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Assessment of Genetic Diversity in Ethiopian Sesame (Sesamum indicum L.) Germplasm using Random Amplified Polymorphic DNA (RAPD) Markers

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ABSTRACT

Genetic diversity among 128 sesame (Sesamum indicum L.) genotypes representing 10 geographically distinct populations in Ethiopia was assessed at DNA level using RAPD analysis. Eleven RAPD primers used amplified a total of 149 bands, of which 142 (95. 45%) were polymorphic. Each primer generated 7 to 23 amplified fragments with an average of 13.5 bands per primer. Percent of polymorphic loci (P%), number of different (Na) and effective (Ne) alleles along with Shannon information index (I) and Nei's gene diversity (He) values suggested that the population of Oromia was the most diverse of all populations, while populations from Afar (cultivars) and AM-NSh were found to be the least diverse. Based on average dissimilarity values obtained with RAPD primers, AM-NG-25, SNNP-7 and SNNP-8 were the most distinct of all genotypes, while genotypes ORO-20 and TIGR-5 showed maximum similarity with others. The UPGMA clustering based on the dissimilarity matrix clustered the genotypes into 3 major groups and 11 subgroups, while three genotypes viz., BENSH-6, ORO-14 and SNNP-5 were found out-grouped from the rest and did not join any of the cluster; they are then most divergent genotypes. Generally, both clustering and PCoA patterns revealed that most genotypes located geographically far apart were found to cluster in the same group, while those genotypes from the same origin dispersed. Overall results indicated that RAPD technique revealed a high level of genetic variation among sesame genotypes collected from diverse ecologies of Ethiopia.

Keywords:

Analysis of molecular variance; genetic distance; principal coordinate analysis; percent polymorphism; Shannon's index.

Abbreviations:

AMOVA = Analysis of Molecular Variance, NJ = Neighbor Joining, PCoA = Principal Coordinate Analysis, UPGMA= Unweighted Pair- group Method with Arithmetic mean.

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1. INTRODUCTION

Sesame (*Sesamum indicum* L.), is one of the most important oil crops in warm temperate to tropical regions. It is widely grown in the central and Northern part of Ethiopia. According to some archaeological findings [1, 2], sesame is thought to have originated in Africa, and there is a great weight of evidence indicating that Ethiopian lowland area is the origin of cultivated sesame [3, 4, 5, 6]. Bedigian [7] also argued that, owing to the wide genetic diversity in Africa, it is reasonable to assume that this subcontinent is the primary center of origin and India would then be thought as a secondary center of origin.

Despite the high nutritional value, health benefits and economic importance in many countries, sesame is largely ignored by plant breeders with a few exceptions [8]. The main challenges associated with sesame cultivation are drought and heat stress at certain periods of the growing season [9, 10, 11, 12] as well as pathogen attacks [13]. Thus, building on genetic diversity to find suitable varieties and breeding material for stressed conditions is of top priority to continue sesame cultivation in traditional areas. Sesame diversity has been investigated using phenotypic markers such as morphological, physiological and phenological traits [14, 15, 16, 17and 18]. However, because of environmental influences and complex genetic structure of many mopho-physiological traits, diversity analyses based only on morphological characters are prone to environmental bias. Therefore, molecular markers have proved to be valuable tools in the characterization and evaluation of genetic diversity between species and among populations. In recent years, several studies have investigated the genetic diversity of sesame using DNA-based markers, including world-based collections [19, 20, 21, 22, 23 and 24].

Despite its nutritional and economic importance, genetic diversity study of sesame germplasm in Ethiopia has been very limited, except with a few attempts on limited accessions [25]. Yet no single work is found particularly based on RAPD markers to evaluate the diversity of sesame populations in Ethiopian. Hence, the lack of sufficient information on the level of genetic variation among the existing local collections has limited to use the germplasm in sesame breeding program.

The use of RAPD marker represents an alternative method in detection of polymorphism in sesame [26]. Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms. Therefore, the present study was designed to investigate the genetic diversity among Ethiopian sesame germplasm using RAPD markers in order to identify highly diverse genotypes for the purposes of broadening the genetic bases of sesame landraces grown in Ethiopia.

2.0 MATERIALS AND METHODS

2.1 Plant material

A total of 128 genotypes of sesame representing different geographical locations in Ethiopia were collected from the Ethiopian Biodiversity Institute (EBI). These materials comprised 119 landraces and nine commercial varieties, which were grouped in this study under ten populations, based on the collection regions/zones (Table 1).

2.2 DNA extraction

Ten seeds from each genotype were planted in plastic pots and maintained in a greenhouse (30ºC/23ºC) day/night. Genomic DNA was isolated from leaves of two weeks-old seedlings following the protocol described by Doyle and Doyle [27] with minor modifications, which included the use of 200 mg per sample polyvinyl pyrollidone (PvP). The quality of DNA after RNase treatment was assessed using 0.8% agarose gel and its concentration was quantified using Nano-Drop spectrophotometer, where OD260nm/OD280nm ratios were above 1.8. Finally DNA samples were diluted in T.E buffer and stored at -20°C for use in PCR amplification.

2.3 Primer selection and optimization

A total of twenty three decamer oligonucleotide primers (Operon Technology) were used for PCR amplification. The 23 primers were initially tested using DNA samples of 10 plants (one from each geographic population). 11 most informative primers (Table 2) that amplified scorable and reproducible bands with the test templates were selected to amplify the DNA of each sesame genotype.

Table 1. List of sesame (*Sesamum indicum*) genotypes grouped in populations based on the regions of collection/ origin

No	Genotype		Origin	No	Genotype		Origin	No	Genotype		Origin
1	AFAR-1	Pop 1	Afar	44	AM-NG-21	3 cont	"	87	ORO-9		"
2	AFAR-2		"	45	AM-NG-22		"	88	ORO-10	8 contn	"
3	AFAR-3		"	46	AM-NG-23		"	89	ORO-11		"
4	AFAR-4		"	47	AM-NG-24		"	90	ORO-12		"
5	AFAR-5		"	48	AM-NG-25		"	91	ORO-13		"
6	AFAR-6		"	49	AM-NG-26		"	92	ORO-14		"
7	AFAR-7		"	50	AM-NG-27		"	93	ORO-15		"
8	AFAR-8		"	51	AM-NSh-1		Amhara North Shoa	94	ORO-16		"
9	AFAR-9		"	52	AM-NSh-2	Pop 4	"	95	ORO-17		"
10	AM-NW-1		Amhara North Wollo	53	AM-NSh-3		"	96	ORO-18		"
11	AM-NW-2		"	54	AM-NSh-4		"	97	ORO-19		"
12	AM-NW-3	Pop 2	"	55	AM-NSh-5		"	98	ORO-20		"
13	AM-NW-4		"	56	AM-NSh-6		"	99	SNNP-1		SNNP
14	AM-NW-5		"	57	AM-SW-1		Amhara South Wollo	100	SNNP-2		"
15	AM-NW-6		"	58	AM-SW-2	Pop 5	"	101	SNNP-3	Pop 9	"
16	AM-NW-7		"	59	AM-SW-3		"	102	SNNP-4		"
17	AM-NW-8		"	60	AM-SW-4		"	103	SNNP-5		"
18	AM-NW-9		"	61	AM-SW-5		"	104	SNNP-6		"
19	AM-NW-10		"	62	AM-SW-6		"	105	SNNP-7		"
20	AM-NW-11		"	63	AM-SW-7		"	106	SNNP-8		"
21	AM-NW-12		"	64	AM-SW-8		"	107	TIGR-1		Tigray
22	AM-NW-13		"	65	AM-SW-9		"	108	TIGR-2	Pop 10	"
23	AM-NW-14		"	66	AM-SW-10		"	109	TIGR-3		"
24	AM-NG-1		Amhara North Gonder	67	AM-SW-11		"	110	TIGR-4		"
25	AM-NG-2	Pop 3	"	68	BENSH-1		Benishangul	111	TIGR-5		"
26	AM-NG-3		"	69	BENSH-2	Pop 6	"	112	TIGR-6		"
27	AM-NG-4		"	70	BENSH-3		"	113	TIGR-7		"
28	AM-NG-5		"	71	BENSH-4		"	114	TIGR-8		"
29	AM-NG-6		"	72	BENSH-5		"	115	TIGR-9		"
30	AM-NG-7		"	73	BENSH-6		"	116	TIGR-10		"
31	AM-NG-8		"	74	BENSH-7		"	117	TIGR-11		"
32	AM-NG-9		"	75	GAMB-1	Pop 7	Gambella	118	TIGR-12		"
33	AM-NG-10		"	76	GAMB-2		"	119	TIGR-13		"
34	AM-NG-11		"	77	GAMB-3		"	120	TIGR-14		"
35	AM-NG-12		"	78	GAMB-4		"	121	TIGR-15		"
36	AM-NG-13		"	79	ORO-1		Oromia	122	TIGR-16		"
37	AM-NG-14		"	80	ORO-2	Pop 8	"	123	TIGR-17		"
38	AM-NG-15		"	81	ORO-3		"	124	TIGR-18		"
39	AM-NG-16		"	82	ORO-4		"	125	TIGR-19		"
40	AM-NG-17		"	83	ORO-5		"	126	TIGR-20		"
41	AM-NG-18		"	84	ORO-6		"	127	TIGR-21		"
42	AM-NG-19		"	85	ORO-7		"	128	TIGR-22		"
43	AM-NG-20		"	86	ORO-8		"				

Key: Benishangul = Benishangul Gumuz, SNNP = Southern Nation and Nationality People.

2.4 PCR amplification and electrophoresis

The PCR conditions for RAPDs were optimized as 5 min at 94°C for initial denaturation, followed by 35 cycles for 1 min at 94°C, 1 min at 37°C for annealing, 2 min at 72°C for extension step and further incubated for 5 min at 72°C for final extension, before cooling to 4°C. Amplification was carried out in 25 µL volumes containing 2.0 µL of dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP), 0.2 µL of *Taq* DNA polymerase (5 U/µL), 2.0 µL DNA template (20 ng/µL), 2 µL of primer, 2.5 µL of 10× PCR buffer, 1.5 µL of $MgCl_2$ (25 mM) and 14.8 µL of sterilized distilled water. The reaction products were fractionated by electrophoresis in 1.5% agarose gel and run in 1×TAE buffer for 3 h at 100 v. The gels were stained

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with ethidium bromide (0.5 µg/ µL) for 10 min followed by de-staining in tap water for 30 min and UV light photographs of the gels with DNA bands were taken using Bio Doc-ItTM gel documentation system (UVP, Cambridge, UK). A 50bp DNA ladder was used as molecular weight standard. Each PCR reaction was performed at least twice and only reproducible bands were scored.

2.5 Scoring and data analysis

The amplified bands in each of the 128 genotypes were scored manually for the presence (1) or absence (0) for each primer combination. Each band was considered to be a locus with the dominant allele present. A binary data matrix was prepared for each primer separately and merged as combined data for overall analysis. For each primer, the number of total bands (TB), polymorphic bands (PB) and polymorphism percentage (P %) were calculated (Table 2).

GENAlex software, version 6.5 [28, 29] was used to analyze the number of different alleles (Na), effective number of alleles (Ne), Nei's expected gene diversity (He), Shannon's information index (I), number of polymorphic band (PB) and percentage polymorphic band (% P) across all the ten populations. The RAPD diploid data for each population were analyzed to calculate Nei's [30] unbiased genetic distance between populations of different regions. GENAlex software was also used for analysis of molecular variance (AMOVA) to assess the inter and intra-population genetic variations [31], and principal coordinate analysis (PCoA) that plots the relationship between distance matrix elements based on the first two principal coordinates. The F value (Fst) among the different populations was estimated using Power-Marker, version 3.2 [32]. The RAPD binary data was further employed to generate genetic dissimilarity matrix for individual sesame genotypes using the Dices coefficient with the help of DARwin, version 5.0 [33]. This was further used to develop a UPGMA tree based on the Neighbor Joining coefficient.

3.0 RESULTS AND DISCUSSION

3.1 Banding pattern and polymorphism of RAPD primers

The genetic diversity of 128 genotypes representing 10 geographically distinct populations was assessed using 11 polymorphic RAPD primers. A total of 149 bands were generated with an average of 13.5 bands per primer, of which 142 (95.48%) were polymorphic (Table 2). The number of bands obtained by individual primers was 7 - 23, the lowest being for primer no. 82 and the highest for primer no. 35. A closer level of polymorphism was observed in the RAPD primers used for the study, where maximum polymorphism (100%) was shown by primer no. 61 and no. 77 and minimum polymorphism (84%) was detected for primer no. 82 (Table 2). The size of the amplified products ranged from 200-2780 bp. The pattern obtained using RAPD-5 and RAPD-6 was depicted in Figure 1.

The level of polymorphism (95.48%), amplified by RAPD primers in the present study was high compared to 84.4% of Adbellatef *et al*. [34] in Sudanese sesame; 78% of Ecran *et al*. [19] in Turkish sesame; 75% of Akbar *et al*. [35] in Pakistani sesame and 66.1% of Tabatabaei *et al*. [36] in Iranian sesame, using 10, 7, 10 and 15 RAPD primers respectively. The higher polymorphism detected in this study may be due to large number of germplasm from different geographic regions and zones of Ethiopia, whereby increasing the chance of including diverse genotypes of sesame. However, our result was comparable with other previous studies [37, 38, 39] who have found highest polymorphisms using RAPD markers.

Table 2. Selected RAPD primes, sequences, total bands, polymorphic bands and other calculated parameters observed in 128 sesame genotypes

Primer No.	Primer Code	Sequence (5'- 3')	AT (^{0}C)	TB	PB	%P
12	RAPD-2	CCTGGGTCCA	34	18	17.0	96.1%
16	RAPD-3	GGTGGCGGGA	34	10	9.8	97.7%
31	RAPD-4	CCGGCCTTCC	34	8	7.0	91.4%
35	RAPD-5	CCGGGGTTAA	32	23	23.0	99.2%
61	RAPD-6	TTCCCCGACC	34	21	21.0	100.0%
77	RAPD-7	GAGCACCAGG	34	17	17.0	100.0%
82	RAPD-8	GGGCCCGAGG	36	7	6.0	84.4%
92	RAPD-9	CCTGGGCTTT	32	14	13.8	98.4%
98	RAPD-12	ATCCTGCCAG	32	10	9.6	96.1%
101	RAPD-13	GCGGCTGGAG	34	12	11.0	93.0%
104	RAPD-14	GGGCAATGAT	32	9	8.0	93.8%
Total	**Mean**			**13.5**	**13.1**	**95.48%**

Note: AT= annealing temp., TB= total bands, PB= polymorphic bands, %P= percent polymorphism

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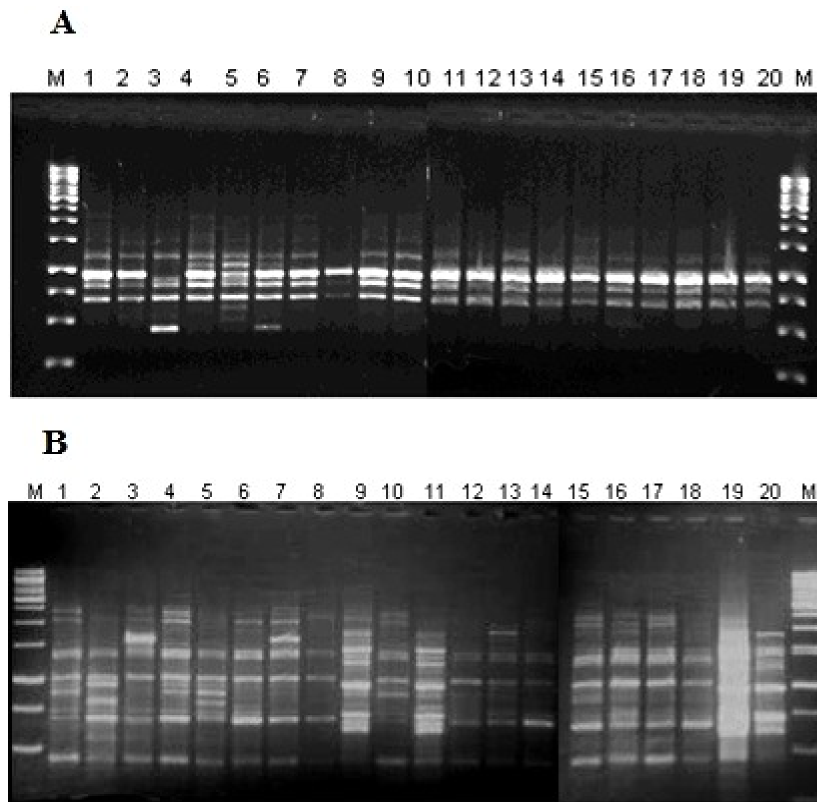

Figure 1. RAPD pattern of 20 sesame genotypes amplified by A= RAPD-5, B= RAPD-6.

3.2 Genetic diversity within populations of sesame germplasm

The percent polymorphic loci observed for the ten populations ranged from 79.2% (AM-NSh) to 97.3% (AM-SW) with an average of 89.4% (Table 3). The population from AM-NSh showed the least number of polymorphic alleles for RAPD primers followed by the Afar population (released cultivars). The different (Na) and effective number (Ne) of alleles along with Shannon information index (I) and Nei's gene diversity (He) also followed the same trend that is all these indices were observed to be the lowest for genotypes from AM-NSh and Afar regions (Table 3). Average Shannon's index and Nei's gene diversity values estimated for all populations showed that the maximum values (I = 0.57, He = 0.39) were observed for genotypes of Oromia region, indicating that they are genetically more diverse than in other regions. These results were in agreement with the findings of Dagmawi [40] and Daniel and Heiko [25] in genetic diversity study of some Ethiopian sesame accessions. They showed Oromia (Welega) to be the most diverse of all populations. The least genetically diverse region was AM-NSh followed by Afar with mean Shannon index of (0.45, 0.46) and gene diversity of (0.30, 0.30) respectively. The average Shannon's index and gene diversity values for the total populations were 0.51 and 0.31 respectively. In contrast, Dagmawi [40] observed lower average Shannon's index (0.26) for Ethiopian sesame collection.

A high level of polymorphism (89.4%) observed in the present sets of sesame population was analogous to the 86.75% polymorphism noticed in Indian and exotic sesame collections [41] and the 84.4% polymorphism in Sudanese sesame collection [34]. Ercan *et al*. [19] also observed 78% polymorphism in evaluation of Turkish sesame. However, compared to other studies conducted with RAPD markers, the diversity in our study was higher than that found by Kumar and Sharma [23] in Indian genotypes, Pham *et al*. [24] in South-East Asian genotypes and by Tabatabaei *et al*. [36] in Iranian sesame accessions.

The genetic dissimilarity values obtained with RAPD have been introduced for measuring genetic relationships in the present sesame genotypes. A wide range (0.004 - 0.98) of dissimilarity values with an average of (0.38) were observed among the genotypes (data not shown). The highest dissimilarity value was recorded between the genotypes AFAR-9 and BENSH-7 followed by AFAR-2 and TIGR-15 (0.94), whereas the minimum dissimilarity was observed between AM-NG-11 and TIGR-5 (0.004) followed by AM-NG-14 and TIGR-12 (0.008). AM-NG-25, SNNP-7 and SNNP-8 had the highest average dissimilarity value of (0.69 each). Hence, they are the most distinct of all genotypes. Pair-wise (Nei's) unbiased genetic distances between populations ranged in the interval 0.00 - 0.20 (Table 4). In general, maximum distance was observed between the population of released cultivars and the landraces, of which, the highest distances were found between Afar and AM-NSh (0.20) followed by Afar and AM-NW (0.19), whereas the lowest distances were observed between Gambella and AM-NG, and between Gambella and Benishangul (Table 4). However, the distances among the populations of the landraces were generally very low, indicating the existence of genetic relationship among the accessions of the different geographic populations. This result supports the results obtained in the AMOVA, PCoA and cluster analyses. The low level of variation among the geographic populations is indicative of the high rates of gene flow between regions due to exchange of sesame germplasm by both human migration and agricultural trade [42].

Table 3. Genetic diversity in sesame populations as detected by 11 RAPD primers

No	Population		N	Na	Ne	I	He	P%
1	AFAR		9.0	1.70	1.54	0.46	0.31	81.2%
2	AM-NW		14.0	1.90	1.65	0.54	0.37	91.3%
3	AM-NG		27.0	1.96	1.65	0.55	0.38	96.0%
4	AM-NSh		6.0	1.68	1.55	0.45	0.31	79.2%
5	AM-SW		11.0	1.97	1.64	0.54	0.36	97.3%
6	BENSH		7.0	1.79	1.56	0.47	0.32	81.2%
7	GAMB		4.0	1.83	1.52	0.46	0.30	87.2%
8	ORO		20.0	1.95	1.70	0.57	0.39	95.3%
9	SNNP		8.0	1.86	1.67	0.53	0.37	91.3%
10	TIGR		22.0	1.94	1.69	0.55	0.38	94.0%
	Total	**Mean**	**12.8**	**1.86**	**1.62**	**0.51**	**0.35**	**89.40%**
		SE	**0.19**	**0.01**	**0.01**	**0.01**	**0.00**	**2.14%**

Note: N= number of samples, Na= number of different alleles, Ne= effective number of alleles, He= Nei's expected gene diversity, I= Shannon's information index, P%= percent of polymorphic loci, SE= standard error. For list of populations see Table 1.

Table 4. Pairwise population matrix of Nei unbiased genetic distance below diagonal and genetic identity above diagonal

Population	AFAR	AM-NW	AM-NG	AM-NSh	AM-SW	BENSH	GAMB	ORO	SNNP	TIGR
AFAR	-	0.824	0.902	0.819	0.891	0.897	0.899	0.889	0.899	0.901
AM-NW	0.194	-	0.916	0.909	0.952	0.908	0.895	0.933	0.926	0.916
AM-NG	0.104	0.088	-	0.879	0.971	0.997	1.000	0.951	0.959	0.977
AM-NSh	0.200	0.095	0.129	-	0.947	0.900	0.861	0.938	0.895	0.925
AM-SW	0.115	0.049	0.029	0.055	-	0.991	0.972	0.991	0.976	0.967
BENSH	0.108	0.096	0.003	0.106	0.009	-	0.995	0.960	0.968	0.974
GAMB	0.107	0.110	0.000	0.149	0.028	0.002	-	0.944	0.963	0.979
ORO	0.118	0.069	0.050	0.064	0.009	0.041	0.058	-	0.952	0.962
SNNP	0.106	0.077	0.042	0.111	0.024	0.033	0.038	0.050	-	0.961
TIGR	0.105	0.087	0.024	0.078	0.034	0.026	0.021	0.039	0.040	-

Note: for abbreviations in the parenthesis, see (Table 1) under 'materials and methods'.

3.3 Analysis of molecular variance (AMOVA)

Genetic variations among the various populations and within populations were assessed using analysis of molecular variance (AMOVA) and were found to be statistically significant ($p < 0.01$). The variation was lowest among the 10 geographic populations and was greatest within the populations, as indicated by the sum of square values calculated in AMOVA (Table 5). Of the total genetic diversity, 6% was attributable to differences among populations and 94% was attributable to within population differences. The average Fst value was 0.058, indicating a lower differentiation among populations (Table 5). Similar results were noted in our investigation of the same samples using ISSR markers. It was also in agreement with the previous reports of Dagmawi [40] in AMOVA analysis of some Ethiopian sesame accessions, and Laurentin and Karlovsky [43] in world sesame collections. The high within population variation in this study could mainly be due to the high out-crossing nature of sesame. Although sesame is mainly self-pollinated, some authors have reported levels of out crossing in it between 5-60% [44, 45, and 46]. Out-crossing plant species tend to present between 10 to 20% of the genetic variation between populations (Hamrick and Godt 1989) and the remaining 80-90% of the total genetic variation is within populations. Hence, some degree of out-crossing could explain the high genetic diversity observed in the present sesame genotypes. On the other hand, one possible reason for the lower differentiation among the

populations could be exchange of sesame germplasm via farmers and sesame trades across regions of Ethiopia due to the increasing demand for sesame export in the country.

Table 5. Analysis of molecular variance (AMOVA) among and within the populations of sesame genotypes

Source	DF	SS	MS	Est. Var.	%	Fst	Prob
Among Pops	9	453.047	50.339	1.755	6%	0.058	0.01
Within Pops	118	3385.531	28.691	28.691	94%		
Total	127	3838.578	79.03	30.446	100%		

Note: DF= degree of freedom, SS= sum of square, MS= mean square, Est. Var.= estimated variance, Fst= fixation index.

3.4 Clustering analysis and relationships among sesame genotypes

The result of cluster analysis revealed that the 128 sesame genotypes clustered into three main groups as shown in the Neighbor Joining (NJ) tree diagram (Figure 2), where the first two groups (I and II) were further sub-divided into 6 and 5 sub-branches respectively. Group-I had the largest number of genotypes (61): five genotypes from Afar, four genotypes from AM-NW, eleven genotypes from AM-NG, four genotypes from AM-NSh, four genotypes from AM-SW, three genotypes from Benishangul, eleven genotypes from Oromia, three genotypes from SNNP and sixteen genotypes from Tigray, with genotypes No. 12 and 31 appearing separately at a sister group position. Group-II contained a total of 57 genotypes: four genotypes from Afar, four genotypes from AM-NW, 16 genotypes from AM-NG, one genotype from AM-NSh, six genotypes from AM-SW, three genotypes from Benshangul, four genotypes from Gambella, seven genotypes from Oromia, two genotypes from SNNP and six genotypes from Tigray with genotypes No. 19, 56, 64 and 86 appearing separately at a sister group position. Group-III contained only seven genotypes from two regions: five genotypes from AM-NW and two genotypes from SNNP. The remaining three genotypes *viz*., BENSH-6, ORO-14 and SNNP-5 were out-grouped from the rest and stand alone (Figure 2). This is an indication of the impact of selection pressure in increasing the genetic diversity.

In the NJ cluster analysis, the grouping pattern demonstrated that most clustered genotypes were not geographically the nearest; genotypes from Tigray and Amhara, for example, were clustered in the same group with genotypes from Benishangul, Gambella and SNNP (Gr. I, II), though these regions are located geographically far apart each other and the genotypes have distinct morphologies. Furthermore, the cultivars (genotypes from Afar) were found to group with the landraces of different regions except with Gambella (Figure 2). These results are in agreement with earlier findings that showed geographical separation did not generally result in greater genetic distance [47, 48, 49 and 50]. The human factor can be responsible for the lack of correlation between genetic and geographical distance in some cases [51]. Similar results have been reported in the evaluation of sesame germplasm collection from Ethiopia by Admas [52] and Dagmawi [40].

The exchange of seeds between farmers of the neighboring regions and sesame trades across regions of the country could be a possible explanation for the spreading of sesame germplasm.

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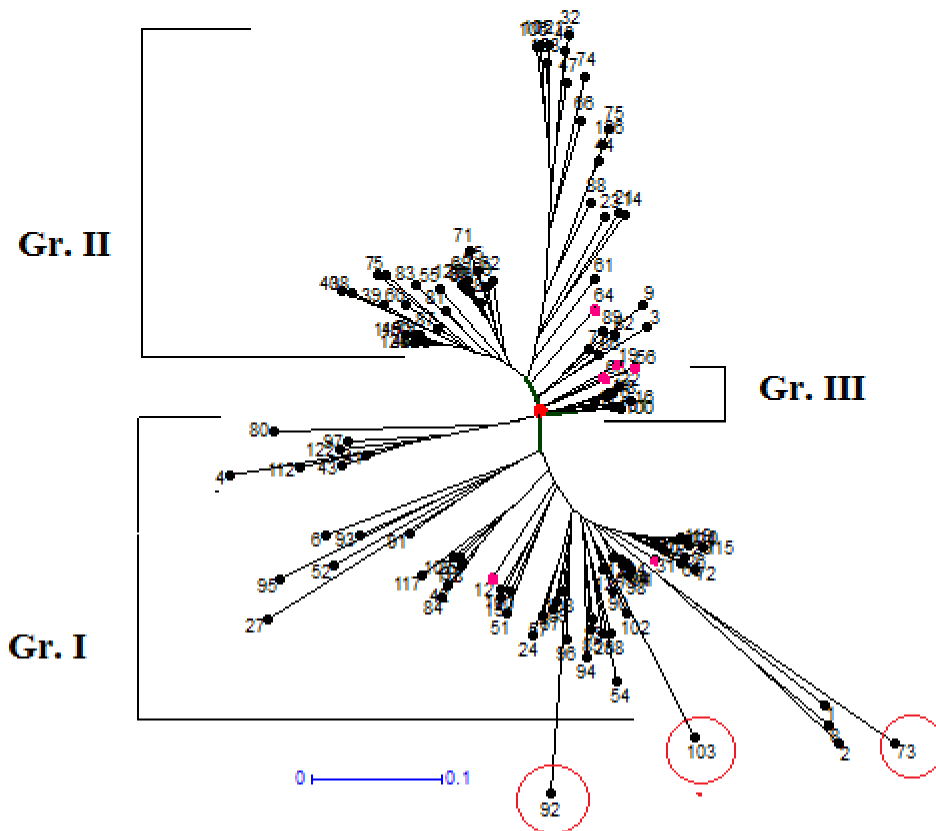

Geographic location

No.	Region	No.	Region
1	Afar	65	Amr-SW
2	Afar	66	Amr-SW
3	Afar	67	Amr-SW
4	Afar	68	Ben shang
5	Afar	69	Ben shang
6	Afar	70	Ben shang
7	Afar	71	Ben shang
8	Afar	72	Ben shang
9	Afar	73	Ben shang
10	Amr-NW	74	Ben shang
11	Amr-NW	75	Gambella
12	Amr-NW	76	Gambella
13	Amr-NW	77	Gambella
14	Amr-NW	78	Gambella
15	Amr-NW	79	Oromiya
16	Amr-NW	80	Oromiya
17	Amr-NW	81	Oromiya
18	Amr-NW	82	Oromiya
19	Amr-NW	83	Oromiya
20	Amr-NW	84	Oromiya
21	Amr-NW	85	Oromiya
22	Amr-NW	86	Oromiya
23	Amr-NW	87	Oromiya
24	Amr-NG	88	Oromiya
25	Amr-NG	89	Oromiya
26	Amr-NG	90	Oromiya
27	Amr-NG	91	Oromiya
28	Amr-NG	92	Oromiya
29	Amr-NG	93	Oromiya
30	Amr-NG	94	Oromiya
31	Amr-NG	95	Oromiya
32	Amr-NG	96	Oromiya
33	Amr-NG	97	Oromiya
34	Amr-NG	98	Oromiya
35	Amr-NG	99	SNNP
36	Amr-NG	100	SNNP
37	Amr-NG	101	SNNP
38	Amr-NG	102	SNNP
39	Amr-NG	103	SNNP
40	Amr-NG	104	SNNP
41	Amr-NG	105	SNNP
42	Amr-NG	106	SNNP
43	Amr-NG	107	Tigray
44	Amr-NG	108	Tigray
45	Amr-NG	109	Tigray
46	Amr-NG	110	Tigray
47	Amr-NG	111	Tigray
48	Amr-NG	112	Tigray
49	Amr-NG	113	Tigray
50	Amr-NG	114	Tigray
51	Amr-NSh	115	Tigray
52	Amr-NSh	116	Tigray
53	Amr-NSh	117	Tigray
54	Amr-NSh	118	Tigray
55	Amr-NSh	119	Tigray
56	Amr-NSh	120	Tigray
57	Amr-SW	121	Tigray
58	Amr-SW	122	Tigray
59	Amr-SW	123	Tigray
60	Amr-SW	124	Tigray
61	Amr-SW	125	Tigray
62	Amr-SW	126	Tigray
63	Amr-SW	127	Tigray
64	Amr-SW	128	Tigray

Figure 2. A Neighbor Joining tree illustrating relationships among 128 genotypes based on genetic distances using 11 RAPD markers

3.5 Principal coordinate analysis (PCoA)

PCoA was performed to provide spatial representation of the relative genetic distances among individual and to determine the consistency of differentiation among populations defined by the cluster analysis. The principal coordinate analysis (PCoA) of the 11 RAPD primers (Figure 3) revealed that the first two principal coordinates, PCA1 and PCA2, accounted for 24.6% and 13.85% of the variation respectively. However, the PCoA plot did not show a similar clustering pattern with the NJ cluster diagram, where the different groups were not clearly separated along the two axes. With the exception of few genotypes representing Amr-NW and Gambella, most of the genotypes were clustered in different groups regardless of their geographic origins and spread along over the two axes. Generally the PCoA result indicated that most of the genotypes did not group together with other genotypes from the same geographical region.

The present study generally suggested the presence of high levels of genetic diversity among the germplasm lines. Although sesame is generally a self-pollinated crop, cross-pollination (5 to 60%) has been reported in it [45]. About 10 to 20% of the genetic diversity among populations is due to cross-pollinations [53]. Hence, some cross-pollination could clarify the high level of genetic diversity examined within the same population. Our results were in agreement to other previous studies who have reported high level of genetic diversity in sesame on the use of closer number of RAPD primers [19, 37, 54, 55 and 56].

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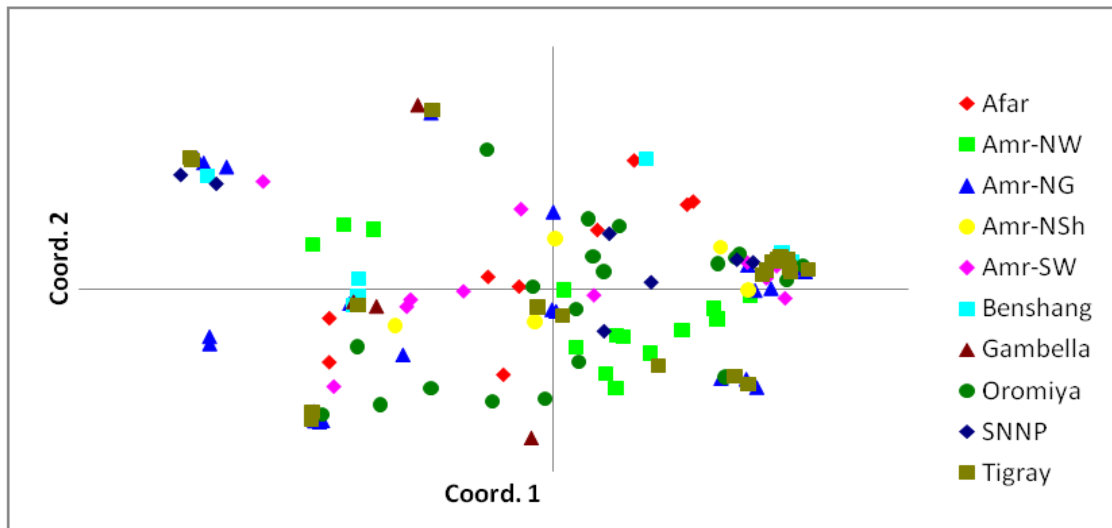

Figure.3. Principal coordinate analysis (PCoA) showing relationship among 10 populations of sesame.

4.0 CONCLUSION

In conclusion, a high level of polymorphism was detected among sesame genotypes from various geographical regions of Ethiopia. This was also supported by earlier RAPD marker results in sesame diversity studies from other countries by applying the OPM primers (100% polymorphism). Furthermore, PCoA and cluster analysis of the present study revealed that most genotypes situated geographically far apart were grouped together in the same clusters, which is an indication of the absence of correlation between geographical separation and genetic distances. This could mainly be a consequence of exchange of sesame seeds among the neighboring farmers as well as the movement of traders across regions in Ethiopia. Genotypes *viz*., BENSH-6, ORO-14 and SNNP-5 exhibited a good amount of genetic divergence and hence they can be used for crossing program in genetic improvement of sesame in Ethiopia

The 11 RAPD primers noticed sufficient genetic diversity among the 128 sesame genotypes to allow for full separation. This signifies that RAPD analysis can reveal high levels of polymorphism in sesame genotypes, even with the use of limited set of primers. Thus, RAPD techniques can be useful for sesame systematic in the maintenance of germplasm banks and the efficient selection of parents in breeding program.

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