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# Assessment of Biodiversity Among Some Sesame Genotypes Using ISSR and Srap Markers

**[81]**

**Nourhan A Aboelnaga[1*], Abodoma A[1], Lamyaa M Sayed[1], Clara R Azzam[2]**

1- Genetics Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

2- Cell Research Dept, Fields Crop Research Institute, Agric Research Center, Postal Code 12619, Giza, Egypt

*Corresponding author: noor_aboelnaga@hotmail.com

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**Abstract**

Biodiversity among 32 sesame (*Sesamum indicum* L.) genotypes; 30 accessions were obtained from various regions of Egypt and two local cultivars were assessed agronomically using different morphometric and yield-related traits. Significant variations were observed for all these traits such as plant height, plant weight, No. of pods, pod weight and oil percentage. The variation between highest and lowest genotype in plant height was almost 50%, as genotype V32 recorded the highest plant height (220 cm), whereas genotype V19 was the shortest one (100 cm). Plant weight ranged from 802.2 to 99.5 g in V6 and V16, respectively. Pod weight varied from 202.6 to 32.2 g in V4 and V15, respectively. No. of pods ranged from 75.7 to 10.3 in V5 and V17, respectively, while oil percent varied from 60% to 40.5% in V11 and V3, respectively. Using morphometric characteristics, the genotypes were grouped into two main clusters with high variation among them. Fifteen out of the 32 genotypes were chosen and subjected to ISSR and SRAP analyses to detect the level of genetic diversity in relation to geographical origins using 11 ISSR and 7 SRAP primers. ISSR primers generated 46 amplified bands. Four out of these primers were resulted in 5 unique markers among the 15 sesame genotypes. Molecular characterization revealed a polymorphism percentage of 46.66% for ISSR markers, while SRAP primers exhibited a total of 22 bands and one out of these primers revealed 3 unique genotype specific marker polymorphism was calculated as 50%. The cluster analysis showed a high genetic diversity among the sesame genotypes and their diversity was consistent with their source pedigrees. The results of principal component analysis (PCA) were closely aligned with those of the cluster analysis. Considering the relatedness of genotypes, geographical origin and their morphological characteristics reflected to the similarity of ISSR and SRAP patterns.

**Keywords**: *Sesamum indicum* L., Biodiversity, Genetic diversity, ISSR, SRAP, PCA

## 1 Introduction

Sesame (*Sesamum indicum* L.) is considered to be one of the ancient oilseed crop, it is a diploid species (2n = 26) dicotyledonous and it has been grown 3050-3500 BC (Bedigian and Harlan 1986). Sesame is highly resistant to drought, grows well in most types of soils, regions and is well investigated for various crop rotations (Çağirgan 2006). It is cultivated in tropical and subtropical regions of the world (Ashri 1998), where 96% of the world sesame seeds are produced by Africa and Asia, with Sudan, India and China are the leading producer countries (Dossa et al 2016). The cultivated areas are 12, 154, 584 and 34000 hectares in the world and Egypt in 2018, respectively, according to FAO statistics (2018). Sesame is a flowering plant, member of the *Pedaliaceae* family, Genus: *Sesamum*, also called benne. There is about 20 species of the genus *Sesamum*, in addition to the only cultivated species; *Sesamum indicum* (Elleuch et al 2011).

Molecular markers have been used by DNA fingerprinting to identify sesame cultivars reliably; it has been widely utilized to check the identity and

purity of cultivars and to assess their genetic diversity in various crops. Genetic variation has been revealed in sesame using markers such as sequence-related amplified polymorphisms; SRAP (Che et al 2009, Zhang et al 2010, Aneja et al 2012) and inter-simple sequence repeats, ISSRs (Reddy et al 2002, Sharma et al 2009, Abate et al 2015). Sesame genotypes characterization using molecular markers are of great value in assisting the design of parental line and breeding techniques.

Genetic characterization of germplasm in sesame are considered significant for conservation and application in breeding programs, to identify the relationship among accessions and to evaluate genetic diversity, which can be defined using morphological parameters. Molecular markers advancements have recently become the primary method of examining plant genetic variability and the genetic basis of specific traits, the advancement of molecular markers work in recent periods has created new opportunities for genetic characterization and biodiversity studies in plants. In addition, the proposal for molecular marker applications has tried to enhance the genetics of agronomic and yield-related traits (Zhang et al 2007, 2010, Wei et al 2008, Cho et al 2011).

In this context, the aims were to determine the variation between sesame genotypes by using morphometric and yield-related traits parameters, assess oil contents among sesame genotypes and evaluate the biodiversity using ISSR and SRAP techniques.

## 2 Material and Methods

### 2.1 Plant materials

Thirty-two sesame genotypes and their origins and barcodes are presented in **Table 1**. Out of them, thirty sesame accessions were kindly obtained from the National Gene Bank and Genetic Resources, Agricultural Research Center (ARC), while the two local cultivars were kindly obtained from Oil Crops Research Department, Field Crops Research Institute, Agricultural Research Center (ARC). These genotypes were cultivated during the successive season at the field of Genetics Dept, Fac of Agric, Ain Shams Univ.

### 2.2 Methods

Molecular experiments were conducted at both the laboratories of Genetics Dept, Fac of Agric, Ain Shams Univ Egypt and the Cell Research Dept., Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

### 2.3 DNA extraction

Leaves samples of the 15 sesame genotypes were collected and stored in liquid nitrogen for DNA extraction, which was carried out using DNeasy plant Mini Kit Isolation protocol (Bio-basic.com).

### 2.4 ISSR-PCR amplification

Eleven ISSR primers sequences **Table 2** were previously reported by Reddy et al (2002), Sharma et al (2009), Abate et al (2015) as a high polymorphism producer of all sesame genotypes, which were utilized to identify the level of genetic variability for sesame genotypes under investigation in relation to geographical origins of sesame. The polymerase chain reaction (PCR) was carried out by using automated thermal cycle (model Techno 512) programmed in 30 µl of mixture containing 3 µl of 10x TBE buffer, 3 µl of $MgCl_2$ 25mM, 3 µl of dNTP's 2.5 mM, 2 µl of ISSR primer 10 pmol, 2 µl of diluted preheated DNA (2 µl of DNA to a 16.80 µl of $H_2O$ and heated for 3 mins at 70ºC), 0.20 µl of Taq polymerase enzyme 5U. The amplification carried out as follows; 94ºC for 4 min followed by 45 cycles of 1 min at 94ºC, 1 min at 57º C, and 2 min at 72ºC. The reaction was finally stored at 72º C for 10 min.

### 2.5 SRAP-PCR amplification

Seven SRAP primer combinations sequences are present in **Table 3** as described in the publications of Che et al (2009), Zhang et al (2010), Aneja et al (2012), AL-Somain et al (2017) were used to assess the polymorphism among the sesame genotypes under investigation. Polymerase chain reaction (PCR) was performed by using DNA Thermal Cycler (model Techno 512, UK) and cycling parameters included 2 min at 94ºC followed by 35 cycles of three steps: 1 min at 94ºC, 30 Sec at 35ºC and 30 Sec at 72ºC. In the following 35 cycles, the annealing temperature was increased gradually up to 50ºC, and for extension, one cycle for 5 min at 72ºC.

### 2.6 Separation and photographing of PCR products

PCR products were separated on 2% agarose gel in 1X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 2.5-3 h. A 100 bp standard DNA ladder as the molecular standard marker was used to confirm the appropriate (ISSR and SRAP bands). The banding patterns were photographed under UV light for further analysis.

**Table 1.** Origins and barcodes number of 32 sesame genotypes

| Sesame landraces | Origin | Barcode No. | Sesame landraces | Origin | Barcode No. |
|---|---|---|---|---|---|
| V1 | Luxor | 1112766 | V16 | Aswan | 1112782 |
| V2 | Luxor | 1112767 | V17 | Al wadi al jaded | 1112783 |
| V3 | Qena | 1112768 | V18 | Qena | 1112784 |
| V4 | Qena | 1112769 | V19 | Qena | 1112785 |
| V5 | Sohag | 1112770 | V20 | Sohag | 1112786 |
| V6 | Asyut | 1112771 | V21 | Sohag | 1112787 |
| V7 | Qena | 1112772 | V22 | Sohag | 1112788 |
| V8 | Sohag | 1112773 | V23 | Qena | 1112789 |
| V9 | Al daqahlya | 1112774 | V24 | Al wadi al jaded | 1112790 |
| V10 | Ismailia | 1112776 | V25 | Beni suef | 1112791 |
| V11 | Ismailia | 1112777 | V26 | Minya | 1112792 |
| V12 | Ismailia | 1112778 | V27 | Minya | 1112793 |
| V13 | Ismailia | 1112779 | V28 | Aswan | 1112794 |
| V14 | Ismailia | 1112780 | V29 | Sohag | 1112800 |
| V15 | Bihara | 1112781 | V30 | Sohag | 1112802 |
| Sesame Cultivars | Origin | Barcode No. | Sesame Cultivars | Origin | Barcode No. |
| V31 | Egypt | Giza 32 | V32 | Egypt | Shandaweel 3 |

**Table 2.** Names and sequences of 11 ISSR primers

| Name of Primer | Sequence 5´→ 3´ | Name of Primer | Sequence 5´→ 3´ |
|---|---|---|---|
| 807 | AGA GAG AGA GAG AGA GT | HB-10 | GAG AGA GAG AGA CC |
| 98A | CA CA CA CA CA CA AC | HB-11 | GTG TGT GTG TGT CC |
| 49B | CAC ACA CAC ACA GG | HB-12 | CAC CAC CAC GC |
| HB-1 | CAA CAA CAA CAA CAA | HB-13 | GAG GAG GAG GC |
| HB-4 | GAC AGA CAG ACA GACA | HB-15 | GTG GTG GTG GC |
| HB-9 | GTG TGT GTG TGT GC | | |

**Table 3.** Names and sequences of 7 SRAP primer combinations

| No. | Name of primer combination | Sequences | |
|---|---|---|---|
| | | Forward primers 5´→ 3´ | Reverse primers 5´→ 3´ |
| 1 | Me-2xEM5 | TGA GTC CAA ACC GGA GC | GACTGCGTACGAATTAAC |
| 2 | Me-5xEM3 | TGA GTC CAA ACC GGA AG | GACTGCGTACGAATTGAC |
| 3 | Me-4xEM4 | TGAGTCCAAACCGGACC | GACTGCGTACGAATTTGA |
| 4 | Me-6xEM6 | TGA GTC CAAA CC GG ACA | GAC TGC GTA CGA ATT GCA |
| 5 | Me-7xEM7 | TGA GTC CAAA CC GGA CG | GACTGCGTACGAATTCAA |
| 6 | Me-9xEM9 | TGAGTCCAAACCGGAGG | GACTGCGTACGAATTCGA |
| 7 | Me-10xEM10 | TGAGTCCAAACCGGAAA | GACTGCGTACGAATTCAT |

### 2.7 Morphometric and yield-related traits

A field experiment was conducted to evaluate thirty-two sesame genotypes for their yield-related traits. The studied yield-related traits were: plant height (cm), plant weight (g.), No. of pods/plant, pods weight (g.) and oil extraction represented by its percentage has been estimated at the National Gene Bank and Genetic Resources, Agricultural Research Center (ARC) as follows: One gram of each genotype was used to extract the oil using a mixture of chloroform: methanol (2:1, v/v) for 8 hrs at 70ºC in a Soxhlet apparatus. The solvent was then evaporated and the residue was dried to a constant weight according to the AOAC (1990). The percentage of oil content was then calculated on a dry weight basis.

### 2.8 Statistical analysis

Morphometric and yield-related traits data were statistically analyzed using MSTAT software program. The analysis of variance (ANOVA) and the least significant differences (L.S.D) analysis were carried out by the software according to (Maxwell and Delaney 1989). The differences were considered significant at $P<0.05$.

### 2.9 Molecular Data analysis

DNA-bands were scored as present (1) or absent (0) for each primer pair. The genetic similarities were calculated according to Dice and the dendrogram was constructed using SPSS windows version 22 (Yang and Quiros 1993). The principal component analysis (PCA) was performed by using the Factominer package in the R software (Development Core Team 2016).

### 2.10 Marker trait association

Analysis of trait marker association of the ISSR and SRAP primers using plant height (PH), plant weight (PW), No. of pods (NP), pods weight (PW), oil content (OC)% were analyzed by Power marker, using f-statistical analysis (Liu and Muse 2005).

## 3 Results and Discussion

### 3.1 Morphometric and yield-related traits

Analysis of variance among the 32 sesame genotypes was investigated to assess some morphometric and yield-related traits. Results revealed significant differences genotypes for all the tested characteristics, as shown in **Table 4.** The dendrogram based on morphometric and yield-related traits data is present in **Fig 1.**

The results indicated that genotype V32 recorded the highest plant height mean (220 cm), whereas genotype V19 was the shortest one (100 cm). V6 recorded the heaviest plant weight mean (802.2 g), while V16 genotype was the lightest one (99.5 g). Genotype V5 recorded the highest pods number/plant mean (75.7), while V17 recorded the lowest value (10.3). On the other hand, V4 scored the heaviest pod weight mean (202.6 g), while genotype V15 was the lightest one (32.2 g). Moreover, V11 and V4 were found to be the highest oil content% (60.17 % and 60%), respectively, while V3 and V27 were the lowest value of oil content% (40.53% and 44.3%). Our findings are in accordance with those reported by Sheoran (2019) who analyzed sixty sesame genotypes using the morphological traits and genetic variability. However, Adu-Gyamfi et al (2019) estimated low variation values in some quantitative and qualitative traits. Overall, the results indicated that some traits could be used as potential germplasm preservation and development programs in sesame.

The dendrogram grouped the 32 studied sesame accessions into 2 major clusters: (A) and (B), the first one contained accessions V5 and V6, meanwhile, the second one was divided into two sub-clusters: (C) and (D), the first one contained V3 and V4, while the second sub-cluster was divided into two sub sub-clusters: (E) and (F), The first one included V1, V9, V8 , V24, V13 and V7, while the second one separated V32 alone (G) and grouped V31,V25,V14,V21,V2,V30 and V10 together (I). While the others genotypes were divided into three groups: (J), (K) and (L), the first one contained V16, V19, V15, V17 and V18, while the second one (K) contained genotypes V11 and V23, meanwhile the remains genotypes grouped into the last one (L).

**Table 4.** Mean values of some morphometric yield-related and oil content% traits for the 32 sesame genotypes

| Sesame No. | Plant height | Plant weight | Pods No. | Pods weight | Oil % |
|---|---|---|---|---|---|
| V1 | 135.5 | 259.4 | 21 | 48.5 | 58.69 |
| V2 | 142.6 | 211.2 | 14.67 | 62.5 | 59.41 |
| V3 | 166.6 | 426.4 | 37.4 | 128.7 | 40.53 |
| V4 | 167.6 | 421.2 | 42.7 | 202.6 | 60 |
| V5 | 170.6 | 765.7 | 75.7 | 149 | 55.48 |
| V6 | 192.3 | 802.2 | 58.4 | 84.2 | 54.65 |
| V7 | 169 | 322.2 | 23.3 | 98.4 | 51.61 |
| V8 | 164 | 300.6 | 27.7 | 46.3 | 50.51 |
| V9 | 155 | 266.2 | 34.3 | 47 | 47.95 |
| V10 | 143.3 | 167 | 17 | 119 | 47.88 |
| V11 | 150.6 | 156.4 | 16.3 | 79.9 | 60.17 |
| V12 | 111.6 | 155.6 | 21.3 | 35 | 54.33 |
| V13 | 166.6 | 338.2 | 50.5 | 37 | 46.25 |
| V14 | 120 | 217.3 | 33.7 | 42.1 | 56.27 |
| V15 | 141.6 | 133.2 | 25.3 | 32.2 | 54.47 |
| V16 | 113 | 99.5 | 16.3 | 49.6 | 52.51 |
| V17 | 145.3 | 120.6 | 10.3 | 33.9 | 58.14 |
| V18 | 128.3 | 121.5 | 11.7 | 48.3 | 56.31 |
| V19 | 100 | 112.5 | 21.3 | 51.2 | 55.68 |
| V20 | 141.6 | 182.3 | 15.3 | 37.6 | 57.29 |
| V21 | 155 | 207 | 16.7 | 50.1 | 49.89 |
| V22 | 143.3 | 160.7 | 12 | 50.5 | 47.68 |
| V23 | 135 | 119.5 | 17 | 83.6 | 55.45 |
| V24 | 165 | 299.3 | 16.7 | 50.7 | 53.81 |
| V25 | 185 | 216 | 14 | 51.2 | 55.27 |
| V26 | 136.6 | 164.6 | 18 | 37.2 | 50.17 |
| V27 | 150 | 162.3 | 13.7 | 38.3 | 44.39 |
| V28 | 143.3 | 177.2 | 20.7 | 56 | 54.35 |
| V29 | 143.6 | 164.4 | 22.3 | 50.5 | 49.66 |
| V30 | 129.3 | 204.2 | 33.3 | 100.5 | 48.97 |
| V31 | 130 | 196.67 | 61.7 | 50 | 53.94 |
| V32 | 220 | 186.67 | 53.3 | 51 | 55.60 |
| LSD | 28.85 | 28.82 | 16.86 | 0.36 | 0.31 |

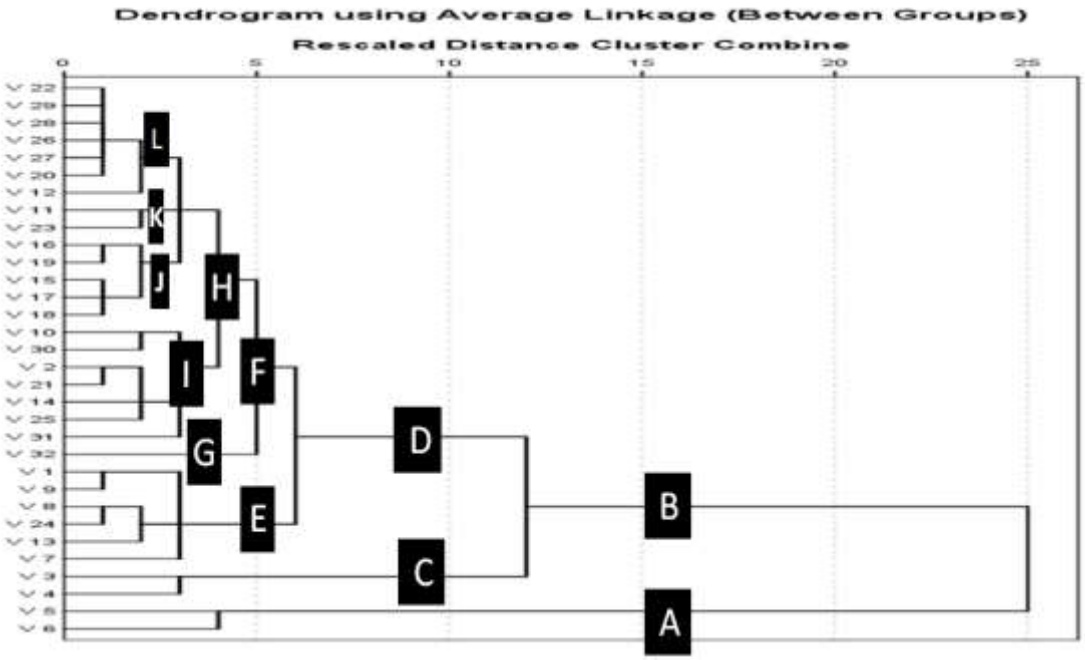


**Fig 1.** Dendrogram illustrating genetic relationship among 32 sesame genotypes generated by UPGMA cluster analysis based on morphometric yield-related traits and oil contents

### 3.2 Molecular identification

ISSR banding patterns was used to access biodiversity among the chosen 15 genotypes V1, V4, V5, V6, V9, V13, V15, V19, V22, V24, V25, V27, V28, V31 and V32 at the molecular level. DNA polymorphism among the sesame genotypes under investigation are shown in **Fig 2** and **Table 5.** The total number of fragments was 46 bands with polymorphism% ranged from 33.33% to 83.33%. Five unique generated bands that could be used as genotype negative markers.

Fragments number per primer varied from 2 (primer HB-10) to 7 (primer HB-1). The overall size of the amplified fragments ranged from 170 bp (primer HB-13) to 1370 bp (primer HB-1). Primer 807 produced six bands with 83.33% polymorphism, while Primer HB-10 and HB-12 resulted in zero polymorphism. In general, 5 negative unique markers were generated over all the 11 ISSR primers. These results confirmed that ISSR-PCR analysis is useful for identification of germplasms and assessment of genetic diversity among the sesame genotypes. These findings are in accordance with the previous study of Reddy et al (2002), Sharma et al (2009) and Abate et al (2015) who reported that ISSR markers are useful method in identifying high genetic diversity in sesame germplasm.

Among the 11 ISSR primers, seven primers generated different positive markers for one of the investigated morphometric and yield-related traits. The results indicated that, primer 98A generated a band with size of 530 bp could be used as positive marker for plant height and No. of pods, another band with size of 760 bp could be used as positive marker for plant height. Primer 49B produced a band with size of 815 bp could be considered as a positive marker for oil content%, another band with size of 560 bp could be used as a positive marker for No. of pods. Primer 807 generated a band with size of 250 bp could be used as a positive marker for oil content% and plant fresh weight, while the bands with sizes of 630 bp and 380 bp could be used as positive markers for plant height, the bands with sizes of 335 bp and 465 bp may be considered as positive marker for pod weight. Primer HB-1 resulted bands with sizes of 925 bp and 1370 bp could be used as positive markers for pod weight. Primer HB-4 resulted in a band with size of 610 bp is considered as positive marker for plant weight. Primer HB-13 produced a band with size of 415 bp could be used as positive marker for plant weight and oil content %, finally, primer HB-15 produced a band with size of 400 bp which considered as positive marker for No. of pods.

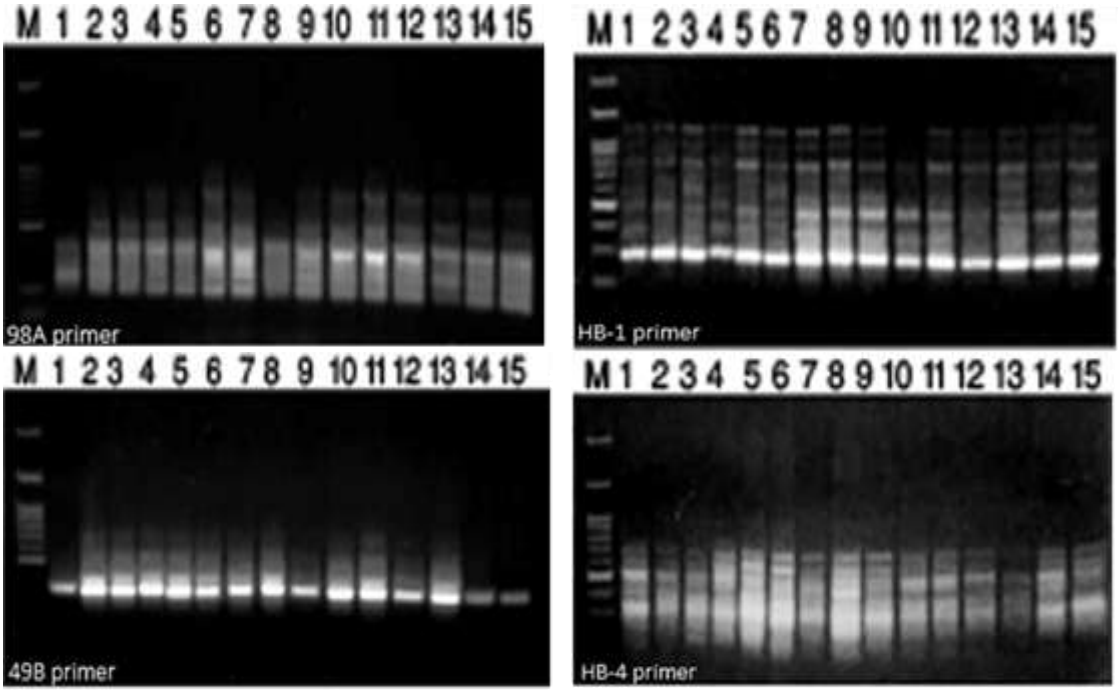


**Fig 2.** An example for patterns of amplified DNA based on ISSR primers: 98A, 49B, HB-1 and HB-4 among the 15 sesame genotypes, M: 100 bp DNA ladder.
1=V1, 2= V4, V3= V5, 4= V6, 5=V9, 6= V13, 7= V15, 8= V19, 9= V22, 10= V24, 11= V25, 12= V27, 13= V28, 14= V31 and 15= V32

**Table 5.** Total bands, monomorphic and polymorphic bands, negative unique bands, polymorphism percentage of the 15 sesame genotypes based on ISSR primers

| Primer Name | Total bands | Monomorphic bands | Polymorphic bands | Negative unique bands | Polymorphism % |
|---|---|---|---|---|---|
| 98A | 6 | 3 | 3 | - | 50% |
| 49B | 4 | 1 | 3 | 1 | 75% |
| 807 | 6 | 1 | 5 | 1 | 83.33% |
| HB-1 | 7 | 3 | 4 | 2 | 57.14% |
| HB-4 | 6 | 5 | 1 | - | 16.66% |
| HB-9 | 3 | 2 | 1 | - | 33.33% |
| HB-10 | 2 | 2 | - | - | - |
| HB-11 | 3 | 2 | 1 | 1 | 33.33% |
| HB-12 | 3 | 3 | - | - | - |
| HB-13 | 3 | 1 | 2 | - | 66.66% |
| HB-15 | 3 | 2 | 1 | - | 33.33% |
| Total | 46 | 25 | 21 | 5 | - |
| Average | 4.1 | 2.2 | 1.9 | 0.4 | 33.33% |

Similarity matrix using Dice coefficient varied from 0.78 between V32 and V25 to 1 between V15 and V13, as shown in **Table 6.** The UPGMA-based dendrogram, based on the DNA profiles using the 11 ISSR primers reveled that two main distinct cluster were created as shown in **Fig 3.** The first cluster included V1 genotype, while the other cluster was separated into two sub-clusters; the first one contained V32, V27 and V31 genotypes, while the second one was divided into two sub sub-clusters, the first one grouped V19, V24, V25, V22, V15. Meanwhile, the second one grouped V28, V5, V13, V9, V6 and V4 genotypes together.

Seven SRAP primer combinations were utilized to access the polymorphism between the 15 sesame genotypes at the molecular level. The results showed that total numbers of 22 bands were generated over all the seven primer combinations, as shown in **Fig 4** and **Table 7.** One negative unique marker and two positive ones were produced as genotype specific markers. Polymorphism percentage ranged between 33.33% and 66.66%.

**Table 6.** Dice similarity coefficient of 15 sesame accessions based on ISSR data analysis

| Sesame accession | V1 | V4 | V5 | V6 | V9 | V13 | V15 | V19 | V22 | V24 | V25 | V27 | V28 | V31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V4 | 0.83 | | | | | | | | | | | | | |
| V5 | 0.86 | 0.90 | | | | | | | | | | | | |
| V6 | 0.83 | 0.90 | 0.90 | | | | | | | | | | | |
| V9 | 0.83 | 0.93 | 0.93 | 0.96 | | | | | | | | | | |
| V13 | 0.83 | 0.93 | 0.93 | 0.96 | 0.86 | | | | | | | | | |
| V15 | 0.87 | 0.91 | 0.84 | 0.91 | 0.83 | 1 | | | | | | | | |
| V19 | 0.85 | 0.89 | 0.81 | 0.86 | 0.86 | 0.91 | 0.91 | | | | | | | |
| V22 | 0.88 | 0.89 | 0.82 | 0.89 | 0.84 | 0.86 | 0.86 | 0.89 | | | | | | |
| V24 | 0.80 | 0.90 | 0.80 | 0.87 | 0.9 | 0.89 | 0.89 | 0.95 | 0.87 | | | | | |
| V25 | 0.85 | 0.92 | 0.85 | 0.89 | 0.87 | 0.87 | 0.87 | 0.90 | 0.88 | 0.89 | | | | |
| V27 | 0.81 | 0.85 | 0.84 | 0.85 | 0.87 | 0.89 | 0.89 | 0.92 | 0.90 | 0.94 | 0.92 | | | |
| V28 | 0.80 | 0.90 | 0.90 | 0.90 | 0.86 | 0.88 | 0.88 | 0.86 | 0.83 | 0.87 | 0.81 | 0.88 | | |
| V31 | 0.81 | 0.85 | 0.84 | 0.85 | 0.88 | 0.90 | 0.90 | 0.88 | 0.86 | 0.83 | 0.84 | 0.93 | 0.92 | |
| V32 | 0.88 | 0.89 | 0.81 | 0.861 | 0.88 | 0.88 | 0.88 | 0.86 | 0.83 | 0.84 | 0.78 | 0.90 | 0.86 | 0.90 |

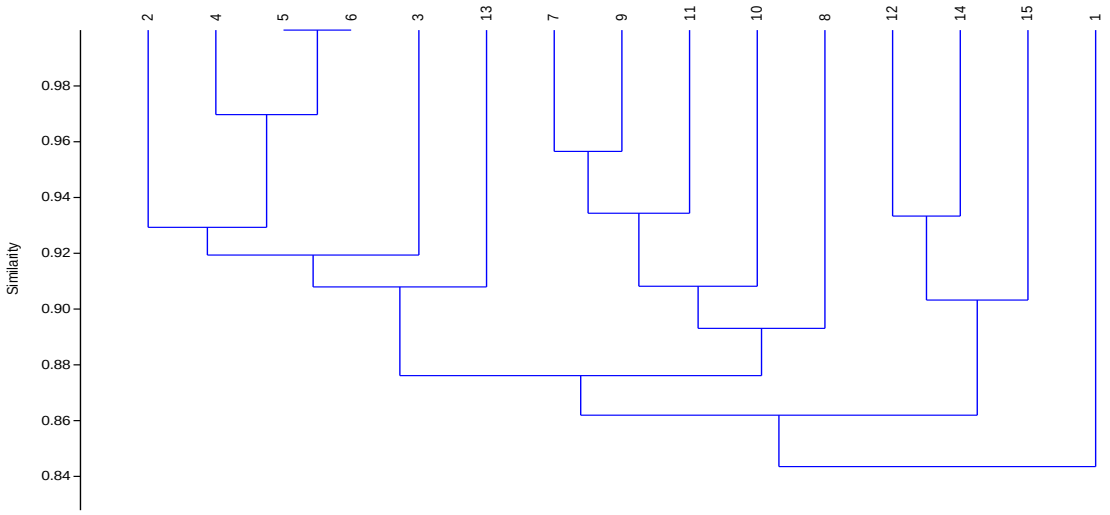


**Fig 3.** Dendrogram tree showed the genetic distance among the 15 studied sesame genotypes based on ISSR data

1=V1, 2= V4, V3= V5, 4= V6, 5=V9, 6= V13, 7= V15, 8= V19, 9= V22, 10= V24, 11= V25, 12= V27, 13= V28, 14= V31 and 15= V32

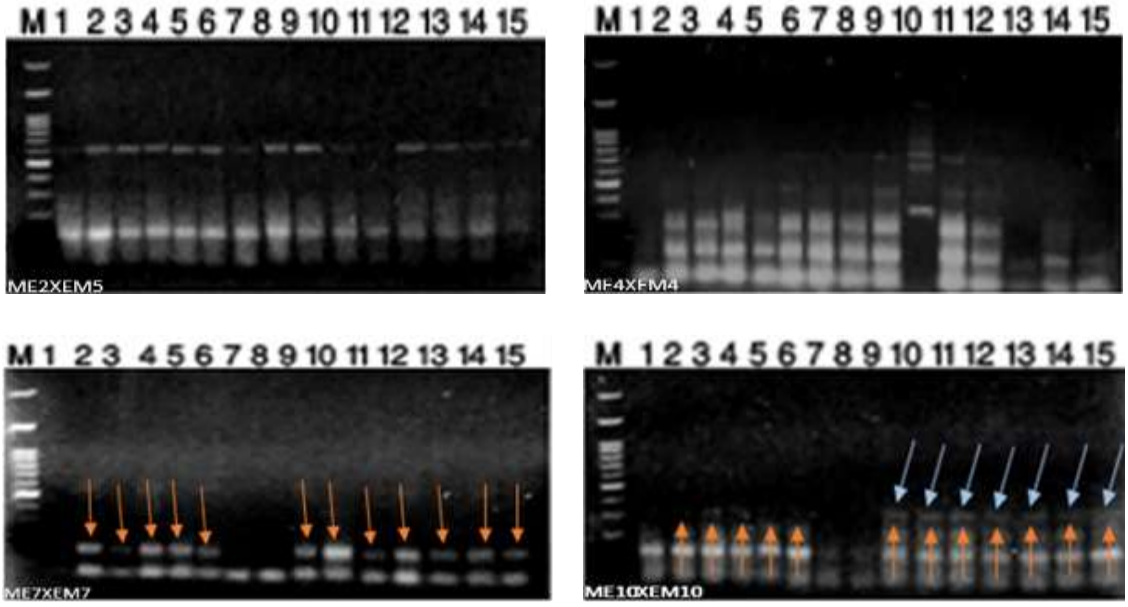


**Fig 4.** An example for patterns of amplified DNA based on SRAP primer combinations: Me 2xEM5, Me-4xEM4, Me-7xEM7 and Me-10xEM10 among the 15 sesame genotypes, M: 100 bp DNA ladder.
1=V1, 2= V4, V3= V5, 4= V6, 5=V9, 6= V13, 7= V15, 8= V19, 9= V22, 10= V24, 11= V25, 12= V27, 13= V28, 14= V31 and 15= V32

**Table 7.** Total bands, monomorphic and polymorphic bands, unique positive, unique negative, polymorphism percentage of the 15 different sesame genotypes based on SRAP primers

| Primer Name | Total bands | Monomorphic band | Polymorphic band | Unique positive | Unique negative | Polymorphism % |
|---|---|---|---|---|---|---|
| Me2xEM5 | 2 | 2 | - | - | - | - |
| ME4XEM4 | 7 | 1 | 6 | 2 | 1 | 62.50% |
| ME5XEM3 | 3 | 3 | - | - | - | - |
| ME6XEM6 | 3 | 2 | 1 | - | - | 33.33% |
| ME7XEM7 | 2 | 1 | 1 | - | - | 50% |
| ME9XEM9 | 2 | 1 | 1 | - | - | 50% |
| ME10XEM10 | 3 | 1 | 2 | - | - | 66.66% |
| Total | 22 | 11 | 11 | 2 | 1 | - |
| Average | 3.2 | 1.5 | 1.5 | 0.3 | 0.1 | 50% |

The number of bands per primer ranged from 2 (primer ME2xEM5, ME7xEM7 and ME9xEM9) to 7 (primer ME4xEM4). The overall size of the amplified products ranged from 120 bp (primer ME6xEM6 and ME7xEM7) to 780 bp (primer ME4xEM4). Primer ME4xEM4 produced 7 bands with a 62.50 % polymorphism, while primer ME2xEM5 and ME5xEM3 produced zero polymorphism. In general, the total negative unique marker recorded 1 negative marker for a particular genotype. Two positive unique markers were recorded. These results confirmed that SRAP are useful for characterization of germplasm and to assess the genetic diversity among sesame genotypes. These findings were in accordance with the previous studies of Che et al (2009), Zhang et al (2010), Aneja et al (2012), and AL-Somain et al (2017) they reported that SRAP analysis is accurate and efficient tool for assessing genetic polymorphisms and sesame genotypes relationships. Among the 7 SRAP primer combinations, 4 primers produced positive marker among the 15 genotypes. Primer ME10XEM10 produced a band with size of

260 bp could be considered a positive marker for oil content % and plant weight, another band with size of 375 bp was generated and considered as positive marker for plant height. Primer ME6XEM6 produced one band with size of 235 bp could be considered as a positive marker for No. of pods and pods weight. Primer ME7XEM7 generated a band with size of 200 bp could be used as positive marker for No. of pods. Primer ME4XEM4 produced a band with size of 500 bp may be considered as positive marker for plant weight.

Similarity matrix values using Dice coefficient varied from 0.70 between V24 and V1 to 1.0 between V6 and V5, as shown in **Table 8.** The UPGMA-based dendrogram based on the DNA profiles using 7 SRAP primer combinations. Two main distinct clusters were created from the analysis of the pooled SRAP data **Fig 5.**

**Table 8.** Dice similarity coefficient of 15 sesame accessions based on SRAP data analysis

| Sesame accession | V1 | V4 | V5 | V6 | V9 | V13 | V15 | V19 | V22 | V24 | V25 | V27 | V28 | V31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V4 | 0.93 | | | | | | | | | | | | | |
| V5 | 0.93 | 0.93 | | | | | | | | | | | | |
| V6 | 0.93 | 0.93 | 1 | | | | | | | | | | | |
| V9 | 0.87 | 0.94 | 0.94 | 0.94 | | | | | | | | | | |
| V13 | 0.81 | 0.88 | 0.88 | 0.88 | 0.94 | | | | | | | | | |
| V15 | 0.86 | 0.87 | 0.81 | 0.81 | 0.88 | 0.94 | | | | | | | | |
| V19 | 0.89 | 0.83 | 0.83 | 0.83 | 0.84 | 0.90 | 0.96 | | | | | | | |
| V22 | 0.87 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.88 | 0.84 | | | | | | |
| V24 | 0.70 | 0.77 | 0.77 | 0.77 | 0.84 | 0.84 | 0.77 | 0.74 | 0.84 | | | | | |
| V25 | 0.82 | 0.88 | 0.88 | 0.88 | 0.94 | 0.94 | 0.88 | 0.85 | 0.94 | 0.9 | | | | |
| V27 | 0.87 | 0.94 | 0.94 | 0.94 | 0.94 | 0.88 | 0.82 | 0.78 | 0.94 | 0.84 | 0.94 | | | |
| V28 | 0.83 | 0.90 | 0.90 | 0.90 | 0.91 | 0.85 | 0.78 | 0.75 | 0.91 | 0.86 | 0.91 | 0.97 | | |
| V31 | 0.90 | 0.90 | 0.96 | 0.96 | 0.91 | 0.85 | 0.78 | 0.81 | 0.91 | 0.81 | 0.91 | 0.97 | 0.94 | |
| V32 | 0.86 | 0.87 | 0.93 | 0.93 | 0.88 | 0.88 | 0.81 | 0.83 | 0.88 | 0.77 | 0.88 | 0.94 | 0.90 | 0.96 |

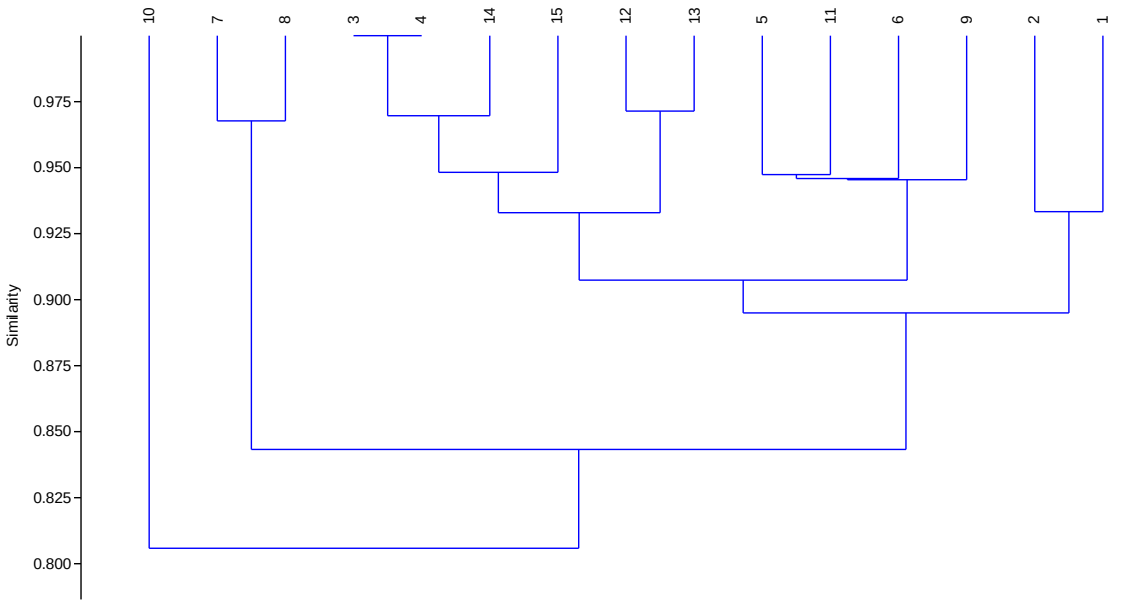


**Fig 5.** Dendrogram tree showed the genetic distance among the 15 studied sesame genotypes based on SRAP data
1=V1, 2= V4, V3= V5, 4= V6, 5=V9, 6= V13, 7= V15, 8= V19, 9= V22, 10= V24, 11= V25, 12= V27, 13= V28, 14= V31 and 15= V32

The first cluster contained V24 genotype, while the second cluster was divided into two sub-clusters, the first one included V15 and V19 genotype, and the second cluster divided into two sub sub-clusters; the first one included V1 and V4 genotypes while the second sub sub-clusters, V22, V13, V25, V9 genotypes lies in the first sub sub-duster, while the second one grouped V27, V28, V32, V31, V6 and V5 genotypes together.

The combination between ISSR and SRAP data revealed that total of 18 primers produced 32 polymorphic fragments out of 68 fragments with 48.33% polymorphism **Table 9**.The ISSR and SRAP data were also combined for UPGMA cluster analysis. The generated dendrogram using pooled of ISSR and SRAP data divided the sesame genotypes into two main clusters, as shown in **Fig 6.** Dice similarity coefficient ranged from 0.76 (V24 and V1) to 0.98 (V13 and V9), as shown in **Table 10.** The first cluster contained V24 genotype, while the second one was divided into two sub-clusters, the first one grouped V1, V19 genotypes, while the second one was divided into two sub sub-clusters, the first one grouped V32, V28, V31 and V27 genotypes. The second sub sub-clusters were divided into two sub sub-clusters; the first one included V25, V22 and V15 genotypes, while the second sub sub-cluster included V4, V5, V6, V9 and V13 genotypes.

**Table 9.** Total bands, band size, Monomorphic and polymorphic bands, unique bands, polymorphism percentage generated with 11 ISSR primers and 7 SRAP primer combinations for 15 accessions.

| Primer Name | Total bands | MS (bp) range of band size | Monomorphic bands | Polymorphic bands | Unique bands | Polymorphism % |
|---|---|---|---|---|---|---|
| ISSR | 46 | 170-1370 | 25 | 21 | 5 | 46.66% |
| SRAP | 22 | 120-780 | 11 | 11 | 4 | 50% |
| Combined | 68 | 120-1370 | 35 | 32 | 9 | 48.33% |

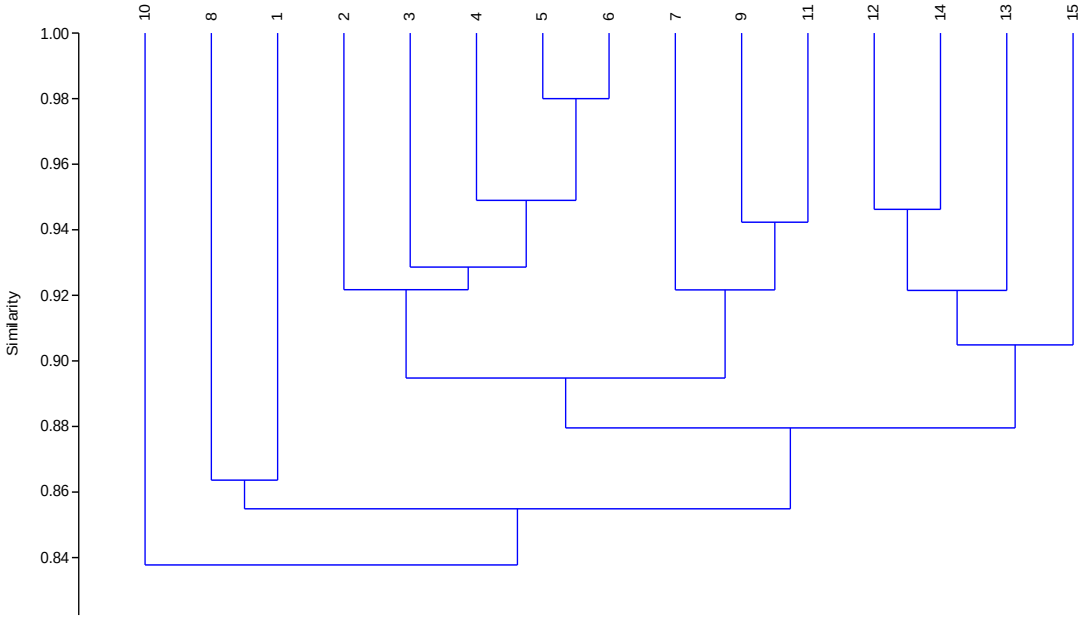


**Fig 6.** Dendrogram tree showed the genetic distance among the 15 studied sesame genotypes based on ISSR and SRAP data
1=V1, 2= V4, V3= V5, 4= V6, 5=V9, 6= V13, 7= V15, 8= V19, 9= V22, 10= V24, 11= V25, 12= V27, 13= V28, 14= V31 and 15= V32

**Table 10.** Dice similarity coefficient of 15 sesame accessions based on ISSR and SRAP data analysis

| Sesame accession | V1 | V4 | V5 | V6 | V9 | V13 | V15 | V19 | V22 | V24 | V25 | V27 | V28 | V31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V4 | 0.87 | | | | | | | | | | | | | |
| V5 | 0.88 | 0.91 | | | | | | | | | | | | |
| V6 | 0.87 | 0.92 | 0.93 | | | | | | | | | | | |
| V9 | 0.85 | 0.94 | 0.94 | 0.96 | | | | | | | | | | |
| V13 | 0.83 | 0.92 | 0.91 | 0.94 | 0.98 | | | | | | | | | |
| V15 | 0.87 | 0.9 | 0.83 | 0.88 | 0.9 | 0.92 | | | | | | | | |
| V19 | 0.86 | 0.87 | 0.82 | 0.85 | 0.85 | 0.88 | 0.92 | | | | | | | |
| V22 | 0.88 | 0.91 | 0.86 | 0.91 | 0.91 | 0.91 | 0.93 | 0.92 | | | | | | |
| V24 | 0.76 | 0.86 | 0.79 | 0.84 | 0.86 | 0.86 | 0.86 | 0.93 | 0.87 | | | | | |
| V25 | 0.84 | 0.91 | 0.87 | 0.89 | 0.91 | 0.91 | 0.91 | 0.86 | 0.94 | 0.91 | | | | |
| V27 | 0.83 | 0.88 | 0.88 | 0.88 | 0.91 | 0.89 | 0.85 | 0.91 | 0.9 | 0.82 | 0.88 | | | |
| V28 | 0.81 | 0.91 | 0.9 | 0.91 | 0.91 | 0.89 | 0.85 | 0.85 | 0.86 | 0.85 | 0.88 | 0.92 | | |
| V31 | 0.84 | 0.87 | 0.89 | 0.89 | 0.9 | 0.88 | 0.83 | 0.85 | 0.87 | 0.79 | 0.85 | 0.95 | 0.93 | |
| V32 | 0.88 | 0.88 | 0.86 | 0.88 | 0.87 | 0.87 | 0.87 | 0.83 | 0.9 | 0.82 | 0.88 | 0.91 | 0.88 | 0.92 |

The genetic distance and similarities among the 15 genotypes of sesame under investigation were estimated using the SRAP and ISSR data set with the average genetic similarities ranged from 24% to 90% **Table 11.** The highest genetic similarity was observed within the two pairs of genotypes (V13 & V9), (V9 &V6) and (V31 & V27) being 90%, 82% and 82%, respectively. However, the lowest genetic similarities were observed within two pairs of genotypes (V31 & V24), (V24 & V1), (V24 & V5), (V27 & V24) and (V24 and V15) as 24%, 27%, 29%, 33% and 36%, respectively.

The principle component analysis (PCA) results based on ISSR+SRAP data separated the 15 studied sesame genotypes into four groups **Fig 7.** The first group included V1, V22, V19, V25, V15, the second group contained V5, V6, V4, V9 and V13. However, the third group contained V31, V27, V28 and V32. Meanwhile, V24 were clustered alone in separate group and distantly related to the three main groups of the two axis of PCA. This result was compatible with the results of UPGMA clustering analysis which distinguished the 15 sesame genotypes, whereas a graphic demonstration of consensus tree which was constructed using Heat Map **Fig 8.** In general, the results revealed that three strongly clades supported that resolved species into distinct branches among the fifteen genotypes of sesame. Out of the 15 sesame genotypes, 14 were sorted together into three clades, while the genotype V24 was clustered jointly as out-group in the basal position of the tree. In details, the first clade comprised jointly two sub-clads, V22 and V25, while V15 and V19 is close to V1. In the second clade, V9 and V13 were close together as sisters to V4, V5 and V6. Meanwhile, the third clade represented that V27 is closely related to V31, at the same time is being sister to V32 and V28. Meanwhile, V1 and V4 were the tallest plant height accession, while V32 and V31 were the shortest accessions, V4 and V6 were the heaviest plant weight accessions, while V32 and V27 were the lightest accession. Moreover, V6 and V31 were the largest No. of pod accessions, while V32 and V15 was the lowest accessions, V9 and V5 was the heaviest pod weight accession, while V13 and V22 were the lightest accession. Genotypes V9 and V28 had the highest oil content% accessions. On the contrary, V22 and V24 recorded the lowest value of oil content%. Adu-Gyamfi et al (2019) detected the genetic variation of 25 sesame genotypes using 38 SSR markers and analyzed the data by using UPGMA method, the UPGMA results based on the genetic distances derived from SSR markers, which were in agreement and consistent with our PCA results. The dendrogram grouped the 25 accessions into five main and two minor clusters, also the PCA analysis divided the sesame genotypes into two main and two minor clusters. Finally they reached that there was a high considerably genetic diversity among the studied sesame genotypes.

**Table 11.** PCA genetic distance and similarities among the fifteen sesame genotypes using SRAP and ISSR data

| Sesame accession | V1 | V4 | V5 | V6 | V9 | V13 | V15 | V19 | V22 | V24 | V25 | V27 | V28 | V31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| V4 | 0.63 | | | | | | | | | | | | | |
| V5 | 0.72 | 0.73 | | | | | | | | | | | | |
| V6 | 0.65 | 0.72 | 0.76 | | | | | | | | | | | |
| V9 | 0.55 | 0.78 | 0.74 | 0.82 | | | | | | | | | | |
| V13 | 0.48 | 0.7 | 0.67 | 0.73 | 0.9 | | | | | | | | | |
| V15 | 0.6 | 0.58 | 0.43 | 0.52 | 0.57 | 0.67 | | | | | | | | |
| V19 | 0.65 | 0.57 | 0.5 | 0.52 | 0.48 | 0.56 | 0.69 | | | | | | | |
| V22 | 0.62 | 0.61 | 0.52 | 0.64 | 0.6 | 0.6 | 0.67 | 0.48 | | | | | | |
| V24 | 0.27 | 0.42 | 0.29 | 0.35 | 0.38 | 0.38 | 0.36 | 0.39 | 0.38 | | | | | |
| V25 | 0.44 | 0.57 | 0.48 | 0.5 | 0.56 | 0.56 | 0.53 | 0.52 | 0.67 | 0.53 | | | | |
| V27 | 0.56 | 0.62 | 0.6 | 0.56 | 0.61 | 0.52 | 0.42 | 0.43 | 0.61 | 0.33 | 0.48 | | | |
| V28 | 0.47 | 0.67 | 0.64 | 0.61 | 0.57 | 0.48 | 0.36 | 0.39 | 0.38 | 0.36 | 0.42 | 0.67 | | |
| V31 | 0.61 | 0.59 | 0.65 | 0.62 | 0.58 | 0.5 | 0.4 | 0.48 | 0.5 | 0.24 | 0.36 | 0.82 | 0.72 | |
| V32 | 0.67 | 0.59 | 0.58 | 0.62 | 0.5 | 0.5 | 0.48 | 0.55 | 0.58 | 0.32 | 0.46 | 0.74 | 0.56 | 0.79 |

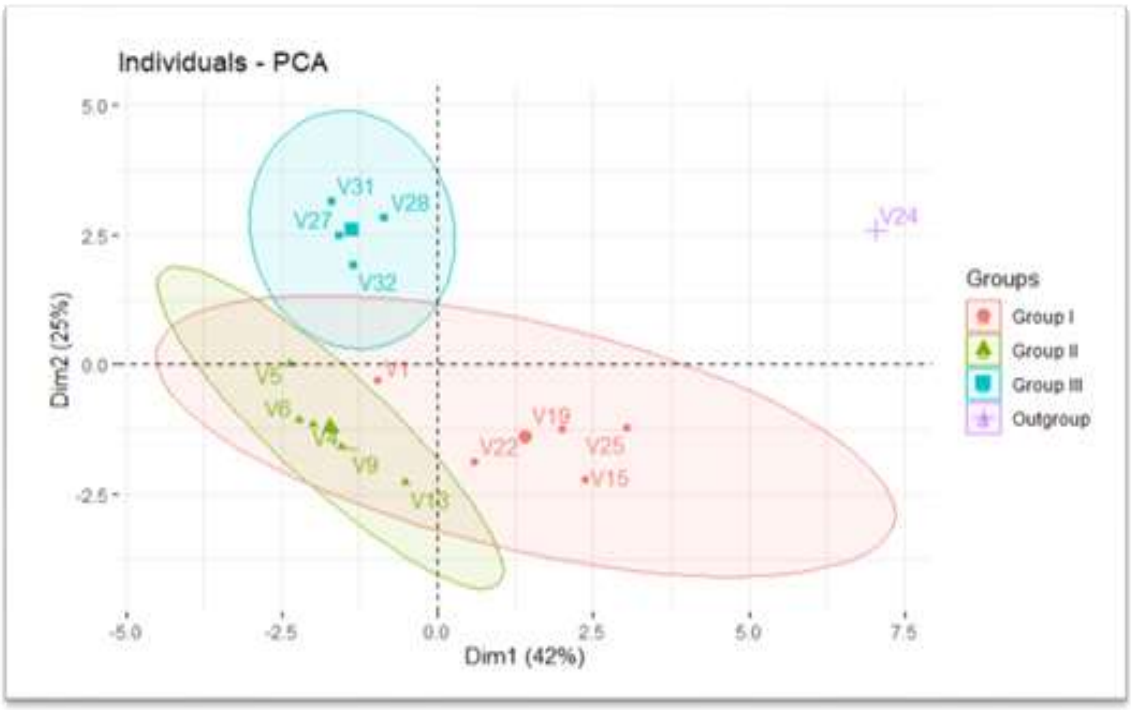


**Fig 7.** PCA analysis showed the genetic relationships among the 15 studied sesame genotypes

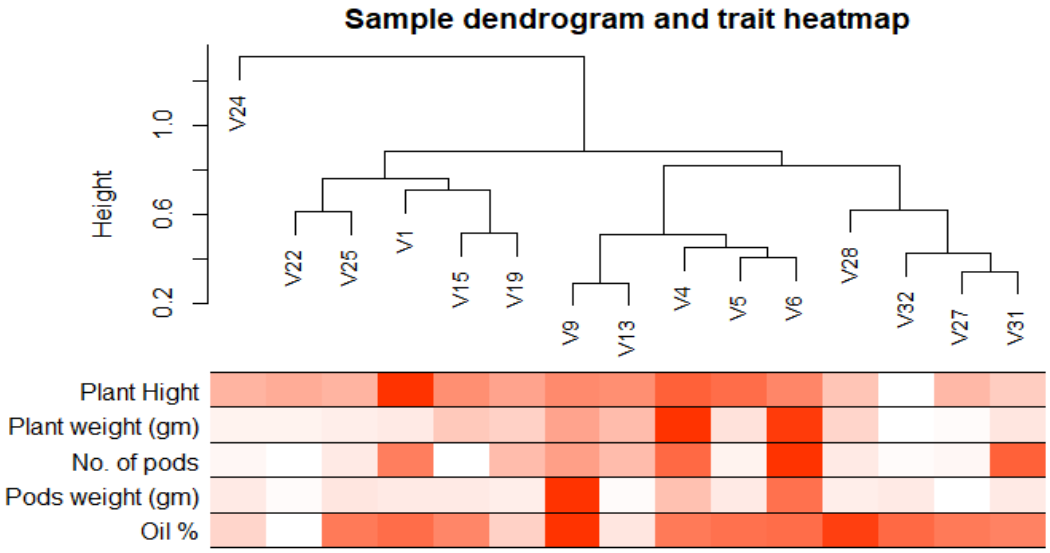


**Fig 8.** Heat Map relationships among 15 genotypes of *Sesamum indicum.* L

## 4 Conclusion

In conclusion, the aim of this analysis was to analyze molecular characterization, marker polymorphisms, morphometric and yield-related traits and genetic variability analysis between 32 genotypes of *Sesamum indcium* L. from different geographical region. Also, ISSR and ISRAP fingerprinting techniques have been used for genetic analysis to provide a valuable method for developing possible marker diagnosis for cultivar research. Eleven ISSR and seven SRAP primers showed clear amplification among the 15 sesame genotypes with polymorphism percentage of 46.66% and 50%, respectively. In addition, genetic diversity was developed using ISSR, SRAP and ISSR+SRAP marker technologies by dendrograms construction among all the various sesame genotypes. Both markers suggested large degree of genetic variations in relation to their distinct regional distributions within 15 separate sesame genotypes, and certain genetic differences were also found with respect to different types of marker technology. Thus, these studies have shown reasonable sources of information to help breeders to determine genetic variability and potentially select economically important traits such as highly oil content %. SRAP proved to be accurate and efficient tool for assessing genetic polymorphisms and sesame accessions relationships. As well as ISSR markers are a useful method in detecting high genetic diversity in sesame germplasm.

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# تقييم التباين الحيوي بين بعض التراكيب الوراثية من السمسم بإستخدام علامات ISSR و SRAP

[81]

**نورهان عاطف أبو النجا[1*] – أحمد أبو دومة[1] – لمياء مصطفى كمال[1] – كلارا رضا عزام[2]**

1– قسم الوراثة – كلية الزراعة – جامعة عين شمس – ص.ب 68 – حدائق شبرا 11241 – القاهرة – مصر

2– قسم بحوث الخليه – معهد بحوث المحاصيل الحقليه – مركز البحوث الزراعية – الرمز البريدي 12619 – الجيزة – مصر

*Corresponding author: noor_aboelnaga@hotmail.com

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**الموجـــــز**

تم دراسه التباين الحيوي بين 32 تركيب وراثي من السمسم (*Sesamum indicum* L.)، 30 منهم تراكيب وراثية تم جمعها من مناطق مختلفة من مصر، بالاضافه الي صنفين محليين وتم تقييمهم من الناحية الزراعية عن طريق السمات الشكلية والإنتاجية. وقد لوحظت اختلافات معنوية لجميع هذه الصفات مثل طول النبات ووزن النبات وعدد القرون ووزن القرون ونسبة الزيت. كان التباين بين أعلى وأدنى تركيب وراثي في ارتفاع النبات حوالي 50٪ حيث سجل التركيب الوراثي V32 أعلى ارتفاع للنبات (220 سم) بينما كان التركيب الوراثي V19 هو الأقصر (100 سم). تراوح وزن النبات من 802.2 إلى 99.5 جرام في V6 و V16، على التوالي. تراوح وزن القرون من 202.6 إلى 32.2 جرام في V4 و V15، على التوالي. تراوح عدد القرون من 75.7 إلى 10.3 في V5 و V17 ، على التوالي، بينما تراوح نسبه الزيت من 60٪ إلى 40.5٪ في V11 و V3 ، على التوالي. باستخدام الخصائص المورفومترية، تم تقسيم الطرز الوراثية في مجموعتين رئيسيتين علي أساس التباين الواضح بينهما. تم اختيار خمسة عشر تركيبا وراثيا من مناطق جغرافية مختلفة من بين التراكيب السمسم الوراثية المختبرة وإخضاعهم لتحليل علي المستوي الجزيئي وتحديد التنوع الجيني فيما يتعلق بالأصول الجغرافية بإستخدام 11 بادئ ISSR و 7 بادئات SRAP . نتج عن بادئات ISSR 46 حزمة مضخمة. أربعة من هذه البادئات نتج عنها 5 علامات فريدة من بين 15 تركيب وراثي من السمسم. أظهر التوصيف الجزيئي نسبة تعدد الأشكال 46.66٪ للواسمات ISSR، بينما أظهرت بادئات SRAP مجموع 22 حزمة وكشف واحد من هذه البادئات عن 3 علامات فريدة محددة خاصة بالنمط الجيني تم حسابه بنسبة 50٪. أظهر التحليل العنقودي تنوعًا وراثيًا عاليًا بين الطرز الوراثية للسمسم وكان تنوعها متسقًا مع أصولها. كانت نتائج تحليل المكونات الرئيسية (PCA) متوافقة بشكل وثيق مع نتائج تحليل التحليل العنقودي. بالنظر إلى العلاقة بين الأنماط الجينية والأصل الجغرافي وخصائصها المورفولوجية التي تنعكس على التشابه بين أنماط ISSR و SRAP.