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Evaluation of Genetic Diversity of Sesame (*Sesamum indicum* L.) Genotypes, Using Agro Morphological and Molecular Markers (RAPD)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to look at the genetic variation in seventeen genotypes of sesame (*Sesamum indicum* L.) from different agro-climatic regions of Sudan, using both agro-morphological traits and RAPD markers. From July 13 to November 2013, the field experiment was conducted on

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the experimental farm of the College of Agricultural Studies, Sudan University of Science and Technology, Shambat. A randomized complete block design (RCBD) with three replications was employed in this investigation. The seventeen sesame genotypes had their nine growths and yield parameters assessed, the number of branches, the height of the first capsules, the number of capsules per plant, and the weight of 1000 seeds all showed a highly significant difference. There were no statistically significant differences between the two groups ($p \geq 0.05$). The plant's height and days to 50% flowering were counted. The number of capsules per plant and seed weight showed the largest genotypic and phenotypic variance. The heritability value was greater when 1000 seeds were used. On the other hand, plant height had the lowest rating. The Highland genotype had the maximum yield (0.2506 ton/ha). Seed yield (ton/ha) was found to be highly associated with seed output per plant, capsule number per plant, and stem diameter. However, there was a non-significant, inverse relationship between height to first capsule and days to flowering. Molecular DNA marker (RAPD) analysis revealed that three RAPD primers chosen for molecular analysis had 170 bands, 130 of which were polymorphic (76.47%). The results based on high genetic variation for agro-morphological traits and polymorphism at DNA level indicating that, the techniques was efficient in determining diversity among sesame genotypes and this information will be useful for collection, conversation and breeding program in the future.

Keywords: Sesame; genetic diversity; morphological; RAPD markers.

1. INTRODUCTION

"Sesame (*Sesamum indicum* L.) is an annual plant in the Pedaliaceae family. It is the oldest of the oilseed crops, having been cultivated in Asia for over 5000 years" [1]. "It is now widely cultivated as an oil crop in tropical and subtropical climates. Sesame is the most important rotation crop in Sudan's rainfed cropping systems. Rainfed conditions are ideal for growing the crop, either as a stand-alone crop or in combination with other crops including sorghum, watermelon, Roselle, cowpea, and maize. Sudan has a very low average yield (0.21 t/ha), compared to the world average of 0.47 t/ha and China's 1.15 t/ha. The level of production is likewise highly changeable. Low productivity and inconsistent total output can be due to several biotic and abiotic issues, the most significant of which is a lack of adequate high-yielding cultivars" [2]. "A thorough understanding of the genetic diversity within and among the genetic resources of accessible sesame germplasm is required for a successful breeding program. Plant breeders can now select parental sources for hybrid creation or distinct populations for selection" [3].

Crop genetic diversity is important for development that is long term and food security [4], as it allows crops to be grown under a variety of environmental stress conditions (including biotic and abiotic). "It is also necessary for the selection of parents for use in plant molecular breeding. Genetic diversity data is available. When it comes to improving plant varieties, this is crucial. (Various methods, such as morphological, biochemical, and molecular markers, are used to study genetic diversity. The most important tool for estimating genetic differences among sesame genotypes is morphology. Several morphological marker-based studies have discovered high genetic diversity in sesame populations" [5,6,1,7]. "Morphological markers, on the other hand, have a limited ability to estimate genetic diversity because they are strongly influenced by environmental factors and thus highly dependent on growing conditions. (Molecular markers get around this limitation.) Molecular markers such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Simple Sequence Repeats (SSR) have been developed, widely used in studies of sesame genetic diversity. However, the goals of this study are to assess the genetic diversity of sesame genotypes using geomorphological and molecular markers (RAPD), to estimate the values of heritability and phenotypic correlation among different sesame genotype traits, and to identify the most promising sesame genotypes for higher-yielding production" [1].

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted of seventeen sesame genotypes obtained from different agro-climatic regions of Sudan and used for both agro morphological and molecular marker characterization (Table 1).

Table 1. Lists the name's of seventeen sesame (*Sesamum indicum* L) genotypes used in this study

Genotype	Origin
Bash	Local variety collected by ARC
High Land	Ethiopian variety introduced by ARC
Caraway	Local variety collected by ARC
DanabAlgamal	Local variety collected by ARC
Soliamani	Local variety collected by ARC
Om Shagara	Local variety collected by ARC
Aldana	Local variety collected by ARC
Abraway	Local variety collected by ARC
Alradom	Local variety collected by ARC
$5_{(3)}$	Inbred line by lob of CAS(SUST)
Harba, 15	Local variety collected by ARC
Thailand	Introduced variety by ARC
J4	Introduced variety by ARC
17	Inbred line by lob of CAS(SUST)
$13_{(3)}$	Inbred line by lob of CAS(SUST)
kenanna	Released variety by ARC Variety
Zagreb	Inbred line by ARC

ARC: Agricultural Research Corporation.
CAS: Sudan University of Science and Technology College of Agricultural Studies

2.2 Field Trials

The field experiments were carried out at the Sudan University of Science and Technology research farm (32º- 35º E and 15º N, 1650 m above sea level) during the summer season of 2008. All genotypes were evaluated geomorphologically in a randomized block trial with three replicates. Each plot consisted of four rows with a length of 4 m and a row spacing of 70 cm with a plant spacing of 7 cm between rows. Before sowing, 80 kg N/ha and 100 kg P/ha were fertilized and four weeks after sowing, 40 kg N/ha were re-fertilized. Days to flowering were recorded visually for each plot. (Plant height, first capsule height, stem diameter per plant, capsules per plant, 1000_seed weight, and seed yield per plant were measured on ten randomly selected plants per plot and their average was used. Two middle rows of each plot were harvested to determine seed yield (ton/ha)).

2.3 Data statistical Analysis

Measuring data for growth and yield were subjected to an analysis of variance for randomized complete block design (RCBD) using (the computer package Statistics-8).

2.4 Coefficient of Variation (C.V)

The coefficient of variation (C.V) was calculated for each trait using the formula below.

$$CV\ percent = (MSE)/\ grand\ mean \times 100$$

Where:

MSE is an abbreviation for mean square error, and G is an abbreviation for grand mean

2.5 Phenotypic (σ^2ph) and Genotypic (σ^2g) Variances

For separate analysis of variance. They were estimated as follows:

$$\sigma^2g=(M2 - M1) /r$$

$$\sigma^2ph= \sigma^2g + \sigma^2e$$

Where:

r= number of replicates = 16
σ^2e= errors or environments
M1, M2= errors and mean squares of the genotypes

2.6 Estimation of Heritability (h^2)

Estimation of heritability (h^2): Heritability was estimated in the broad sense separately for both seasons using the formula proposed by [8] as follows:

Based on the separate ANOVA:

$h^2 = \sigma^2g/ \sigma^2ph$ where σ^2g denotes genotypic variance and σ^2ph denotes phenotypic variance.

The phenotypic and genotypic coefficients of variation were calculated using [1] formula

$$PCV\ (phenotypic\ coefficient\ of\ variation) = \sigma^2Ph/\ Grand\ mean \times 100$$

$$GCV\ (genotypic\ coefficient\ of\ variation) = \sigma^2g\ /\ Grand\ mean \times 100$$

2.7 Correlation Coefficients

They were used to calculate phenotypic covariance. It was then used to calculate the phenotypic correlation between various traits using [9] formula

> The phenotypic correlation coefficient (r p) is equal cov p (xy) / √ (σ^2px × σ^2py)

r p (xy) phenotypic correlation coefficient
cov p (xy)= phenotypic co variance of traits x , y
σ^2px = phenotypic covariance of trait x
σ^2py= phenotypic covariance of trait y

2.8 DNA Molecular Analysis

2.8.1 DNA extraction

"DNA was extracted from young leaves of 3- to 4-week-old seedlings following a modified protocol described in" [10]. "The DNA pellet was washed twice in ethanol (75%) and allowed to dry for 20-30 min. After dissolving the DNA pellet in 30 µl TE (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0) buffer containing RNAse (2 µl 20 mg/ml RNAse in 1 ml TE), Concentration of DNA obtained were determine through 260/280 absorbance measure using nm the NanoDrop spectrophotometer 2000" [11]. DNA samples were stored at -20°C until further use).

2.9 RAPD Assay

"The PCR-RAPD procedure for sesame was performed" as described by [12]. "The amplification reaction was performed in a 25 µl volume containing 1X PCR reaction buffer (10 mM Tris-HCl, pH 8.3, and 50 mM KCl), 0.2 mM dNTP. Respectively)". [12] "The PCR reaction was conducted in 50 µl reaction volume containing 1x PCR buffer, 1.5 mM MgCl , 0.2 mM of each dNTPs, 1 mM of primer, 1 U Taq DNA (promega) polymerase and 10 ng genomic DNA. DNA amplification was performed using a thermal cycler programmed for first cycle of 5 min at 94°C (initial strand separation); followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing) and 2 min at 72°C and a final cycle at 72°C for 10 min. The PCR product were mixed with 2.5 µl of 10 X loading dye (0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose, w/v) and spun briefly in a microfuge before loading. The PCR products and 1 kb DNA ladder were electrophoresed on 2% agarose gel at 100 V followed by staining with ethidium bromide and photographed on Polaroid 667 film under ultra-violet light" [13].

2.10 Data Analysis

"The experiments were repeated for a minimum of three times to confirm the banding patterns and only those consistent bands on the gels were scored for data analysis. For each primer, the number of polymorphic and monomorphic bands was determined. Bands clearly visible in at least one genotype were scored (1) for present and (0) for absent, and entered into a data matrix. Fragment size was estimated by interpolation from the migration distance of marker fragments. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The genetic dissimilarity (D) matrix among genotypes was estimated" according to [12]. "The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA)" according [14].

3. RESULTS AND DISCUSSION

3.1 Morphological Characterization

Analysis of the variance of this study revealed that highly significant differences were observed among genotypes for most of the traits studied (Table 2 and Table 3). A wide range of the genetic variability in most of the traits indicates potential genetic variability in the sesame genotypes studied. Similarly, a significant difference among a large number of Sudanese sesame genotypes was observed [15].

The highest yielding genotypes were High land, Harba15, and Alradom with seed yields of 43.86, 42.53, and 41.33 kg/plant, respectively (Table 3). The high genetic variability among genotypes observed in seed yield offers great potential for future sesame breeding programs. The estimation of genotypic (σ²g), phenotypic (σ²ph), phenotypic coefficient of variation (PCV), and genotypic coefficient of variation (GCV) are given in Table 4). (The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) for all traits studied). The highest values of the phenotypic coefficient of variation (170.229%-340.582%) were obtained by first capsule height and grain yield per plant (g), and the lowest values (43.714-6.5109) were obtained by stem diameter and seed per plant (t/ha), respectively (Table 4). These results indicate the low level of selection for improvement based on these traits. Similar results were reported by [15-16]. High broad-based heritability was estimated for the traits

Table 2. Mean squares of individual analysis for growth and yield traits of seventeen genotypes of sesame (*Sesamum indicum* L.) evaluated at Shambat during the season (2013-2014)

Characters	Mean squares of blocks	Mean squares of genotypes	Mean squares of error
Flower 50%	0.41	85.37*	47.18
Plant height(cm)	160.80	307.73^{Ns}	183.52
Height first capsule (cm)	550.55	386.84**	129.124
Stem diameter(cm)	1.658	1.446*	0.567
No. of branches	3.90	8.35**	1.84
No. of capsules	223.46	1574.74**	550.65
Weight of 1000 seed (g)	9.35	0.733**	1.97
Seed yield per plant (g):	28.42	283.44^{Ns}	199.08
Seed yield (t/ha)	0.00102	0.00954^{Ns}	0.00646

*N_S =not significant difference **highly significant difference*
**significant difference*

Table 3. Means of different traits of seventeen sesame genotypes evaluated at shambat, during seasons (2013-2014)

Genotypes	Flower 50%	Plant height (cm)	Height. F.capsules (cm)	Stem diameter (cm)	No. of branches	No. of capsules	Seeds yield (g)	Seeds yield (t/ha)	Weight of 1000 seed
Abraway	48.33	130.43	73.43	5.87	4.7	78.03	38	0.2171	3.2
Om Shagara	61	141.53	85.39	5.32	7.44	123.53	29.63	0.1693	2.8
Alsodana	49	131.90	87.40	4.60	5.73	58.90	27.6	0.1577	2.6
DanabAlgamal	55.33	137.33	93.41	4.85	7.46	57.97	12.63	0.0721	2.9
Kenana()	44	135.50	81.35	4.13	4.56	73.60	20.20	0.1154	2.4
Alradom	45.33	141.27	82.05	5.57	6.73	131.27	41.33	0.2360	3
$13_{(3)}$	43	121.27	64.68	3.52	1.33	57.10	24.10	0.1377	3.4
Carawy	47.33	144.27	89.14	4.95	5.8	95.13	39.06	0.2232	2
Bash	40	123.57	62.16	4.06	2.3	73.63	38.46	0.2198	3.2
J4	51	131.23	67.91	5.17	4.6	89.83	38.40	0.2194	3.8
Harba15	42	119.50	53.63	4.29	4.43	86.33	42.53	0.2440	3.4
Highland	51	138.10	76.83	4.55	4.13	80.93	43.86	0.2506	3
Tailandi	42.66	111.63	61.16	3.89	3.76	59.10	25.53	0.1459	2.5
17	45	113.43	64.33	3.80	3.5	70.20	39.23	0.2242	3.4

Genotypes	Flower 50%	Plant height (cm)	Height. F.capsules (cm)	Stem diameter (cm)	No. of branches	No. of capsules	Seeds yield (g)	Seeds yield (t/ha)	Weight of 1000 seed
Soliamani	46.66	116.28	66.03	3.85	3.66	62.03	19.80	0.1131	3
$5_{(3)}$	44.33	126.73	78.53	3.72	3.27	57.17	27.10	0.1549	2.8
Tagrib	52	129.53	73.96	4.80	5.08	52.53	19.66	0.1024	2
Grand Mean	47.52	129.03	74.20	4.52	4.61	76.9	31.01	0.1766	2.92
C.V	14.45	10.50	15.31	16.64	29.41	30.51	30.40	45.51	3.83
SE±	5.6085	11.061	9.2781	0.6152	1.1093	19.16	11.52	0.656	2.54
LSD	11.424	22.531	18.899	1.2532	2.2595	39.027	23.467	0.1337	1.32

Table 4. Phenotypic (PCV %) and Genotypic coefficient of variation (GCV %), phenotypic and genotypic variance for the different characters studied on seventeen genotypes of sesame evaluated at Shambat during the season (2013-2014)

Traits	Phenotypic variance (δ^2ph)	Genotypic variance (δ^2g)	Genotypic coefficient of variation (GCV %)	Phenotypic coefficient of variation (PCV %)	Heritability$(h)^2$%
Flowering	59.914	12.731	51.754	112.27	21.248
Plant height	224.926	41.399	56.64	132.03	18.405
Height. F.capsules	215.029	85.905	107.59	170.229	39.950
Stem diameter	0.86550	0.29777	25.637	43.714	34.404
No. of branches	4.0152	2.169	68.541	93.229	54.019
No. of capsules	892.0133	341.36	210.68	340.582	38.268
Seed yield(g)	227.2063	28.119	95.224	270.681	12.375
Grain yield (t/ha)	0.00074866	0.00010266	2.4110	6.5109	13.71
Weight of 1000 seed	4.294	3.892	115.419	121.233	90.63

Table 5. Estimate of phenotypic correlation (above the diagonal) and phenotypic correlation (bellow the diagonal) of seventeen sesame genotypes

Traits	Flowering	Plant height	Height. F.capsules	Stem diameter	No. branches	No. capsules	Seed yield(g)	Grain yield (t/ha)	Weight of 1000 seed
Flowering	-	0.1636^{Ns}	0.3919**	0.1700^{Ns}	0.4456**	-0.0056^{Ns}	-0.2667*	-0.2693*	-0.1103**
Plant height	0.1636^{Ns}	-	0.7661**	0.5084**	0.5095**	0.4978**	0.1800^{Ns}	0.1797^{Ns}	-0.2255^{NS}
Height. F.capsules	0.3919**	0.7661**	-	0.3947**	0.4824**	0.2052^{Ns}	-0.1356^{Ns}	-0.1365^{Ns}	-0.3800**
Stem diameter	0.1700^{Ns}	0.5084**	0.3947**	-	0.5399**	0.5791**	0.3256*	0.3246*	-0.3892^{Ns}
No. branches	0.4456**	0.5095**	0.4824**	0.5399**	-	0.5297**	0.0594^{Ns}	0.0635^{Ns}	-0.2791*
No. capsules	-0.0056^{Ns}	0.4978**	0.2052^{Ns}	0.5791**	0.5297**	-	0.5134**	0.5160**	0.1003^{Ns}
Seed yield(g)	-0.2667*	0.1800^{Ns}	-0.1356^{Ns}	0.3256*	0.0594^{Ns}	0.5134**	-	0.9991**	0.2569*
Grain yield (t/ha)	-0.2693*	0.1797^{Ns}	-0.1365^{Ns}	0.3246*	0.0635^{Ns}	0.5160**	0.9991**	-	0.2695**
Weight of 1000 seed	-0.1103**	-0.2255^{Ns}	-0.3800**	-0.3892^{Ns}	-0.2791*	0.1003^{Ns}	0.2569*	0.2695**	-

Table 6. Matrix of RAPD dissimilarity among eighteen *sesame* genotypes based on coefficient was used to construct a dendrogram by unweighted pair group method with arithmetic average (UPGMA) according to Rohlf (1993)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
S1	1.00																	
S2	0.92	1.00																
S3	0.50	0.52	1.00															
S4	0.73	0.79	0.59	1.00														
S5	0.67	0.71	0.55	0.93	1.00													
S6	0.71	0.77	0.50	0.86	0.92	1.00												
S7	0.67	0.71	0.55	0.93	1.00	0.92	1.00											
S8	0.50	0.53	0.56	0.60	0.63	0.67	0.63	1.00										
S9	0.43	0.45	0.68	0.52	0.55	0.57	0.55	0.56	1.00									
S10	0.14	0.14	0.26	0.17	0.18	0.19	0.18	0.24	0.21	1.00								
S11	0.44	0.46	0.61	0.52	0.48	0.44	0.48	0.40	0.45	0.42	1.00							
S12	0.23	0.24	0.46	0.26	0.27	0.28	0.27	0.31	0.41	0.43	0.57	1.00						
S13	0.33	0.35	0.41	0.42	0.38	0.39	0.38	0.36	0.41	0.57	0.63	0.48	1.00					

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
S14	0.04	0.05	0.14	0.04	0.00	0.00	0.00	0.07	0.10	0.39	0.33	0.28	0.28	1.00				
S15	0.31	0.32	0.54	0.38	0.35	0.36	0.35	0.38	0.54	0.40	0.64	0.62	0.68	0.26	1.00			
S16	0.30	0.31	0.52	0.37	0.33	0.35	0.33	0.37	0.52	0.38	0.62	0.65	0.72	0.25	0.80	1.00		
S17	0.50	0.53	0.43	0.63	0.56	0.60	0.56	0.43	0.50	0.25	0.38	0.33	0.52	0.09	0.55	0.52	1.00	
S18	0.11	0.12	0.26	0.10	0.11	0.11	0.11	0.13	0.26	0.17	0.23	0.33	0.17	0.18	0.30	0.29	0.18	1.00

OPR10: CCATTCCCCA
OPL18: ACCACCCACC
Primers= OPL18; OPR10
Jaccard's similarity dendrogram:

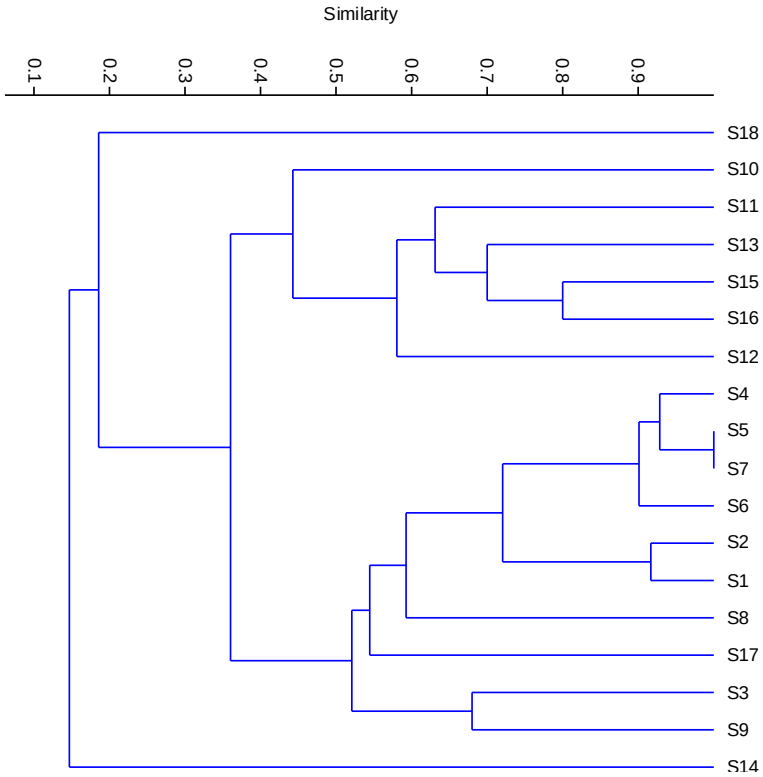

Fig. 1. Dendrogram constructed for eighteen sesame genotypes based on genetic distances using three RAPD Primers

studied, ranging from 12.38% for seed yield/plant (g) to 90.63% for thousand weight (Table 2). A significant correlation coefficient was observed between each seed weight, capsules/plant, and stem diameter with seed yield (ton/ha) (Table 5). There was a significant and positive correlation between the height of the first capsule, stem diameter, number of branches, and number of capsules with plant height (Table 5). These results are in agreement with the findings of [17-20].

3.2 Molecular Genetic Diversity of Sesame

Two RAPD primers were used for the analysis of the seventeen sesame genotypes studied. These primers were used to generate 22 polymorphic bands out of a total of 22 amplified bands, 22 being polymorphic. The RAPD primer OPL18 had the highest percentage of polymorphic bands at 100% (Table 6). This was also confirmed by previous RAPD marker results from other sesame studies using the OPM-06 primers (100% polymorphism). The high level of polymorphism observed in our study (75%) is consistent with the 78% polymorphism found in the genetic diversity assessment of Turkish sesame [21-22]. also observed a polymorphism of 86.75% in a study on the genetic diversity of Indian and exotic sesame germplasm. The similarity coefficient of seventeen sesame genotypes based on the RAPD marker ranges from 0.00 to 100 (Table 6). Among the genotypes, Soliamani and Alsodana showed the highest similarity index (100%), while genotype 17 with Solianani, Omshagara, and Alsodana had the lowest index (Table 6). Cluster analysis was carried out using an RAPD marker, which produced a dendrogram that divided the genotypes into two distinct clusters. The first cluster was further subdivided into subgroups. The first cluster contained eight genotypes as sisters, namely the genotypes (Kenana and Tagrib) and the genotypes (Soliamani and Alsodana) as second sisters. Also, the genotypes (Bash and Highland) as third sisters and (Caraway and Alradom) (Fig. 1). The second cluster contained only one genotype (17). “The results of this study are in agreement with previous studies based on morphological, agronomic traits, and molecular markers which showed high genetic diversity in sesame germplasm” [23,22,21,24,25,26].

4. CONCLUSION

It concluded that high genotypic variability was detected among the seventeen sesame genotypes from different geographical regions and localities which would be useful for plant breeding and conservation. The agro morphological and molecular markers complemented each other in assessing the extent of genetic variation in sesame and the combined application of these methods helps to better understand the genetic diversity and relationships within and among sesame genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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