



MORPHOLOGICAL AND MOLECULAR VARIABILITY FOR SOME SESAME GENOTYPES

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ABSTRACT: Eight sesame (*Sesamum indicum* L.) genotypes were evaluated using a randomized complete block design in field experiment with three replications for their diversity in seven agronomic and morphological traits at Ismailia Agricultural Research Station, ARC, Ismailia Governorate, Egypt during the two successive seasons 2016 and 2017. Results showed that the most promising genotypes for seed yield and its attributes were Zahar₁₄, Shandwell₃, H_{88A2}, Zahar₁₈, B₄₋₂, and Zahar₁₈. For all traits, estimates of phenotypic coefficient of variation (PCV) were slightly higher than those of genotypic coefficient of variation (GCV). High heritability coupled with high genetic advance (as % of the mean) was recorded for all studied traits. Five ISSR primers were used for fingerprinting of the eight sesame genotypes produced 29 bands, 23 of them were polymorphic with 79% polymorphism, while primer ISSR 1 produced two positive markers in shandweell 3 and line M₄₋₅₅. Primer ISSR 6 and ISSR 8 produced one band positive marker in the same line M₄₋₅₅.

Key words: *Sesamum indicum* L., genetic variability, heritability, inter-simple sequence repeats (ISSR).

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important oilseed crop, not only in Egypt but also all over the world. It plays an important role as an industrial and food crop. In Egypt, there is a large gap between oil production and consumption. High seed and oil yield potential, therefore is considered necessary to narrow the gap between production and demand. Consequently, great attention must be given to genetic improvement of yield, which could be achieved by identifying the nature and magnitude of genetic variability in the breeding materials that helps the breeder for selecting the appropriate breeding methodology in the crop improvement program.

Many sesame investigators such as **Tamina and Dasgupta (2003)**, reported that phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for plant height, days to flowering, flower duration,

days to maturity, number of branches/pl., number of capsules/pl, number of seeds/cap., capsule length, seed index, protein percentage, harvest index and seed yield/fad. **Raghuwanshi (2005)** estimated genetic variability for days to 50% flowering, days to maturity, plant height, number of branches/pl., number of capsules/pl., seed yield, seed index and oil content in 100 genotypes of sesame. He found wide range and high variability for seed yield and its components, except for seed index that showed low to moderate variability. **El-Shakhess et al. (2003 and 2008)**, **Babu et al. (2005)**, **Ganesan (2005)**, **Mothilal (2006)** and **Iwo et al. (2007)** recorded high heritability combined with high genetic advance for plant height, number of branches/pl., number of capsules/pl. and seed index. They added that high heritability combined with high genetic advance for these traits, indicated that additive gene action of high magnitude and phenotypic selection could be effective for improving these characters. Genetic

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diversity in crop species can be determined using morphological and agronomic characteristics as well as isozyme and DNA marker analyses (Liu, 1997). Different molecular markers have been used to study the genetic diversity of sesame germplasm collections in different countries. To study genetic relationships of sesame germplasm, Daniel and Parzies (2010) used SSR markers to investigate the genetic variability of Ethiopian sesame landraces. Isshiki and Umezaki (1997) and Nanthakumar *et al.* (2000), employed isozyme markers. Kim *et al.* (2002) used ISSR marker to study the genetic relationships of sesame germplasm collected from Korea. Arriell *et al.* (2007) assessment the genetic divergence of 30 morphological traits in 108 sesame genotypes through multivariate analysis. Several molecular marker based studies on sesame also revealed detailed information on its genetic diversity (Laurentin and Karlovsky, 2006; Salazar *et al.*, 2006; Pham *et al.*, 2009).

The aims of the current study were to, 1-study morphological and yield characteristics of eight sesame genotypes, 2-estimate some genetic parameters, which help achieving a successful breeding program, 3-utilize molecular marker using PCR-based ISSR (Inter-Simple Sequence Repeats DNA) to differentiate among genotypes under study

MATERIALS AND METHODS

To genetically improve yield of sesame, 8 sesame (*Sesamum indicum* L.) genotypes *i.e.* Shandwell 3, M₂A₂₄, Zahar₁₂, Zahar₁₄, Zahar₁₈, H₈₈A₂, B₄₋₂, and M4-55 were received from Oil Crops Research Dept., FCRI, ARC, Giza, Egypt. These materials were evaluated at Ismailia Agricultural Research Station, ARC, Ismailia Governorate, Egypt during the two successive summer seasons 2016 and 2017 to identify morphological and molecular variability among sesame genotypes for improving yield of sesame.

In both seasons, the same materials were planted in a randomized complete block design with three replications. Each experimental plot consisted of five ridges, 4 m long, 60 cm apart and 10 cm between hills, resulting in a plot size of 12 m². All other agricultural practices were applied as recommended for sesame crop. Five

competitive plants were randomly taken from the second ridge of each plot to measure, plant height (cm), first capsule height (cm), fruiting zone length (cm), number of capsules/plant, 1000 seed weight (g) and seed yield plant⁻¹ (g). However, seed yield per metersquare was recorded from plants in the two central (third and fourth) ridges; then converted to yield per faddan.

Standard statistical techniques for all traits were performed using randomized complete block design with three replications according to Gomez and Gomez (1984). The combined analysis of variance (across the two seasons) was done according to Snedecor and Cochran (1989) after confirmation of homogeneity test by Bartlett's test. Genotypic and phenotypic coefficients of variation were computed according to Burton and De Vane (1953), broad sense heritability (H²b) (Hansen *et al.*, 1956) and genetic advance as percent of the mean (Johnson *et al.* 1955).

ISSR-PCR Analyses

Six ISSR primers were evaluated for eight sesame genotypes. Name and sequences of the primers are shown in Table 1.

Polymerase Chain Reaction (PCR) Conditions

ISSR-DNA amplification was carried out in PCR tubes containing 25 µl reaction mixtures, having 1 µl template DNA, 1 µl ISSR primer, 15 µl of dd H₂O and 7 µl PCR mix. Amplification was carried out in a PTC- 200 thermal cycle (MJ Research, Watertown, USA) programmed as follows: Denaturation, 94°C for 3 minutes, then for 40 cycles. Each cycle consisted of 30 second at 94°C, 1 minute at 40°C, 2 minutes and one minute at 72°C, followed by a final extension time of 12 minutes at 72°C and 4°C (infinite)

Gel Electrophoresis

Gel electrophoresis was applied according to Sambrook *et al.* (1989). The run was performed for one hr., at 80 volt in pharmacia submarine (20 × 20 cm). Bands were detected on UV-transilluminator and photographed by gel documentation 2000, Bio- Rad. Fragment sizes of ISSR were estimated from the gel by comparison with the 100+1.2 kb ladder marker. The bands were recorded as either present (+) or absent (-) into a database of + and -.

Table 1. Name and sequences of the 6 primers used for ISSR-PCR analyses

Primer name	Sequence
ISSR 1	TTA ACC GGG G
ISSR 2	TTC CCC GAG C
ISSR 3	GAG CAC CTG A
ISSR 4	GGG CCC GAG G
ISSR 6	GCC CGG TTT A
ISSR 8	CCC CCC TTA G

Data Analysis

The data of PCR systems were analyzed to detect the similarity matrices using Gel/works 1D- advanced software UVP-England program. The relationships among different eight genotypes as revealed by dendrograms resolved using SPSS Windows (Version 16) program were estimated. Possible molecular markers for different qualitative and quantitative characteristics were detected for subsequent linkage and genome analyses.

RESULTS AND DISCUSSION

Mean Performance

Highly significant differences were detected among sesame genotypes for all studied traits in both seasons and their combined analysis as presented in Table 2, indicating the presence of sufficient magnitude of genetic variability for effective selecting the superior genotypes. Similar conclusion has been reported earlier by **El-Shakhess *et al.* (2008)**.

Combined analyses in Table 2, show that the genotypes Zahar₁₂, Zahar₁₄, Shandwell₃, H₈₈A₂, Zahar₁₈, B₄₋₂, Zahar₁₈ were considered as promising ones had shortest plant height and first capsule height, the longest fruiting zone, more capsule number, the heaviest weight of 1000-seed, seed yield plant⁻¹ and seed yield fad.⁻¹, in the same order.

Estimation of Genetic Parameters

To better evaluate the amount of genetic variability for tested sesame genotypes, the range, mean, phenotypic (PCV) and genotypic

(GCV) coefficient of variation, heritability in broad sense (H^2_b), selection index (SI) and genetic advance (GA) were computed for seven traits (Table 3).

It was apparent from Table 3, as seen in combined analysis that all sesame genotypes tested exhibited broad-range values for all traits. Plant height varied from 146.20 to 191.68 with a mean of 174.46 cm; first capsule height 41.92 to 84.07 with a mean of 67.22 cm; fruiting zone length from 77.78 to 129.08 cm with a mean height of 106.99 cm; number of capsules per plant from 55.53 to 87.42 with an average of 71.86; 1000-seed weight from 3.08 to 4.57 cm with an average weight of 4.19 cm; seed yield plant⁻¹ ranged from 13.52 to 24.60 g with an average weight of 18.56 g; seed yield per fad.⁻¹ from 312.67 to 599.02 kg with an average weight of 482.46 kg.

Phenotypic coefficient of variation (PCV) was slightly higher than genotypic coefficient of variation (GCV) for all studied traits (Table 2) indicating negligible influence of environment on the expression of all traits. Consequently, the selection would be effective to genetic improvement of the studied traits for these materials. Similar results were observed by **Laurentin and Montilla (2002)**, **Babu *et al.* (2005)**, **Ganesan (2005)** and **El-Shakhess *et al.* (2008)**. High PCV and GCV as shown in combined analysis, were observed for seed yield/fad., (PCV = 1210.52, GCV = 816.21), fruiting zone length (PCV = 131.03 and GCV = 99.86) and first capsule height (PCV=109.36 and GCV = 60.48), and dropped to moderate for capsules number (PCV = 59.08, GCV = 41.68), plant height (PCV =54.41, GCV = 30.71), while,

Table 2. Mean performance of 8 sesame genotypes for seven quantitative traits in both 2016 (1st) and 2017 (2nd) seasons and their combined analysis (Com.)

Character	Plant height			First capsule height			Fruiting zone length			Capsules number		
	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.
Shandwell 3	180.00	192.13	186.07	57.77	61.57	59.67	122.23	135.93	129.08	76.80	79.03	77.92
M₂A₂₄	157.80	173.63	165.72	63.83	75.43	69.63	93.97	90.23	92.10	62.40	57.70	60.05
Zahar₁₂	145.27	147.13	146.20	70.47	72.33	71.40	74.80	80.77	77.78	75.33	78.63	76.98
Zahar₁₄	167.87	181.17	174.52	42.00	41.83	41.92	126.10	126.80	126.45	50.87	60.20	55.53
Zahar₁₈	181.93	181.07	181.50	74.73	77.50	76.12	107.20	106.53	106.87	74.07	70.17	72.12
H₈₈A₂	193.53	189.83	191.68	66.40	69.03	67.72	127.03	120.03	123.53	86.60	88.23	87.42
B₄₋₂	176.17	184.60	180.38	69.57	64.97	67.27	106.83	111.43	109.13	75.60	72.37	73.98
M₄₋₅₅	169.13	170.10	169.62	83.67	84.47	84.07	85.47	96.43	90.95	67.33	74.43	70.88
LSD_{5%}	23.65	17.19	19.50	17.19	19.50	21.99	15.74	17.39	24.38	16.14	17.52	14.03
LSD_{1%}	32.82	17.19	19.50	23.86	27.07	30.52	21.85	24.13	33.84	22.40	24.31	19.47
	1000-seed weight			Seed yield plant ⁻¹			Seed yield fad. ⁻¹					
	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.			
Shandwell 3	4.47	4.63	4.55	20.23	20.30	20.27	494.70	656.60	575.65			
M₂A₂₄	4.23	4.20	4.22	18.50	18.63	18.57	527.57	565.23	546.40			
Zahar₁₂	4.77	4.23	4.50	15.10	11.93	13.52	296.53	328.80	312.67			
Zahar₁₄	3.97	3.80	3.88	20.10	13.60	16.85	373.80	359.57	366.68			
Zahar₁₈	4.33	4.80	4.57	12.10	21.00	16.55	653.63	544.40	599.02			
H₈₈A₂	3.93	4.47	4.20	18.83	19.90	19.37	507.20	535.63	521.42			
B₄₋₂	3.03	3.13	3.08	24.53	24.67	24.60	565.23	598.57	581.90			
M₄₋₅₅	4.33	4.73	4.53	17.03	20.50	18.77	359.30	352.53	355.92			
LSD_{5%}	14.56	10.73	0.73	0.84	0.45	4.01	2.98	2.56	132.30			
LSD_{1%}	20.21	14.89	1.02	1.17	0.62	5.57	4.13	3.55	183.62			

Table 3. Phenotypic and genotypic parameters of 8 sesame genotypes for all traits in both seasons 2016 (1st) and 2017 (2nd) and their combined analysis (com.)

Parameter	Plant height (cm)			First capsule height (cm)			Fruiting zone length (cm)			Capsules number (cm)			
	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.	
SD	13.50	9.82	11.14	12.56	8.99	9.93	13.92	9.21	10.00	8.01	8.31	6.13	
SE	11.03	8.01	9.09	10.25	7.34	8.11	11.37	7.52	8.17	6.54	6.79	5.00	
CV	7.88	5.53	6.38	19.01	13.14	14.77	13.20	8.49	9.35	11.26	11.45	8.52	
Range	Min	145.27	147.13	146.20	42.00	41.83	41.92	74.80	80.77	77.78	50.87	57.70	55.53
	Max	193.53	192.13	191.68	83.67	84.47	84.07	127.03	135.93	129.08	86.60	88.23	87.42
mean	171.46	177.46	174.46	66.05	68.39	67.22	105.45	108.52	106.99	71.13	72.60	71.86	
PCV	67.60	50.56	54.41	130.17	107.76	109.36	160.50	126.27	131.03	74.89	67.55	59.08	
GCV	32.15	32.46	30.71	50.58	68.37	60.48	99.22	100.19	99.86	44.80	35.82	41.68	
H ²	0.73	0.84	0.80	0.66	0.84	0.79	0.83	0.92	0.91	0.82	0.77	0.88	
SI 5%	38.47	33.85	34.81	33.13	30.68	30.64	46.49	41.83	42.31	26.08	25.02	23.28	
GA	524.53	468.30	467.28	349.08	382.65	358.43	868.65	780.36	785.87	269.36	234.30	230.66	
GAM(% of mean)	305.91	263.89	267.84	528.47	559.50	533.20	823.73	719.09	734.55	378.71	322.75	320.99	
GV	165.35	172.84	160.73	100.24	140.28	121.98	313.88	326.18	320.53	95.60	78.01	89.85	
PV	347.73	269.18	284.77	257.96	221.10	220.54	507.75	411.09	420.57	159.79	147.12	127.37	
Parameter	1000-seed weight (g)			Seed yield plant ⁻¹ (g)			Seed yield fad. ⁻¹ (kg)						
	1 st	2 nd	Com.	1 st	2 nd	Com.	1 st	2 nd	Com.				
SD	0.42	0.48	0.26	1.46	1.70	1.46	94.17	75.72	75.55				
SE	0.34	0.39	0.21	1.19	1.39	1.19	76.89	61.83	61.68				
CV	10.11	11.29	6.09	7.86	9.04	7.86	19.94	15.37	15.66				
Range	MIN	3.03	3.13	3.08	13.52	11.93	13.52	296.53	328.80	312.67			
	MAX	4.77	4.80	4.57	24.60	24.67	24.60	653.63	656.60	599.02			
mean	4.13	4.25	4.19	18.56	18.82	18.56	472.25	492.67	482.46				
PCV	3.10	3.65	2.40	21.15	33.74	21.15	1422.23	1341.97	1210.52				
GCV	1.70	1.84	1.88	17.32	28.62	17.32	796.24	954.03	816.21				
H ²	0.78	0.75	0.92	0.93	0.94	0.93	0.79	0.88	0.86				
SI 5%	1.28	1.41	1.13	7.08	9.00	7.08	292.84	290.54	273.07				
GA	0.62	0.72	0.57	22.63	37.08	22.63	32936.49	36033.99	31131.82				
GAM (%) of mean	15.05	17.02	13.58	121.91	197.05	121.91	6974.44	7314.07	6452.78				
GV	0.21	0.23	0.24	9.65	16.15	9.65	11280.61	14100.52	11813.54				
PV	0.38	0.47	0.30	11.77	19.05	11.77	20149.22	19834.36	17520.62				

PCV: Phenotypic Coefficient of Variation, GCV: Genotypic Coefficient of Variation, H²%; Broad sense heritability, Si: Selection index, GA: Genetic advance, GAM%: Genetic advance as percent of mean

drastically reduced for seed yield plant⁻¹ (PCV = 21.15, GCV = 17.32) and 1000 seed weight (PCV = 2.40, GCV = 1.88). High heritability in broad sense was recorded for all studied traits, which supported selection for improving these traits, and has been observed in earlier studies by **Prasad *et al.* (2007)** and **El-Shakhess *et al.* (2008)**.

It could be emphasized that without considering genetic advance, the heritability in broad sense would not be practically important in selection based on phenotypic form. This was confirmed by **Johnson *et al.* (1955)** who discovered efficient use of heritability in broad sense along with genetic advance, which would give a more reliable index of selection value. High values of heritability coupled with high values of genetic advance (as % of mean) were detected for all studied traits (Table 3), indicating the importance of additive gene effects in the inheritance of these traits, thus, selection for these traits would be effective. This was reported also by **Velu and Shunmugavalli (2005)**, **Mothilal (2006)**, **Prasad *et al.* (2007)** and **El-Shakhess *et al.* (2008)**.

Molecular Marker Combined Analyses for Eight Sesame Genotypes

In the present study six primers of ISSR were selected to differentiate among eight genotypes of sesame; these primers produced multiple bands, which ranged between nine bands for primer ISSR 1, to three bands for primer ISSR2, ISSR4 and ISSR8. The total number of bands was 29, 23 of them were polymorphic (79% polymorphism), the highest level of polymorphism was observed in primer ISSR1 which showed 100% polymorphism, while the lowest polymorphism was 33% in primer ISSR8 as show in Table 4 and Fig. 1. Primer ISSR1 produced 9 bands with the fragment sizes ranging from 960 bp to 455 bp which giving, 100% polymorphism, this primer ISSR1 produced two positive markers in two different genotypes; Shandweell 3 at Molecular weight (MW) 455pb and Line M₄₋₅₅ at MW 830pb. Primer ISSR2 produced 3 bands with the fragment sizes ranging from 860 bp to 605 bp, 2 of them were polymorphic (67% polymorphism). ISSR3 produced 6 bands with the fragment sizes ranging from 1050 bp to 310 bp, 5 of them were

polymorphic (83% polymorphism). Primer ISSR4 showed 3 bands with the fragment sizes ranging from 800 bp to 300 bp, 2 of them were polymorphic (67% polymorphism). Primer ISSR6 produced 5 bands with the fragment sizes ranging from 1250 bp to 450 bp, 4 of them were polymorphic (80% polymorphism), this primer ISSR6 produced one marker for Line M₄₋₅₅ at MW 450 pb. Primer ISSR 8 produced 3 bands with the fragment sizes ranging from 850 bp to 550 bp, one band of them polymorphic (33% polymorphism) this primer ISSR 8 produced one marker for Line M₄₋₅₅ at MW 850 pb. **Admas *et al.* (2013)** studied cultivars and varieties of sesame from north western Ethiopia each consisting of ten individual samples were analyzed using four ISSR markers with the aim of assessing the genetic study area in 2011 main cropping season and dried by in a silica gel of DNA extraction and the samples were moved to the genetics laboratory of Addis Abab University for further laboratory investigation. The four ISSR yielded 37 amplification products of which 36 bands (97.30%) exhibited polymorphism, the maximum similarity was observed for the cultivars. ISSR technique was also performed for sesame to study the genetic relationship of a sesame germplasm in Korea by **Kim *et al.* (2002)**.

Anitha *et al.* (2010) used 14 ISSR primers to estimate the diversity of 10 sesame varieties cultivated in Tamil Nadu (India). On the other hand, **Kim *et al.* (2002)** used 14 ISSR markers to determine genetic relationships of 75 Korean and exotic sesame accessions recorded 79 bands of which only 26 (33%) were polymorphic.

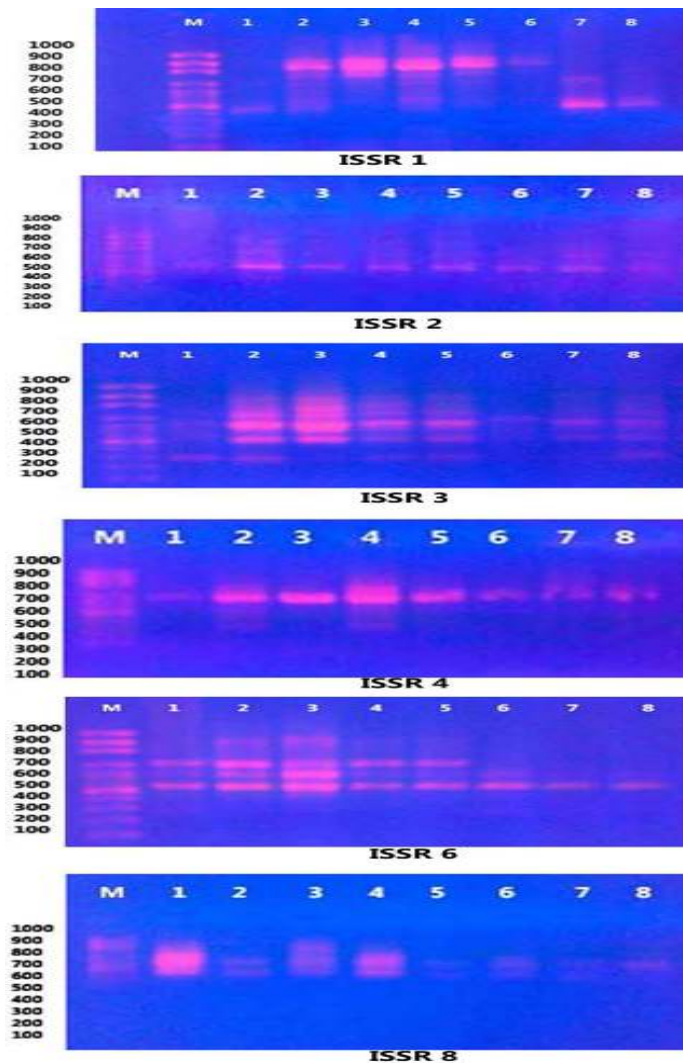
Combined Analysis for Eight Sesame Genotypes

Similarity index and dendrogram across the eight sesame genotypes under investigation based on ISSR analyses are shown in Table 5 and Fig. 2. The comparison revealed that the highest closely related in Line M2A24/LineH₈₈A₂ (similarity matrix of 0.901), followed by LineM2A24/Line B₄₋₂ (similarity matrix of 0.884). The lowest relationships were recorded for Shandweell 3/Zahra₁₄ (similarity matrix of 0.481). The resulted produced in two main clusters. One of them involved the Shandweell 3/

Table 4. DNA polymorphic in eight sesame genotypes using PCR with six ISSR primers

Primer	MW (bp)	Mono-morphic	Poly-morphic	NB	P (%)	Genotype	MW PM
ISSR 1	960:455	0	9	9	100	Line M ₄₋₅₅	830
						Shandweell3	455
ISSR 2	860:605	1	2	3	67		
ISSR3	1050:310	1	5	6	83		
ISSR 4	800:300	1	2	3	67		
ISSR 6	1250:450	1	4	5	80	Line M ₄₋₅₅	450
ISSR8	850:550	2	1	3	33	Line M ₄₋₅₅	850
Total		6	23	29	79		

NB, number of bands MW, molecular weight PM, positive marker G, genotype P(%), % polymorphism

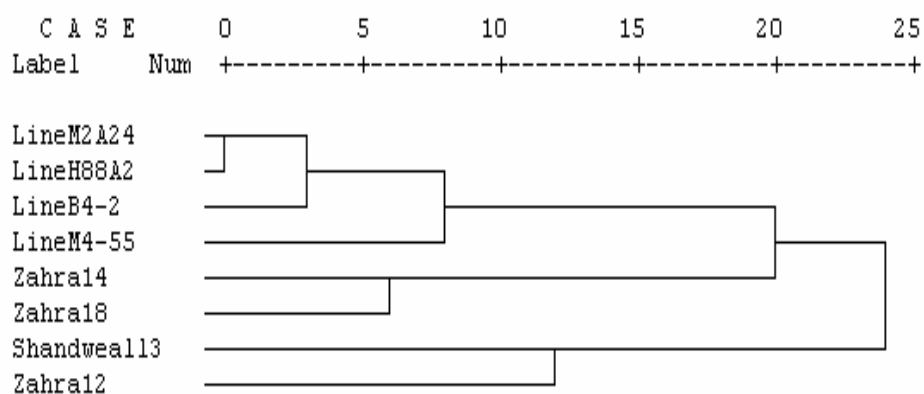


1-Shandweell3, 2-M₂A₂₄, 3-M₄-55, 4-B₄₋₂, 5- H₈₈A₂, 6-Zahar₁₂, 7-Zahar₁₄, 8- Zahar₁₈

Fig. 1. ISSR banding patterns amplified with 6 primers for eight sesame genotypes

Table 5. Similarity matrix among the eight sesame genotypes used ISSR analyses

Genotype	Shandweall3	Line M2A24	Line M4-55	Line B4-2	Line H88A2	Zahra12	Zahra14
Line M2A24	0.625						
Line M4-55	0.581	0.780					
Line B4-2	0.606	0.884	0.810				
Line H88A2	0.601	0.901	0.769	0.829			
Zahra12	0.736	0.552	0.571	0.533	0.593		
Zahra14	0.481	0.629	0.529	0.611	0.727	0.545	
Zahra18	0.666	0.647	0.606	0.686	0.625	0.571	0.815

**Fig. 2. Dendrogram of the genetic distances among the eight sesame genotypes using ISSR analyses**

Zahra₁₂, while the second cluster involved the rest of the genotypes. The second cluster was divided into two sub clusters, one included Zahra₁₄/ Zahra₁₈, while the other sub cluster involved four genotype (Line M2A24, Line H_{88A2}, Line B₄₋₂ and Line M₄₋₅₅).

Generally, it was concluded that there was a significant morphological and molecular variability or divergence among the evaluated genotypes. Thus, there is enormous opportunity in the improvement program of the sesame through direct selection and crossing or hybridization involving divergent genotypes, which can be done to produce viable and potential segregant populations for the subsequent breeding work and making potential variability for producing new genetic recombinations.

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التباين المورفولوجي والجزيئي لبعض التراكيب الوراثية من السمسم

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أقيمت هذه التجربة لدراسة قيم التباعد الوراثي لثمانية تراكيب وراثية من السمسم في تصميم قطاعات كاملة العشوائية في ثلاث مكررات لسبع صفات محصولية ومورفولوجية بمحطة بحوث الإسماعيلية، مركز البحوث الزراعية، محافظة الإسماعيلية، مصر، خلال موسمي الزراعة 2016 و2017، وأظهرت النتائج أن أفضل التراكيب الوراثية الواعدة للمحصول ومساهماته هي Zahar₁₄، شندويل^٣، H_{88A2}، Zahar₁₈، B₄₋₂ و Zahar₁₈، كما أظهرت النتائج أن تقديرات معامل التباين المظهري أعلى قليلا من معامل التباين الوراثي لكل الصفات تحت الدراسة، وكذلك تم تسجيل ارتفاع قيمة كفاءة التوريث بالمعنى الواسع إلى جانب ارتفاع قيمة التقدم الوراثي بالانتخاب (كنسبة مئوية للمتوسط) لكل الصفات تحت الدراسة، استخدام تكنيك ISSR باستخدام 6 بادئات و أظهرت النتائج 29 علامة جزيئية منهم 23 مختلفة بنسبة 79%، البادئ ISSR1 قدم علامتين جزيئيتين موجبتين في التركيب الوراثي شندويل 3 والتركيب الوراثي M₄₋₅₅ على التوالي، بينما البادئ ISSR6 والبادئ ISSR 8 كلاهما قدم علامة جزيئية واحدة في التركيب الوراثي M₄₋₅₅.

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