British Biotechnology Journal
8(4): 1-13, 2015, Article no.BBJ.18481
ISSN: 2231–2927

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Assessment of Genetic Diversity in Ethiopian Sesame (*Sesamum indicum* L.) Germplasm Using ISSR Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author MA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FM and Author AA managed the analyses of the study. Author MN managed the literature searches. Author MA carried out edited corrections and all e-mail correspondences. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/18481
Editor(s):
(1) Mahalingam Govindaraj, ICRISAT, Patancheru, India.
Reviewers:
(1) Anonymous, Chapingo Autonomous University, Mexico.
(2) Anonymous, University of Wisconsin-Madison, USA.
(3) David Ojo, Research and Consult Dept, National Horticultural Research Institute, Ibadan, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/10333

Original Research Article

Received 24th April 2015
Accepted 8th June 2015
Published 26th July 2015

ABSTRACT

Aim: This study aimed to uncover the diversity and population structure of 128 sesame genotypes using ISSR markers and identify highly diverse genotypes for the purposes of broadening the genetic base of sesame landraces grown in Ethiopia.
Place and Duration of Study: The study was conducted in Botany research laboratory of Kasetsart University, Thailand, from April to July, 2013.
Methodology: Genomic DNA of 128 sesame genotypes were subjected to PCR amplification and electrophoresis using seven ISSR markers and a binary data matrix prepared for each primer by scoring clear bands. The data generated were used to calculate the number of total bands (TB),

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polymorphic bands (PB), polymorphism percentage (P %) and polymorphic information content (PIC) for each locus. The number of different (Na) and effective (Ne) alleles, polymorphic loci (%), Shannon's information index (I) and Nei's gene diversity (He) for each population were calculated using GenAlEx 6.5 software. The data were also subjected to analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) via distance matrix. Fixation index (Fst) was computed to measure genetic differentiation among populations. Genetic associations among individual genotypes were determined based on dissimilarity matrix using Darwin version 5.0 and a Neighbour-Joining hierarchal tree was constructed based on UPGMA.

Results: The 7 ISSR primers in 128 sesame genotypes yielded 96 reproducible amplified bands. The number of amplified bands varied from 7 to 19. Out of 96 bands, 89 (92.2%) were polymorphic. Average number of bands and polymorphic bands per primer were 14 and 12.6 respectively. The polymorphic information content (PIC) value ranged between 0.26 and 0.76, showing the high informativeness of the selected primers. The overall gene diversity and Shannon's information index were 0.37 and 0.54 respectively. Average dissimilarity value among the genotypes was 0.39. Maximum dissimilarity (0.88) was observed between genotypes Amr-NW6 and Amr-NG9 and less dissimilarity (0.014) was recorded between Amr-NW1 and Amr-NG1. SNNP-7 was the most diverse of all genotypes with highest average dissimilarity value of 0.77. AMOVA showed lower genetic divergence between populations (6%) than within population (94%) with average Fst of 0.061 across populations. The high intra-population variation could be because of large number of genotypes included and due to high out-crossing nature of sesame. Clustering and PCoA analyses clustered the genotypes into individual groups where most of the landraces were grouped in separate clusters irrespective of their geographic origins, while the cultivars were grouped in one cluster, suggesting less variability within the released varieties than the landraces. Accessions no. 56, 73, and 105 were out grouped from the rest.

Conclusion: There exist considerable variations among sesame genotypes collected from different geographical regions of Ethiopia. Genotypes Amr-NSh-6, Benishangul-6 and SNNP-7 exhibited a good amount of genetic divergence and hence can be used in crossing program for genetic improvement of sesame in Ethiopia.

Keywords: AMOVA; clustering; fixation index; genetic distance; PCoA; PIC.

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crops [1,2]. Sesame seeds are important source of oil (44 - 58%), protein (18 - 25%), and carbohydrate (14%) [3]. Ethiopia is considered as the basic diversity centre of cultivated sesame [4-8]. Among oilseeds, sesame, locally called *selit* is the leading export crop in Ethiopia [9]. It grows well in the lowlands either as sole crop or intercropped with millet or sorghum; most sesame growing areas of Ethiopia receive 300 to 700 mm of rain during the growing season [10].

Despite its nutritional value and economic importance, sesame yield in Ethiopia is very low. The national average in 2012/13 was 757 kg/ha [11] which is much lower compared to research seed yield of 1800 kg/ha [12]. Total area under sesame cultivation in Ethiopia has been grown faster from 23,000 ha in 1994/95 to 315,000 ha in 2008/09 [13], with concomitant low average seed yield per hectare. One reason for low seed yield and reduced acreage under cultivation is the persistent use of traditional practices and lack of improved varieties for use by the farmers [9,14]. This situation can be corrected by selecting varieties of good quality and high adaptive potential to the diverse climatic conditions. With an increasing demand for sesame over time, movement of sesame seeds through traders across regions has become apparent since the last decade.

Genetic diversity in sesame, based on morphological, biochemical and molecular markers, has been reported by many researchers worldwide [2,15-26]. However, there are very few published works on genetic diversity of the local sesame collections of Ethiopia based on molecular markers.

ISSR marker is more reproducible and cost effective for researchers in developing countries like Ethiopia. The technique does not need any prior information about DNA sequence and overcomes many of the technical limitations of RAPD and AFLP [27,28]. The ISSR techniques have been used in Ethiopia to detect genetic

diversity and population structure of Tef [29], Coffee [30,31], Lentils [32], Rice [33] and sesame [34,35].

Therefore, this study was aimed to determine the extent of genetic diversity in Ethiopian sesame germplasm using ISSR markers and identify highly diverse genotypes for the purposes of broadening the genetic base of sesame landraces grown in Ethiopia.

2. MATERIALS AND METHODS

2.1 Plant Material

A total of 128 sesame genotypes collected from 20 Administrative Regions and Zones, which represent different geographical regions in Ethiopia were obtained from Ethiopian Biodiversity Institute (EBI). The material comprised 119 landraces and nine commercial varieties, which were grouped in this study under ten populations (Table 1) based on the collection regions and/or zones.

2.2 DNA Extraction

Ten seeds from each genotype were planted in plastic pots and maintained in a greenhouse (30°C /23°C) day/night at Botany research laboratory of Kasetsart University, Thailand, in 2013.

Genomic DNA was isolated from leaves of two weeks-old seedlings following the protocol described by Doyle and Doyle [36]. The quality of DNA after RNase treatment was assessed using 0.8% agarose gel and finally its concentration was quantified using Nano Drop spectrophotometer (ND-1000, version 3.1.1 USA), where OD-260 nm/OD-280 nm ratios were above 1.8. DNA samples were diluted at a concentration of 25 ng/µL by adding TE buffer and stored at -20°C for final use in PCR amplification.

2.3 Primer Selection and Optimization

A total of 17 ISSR primers were selected from literature [17] and a primer kit was obtained from the University of British Colombia (primer kit UBC 900). A few representatives of each sesame population were initially screened with these primers and seven primers (Table 2) that produced clear DNA bands and consistent polymorphisms were selected to amplify the DNA of each genotype. A total of ten DNA samples were randomly selected from genotypes of each population to test the reproducibility of PCR banding patterns and optimize the reaction conditions.

2.4 PCR Amplification and Electrophoresis

PCR amplification was carried out as described by Uzun and Cagirgan [24] with minor modification in annealing temperature and reaction volume. The reaction was performed 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 PCR reaction was optimized as initial denaturation temperature of 94°C for 5 min followed by 35 cycles for 1 min at 94°C, 1 min for annealing at 58^0C and 2 min at 72°C for extension step followed by 7 min at 72°C for final extension before cooling to 4°C. ISSR bands were separated on 1.8% agarose gel and run in 1×TAE buffer (40 mM Tris acetate, pH 7.5, 1 mM EDTA) for 3 h, at 100 v. The gels were stained with ethidium bromide (0.5 µg/ µL) for 10 min followed by de-staining in tap water for 30 min. DNA bands were detected using UV trans-illumination and photographed using Bio Doc-ItTM gel documentation system (UVP, Cambridge, UK).

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. The molecular size of the DNA fragments was estimated using 50bp DNA ladder (Fermentas). The 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). The number of different alleles (Na), number of effective alleles (Ne), polymorphic loci (%), Shannon's information index (I) and Nei's gene diversity (He) of phenotypic diversity for the binary data were calculated using GenAlEx software version 6.5 [37,38]. GenAlEx was also used for computing molecular variance analysis (AMOVA) and principal coordinate analysis (PCoA) via distance matrix. Genetic associations

among individual genotypes were determined with the help of Darwin version 5.0 [39] using the dissimilarity matrix and a Neighbour-Joining (NJ) hierarchal tree was constructed based on UPGMA. The fixation index (Fst) to measure genetic differentiation among the populations, and polymorphic information content (PIC) for each ISSR locus were calculated using the genetic analysis package Power-Marker, version 3.2 [40].

Table 1. List of Ethiopian sesame (*Sesamum indicum*) germplasm with regions of collection

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

Key: SNNP= southern nation and nationality people

3. RESULTS AND DISCUSSION

3.1 Banding Pattern and Polymorphism of the ISSR Primers

The genetic diversity among 128 genotypes representing 10 geographically distinct populations was assessed using 7 polymorphic ISSR primers. The number of bands ranged from 9 (ISSR-7) to 19 (ISSR-1) for each of the DNA sample. Average number of bands and polymorphic bands per primer were 14 and 12.6 respectively. The seven primers amplified a total of 96 scorable alleles, of which 89 (92.2%) were polymorphic (Table 2). Level of polymorphism ranged from 75.0 to 98.4, where primers ISSR-2, 3, and 6 exhibited the highest percent of polymorphic band, while ISSR-7 detected the least percent of polymorphism (Table 2). The level of polymorphism obtained in this study (92.2%) was high as compared with the 70.6% of Benson et al. [16] in molecular diversity of East African sesame and related wild species, 75.86% of Dagmawi [30] in genetic diversity of Ethiopian sesame germplasm collection, and 66.87% of Sharma et al. [22] in comparative analysis of RAPD and ISSR for characterization of sesame. In contrast, a lower level of polymorphism (33%) was detected in previous studies of Korean sesame genotypes [19] and (57%) in Indian sesame varieties [25]. However, our results were comparable with the 98.5% of polymorphism reported by Anitha et al. [28] and the 100% of polymorphism reported by Salazar et al. [41] in genetic diversity analysis of Tamil Nadu and Venezuela sesame varieties respectively. The higher percent of polymorphism detected in this study could be because of large number of samples from several regions of the country and also because Ethiopia is considered as the origin and centre of diversity of cultivated sesame [42]. According to Sanchez et al. [43], the difference in polymorphism may be due to the genotypes used, nature of ISSR primers and annealing temperatures used. Low annealing temperature may increase non-specific amplification, leading to artefact bands. The modification of annealing temperature has a great impact on the richness and legibility of fingerprints [44].

The PIC value, as a relative measure level of polymorphism varied from 0.26 (ISSR-7) to 0.76 (ISSR-2) with an average value of 0.43 (Table 2). The higher PIC value indicated the informativeness of the primers. The most informative primer in the present study was UBC848 with PIC value of 0.76, which is similar to previous result reported by Young et al. [45] in sesame collections from Korea and 22 countries. The fragment size ranged from 250 to 3000 bp. A representation of the ISSR band profile obtained with primer UBC835 and UBC848 are shown in (Fig. 1).

3.2 Genetic Diversity within Populations of Sesame Genotypes

The evaluation of the 10 sesame populations with seven ISSR primers resulted in overall species diversity of 0.39 and gene diversity of 0.36 (Table 3). This result is in agreement with the previous studies of Ashri [46], Bhat et al. [47] and Ercan et al. [48] who have reported high genetic diversity in sesame germplasm. A wide range (0.01 - 0.88) of dissimilarity value was observed among the genotypes (data not shown). The maximum dissimilarity (0.88) was observed between Amr-NW6 and Amr-NG9 while the minimum dissimilarity (0.014) was recorded between Amr-NW1 and Am-NG1 (the closest accessions). SNNP-7 was the most distinct of all genotypes followed by Amr-NG25 with average dissimilarity value of 0.77 and 0.75, respectively, while Oromia-16 showed maximum similarity with

Table 2. Selected ISSR primes, sequences, total bands and polymorphic bands observed in 128 sesame genotypes

Primer code	Primer name	Sequence (5'- 3')	AT (°C)	TB	PB	%P	PIC
ISSR-1	UBC835	AG_8YC	58	19	18.6	97.7%	0.42
ISSR-2	UBC848	CA_8RG	58	18	17.7	98.4%	0.76
ISSR-3	UBC847	CA_8RC	58	15	14.8	98.4%	0.48
ISSR-4	UBC850	GT_8YC	58	13	11.4	87.5%	0.32
ISSR-5	UBC855	AC_8YT	58	12	10.8	89.8%	0.36
ISSR-6	UBC873	$GACA_4$	49	10	9.8	98.4%	0.42
ISSR-7	UBC846	CA_8RT	58	9	6.8	75.0%	0.26
Mean				13.7	12.6	92.2%	0.43

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

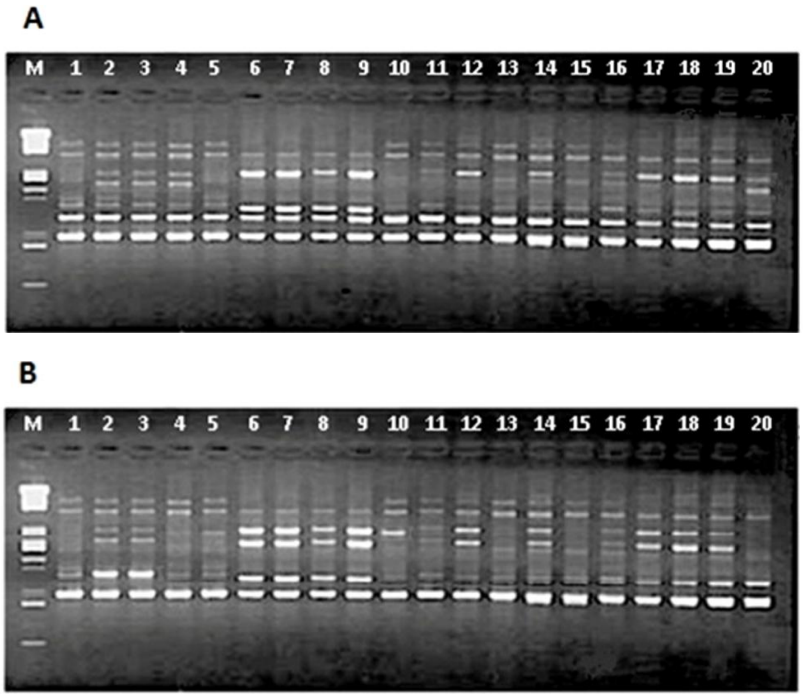

Fig. 1. PCR products from twenty sesame genotypes (lane 1 - 20) amplified with (A) primer UBC835 and (B) primer UBC847. M = 50 bp molecular weight marker

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

Population		N	Na	Ne	I	He	P%
Afar		9.00	1.64	1.49	0.42	0.28	77.08%
Amr-NW		14.00	1.95	1.74	0.58	0.40	94.79%
Amr-NG		27.00	1.95	1.67	0.56	0.38	94.79%
Amr-NSh		6.00	1.77	1.59	0.47	0.32	79.17%
Amr-SW		11.00	1.94	1.59	0.50	0.34	94.79%
Benshang		7.00	1.70	1.55	0.47	0.32	83.33%
Gambella		4.00	1.92	1.56	0.48	0.32	91.67%
Oromia		20.00	2.00	1.71	0.59	0.41	100.00%
SNNP		8.00	2.00	1.78	0.60	0.42	100.00%
Tigray		22.00	1.94	1.71	0.56	0.39	93.75%
Total	Mean	**12.80**	**1.88**	**1.64**	**0.52**	**0.36**	**90.94%**
	SE	0.237	0.013	0.011	0.007	0.005	2.59%

Note: N= number of samples, Na= number of different alleles, Ne= effective number of alleles, He= Nei's gene diversity Nei (1978), I= Shannon's information index, P%= percent of polymorphic loci

others at 0.20 average dissimilarity value. Based on pair-wise population analysis, the SNNP were found to be the most diverse population with average Nei's gene diversity and Shannon index values of 0.42 and 0.60, respectively; whereas, Afar was the least diverse followed by Benishangul with average gene diversity of (0.28 and 0.31) and Shannon index of (0.42 and 0.47), respectively (Table 3). The different (Na) and effective number (Ne) of alleles also followed the same trend that is all these values were highest for SNNP and lowest for Afar and Benishangul populations (Table 3). This is in contrast with the patterns reported by Dagmawi [35] and Daniel and Heiko [49] in genetic diversity study of sesame germplasm collection in Ethiopia. They showed Oromia to be the most diverse of all populations. The proportion of polymorphic loci for the present set of population ranged from 77.1% (Afar) to 100% (Oromia and SNNP) with average of 90.94%. Moderate polymorphism (83.3%) was detected for Benishangul population

(Table 3). This result was higher compared to the previous results of Dagmawi [35] and Daniel & Heiko [49], who have reported 43.41 and 74.04% of average polymorphism respectively for sesame populations of Ethiopia.

Pair-wise distance analysis (Nei unbiased genetic distance) between populations (Table 4) ranged from 0.00 (Gambella and Amr-NG) to 0.27 (Amr-NSh and Afar). The highest distances were observed between the population of Afar (released cultivars) and the populations representing the landraces. However, the distances between the populations of the landraces were much lower indicating the genetic relatedness of the various geographic populations which may be caused by the high rates of gene flow due to exchange of germplasm among farmers and sesame trades across the regions. This was consistent with the result obtained by Dagmawi [35] in evaluation of 82 Ethiopian and 38 exotic sesame accessions.

The higher proportion of polymorphism in the present study could suggest the extent of genetic diversity among the various sesame germplasm of Ethiopia. The diversity could mainly be attributed to diverse agro-climatic conditions in the country. Accessions from different regions were sometimes closely related and accessions from the same region had different genetic background. Even so, the intraregional diversity could be as a valuable source as interregional diversity for sesame improvement [16].

3.3 Analysis of Molecular Variance (AMOVA) and Partitioning of Genetic Diversity

The AMOVA test, calculated to examine the differences among and within geographical populations was found to be statistically significant ($p < 0.001$). The test showed highest genetic variation within population (94%), whereas the variation among geographic populations was only 6% (Table 5). The average Fst value was 0.061, indicating a lower differentiation among populations. This result showed a great intra-population genetic diversity but no significant difference was detected among the different populations. The result of this study is in complete agreement with some of the previous studies in AMOVA of Ethiopian sesame germplasm using ISSR markers [35], who obtained 94.1% within population and 5.9% among populations variation with average Fst value of 0.06 across populations. Similar result was also found in AMOVA of sesame genotypes from 5 different countries [50], where 95% of the variance was due to differences within population. In contrast, Daniel and Heiko [49] found 58.8% of the total genetic variation within population and 41.2% between populations, in AMOVA of 50 landraces of sesame from Ethiopia.

The high within population variation in this study could mainly be because of the large number of accessions included and due to high out-crossing nature of sesame. Although sesame is mainly self-pollinated, some authors have reported levels of out crossing between 5-60% in this species [51-53]. Out-crossing plant species tend to present between 10 and 20% of the genetic variation between populations [54] and the remaining 80-90% of the total genetic variation within populations. Hence, some degree of out-crossing could explain the high genetic diversity observed in the studied sesame accessions. The lower variance among populations in this study could be explained by exchange of sesame seeds among nearby districts through farmers that may enhance gene flow across regions of Ethiopia.

3.4 Principal Coordinate Analysis (PCoA)

The genetic distance matrix obtained using seven ISSR primers were used for PCoA analysis among populations of Ethiopian sesame germplasm (Fig. 2). The result indicated that the first two principal coordinates, PCoA1 and PCoA2 explained 33.18 and 16.16% of the variation respectively and together explained 49.34% of the total variation. In the PCoA graph, the released cultivars, unlike the land races were found to aggregate in one group and positioned around the centre of the plot, showing that these genotypes are genetically closer than the others. This result confirms the result obtained in all the above diversity indices. However, with the exception of few genotypes representing Oromia and Tigray, most of the landraces that represent different geographical regions were found to form distinct groups and spread all over the plot (Fig. 2).

Generally, the PCoA result indicated that most of the landraces did not group together with other genotypes from the same geographical region. One possible reason for this could be the exchange of sesame germplasm among farmers across regions. This result was in congruence with the result reported by Dagmawi [35].

Table 4. Nei unbiased genetic distance below diagonal among sesame populations tested by seven ISSR primers

Population	Afar	Amr-NW	Amr-NG	Amr-NSh	Amr-SW	Benshang	Gambella	Oromia	SNNP	Tigray
Afar	-									
Amr-NW	0.223	-								
Amr-NG	0.165	0.045	-							
Amr-NSh	0.270	0.034	0.110	-						
Amr-SW	0.195	0.004	0.029	0.049	-					
Benshang	0.158	0.048	0.023	0.108	0.008	-				
Gambella	0.157	0.070	0.000	0.148	0.032	0.019	-			
Oromia	0.173	0.014	0.020	0.045	0.001	0.014	0.028	-		
SNNP	0.163	0.008	0.031	0.055	0.018	0.014	0.035	0.008	-	
Tigray	0.200	0.030	0.026	0.050	0.049	0.068	0.022	0.035	0.021	-
Average distance = 0.038										

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

Source	Df	SS	MS	Est. Var.	Var.%
Among Pops	9	307.04	34.12	1.26	6%
Within Pops	118	2196.97	18.62	18.62	94%
Total	127	2504.02		19.87	100%
Fst = 0.061					

Note: Df = degree of freedom, MS = mean square, SS = sum of square, Est. Var = estimated variance

3.5 Clustering Analysis and Relationships among Sesame Genotypes

The 128 genotypes of sesame were divided into 3 major groups that were sub-grouped into 9 clusters (Fig. 3). Genotypes of sesame landraces were widely distributed and formed separate clusters, whereas the cultivars were seen grouped together under the same cluster. The most distinct genotypes were accession No. 56, No. 73 and No. 105 (Fig. 3). Genetic distances among the landraces were larger than distances between the released cultivars. In general, there was no clear clustering in the UPGMA tree. The majority of the landraces from different geographical regions were inter-mixed and grouped together in the same clusters with the exception of some genotypes, Amr-NSh-6, Benishangul-6 and SNNP-7, which were found to out-group from any of the population and stand alone (Fig. 3).

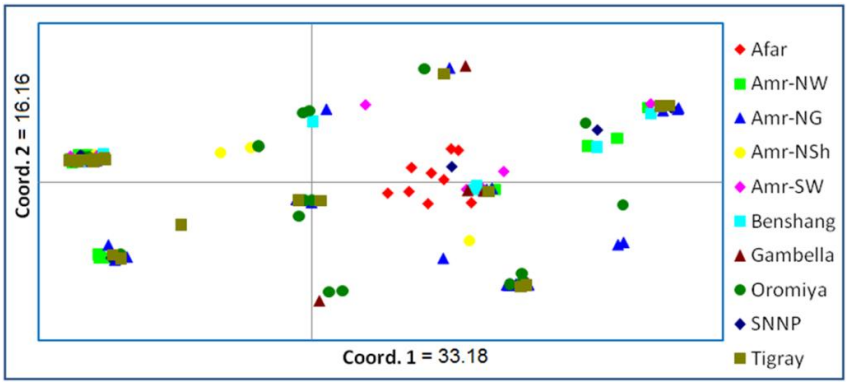

Fig. 2. PCoA scatter plot diagram showing relationships among populations of sesame

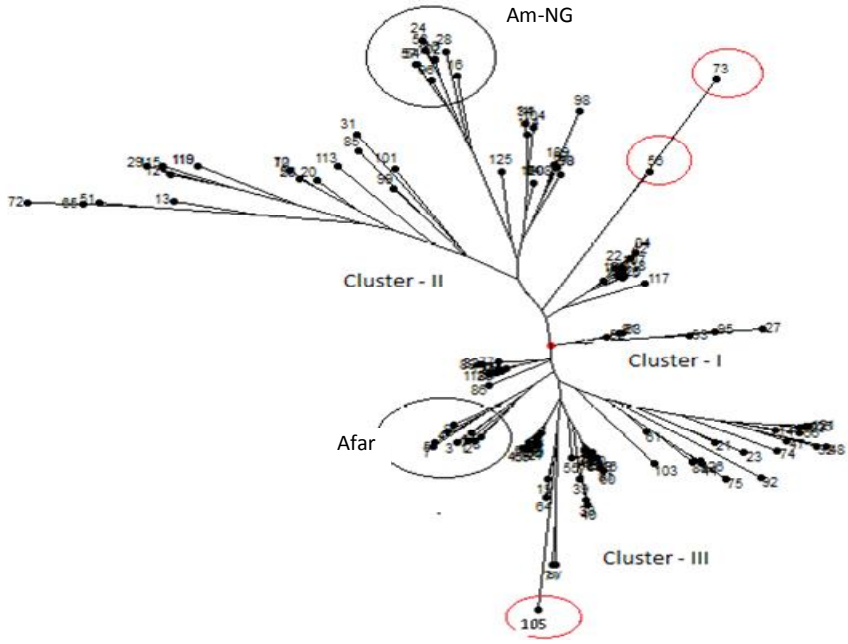

Fig. 3. UPGMA tree illustrating relationships among 128 sesame genotypes based on genetic distances using 7- ISSR primers

Note: Numbers on the tree correspond to the accessions' number as presented in Table 1. Amr-NW = amhara north wollo, Amr-NG = amhara north gonder, Amr-NSh = amhara north shoa, Amr-SW = amhara south wollo, Benshang = benishangul gumuz, SNNP = southern nation and nationality people

Although the present study indicated the presence of a relatively high genetic diversity among landraces of sesame from various geographical locations of Ethiopia, most genotypes situated geographically far apart were grouped together in the same cluster. These results are in agreement with earlier studies which showed that geographical separation did not generally result in greater genetic distance [20,21,26,55]. This could be a consequence of exchange of genetic materials among the neighbouring farmers as well as traders in the region. The human factor has been previously shown to be responsible for the lack of correlation between genetic and geographical distance [56]. The increasing demand for sesame by exporters in Ethiopia, the movement of traders and exchange of germplasm across different regions could be a possible explanation for the spreading of sesame seeds.

4. CONCLUSION

The present study detected a high level of polymorphism among the different sesame genotypes of Ethiopia. Accessions viz., no.56 (Amr-NSh-6), no.73 (Benishangul-6) and no. 105 (SNNP-7) showed a good amount of genetic divergence and would therefore be useful in broadening genetic base of landraces of sesame in Ethiopia. The size and number of polymorphic fragments, percentage of polymorphic loci, together with the overall species and gene diversity indices reported in the study indicated high genetic diversity among the germplasm lines. The most diverse accessions identified in this study should be given a due emphasis to include them in sesame breeding program of the country.

In this study, we demonstrated that the ISSR primers revealed high genetic variation among individual genotypes and also revealed low genetic differentiation among the ten populations studied. These results could suggest that the homogeneity among the ten (geographic region based) populations could be due to genetic flux or that they may had a common origin, alternatives that needs to be further explored. However, the study generally showed that the Afar population is genetically different from the other populations and hence there was a relatively greater distance between Afar (the cultivars) and the population of the landraces, though, genotypes of the Afar population are the most genetically related than genotypes of any other group. However, the present result needs to be explored further by the use of species specific markers such as SSR, SNP and GBS.

Cluster analyses of the present study showed that most genotypes of the landraces did not group together with other genotypes from the same geographical region. The close relationship between some sesame genotypes from the different populations might be due to gene flow caused by the exchange of germplasm through farmers and traders across regions of Ethiopia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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