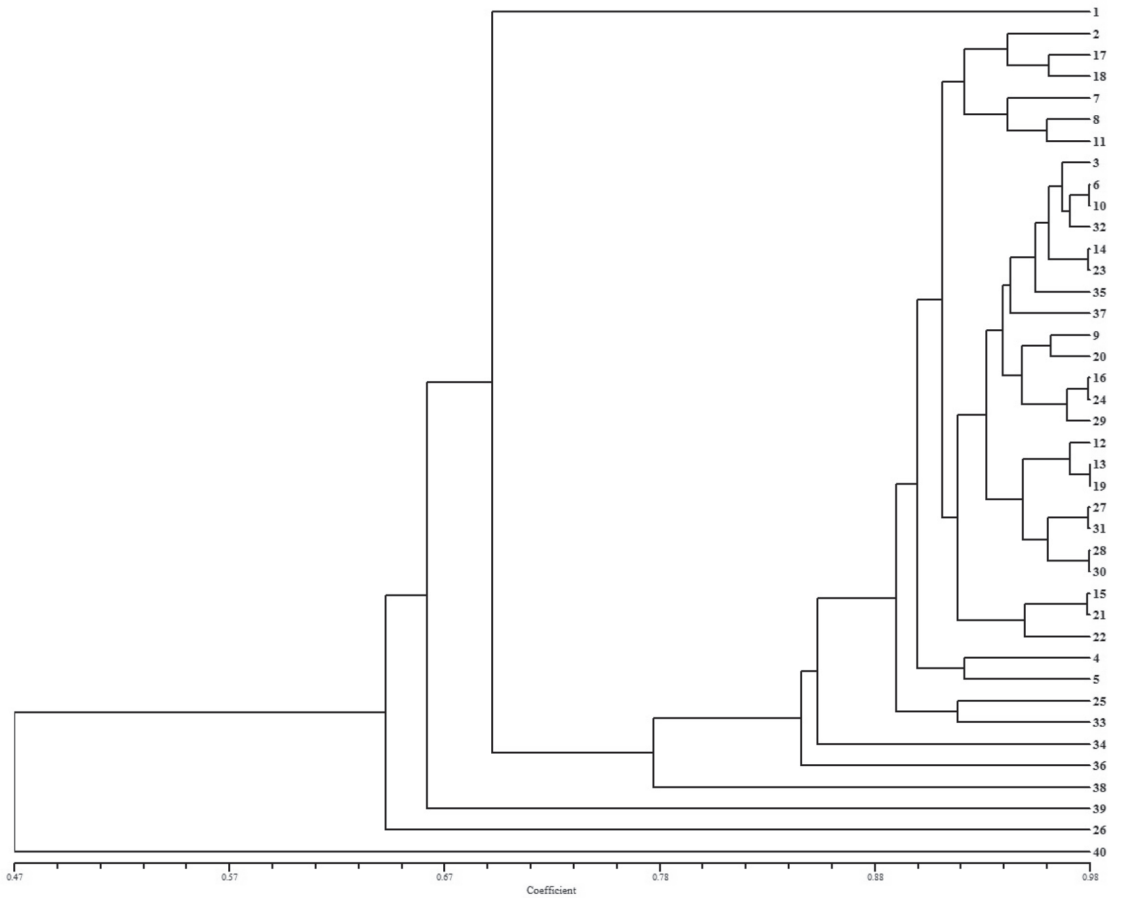

Fig. 3: Dendrogram based on UPGMA analysis demonstrating the relationships among 40 sesame accessions based on RAPD data.

Table 3: Details of RGA primers.

S.No	Marker	Primer Sequence	Annealing temperature (T_a)	Fragment size (bp)
1	h2_13m22a-**F**	TCAAACTCAAGCCACCACAA	47°C	280
	h2_13m22a-**R**	GCTCGAGTCATGGAGGGTAA		
2	Pto-kin1IN-**F**	AAGTGGAACAAGGTTACG	45°C	300
	Pto-kin2IN-**R**	GATGCACCACCAGGGGG		

Cluster II (20), Cluster III (3), Cluster IV (2), Cluster V (2) accessions but remaining seven accessions viz., IC 132680, LC SVPR1, LC RT351, LC JCS1020, LC MT10, EC 357019 and LC DS01 were not included in any of the cluster. An assessment of the proportion of diversity present within accessions indicated that most of the diversity (greater than 65%) was detected. Among all the major clusters, Cluster II is the biggest consisting 20 accessions followed by Cluster I and III, while Cluster IV and V are small comprising two Indian accessions for each. Pair wise similarity coefficient for the fourty sesame accessions ranged from 0.40 to 0.98. The maximum similarity index (0.98) was observed between IC 132659 and LC Shekar, EC 334963 and EC 358989 respectively and the lowest similarity index 0.40 was observed between IC 201123 and LC DS01.

Characterization of RGA markers for disease resistance

Specificity to resistance among a total of forty genotypes of sesame from different geographical regions of the world and its four diploid wild species were examined with twenty RGA markers. The PCR amplification with RGA primers produced single specific band of expected size (in bp), which confirms the presence of disease resistance locus in the tested sesame accessions and its wild taxa. Among the twenty RGA markers used in the present study only two markers were amplified successfully while eighteen markers producing no amplification which reflects susceptibility towards disease. Only two markers were found specific to YMV (Yellow Mosaic Virus) resistance (Table 3). RGA marker h2_13m22a produced an amplicon of size

280 bp was observed in the resistant genotypes, which includes an accession IC 413234 and two wild taxa viz., *S. mulayanum* and *S. orientale* var. *malabaricum.* Similarly, Pto-kin1IN produced amplicon of size 300 bp in resistant genotypes which consists of IC 132680, EC 361722, IC 132692, IC 31843, IC 201123, EC 334963, EC 361725, IC 413234, EC 358989, IC 132702, IC 132362, IC 96221, IC 141169, EC 361724, IC 96234, EC 350653, EC 359900, IC 413262, EC 359013, EC 334956, IC 413239, IC 132692, IC 132692, EC 357019, IC 132659, IC 132174, IC 132671, LC Shekar, LC MT16, LC SSD21, LC SVPR1, LC SSD22, LC RT351, LC DS05, LC JCS1020, LC MT10, LC DS01 (36 sesame accessions) and one of its wild species viz., *S. mulayanum*, while other diploid species like *S. alatum* and *S. capense* did not showed positive test for the resistant locus for the disease (Fig. 2).

DISCUSSION

Varietal characterization using morphological characters possess several undesirable features like seasonal dependence, large space requirement, time consuming, tedious and environmental influence. The alternative way to overcome these limitations and to speed up the testing procedures is the use of RAPD markers in addition to morphological markers. PCR based RAPD analysis has profoundly increase the potential to easily detect genetic polymorphism among organisms, particularly for those in which DNA sequence information is unknown [11]. Measurement of pattern and extent of genetic variability in natural populations of various species is a major thrust area of population genetics and plant breeding. Out crossing species tend to have higher levels of variability within populations, but smaller degree of differentiation among populations than self pollinated species [50]. Sesame is naturally a self pollinated oilseed crop. However, floral morphology attracts insects and their activity can lead to different rates of out crossing from few to as high as 65% [51, 52, 53]. Our results support the previous studies based on RAPD technique which have reported high level of genetic variability in sesame germplasm [13, 15, 14].

RAPD markers are widely used in deciphering genetic relationships among species cultivars and accessions and are highly efficient in evaluating genetic similarities and dissimilarities between cultivars and accessions even when morphological description is blurred for the selection of a better genotype in breeding progamme. Therefore, morpho-agronomic markers may not reflect true genetic identities and diversities [54, 55].

The high level of genetic variability was displayed among tested sesame germplasm. Our results are in agreement with the previous findings with high level of genetic polymorphism studied by RAPD markers [13, 14, 15]. In contrast, low level of genetic diversity was observed with the use of different markers like isozymes [33]. They have studied fourty one sesame accessions from three countries namely Japan, Thailand and Korea using isozymes, out of seven selected enzymes for their study, only an enzyme *Iso-citrate dehydrogenase* showed variation among the accessions. This study confirms the narrow genetic base of accessions in these countries. Similarly, a very low level of genetic variability was also noticed in thirty two sesame genotypes with the use of AFLP markers by Laurentin and Karlovsky [22] because this technique is very expensive as series of chemical steps are needed until polymorphisms can be detected and it requires prior knowledge of sequence and probes. The extent of genetic polymorphism detected by RAPD is greater than RFLP [11].

All the accessions studied were grouped into five main close knit clusters in UPGMA based dendrogram (Fig. 3). The cluster I is the second largest cluster with six accessions which comprises exotic (4) and Indian (2) accessions. The highest genetic similarity 0.96 was recorded between EC 350653 and EC 359900 and lowest 0.94 was observed between IC 132392 and IC 132702. Cluster II was the largest one and it is further divided into two sub clusters allotted with twenty accessions which included exotic (3), Indian (12) and local (5) accessions. The highest similarity index was 0.98 observed between IC 132659 and LC Shekar, EC 334963 and EC 358989 respectively and the lowest similarity index 0.40 was observed between IC 201123 and LC DS01. However, among the genotypes in this major cluster, the genetic similarity ranges from 0.40 - 0.98. Cluster III includes three accessions (2 exotic and 1 Indian) with the genetic similarity ranges from 0.94 - 0.97. It is followed by cluster IV and V with two Indian accessions having 0.92 (IC 31843 and IC 201123) and 0.91 (IC 132692 and LC SSD21) similarity index respectively. Therefore, the high level of genetic variability was reflected in the study may be due to different rates of out crossing in sesame [51, 52].

The definitive intent of majority of the breeding programs in recent years has been to develop the multiple disease resistant varieties along with an increase in yield per unit area and unit time. In order to develop the disease resistant varieties, evaluation of existing genotypes for the presence of disease resistance and genetic diversity is a must. Due to the lack of uniform screening techniques and time required for the selection of resistant genes on phenotypic scale, indirect selection using tightly linked markers is an efficient and a better strategy [56]. Apart from Sequence Related Amplified Polymorphism (SRAP), Resistance gene analog (RGA) primers are also useful markers to identify markers linked to disease resistant genes and designed from conserved repeat motifs of the "Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR)" resistant gene family [57, 58].

Screening of the available genotypes for the presence of resistant gene sources is a prerequisite in any breeding program for resistant genotypes. This is also a prerequisite in order to substantiate the results obtained by molecular analysis. Kooner *et al.* [59] found just two genotypes were resistant to MYMV out of the fifty seven genotypes of mungbean. Bainade *et al.* [60] have studied the association of ISSR markers linked to powdery mildew resistant gene in green gram genotypes. Among the 75 ISSR markers studied, 4 markers were showed polymorphism and only one marker ISSR 834 generated an amplicon size of 698 bp in resistant genotypes but it was absent in susceptible genotypes. As the Resistant Gene Analogues are derived from the NBS-LRR disease resistant motifs it offers resistance to fungal, bacterial and viral pathogens [61]. The characterization of NBS-LRR derived markers revealed that only two markers viz., h2_13m22a and Pto-kin1IN, among the tested RGA markers were specific to yellow mosaic virus in sesame. Primer Pto-kin1IN generated an amplicon of size 300 bp in most of the species and its two wild taxa *S. mulayanum* (Fig. 2) and primer h2_13m22a generated an amplicon of size 280 bp in only one genotype and in two of its wild taxa viz., *S. mulayanum* and *S. orientale* var. *malabaricum.* The wild species of genus *Sesamum* possesses multiple disease resistance [6, 62, 63, 64, 4]. As there is ease of crossability between these two wild taxa and cultivated sesame so these two wild taxa can be used to transfer the genes into cultivars by conventional breeding method [4].

CONCLUSION

Varietal identification of cultivated plants based on morphological traits would necessarily require the plant to grow to a flowering and fruiting stage. RAPD fingerprints can characterize and identify the variety or genotypes at faster rate without waiting for the plant to reach maturity and RGA markers are closely linked to known resistant genes and plays an important role in selection of better parents in marker assisted selection, mapping and isolation of respective resistant gene. Hence, combination of RAPD and RGA markers are found to be very promising and reliable to characterize the sesame germplasm and assessment of genetic base to select the parents for future breeding strategy.

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