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ARTICLES

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Full Length Research Paper

Genetic diversity of sesame germplasm collection (*SESAMUM INDICUM* L.): implication for conservation, improvement and use

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Genetic diversity assessment of genetic resources maintained at Gene-Banks has important implication for future improvement, conservation and collection activities. However, such information is not available for sesame collected by IBC, Ethiopia. Inter simple sequence repeat (ISSR) marker was used to assess the level of genetic diversity, genetic structure and genetic distance, and to indirectly estimate the level of gene flow among populations of sesame in Ethiopia. A total of 120 (82 Ethiopian and 38 exotic) sesame accessions and six ISSR primers were used. DNA was extracted using a triple CTAB extraction method from silica gel dried bulked sample of five randomly selected individual plants per accession at the stage of three to four weeks after planting. The presence of higher polymorphism was revealed among accessions collected from Ethiopia (75.85) than the exotic accessions (65.52). The average gene diversity relative to the overall population was 0.24. Samples from Welega was the most diverse, with gene diversity value of 0.26 followed by samples from Tigray (0.20) and Shewa (0.20). Samples from Gojam (0.10) and Sudan (0.12) were the least diverse. Inter-population genetic distance (D) ranged from 0.031 to 0.165 for the overall population. From the exotic accession, samples of South East Asia are distantly related to most of the Ethiopian accessions. Unweighted pair group method with arithmetic mean analysis (UPGMA) of Ethiopian sesame populations revealed two major groups and three outliers (Cultivated, Welega and Illubabore).

Key words: Bulk sampling, gene flow, genetic differentiation, genetic distance, inter simple sequence repeat (ISSR), genetic diversity and sesame landrace accessions.

INTRODUCTION

The cultivated sesame, *Sesamum indicum* L., belongs to the family Pedaliaceae. Even though the origin of sesame is still in debate, Mehra (1967) and Mahajan (2007)

stated that Ethiopian region is accepted as the origin of cultivated sesame. In addition, Bedigian (1981) argues that, owing to the wide genetic diversity in Africa, it is

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reasonable to assume that this subcontinent is the primary center of origin and India would then be thought of as a secondary center for sesame. It is perhaps one of the oldest crops cultivated by man, having been grown in the Near East and Africa for over 5,000 years for cooking and medicinal needs. Generally, 65% of world sesame production is used for edible oil extraction and 35% for confectionary purpose. The fatty acid composition is rather attractive, due to the high level of unsaturated fatty acids.

Sesame is grown in more than 60 countries in the world. According to FAO (2011) Ethiopia is ranked 6th in sesame production with 327,741 tons (10%) of production per year. Sesame seed is by far the leading crop in the countries oil seeds export where by more than 90% of the production is directed to export followed by Niger seed CSA (2010). The Ethiopian whitish Humera type is known for its taste (sweet) in the world market hence it is exported to the confectionary market where white seeded types are demanded by the consumers (Wijnands et al., 2009).

The main objective of sesame groups in National Agricultural Research Systems of Ethiopia is to develop cultivars concentrating on achieving a variety that combines disease resistance, white seeded and high yielding characters and works towards increased market values. The research in the country is geared towards evaluation of new collected accessions and exotic materials to develop a sesame variety with desirable characteristics with a combination of trait of interest including considerable oil content which are important for the export market (Tadele, 2005).

To strengthen the research program and broaden the genetic bases in sesame breeding, Ethiopian Institute of Agricultural Research has assembled 221 germplasm collections during 2002-2004 from different sesame growing regions in Ethiopia and preserved at Werer Agricultural Research Center. This makes the total sesame accessions preserved in Ethiopia to around 870 when it adds up with the IBC collection. However genetic variability studies, using any marker system, have not yet been conducted for the IBC collection. To meet the objectives of the national sesame improvement, information on genetic diversity and their relationship within and among collected accessions is inevitable.

Genetic markers are widely used by breeders and conservationists to study genetic diversity. A number of markers exist that can be used as tool to discern genetic variability within among populations. Each marker system has its own advantages and disadvantages in terms of ease of use and/or the degree of information provided. One of the genetic marker, molecular markers, are identifiable DNA sequence, found at specific locations of the genome, and transmitted from one generation to the next. The presence of various types of molecular markers, and differences in their principles, methodologies, and applications require careful consideration in choosing one

or more of such methods. No molecular markers are said to be ideal and fulfill all requirements needed by researchers. Thus, one can choose among the variety of molecular techniques, according to the kind of study to be undertaken. To be called a suitable molecular marker it should be highly polymorphic, co-dominant inheritance, frequent occurrence and even distribution throughout the genome, selectively neutral behavior, easy access, easy and fast assay, low cost and high throughput, high reproducibility, and transferability between laboratories, populations and/or species (Weising et al., 2005).

Inter-simple sequence repeat (ISSR) is amplification of DNA segments present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction (Zietkiewicz et al., 1994). ISSR marker, in addition to its suitability to genetic diversity study, is highly polymorphic, reproducible, cost effective, requires no prior information of the sequence (Bornet et al., 2002). These facts suggest that ISSR could be an unbiased tool to evaluate the changes of diversity in agronomically important crops (Brantestam et al., 2004).

ISSR markers are now being applied for cultivar identification, assessment of genetic diversity in various plant species and in determining genetic diversity and phylogenetic relationships within and among cultivated crops (Hou et al., 2005; Joshi et al., 2000). In agronomically important crops such as sesame this marker is used to study the patterns and level of diversity. Hence, the ISSR marker assay has been chosen to study the level of diversity and patterns of distribution of sesame genetic resources in Ethiopia.

MATERIALS AND METHODS

Experiment material and DNA extraction

Sesame germplasm sample consisting of 120 accessions (82 Ethiopian and 38 exotic) were drawn from the over 636 accessions currently maintained at the gene bank at the institute of Biodiversity Conservation, Addis Ababa, Ethiopia (Table 1 and Figure 1). The sampling procedure was set in such a way that accessions from the major sesame growing regions were sufficiently represented in the sample. Germplasm passport data were used to make sure that the major regions are represented. Additional sesame samples from Africa and other parts of the world were included for comparative analysis. The samples were laid out in augmented randomized block design in Werer Agricultural Research Center, Ethiopia on station field plots. The accessions were grown in 2 row plots of 4 m length.

Young leaves (3-5 in number) were collected separately from 5 randomly selected individual plants per accession three to four weeks after planting and dried in silica gel. Approximately equal amounts of the dried leaf samples were bulked for each accession and ground with sterile pestle and mortar with addition of liquid nitrogen. Total genomic DNA were isolated from about 0.2 g of the pulverized leaf sample using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique as describe by Borsch et al. (2003). The isolated DNA samples were visualized using 1% ethidium bromide stained agarose gel under UV light. Based on a test gel result of DNA quantity (band intensity) and

Table 1. List of Sesame accessions, altitude and location of collection used in the study (Source: IBC).

No.	Accession #	Altitude	Former Administrative Region/ Exotic	No.	Accession #	Altitude	Former Administrative Region/ Exotic	No.	Accession #	Altitude	Former Administrative Region/ Exotic	No.	Accession #	Altitude	Former Administrative Region/ Exotic
1	111501	NA	Gamo Gofa	32	222876	NA	Illubabor	64	202353	1640	Welo	96	231410	700	Exotic
2	208888	NA	Gamo Gofa	33	202285	1490	Shewa	65	202370	1590	Welo	97	205194	700	Exotic
3	208889	1050	Gamo Gofa	34	202286	1490	Shewa	66	215260	NA	Welo	98	205195	800	Exotic
4	212993	1290	Gamo Gofa	35	202374	1395	Shewa	67	203631	NA	Exotic	99	205191	900	Exotic
5	212994	1270	Gamo Gofa	36	203099	1240	Shewa	68	203633	NA	Exotic	100	205192	1100	Exotic
6	212995	1290	Gamo Gofa	37	212536	1580	Shewa	69	203634	NA	Exotic	101	203595	760	Exotic
7	214976	NA	Gamo Gofa	38	212537	NA	Shewa	70	203637	NA	Exotic	102	203597	NA	Exotic
8	111502	NA	Gojam	39	212540	1600	Shewa	71	203638	NA	Exotic	103	203599	700	Exotic
9	111503	NA	Gojam	40	234015	1090	Tigray	72	227862	NA	Exotic	104	203600	750	Exotic
10	111513	NA	Gojam	41	210871	NA	Eritrea	73	227864	NA	Exotic	105	203627	NA	Exotic
11	211921	1600	Gojam	42	214202	NA	Eritrea	74	227865	NA	Exotic	106	Abasena	1050	Released
12	230260	NA	Gojam	43	235405	1650	Tigray	75	227866	NA	Exotic	107	E	NA	Released
13	205173	NA	Gonder	44	237523	900	Tigray	76	227867	NA	Exotic	108	Hir Hir or T- 85	NA	Released
14	235769	900	Gonder	45	238269	820	Tigray	77	227873	NA	Exotic	109	Mehedo	NA	Released
15	235903	1150	Gonder	46	238270	710	Tigray	78	227874	NA	Exotic	110	Adi	1050	Released
16	241341	600	Gonder	47	238271	710	Tigray	79	227875	NA	Exotic	111	Acc-BG-006	1290	Benishangulgumuz
17	241342	600	Gonder	48	238272	710	Tigray	80	227876	NA	Exotic	112	Acc-BG-002	1270	Benishangulgumuz
18	241334	750	Gonder	49	241305	1200	Tigray	81	227879	NA	Exotic	113	Acc-BG-001	1290	Benishangulgumuz
19	241344	980	Gonder	50	241311	1120	Tigray	82	227903	NA	Exotic	114	Acc-BG-003	1050	Benishangulgumuz
20	241346	1890	Gonder	51	111505	1440	Welega	83	227904	NA	Exotic	115	Acc-BG-010	NA	Benishangulgumuz
21	241347	1700	Gonder	52	202517	1420	Welega	84	227906	NA	Exotic	116	Acc-BG-012(2)	1050	Benishangulgumuz
22	202512	1600	Harerge	53	202518	1360	Welega	85	227907	NA	Exotic	117	Acc-BG-003(2)	NA	Benishangulgumuz
23	208670	1830	Harerge	54	207953	1300	Welega	86	227908	NA	Exotic	118	Acc-BG-019(1)	1600	Benishangulgumuz
24	208671	1900	Harerge	55	207954	1200	Welega	87	227933	NA	Exotic	119	New (EW-004)	NA	Bako
25	208672	1350	Harerge	56	208752	NA	Welega	88	227934	NA	Exotic	120	New (EW-015)	NA	Bako
26	208673	1840	Harerge	57	237994	1290	Welega	89	231397	NA	Exotic				
27	228816	1500	Harerge	58	215816	1435	Welega	90	231399	NA	Exotic				
28	223340	NA	Harerge	59	212632	1460	Welo	91	227855	NA	Exotic				
29	207956	NA	Illubabor	60	202318	1525	Welo	92	231406	NA	Exotic				
30	207957	NA	Illubabor	62	202340	1555	Welo	93	231407	NA	Exotic				
31	216733	600	Illubabor	63	202345	1565	Welo	95	231409	NA	Exotic				

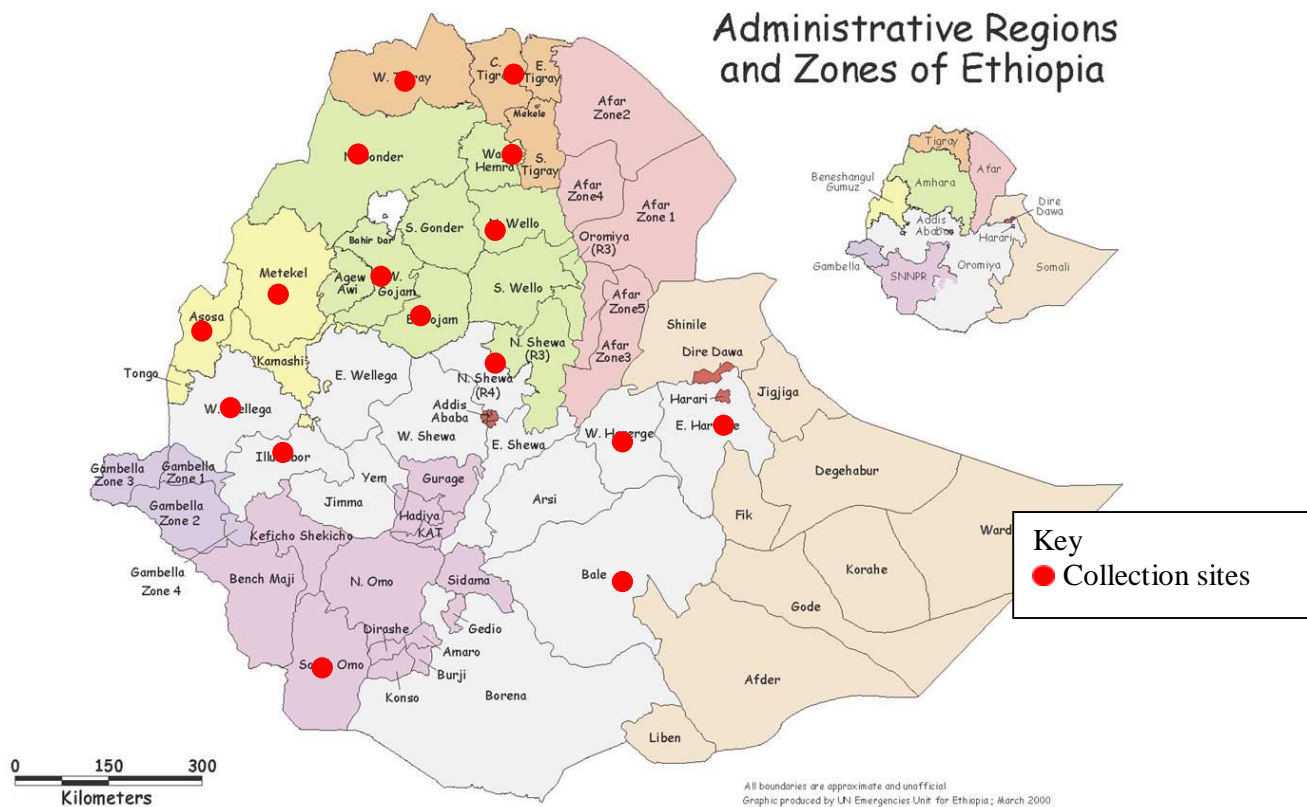


Figure 1. Former administrative region of Ethiopia showing where the sesame accessions used in this study were collected (Source: Map of the world).

Table 2. Banding patterns generated using the six primers, their annealing temperature, repeat motifs, amplification patterns and number of scored bands.

Primer	Annealing temperature (°C)	Repeat motif ¹	Amplification pattern	Number of scored bands
810-H	45	(GA) ₈ T	Good	9
818-H	45	(CA) ₈ G	Good	9
834-H	45	(AG) ₈ YT	Good	10
844-H	45	(CT) ₈ RC	Good	10
860-H	45	(TG) ₈ RA	Good	9
880-H	48	(GGAGA) ₃	Good	11
Total				58

¹Single-letter abbreviations for mixed base positions: R = (A, G); Y = (C, T).

quality (absence or presence of minimum smear) the second extraction of most and the first extraction of some samples were selected for further PCR amplification. The selected genomic DNA samples were diluted with sterile distilled water in 1:5 ratios.

ISSR analysis

The ISSR marker assay was conducted at Genetics Research Laboratory of the Microbial, Cellular and Molecular Biology Program Unit, Faculty of Life Sciences, Addis Ababa University, Addis Ababa. A set of primer kit was obtained from the University of British Columbia (primer kit UBC 900). Based on published work of Kim et al. (2002) and lab experience, a total of 12 primers were

used for the initial testing of primers variability and reproducibility. A total of eight sesame DNA samples from geographically distant localities were selected to screen primers and optimize the reaction conditions. Finally, a total of six polymorphic and reproducible ISSR primers were selected for the final analysis (Table 2).

The polymerase chain reaction was conducted in Biometra 2000 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1 µl template DNA, 14.0 µl H₂O, 5.0 µl dNTP (1.25 mM), 2.5 µl Taq buffer (10xThermopol reaction buffer), 2.0 µl MgCl₂ (2 mM), 0.3 µl primer (20 pmol/µl) and 0.2 µl Taq Polymerase (5 u/µl). The amplification program was set as 4 min preheating and initial denaturation at 94°C, followed by 40 cycles of 15 sec 94°C, 1 min primer annealing at (45°C/48°C) based on primers used, 1 min and 30 sec extension at 72°C and the final

extension for 7 min at 72°C. The PCR products were stored at 4°C until loading on gel for electrophoresis. The lid temperature was held at 105°C. The PCR products were stored at 4°C until loaded on gel for electrophoresis. The amplification products were differentiated by electrophoresis using an agarose gel (1.67% agarose with 100 ml 1xTBE) and 8 µl amplification product of each sample with 2 µl loading dye (6 times concentrated) was loaded on gel. DNA marker 1 k bp (molecular ladder) was used to estimate molecular weight and size of the fragments. Electrophoreses was done for 2 h at constant voltage of 100 V. The DNA was stained for 30 min with (10 mg/ml) ethidium bromide (EtBr) which was mixed with 450 ml distilled water and distained for 30 min with 450 ml of distilled water.

Statistical analysis

Clearly resolved, unambiguous bands were scored visually for their presence or absence for each primer and sample. ISSR profiles/bands were scored manually for each individual accession from the gel photograph. The bands were recorded as discrete characters, presence '1' or absence '0' and '?' for missing data. To analyse the scored '1' '0' fragment data assembled in data matrix. The total number of bands, distribution of bands across accessions, number of polymorphic bands in a set of accessions, and average number of bands per primer were calculated. Similarity matrix was generated based on the simple-matching coefficient (Jaccard's), using the presence/absence data for individual ISSR fragment.

Based on recorded bands different software's were used for analysis. POPGENE version 1.32 software (Yeh et al., 1999) was used to calculate genetic diversity for each population as number of polymorphic loci and percent polymorphism, Shannon index, coefficient of gene differentiation (G_{ST}) (Nei, 1973), Nei's standard genetic distance (GD) (Nei, 1972) and gene flow estimates (McDermott and McDonald, 1993). Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006). NTSYS- pc version 2.02 (Rohlf, 2004) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) software's were used to calculate Jaccard's similarity coefficient which is calculated with the formula:

$$S_{ij} = \frac{a}{a+b+c}$$

Where, 'a' is the total number of bands shared between individuals i and j, 'b' is the total number of bands present in individual i but not in individual j and 'c' is the total number of bands present in individual j but not in individual i.

The unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare the population and generates phenogram using NTSYS- pc version 2.02 (Rohlf, 2004). The Neighbor Joining (NJ) method (Saitou and Nei 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek et al., 1999).

RESULTS

Banding patterns of the ISSR primers used

Out of the 12 primers tested initially, six primers (five dinucleotide and one penta-nucleotide) that gave relatively clear banding pattern were selected and used in this study (Table 2). The molecular weight of the bands amplified using the primers were in the range of 450 to 4000 bp. A total of 58 clear bands were scored, from 82

and 38 exotic sesame accessions. Out of the total 58 ISSR fragments, 44 were found to be polymorphic. The least polymorphic bands (four) were scored from primer 834_H and the remaining five primers all showed equal polymorphic bands (eight). The average number of bands and polymorphic bands per primer were 9.67 and 7.33, respectively.

Polymorphism based on ISSR analysis

In all populations or individuals the number of polymorphic loci ranges from four for primer-834 to eight for all other primer. Of the total 58 loci scored, 44 (75.86%) were observed to be polymorphic. From all the populations (considering Ethiopian accessions and exotic accessions as independent population) studied Welega (58.62%), Tigray (50.00%) and Gonder (44.83%) were found to have higher percent polymorphism. Gojam (25.86%) and Sudan (27.95%) on the other hand showed the least percent polymorphism.

Ethiopian accessions (considering as a single large population) when compared to exotic accessions they showed high percent polymorphism 75.85 and 65.52%, respectively (Table 3). Among the exotic accessions (considering samples from different country as independent population) accessions from Sudan (27.95%) were the least polymorphic and the South East Asian ones (43.1%) showed high present polymorphism. No unique bands were observed for either the accessions or the populations.

As to the primers used, none of the primers showed 100 per cent polymorphism. Primer 810, 818 and 860 showed the highest percent polymorphism (88.89%) and the least polymorphic band was scored by primer 834 which is 40.00 % and this primer also showed higher standard deviation for the genetic diversity and Shannon index which goes with its least polymorphism. The rest primers namely, 844 and 880 showed 80.00 and 72.73 per cent polymorphism, respectively (Table 4).

Genetic diversity

Grouping Ethiopian accessions into administrative region based population and exotic accessions in to country based population showed that samples from Welega were the most diverse (0.26) followed by samples from Tigray (0.20) and Shewa (0.20). Samples from Gojam (0.10) and Sudan (0.12) were the least diverse. The average gene diversity relative to the overall population was 0.24, considering accession from different administrative region of Ethiopia and exotic countries as independent population. The overall diversity index values for the total population and the Ethiopian sesame accessions were found to be 0.37 (Table 3). The extent of gene differentiation relative to the total Ethiopian population

Table 3. Number of Polymorphic Loci (NPL), percent polymorphism (PP) genetic diversity (GD) Shanon Index (I), gene differentiation (G_{ST}) and gene flow (Nm) with 120 sesame accessions and all primer.

Population	NPL	PP	GD \pm SD	I \pm SD	G_{ST}	Nm
Benishangul Gumuz	25	43.1	0.18 \pm 0.22	0.26 \pm 0.31		
Gamo Gofa	24	41.38	0.17 \pm 0.22	0.25 \pm 0.31		
Gojam	15	25.86	0.1 \pm 0.18	0.15 \pm 0.26		
Gonder	26	44.83	0.19 \pm 0.22	0.27 \pm 0.31		
Harerge	22	37.93	0.14 \pm 0.2	0.21 \pm 0.29		
Illubabor	22	37.93	0.17 \pm 0.23	0.25 \pm 0.32		
Shewa	28	48.28	0.2 \pm 0.21	0.28 \pm 0.31		
Tigray	29	50	0.2 \pm 0.21	0.27 \pm 0.3		
Welega	34	58.62	0.26 \pm 0.23	0.37 \pm 0.32		
Welo	23	39.66	0.17 \pm 0.22	0.24 \pm 0.31		
Cultivated	29	50	0.22 \pm 0.23	0.31 \pm 0.32		
Ethiopian average	25.18	43.41	0.18	0.26		
Ethiopia total	44	75.86	0.24 \pm 0.19	0.37 \pm 0.27	0.25	1.49
Egypt	21	36.21	0.14 \pm 0.21	0.21 \pm 0.30		
Mexico	22	37.93	0.14 \pm 0.20	0.21 \pm 0.28		
Somalia	23	39.66	0.18 \pm 0.23	0.26 \pm 0.32		
S. E. Asia	25	43.1	0.19 \pm 0.23	0.27 \pm 0.32		
Sudan	16	27.95	0.12 \pm 0.20	0.17 \pm 0.29		
Tanzania	22	37.93	0.15 \pm 0.21	0.23 \pm 0.30		
Zambia	24	41.38	0.18 \pm 0.22	0.25 \pm 0.31		
Zimbabwe	24	41.38	0.18 \pm 0.22	0.25 \pm 0.31		
Exotic average	22.12	38.1925	0.16	0.23125		
Exotic Total	38	65.52	0.23 \pm 0.20	0.34 \pm 0.28	0.29	1.19
Average population	23.89	41.21				
Total Population	44	75.86	0.24 \pm 0.19	0.37 \pm 0.27	0.28	1.28

Table 4. Number of Scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP) genetic diversity (GD) Shanon index (I), for each primer.

Primer	NSB	NPL	PP	GD	I
810	9	8	88.89	0.23 \pm 0.18	0.36 \pm 0.25
818	9	8	88.89	0.35 \pm 0.17	0.51 \pm 0.23
834	10	4	40.00	0.12 \pm 0.18	0.19 \pm 0.27
844	10	8	80.00	0.23 \pm 0.17	0.37 \pm 0.24
860	9	8	88.89	0.26 \pm 0.2	0.40 \pm 0.27
880	11	8	72.73	0.27 \pm 0.21	0.39 \pm 0.29
Total	58	44	75.86	0.24 \pm 0.19	0.37 \pm 0.27

was (G_{ST}) 0.25 and the extent of gene flow (Nm) among populations of Ethiopian sesame accessions (considering administrative region as independent population) was 1.49. For the exotic accessions the extent of gene differentiation was 0.29 and the extent of gene flow was 1.19 (Table 3).

Analysis of molecular variance

Analysis of molecular variance was carried out on the

overall ISSR data score of sesame accession without and with grouping (Tables 5 and 6). AMOVA without grouping revealed that higher percentage of variation (94.09%) is attributed to the within population variation while the remaining variation is due to the among population variation (5.90%). The grouping AMOVA analysis, considering the Ethiopian accessions as one group and the exotic ones as another, also showed 5.88% among populations within group variation, 94.09% within group variation and 0.026% among group variation (Table 6).

Table 5. Analysis of Molecular Variance (AMOVA) of sesame accessions in Ethiopia without grouping.

Source of variation	Sum of squares	Variance components	Percentage variation	Fixation	P
Among populations	62.647	0.15786	5.90	0.05903	0.00
Within population	240.021	2.51660	94.09		0.00
Total	302.667	2.67446			

Table 6. Analysis of Molecular Variance (AMOVA) of sesame accessions in Ethiopia with grouping.

Source of variation	Sum of squares	Variance components	Percentage variation	Fixation	p
Among groups	3.481	0.00069	0.02577	0.05903	0.00
Among populations within groups	59.166	0.15749	5.88792		0.00
Within populations	240.021	2.5166	94.08632		0.00
Total	302.667	2.67477			

The variations was found to be highly significant at $P=0.00$, 1000 permutations.

Genetic distance

Inter-population genetic distance (D) ranged from 0.031 to 0.165 for the total 19 population (Table 7). Administrative region based population classification of the Ethiopian sesame accessions revealed inter population genetic distance ranging from 0.041 to 0.16. Samples from Illubabore were distantly related to samples of Wello (0.150), Shewa (0.116) Harerge (0.144) and Gamo Goffa (0.134) (Table 8). Among the pairwise population comparisons made within Ethiopian population, samples from Welega and Harerge showed the highest genetic distance (0.160) and samples from Shewa and Harerge showed the least genetic distance (0.041). In general from the exotic accessions samples of South East Asia is distantly related to most of the Ethiopian accession. Genetic distance between the other pairwise combinations of populations was very low with the least genetic distance between samples of Mexico and Tanzania (0.031).

Clustering analysis

Cluster analysis of Ethiopian sesame populations revealed two major groups and three outliers (Cultivated, Welega and Illubabore). The first major cluster again forked into two sub groups the first containing Tigray, Harerge and Shewa populations, while the second contained Gamo Goffa, and Wello populations which are characterized as the lowest sesame producers of the country.

More over the migration of the welo people to the south may have its own impact on the clustering of Gamo Goffa

and Welo (Baker, 2001). Seeds of some sesame germplasm might have been transported from Welo to Gamo Gofa along with human (Figure 2). The second major cluster comprise of Gojam, Benishangul Gumuz and Gonder populations which embodies the north western parts of the country.

DISCUSSION

Genetic diversity and application of ISSR marker

Understanding of the extent and pattern of genetic variation can be useful for several purposes. Such information can be used to design effective germplasm conservation and for setting germplasm collection mission as well as to estimate or predict the risk of genetic erosion in certain area.

From breeding point of view, knowledge of pattern of genetic variability is useful for defining heterotic patterns in hybrid breeding and for relating the observed pattern with presence of certain economically important traits. Pattern of genetic variability can be studied by morphological, isozyme or molecular markers. Among the markers ISSR markers are important to study genetic variations in plant species, as they are effective in detecting very low levels of genetic variation (Zietkiewicz et al., 1994).

Generally, ISSR primers have high resolution power in diversity analysis of different crops. This marker were observed to be very useful in detecting genetic diversity and population structure of Tef (Assefa, 2003), Coffee (Aga, 2005; Tesfaye, 2006), Lentils (Edosa et al., 2007), and rice (Gezahegne et al., 2007) collected from different parts of Ethiopia.

In addition to the advantages (inexpensive, easy to generate), ISSRs are powerful in detecting polymorphisms with high reproducibility. ISSR can detect even more polymorphism than RFLPs in maize (Kantety et

Table 7. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) in 19 populations of Ethiopian and some exotic sesame accessions.

POP	BG	CU	GD	GG	GJ	HR	IL	SH	TG	WL	WO	SEA	MX	ZA	ZW	SO	SU	TZ	EG
BG	****	0.9377	0.9539	0.9073	0.9384	0.9117	0.9111	0.9302	0.9388	0.9142	0.9253	0.8799	0.9205	0.9311	0.9191	0.9078	0.9283	0.9401	0.9376
CU	0.0643	****	0.9319	0.9229	0.9161	0.9050	0.9088	0.9261	0.9381	0.9088	0.9067	0.8875	0.9131	0.9245	0.9132	0.9216	0.9068	0.9020	0.9093
GD	0.0472	0.0705	****	0.9497	0.9329	0.9336	0.9214	0.9405	0.9430	0.9067	0.9372	0.9389	0.9428	0.9421	0.8975	0.9212	0.9285	0.9389	0.9355
GG	0.0972	0.0803	0.0516	****	0.9436	0.9435	0.8746	0.9393	0.9306	0.9043	0.9482	0.9096	0.9512	0.9416	0.8843	0.9246	0.8960	0.9066	0.9165
GJ	0.0635	0.0876	0.0695	0.0581	****	0.9332	0.8939	0.9149	0.9383	0.8866	0.9215	0.8808	0.9108	0.9161	0.8942	0.9334	0.9224	0.9314	0.9171
HR	0.0924	0.0998	0.0687	0.0582	0.0691	****	0.8656	0.9594	0.9515	0.8524	0.9527	0.9014	0.9219	0.9149	0.8604	0.9259	0.9058	0.9131	0.9422
IL	0.0931	0.0957	0.0819	0.1340	0.1121	0.1443	****	0.8644	0.9132	0.8903	0.8605	0.8904	0.8682	0.9026	0.8584	0.8933	0.9153	0.9164	0.8479
SH	0.0723	0.0768	0.0613	0.0626	0.0890	0.0414	0.1457	****	0.9413	0.8983	0.9416	0.9214	0.9435	0.9277	0.8787	0.9386	0.9089	0.9112	0.9565
TG	0.0631	0.0639	0.0587	0.0720	0.0637	0.0497	0.0908	0.0605	****	0.9004	0.9187	0.9001	0.9091	0.9218	0.9205	0.9424	0.9445	0.9535	0.9445
WL	0.0898	0.0956	0.0980	0.1005	0.1203	0.1597	0.1162	0.1073	0.1050	****	0.8781	0.8743	0.8963	0.8988	0.9068	0.9051	0.8627	0.8933	0.8880
WO	0.0777	0.0980	0.0649	0.0532	0.0817	0.0485	0.1502	0.0602	0.0848	0.1300	****	0.8995	0.9428	0.9198	0.8806	0.9062	0.9024	0.9006	0.9353
SEA	0.1279	0.1194	0.0630	0.0948	0.1270	0.1038	0.1161	0.0819	0.1052	0.1344	0.1059	****	0.9177	0.8928	0.8630	0.8839	0.8927	0.8743	0.8941
MX	0.0828	0.0909	0.0589	0.0500	0.0934	0.0814	0.1413	0.0582	0.0953	0.1095	0.0590	0.0859	****	0.9692	0.8801	0.8983	0.8831	0.9117	0.9427
ZA	0.0714	0.0785	0.0596	0.0602	0.0876	0.0890	0.1025	0.0750	0.0815	0.1067	0.0836	0.1134	0.0313	****	0.8825	0.9071	0.9009	0.9188	0.9179
ZW	0.0844	0.0909	0.1081	0.1230	0.1119	0.1504	0.1526	0.1293	0.0828	0.0978	0.1271	0.1474	0.1277	0.1249	****	0.9033	0.9234	0.9012	0.9250
SO	0.0967	0.0817	0.0820	0.0784	0.0689	0.0770	0.1128	0.0633	0.0593	0.0998	0.0985	0.1234	0.1073	0.0975	0.1017	****	0.9271	0.9221	0.9253
SU	0.0744	0.0979	0.0741	0.1099	0.0808	0.0989	0.0885	0.0955	0.0571	0.1477	0.1027	0.1135	0.1243	0.1043	0.0797	0.0756	****	0.9040	0.9262
TZ	0.0617	0.1031	0.0631	0.0981	0.0711	0.0909	0.0873	0.0930	0.0476	0.1128	0.1046	0.1343	0.0925	0.0847	0.1040	0.0810	0.1009	****	0.9199
EG	0.0645	0.095	0.0666	0.0872	0.0865	0.0595	0.165	0.0444	0.0571	0.1188	0.0669	0.1119	0.059	0.0856	0.0779	0.0777	0.0766	0.0835	****

al., 1995) and more than AFLPs in rice (Blair et al., 1999).

In this study the extent and pattern of genetic variability among 82 Ethiopian and 38 exotic samples of sesame accessions were estimated using 5 di-nucleotide and one penta-nucleotide ISSR primer markers. The large number of accessions held in this study dictates the approach that can be employed. A quick, simple but reliable molecular protocol must be combined with an appropriate strategy for handling large sample sizes (Edosa et al., 2007; Edosa et al., 2010; Edosa et al., 2011 Gilbert et al., 1999). In this study, bulk sampling approach was chosen because it permits representation of the vast accession by optimum number of plants. Yang

and Quiros (1993) reported that bulked samples with 10, 20, 30, 40 and 50 individuals had resulted in the same RAPD profiles as that of the individual plant constituting the bulk sample. Edosa et al. (2007) used bulked samples for diversity assessment in lentil collected from Ethiopia. The technique revealed higher genetic diversity, and, therefore, validated the usefulness of bulk sample analyses.

This result also confirms that bulked leaf samples and ISSR marker is efficient in detecting polymorphism within and among populations and accessions of sesame. The present study suggested the existence of moderate level of diversity (0.23) among sesame accessions collected from Ethiopian Institute of Biodiversity Conservation.

Thus, ISSR marker systems will provide a useful tool in the future design of collection strategies for conservation and use of sesame accessions in Ethiopia. The ISSR technique was previously performed in sesame to study the genetic relationship of sesame germplasm in Korea by Kim *et al.* (2002). He used 14 reliable ISSR primers and found 33% polymorphism from 79 amplification products among the 75 sesame accessions. This is low when it is compared with this results percent polymorphism (75.86%). RAPD marker was also used by Abdellatif et al. (2008) in a set of 10 sesame germplasm collected from different regions of Sudan.

A total of 64 polymorphisms (6.4 polymorphic markers per primer) out of 75 reproducible products

Table 8. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) in 11 populations of Ethiopian sesame accessions.

Pop	BG	CU	GD	GG	GJ	HR	IL	SH	TG	WL	WO
Pop	****	0.9377	0.9539	0.9073	0.9384	0.9117	0.9111	0.9302	0.9412	0.9142	0.9253
BG	0.0643	****	0.9319	0.9229	0.9161	0.9050	0.9088	0.9261	0.9326	0.9088	0.9067
CU	0.0472	0.0705	****	0.9497	0.9329	0.9336	0.9214	0.9405	0.9384	0.9067	0.9372
GD	0.0972	0.0803	0.0516	****	0.9436	0.9435	0.8746	0.9393	0.9284	0.9043	0.9482
GJ	0.0635	0.0876	0.0695	0.0581	****	0.9332	0.8939	0.9149	0.9373	0.8866	0.9215
HR	0.0924	0.0998	0.0687	0.0582	0.0691	****	0.8656	0.9594	0.9536	0.8524	0.9527
IL	0.0931	0.0957	0.0819	0.1340	0.1121	0.1443	****	0.8644	0.9055	0.8903	0.8605
SH	0.0723	0.0768	0.0613	0.0626	0.0890	0.0414	0.1457	****	0.9437	0.8983	0.9416
TG	0.0606	0.0698	0.0636	0.0743	0.0647	0.0475	0.0993	0.0579	****	0.9029	0.9152
WL	0.0898	0.0956	0.0980	0.1005	0.1203	0.1597	0.1162	0.1073	0.1021	****	0.8781
WO	0.0777	0.0980	0.0649	0.0532	0.0817	0.0485	0.1502	0.0602	0.0886	0.1300	****

BG, Benishangul Gumuz; CU, cultivated; GD, Gonder; GG, Gamo Goffa; GJ, Gojam; HR, Harerge; IL, Illibabore; SH, Shewa; TG, Tigray; WL, Welega; WO, Welo; SEA, South East Asia; MX, Mexico; ZA, Zambia; ZW, Zimbabwe; SO, Somalia; SU, Sudan; TZ, Tanzania; EG, Egypt.

(7.5 fragments per primer) were obtained from the 10 primers used and low level of genetic similarity among accessions (Abdellatef et al., 2008). Seleshi (2008) studied the genetic divergence of 100 sesame accessions using 13 agro-morphological traits from EIAR collection and found wide variability for all the measured characters which agrees with this result.

The success of a crop-improvement program largely depends on the availability and knowledge of the genetic resources in a germplasm collection. In the present study all the diversity parameters confirmed that there is higher gene diversity in Ethiopian accessions than exotic accessions. Since areas of high genetic diversity contribute more accessions than those with a low diversity for further and future collection, breeding and conservation activities high priority should be given to areas with high genetic diversity. The result of this study exhibited among the ten Ethiopian areas moderate to higher genetic diversity is revealed by the accessions from the Welega, Tigray and Shewa areas. Hence, for selection based population improvement these areas have a high potential as compared to areas with lower genetic diversity like Gojam and Harerge.

Genetic distance and implication for improvement

Genetic distance is a measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations or species. In this study, inter-population genetic distance for the whole population (D) ranged from 0.031 to 0.165. From the Ethiopian populations comparatively samples from Illubabore, Harerge, Gamo Goffa, Gojam and Wello showed moderate to high genetic distance related to their respective pair wise comparison. Samples from Benishangul Gumuz, Gonder and Tigray relatively found to be more

related with each other and with other pair wise comparison showing low to very low genetic distance. Since the bulk of Ethiopian sesame production is coming from the later places this close similarity can be explained by exchange of the sesame seed among neighboring localities in the north and northwest. Although the distance between exotic accessions and local accessions varied from population to population on the average, the local accessions were separated from the exotic ones by considerable amount the south eastern Asian accessions being the most distant.

Genetic structure, patterns of distribution and implication for conservation

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation, and population isolation), genetic drift, mating system, gene flow and selection (Schaal et al., 1998). In the present study there was moderate level of genetic differentiation ($G_{ST} = 0.25$) among the Ethiopian sesame accessions.

Analysis of molecular variance (AMOVA) using 32 AFLP marker for sesame accessions originate from five different geographical regions representing the proposed diversity centers for sesame: India, Africa, China-Korea-Japan, Central Asia and Western Asia indicates that 5% of the variance among the patterns was due to differences among groups and 95% was due to differences within groups (Laurentin and Karlovsky 2006).

In another study, Endale et al. (2008), using SSR marker, also found 41.2% of the total genetic variation between population and 58.8 % within population for 50 sesame landraces of Ethiopia. Similarly, the result of this study is also in agreement with the above AFLP and SSR

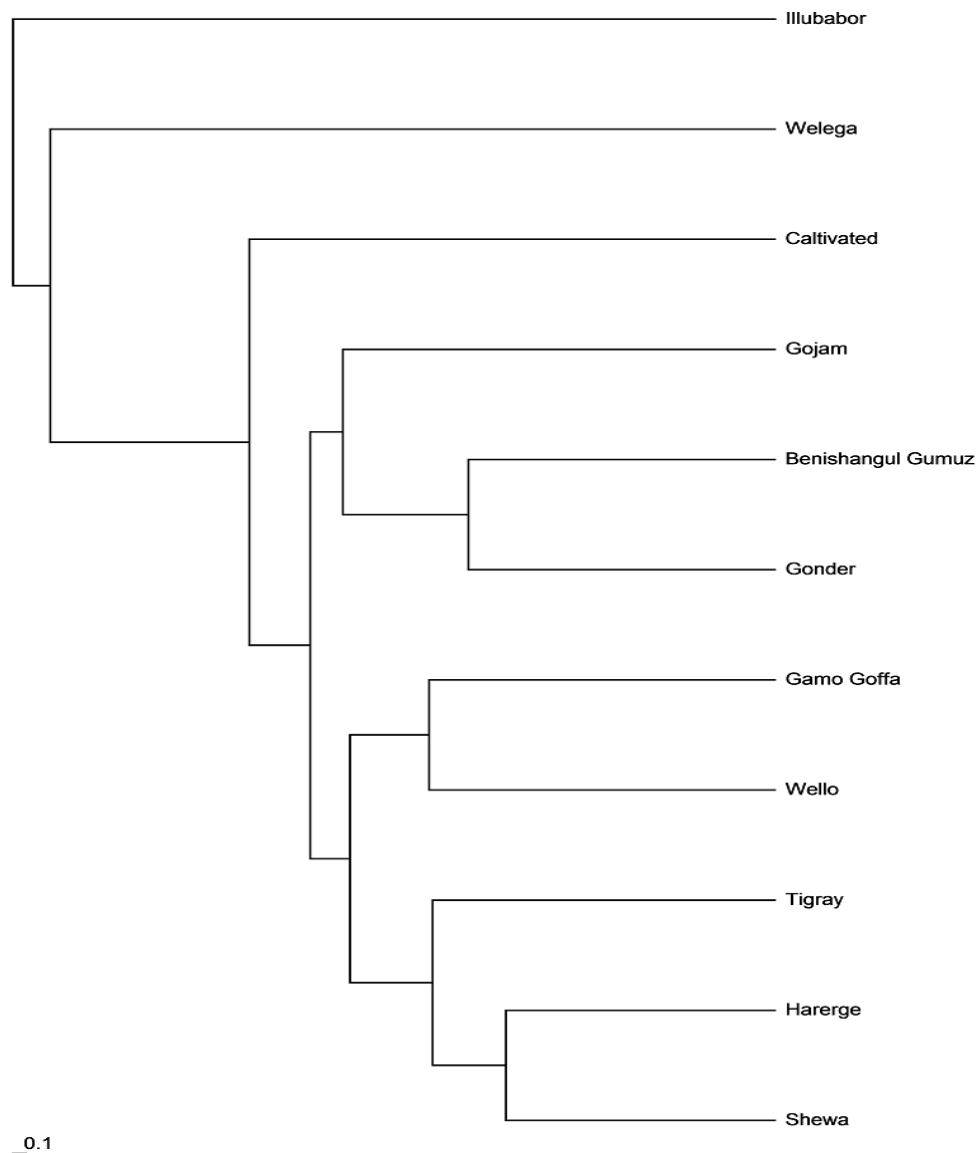


Figure 2. NJ based dendrogram for 11 Ethiopian sesame populations using 6 ISSR (5 di and 1 penta nucleotide) primers.

analysis.

In this study, the AMOVA analysis showed highly significant ($P=0.00$) genetic differences among populations and within populations. Of the total variation, 5% attributed to among population and 95% attributed to within populations. Even though based on AMOVA analysis, a high estimate of genetic differentiation between populations of inbred species is expected this may not be a general truth if there is high gene flow represented by seed movement through human involvement (Edossa et al., 2007).

Moreover, pollen movement facilitated by insect could also have a role for the observed pattern. The lower among group variance of this study can be explained in line with the above argument. Gene flow is the exchange or movement of gametes, individuals, and populations on

a geographic scale (Joseph and Bruce 1993). Gene flow, in conjunction with other evolutionary forces, can result in the spread of single genes (or DNA sequences), genotypes, and even the establishment of whole populations in different regions. The movement of one individual per generation between populations is sufficient to prevent substantial differentiation between those populations (Joseph and Bruce, 1993).

The values obtained from Nm , the product of the effective size of individual populations (N) and the rate of migration among them (m); show the approximate number of individuals migrating from one population to the other, in a typical island model. Generally, if $Nm < 1$ local differentiation of populations will result, and if $Nm > 1$ there will be little differentiation among populations (Wright, 1951). The overall Nm (1.28) value of this study

is considered to be higher according to Slatkin (1981, 1985), Caccone (1985) and Waples (1987) grouping. They grouped Nm values into three categories: high ($Nm > 1.000$), intermediate, (0.250 - 0.990), and low (0.000 - 0.249). The fact that Nm value of this study is higher indicates gene flow between populations is obvious which will agree with the AMOVA result showing there is low variation among population.

The G_{ST} value of this result also shows the lack of population differentiation which goes with Laurentin and Karlovsky (2006) work. They found a G_{ST} value of 0.20 (below this study 0.25) and suggested the lack of association between geographical origin and population differentiation. In their finding particularly Indian, African and Chinese-Japanese-Korean accessions are distributed throughout clusters in UPGMA analysis and the whole two-dimensional space in PCA. Toan et al. (2011) also found an intermediate estimate of gene flow (Nm) value of 0.38 on average in their study. This lack of association between geographical distribution and classification based on molecular markers in sesame was explained by the exchange of sesame seeds among widely separated locations which could be due to movement/migration of peoples from one place to another along with their own seed or the purchase of seeds from other places for cultivation. These results also became apparent from the cluster analysis.

Conclusion

This study shows that bulk sampling strategy for DNA analysis and ISSR marker are important for genetic diversity study in sesame. The study also shows, among the sesame accessions held by IBC, in comparison, Ethiopian accessions are more diverse than the exotic ones. Separate analysis of the Ethiopian accession revealed there are places with higher genetic diversity which will be valuable for the future collection, conservation and improvement strategies. Regions that showed relatively high level of genetic diversity should be the main focus for future collection and population improvement based on selection. The present study also showed weak association between genetic variation of sesame accessions and their ecological regions of origin. The most likely important factor affecting the genetic structure of sesame in this region is possibly human activities. This shows there could be a possibility of sampling plants with the same genetic constitution from different administrative regions. This problem becomes severe if an administrative region is represented by small sample as in the case of IBC.

Conflict of interest

The authors have not declared any conflict of interest.

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