GENETIC VARIABILITY AMONG LANDRACES OF SESAME IN ETHIOPIA

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ABSTRACT

Sesame (*Sesamum indicum* L.) is an ancient oil crop that has been referred to as the 'Queen of oilseeds' by virtue of its high quality oil. Ten Simple Sequence Repeats (SSRs) markers were used to study patterns of genetic variation within and among 50 sesame populations representing the existing Ethiopian collections. The number of alleles ranged from 6 to 17, with an average of 12.1 per locus. The Polymorphism Information Contents (PICs) of the markers ranged from 0.393 to 0.820. The observed heterozygosity (Ho) for landraces and cultivars ranged from 0.060 to 0.440 and 0.120 to 0.260, with a mean of 0.234 and 0.193, respectively, indicating the existence of higher heterozygosity within the landraces than cultivars. Landraces (He=0.377) showed higher Nei's average genetic diversity than the cultivars (He=0.305). The fixation index (Fst) values for the landraces and cultivars were 0.427 and 0.26, respectively, indicating that genetic divergence between populations was smaller than genetic divergence within. High outcrossing of 45 and 46.4% for landraces and cultivars, respectively, indicates that both landraces and cultivars are line mixtures and segregants of past outcrossing results. A Neighbour-Joining dendrogram based on Modified Rogers' Distances (MRD) and a principal coordinate analysis based on a simple dissimilarity matrix, points towards some grouping based on geographical origin. The established SSR markers will be very useful for further diversity analyses among a large landrace collection of Ethiopian sesame and in the establishment of a core collection. Moreover, existence of great genetic variability between the landraces is a good starting point of sesame crop improvement programs in Ethiopia.

Key Words: Markers, *Sesamum indicum*, simple sequence repeat

RÉSUMÉ

La Sésame (*Sesamum indicum* L.) est une culture à huile anciennement dénomée "Reine de graines oléagineuses", en vertu de son huile de haute qualité. Dix marqueurs à répétition simple de séquences (SSR) étaient utilisés pour étudier le modèle de variation génétique, parmi et au sein de 50 populations de sésames éthiopiennes représentant les collections existantes locales. Le nombre d'allèles variait de 6 à 17 avec une moyenne de 12,1 par locus. Le contenu d'informations polymorphiques (PICs) des marqueurs variait entre 0.393 et 0.820, ce qui montre une nature hautement informative des SSR ici présentées. L'hétérozygosité observée (Ho) pour les races locales et les cultivars variait de 0,060 à 0,440 et de 0,120 à 0,260, avec une moyenne de 0,234 et 0,193 respectivement, indiquant l'existence d'une héterozygosité plus élevée dans les races locales que dans les cultivars. Les races locales (He=0.377) ont montré une Nei avec une moyenne de diversité génétique plus élevée que celle des cultivars (He= 0.305). Les valeurs d'index de fixation (Fst) pour les races locales et les cultivars étaient de 0.427 et 0.26 respectivement, montrant que la divergence génétique entre les populations était plus petite que la divergence génétique parmi les populations. De façon surprenante, 45 et 46.4% des hauts croisements étaient estimés pour les races locales et les cultivars respectivement, montrant ainsi que les races locales et les cultivars sont des lignées mixtes séparant les résultats des anciens croisements. Un dendogramme développé sur base des Distances modifiées

de Rogers (DMR), et l'analyse de principales coordonnées basée sur une simple dissemblance matricielle montrent quelques groupements sur base de l'origine géographique. Les marqueurs (SSR) établis seront très utiles pour des analyses supplémentaires de diversité parmi une large collection de races locales de sésames ethiopiennes et dans l'établissement d'une collection de base. En outre, l'existence d'une grande variabilité génétique entre les races locales est un bon point de départ pour des programmes d'amélioration de la culutre de sésame en Ethiopie.

Mots Clés: Marqueurs, *Sesamum indicum*, répétition de séquences simples

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an ancient oil crop that has been referred to as the 'Queen of oilseeds' by the virtue of its high quality oil (Salako and Falusi, 2001). Sesame belongs to the *Pedaliaceae* family, which contains 60 species organised into 16 genera (Rendle, 1963; Ashri, 1998). The chromosome number of the cultivated sesame was reported as 2n = 26 (Toan *et al.*, 2009). Sesame is generally considered a self-pollinated crop despite varying degrees of natural crosses ranging from less than 10% (Khidir, 1972) to over 50% (Rheenen, 1980). Sesame is basically a crop of the tropics and subtropics, but whose origins are still debated. Ethiopia is considered to be the original home, although there are considerable arguments in favour of India as the other possible centre, of origin (Seegeler, 1983).

In Ethiopia, sesame breeding based on introduction and pure line selection was started in the late 1960s by Werer Agricultural Research Centre (WARC) involving materials from indigenous landraces as well as exotic sources (Tadele, 2005). Since then ten cultivars have been released and more than 300 landraces collected.

However, variability among these collections is unknown; information about their morphological traits is available (WARC, 2006). Information about genetic diversity and relationships among sesame landraces is vital for crop improvement. Information on the use of molecular markers for the characterisation of genetic diversity in sesame is limited. Little information is available on the use of molecular markers such as isozyme (Isshiki and Umezaki, 1997), RAPD (Bhat *et al*., 1999), ISSR (Kim *et al*., 2001), SSRs (Dixit *et al*., 2005) and AFLP (Laurentin and Karlovsky, 2006; Ali *et al.*, 2007).

SSRs are clusters of short tandemly repeated nucleotide bases and have proved the most valuable polymerase chain reaction (PCR) based DNA markers in genetic diversity analysis of many crop species. They are characterised by high variability, co-dominant nature, great abundance and even distribution throughout genomes. For SSRs, each location in a chromosome that contains core repeats may have a different number of copies of the repeat. Polymorphism is based on the number of tandem repeats and, therefore, the length of PCR products (Dreisigacker *et al*., 2005; Blair *et al*., 2006; Milee *et al.,* 2008). The development of SSRs markers could be based on the screening of genomic DNA libraries specifically constructed for the discovery of repeated sequences in the genome.

Ten SSR markers have been developed (Dixit *et al*., 2005). However, they have not yet been used to assess the genetic diversity of different sesame landraces. The objective of this work was, therefore, to study the genetic diversity of cultivated sesame in Ethiopia based on SSR markers.

MATERIALS AND METHODS

Plant materials. Seeds of 47 landraces of sesame collected from different sites (Fig. 1) covering major sesame growing areas in Ethiopia and three nationally released cultivars named K74 (Kelafo 74), M80 (Mehado- 80) and Tate (Table 1) were used in the study. Five seeds from each population (landraces/cultivars) were planted separately in soil in a 77 cell tray in a green house. Young leaves (2-3 leaves) free of
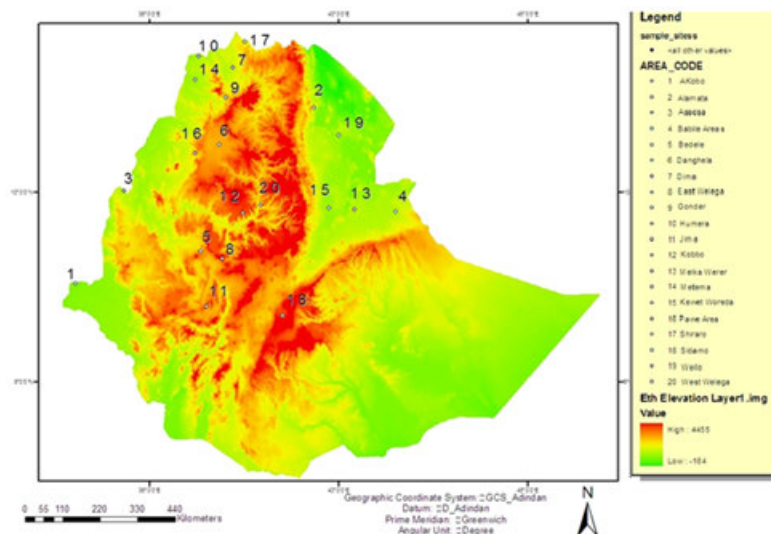
Figure 1. Collection sites of sesame landraces. The forty seven landraces are collected from twenty different collection sites shown in this Figure.

TABLE 1. Sesame landraces/cultivars collected from various parts in Ethiopia

Entry no.	Landraces/ cultivars name	Collection area	Region	Entry no.	Landraces/ cultivars name	Collection area	Region
1	BG019	Danghela	Amara	26	Acc202433	Wello	Amara
2	GA005	AKobo	Gambella	27	J02	Jima	Oromia
3	EW011	East Welega	Oromia	28	Mikadra	Humera	Tigray
4	EW009	East Welega	Oromia	29	Tate*		
5	WW001	West Welega	Oromia	30	AccBG001	Assosa	Benishangul -Gumuz
6	Shiraro	Shiraro	Tigray	31	GA009	Akobo	Gambella
7	Niguara	Alamata	Tigray	32	J01	Jima	Oromia
8	WoldyaKobo	Kobbo	Amara	33	Tejahir	Metema	Amara
9	KeySelit	Metema	Amara	34	Tajareb	Metema	Amara
10	BCS043	Melka Werer	Afar	35	Hirhir	Humera	Tigray
11	Acc11323	Danghela	Amara	36	J03	Jima	Oromia
12	BM52	Melka Werer	Afar	37	G03	Akobo	Gambella
13	Beshbesh	Humera	Tigray	38	GojamAzene	Pawe	Benishangul-Gumuz
14	Gobyo82	Wello	Amara	39	B02	Bedele	Oromia
15	Acc07	Humera	Tigray	40	BG004	Danghela	Amara
16	BCS010	Melka Werer	Afar	41	AccN5003	Kewet Woreda	Amara
17	Tejin	Gonder	Amara	42	Acc18	Wello	Amara
18	Acc212992	Sidamo	SNNPR	43	Key Selit 2	Wello	Amara
19	K74*			44	Hirhir2	Metema	Amara
20	Dima	Dima	Tigray	45	M80*		
21	Acc205173	Gonder	Amara	46	BM50	Melkawerer	Afar
22	Acc08	Metema	Amara	47	Assosa	Assosa	Benishangul-Gumuz
23	7B	Melka Werer	Afar	48	BCS022	Melka Werer	Afar
24	Acc111502	Danghela	Amara	49	Acc09	Humera	Tigray
25	Abunam	Metema	Amara	50	Acc 208672	Babile area	Oromia

*Cultivars

disease infestation were collected and dried in filter paper bags on the silica gel for 48 hours.

DNA extraction, primer selection and polymerase chain reaction. Genomic DNA was extracted from dried leaf tissues employing a modified CTAB procedure (Saghai Maroof *et al.*, 1984). DNA samples were stored at –20 °C in TE buffer until the measurement of concentration. The concentration of the DNA fragments was estimated against lambda DNA standards at concentrations of 10 and 50 μl and DNA samples were diluted accordingly. A total of 13 SSR markers were selected for this study. Three primer pairs for novel SSR markers (Si-ssr8910, Si-ssr8914 and Si-ssr8920) have been developed employing Primer Premier 5.0 Software (Lalitha, 2000) using publicly available Expressed Sequence Tags (ESTs) for sesame obtained from the National Centre for Biotechnology Information (NCBI).

Ten sesame SSR markers were selected from literature (Dixit *et al.*, 2005). The forward primers of each of these 13 primers were labeled with fluorescent dyes, namely tetrachloro-6-carboxyfluorescien (TET; green colour), Hexachloro-6-carboxyfluorescein (HEX; black colour) or 6-carboxyflourescein (6-FAM; blue) at the 5' end of the primer. The 10 SSRs primers that produce amplified products are listed in Table 2. PCR conditions were maintained and each PCR reaction was carried out in a 10 μl reaction volume containing bidest water, 1.5 mM MgCl2, 1 x 1.5 mM MgCl2 of 10 x PCR buffer, 0.2 mM of deoxyribonucleotides, 250 nM of each forward and reverse primers, 0.5 units of Taq DNA polymerase and 10 ng of DNA. The temperature profile used for PCR amplification comprised a denaturation step at 94 °C for 1 minute, followed by primer annealing temperature at 45.2 -53 °C for 1 minute, and elongation at 72 °C for 1 minute. After 34 cycles, the reaction was terminated with 10 min at 72°C for the final extension.

The PCR reaction was carried out under the same conditions for all the primers except for the annealing temperatures. The PCR products were electrophoresed on 3% Metaphor Agarose Gel (9 g of metaphor and 300 ml of 1xTBE) at

TABLE 2. Simple sequence repeats markers and their characteristics

SSR locus	Gene bank accession no.	Primers (5'-3')	Dye type	Repeat motif/core	Annealing Temp (°C)
GBssr-sa-05[1]	AY838904	F-TCATATATAAAAGGAGCCCAACR-GTCATCGCTTCTCTCTTCTTC	HEX	$(CT)_{13}$	45.2
GBssr-sa-08[1]	AY838905	F-GGAGAAATTTTCAGAGAGAGAAAAR-ATTGCTCTGCCTACAAATAAAA	FAM	$(AG)_{17}$	53.0
GBssr-sa-09[1]	AY838907	F-CCCAACTCTTCGTCTATCTCR-TAGAGGTAATTGTGGGGGA	TET	$(CT)_{18}$	46.2
GBssr-sa-72[1]	AY838913	F-GCAGCAGTTCCGTTCTTGR-AGTGCTGAATTTAGTCTGCATAG	FAM	$(CT)_{9}$	55.9
GBssr-sa-108[1]	AY838915	F-CCACTCAAATTTTCACTAAGAAR-TCGTCTTCCTCTCTCCCC	TET	$(GA)_{7}{}^{*}(GA)_{15}$	51.0
GBssr-sa-123[1]	AY838916	F-GCAAACACATGCATCCCTR-GCCCTGATGATAAAGCCA	HEX	$(TC)_{21}{}^{*}(TC)_{15}$	51.0
GBssr-sa-182[1]	AY838921	F-CCATTGAAAACTGCACACAAR-TCCACACACAGAGAGCCC	FAM	$(AT)_{11}{}^{*}(TC)_{18}{}^{*}(TG)_{12}$	53.0
GBssr-sa-184[1]	AY838922	F-TCTTGCAATGGGGATCAGR-CGAACTATAGATAATCACTTGGAA	HEX	$(TC)_{20}$	51.0
Si-ssr8910[2]	AY838910	F-CGATGGGCTCGGAATGAAR-CCACCGTCAGCGACTTCA	TET	$(TTG)_{x}$	45.2
Si-ssr8920[2]	AY838920	F-GACCCTAAATCTCAACCAR-ACTATGGCCTTGAAACT	FAM	$(GA)_{12}$	45.2

1 = SSR loci selected from literature, Dixit et al. (2005); 2 = Newly developed EST derived SSR loci

140 V for 2h, DNA ladder (50 bp) of 4 µl was used for comparison. The gels were stained consequently with ethidium bromide (5 µg ml^{-1}) for 10 minute, de-stained for 15 minute in distilled water and visualised under a UV transilluminator and the picture captured with a digital camera.

Capillary electrophoresis. A MegaBACE sequencer (Amersham Biosciences) was used to separate PCR amplification products. Individual PCR products were pooled (1-3 markers run^{-1}) according to their fluorescent label to permit discrimination of the individual markers after electrophoretic separation. A 96 well PCR plate format and transfers using eight channel pipettes were used throughout. The internal size standard was labeled with ET-ROX (ET 400-R, Amersham biosciences). The final cocktail of the samples contained 0.5 µl of multiplexed PCR products and 5 µl of diluted ET-ROX standard (1:20 dilution with loading solution). Samples were incubated for 1 minute at 94 °C and cooled down before quick centrifuging, and then successively loaded in the MegaBACE and run for approximately 2 hours.

Statistical analysis. The fragment sizes were assessed using MegaBACE Fragment Profiler (Amersham Biosciences) software. The resulting SSR data were analysed statistically using the software 'Tools for Population Genetic Analysis'' (TFPGA) version 1.3 (Miller, 2000) to calculate a distance matrix between pairs of sesame population based on modified Rogers' distance (MRD) measure (Goodman and Stuber, 1983). The genetic diversity of each SSR marker was measured in terms of number of alleles per locus, allele frequencies, observed heterozygosity (Ho) and Expected heterozygosity (He) also known as Nei's average gene diversity using the "Population Genetics" (PopGene) software version of 1.31 (Yeh et al., 1999).

The polymorphic information content (PIC) for each marker was determined as described by Anderson et al. (1992):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2$$

Where $p^2 i$ is the frequency of *ith* allele.

Cluster analysis was performed by using MRD estimate values and dendrogram constructed to visualise the relationship among the 50 population based on the Neighbour-Joining method using the software package "Molecular Evolutionary Genetics Analysis'' (MEGA, Kumar et al., 2004). For better comparability, a principal coordinate analysis (PCoA) was performed using DARWIN V5 software (Perrier et al., 2003). The fixation index (*F*st) which characterises the partitioning of diversity between and within population was calculated as described by Wright (1978) as follows:

$$F_{st} = \frac{H_T - H_S}{H_T}$$

Where H_T is the expected heterozygosity in the total population and H_s the mean expected heterozygosity within subpopulations.

Allele frequencies of SSR loci were used to calculate mean expected heterozygosity within sub populations (H_s) and expected heterozygosity in the total populations (H_T). The outcrossing rate was estimated based on the equilibrium inbreeding coefficient (F$_e$) under partial selfing:

$$F_e = \frac{1-t}{1+t}$$ (Crow and Kimura, 1970). This equation can be transformed to: $t = \dfrac{1-F_e}{1+F_e}$

$$F_e \text{ equals } 1 - \frac{H_e}{H_r}$$

where H_e is the equilibrium heterozygosity at a given locus, and H_r the heterozygosity expected under random mating (Hardy - Weinberg Equilibrium).

For estimating the outcrossing rate, H_e was replaced by the observed heterozygosity, and H_r replaced by Nei's average gene diversity, which equals the expected heterozygosity assuming Hardy-Weinberg Equilibrium. The resulting estimate is designated outcrossing at inbreeding equilibrium (t$_e$).

RESULTS AND DISCUSSION

Levels of polymorphism for SSR markers. Screening of publicly available ESTs data base for sesame resulted in the identification of 3 SSRs. Ten additional SSRs were selected from literature. All 13 SSRs markers were screened using 4 DNA samples of sesame landraces. Two markers (GBssr-sa-33 and GBssr-sa-173) from literature and one makers developed from publicly available ESTs for sesame Si-ssr8914 failed to produce amplification products and were discarded from the study. The two SSRs developed from publicly available ESTs database that produced amplification products were Si-ssr8910 and Si-ssr8920 and together with the 8 SSRs selected from literature are listed in Table 3. All SSR markers showed polymorphism. Overall, 121 alleles were detected using the 10 SSR markers (Table 3). The observed number of alleles (NA) per SSR marker ranged from 6 to 17 with a mean of 12.1 alleles per marker (Table 3). The maximum number of alleles was detected at marker Si-ssr8920 (NA=17). A wide range of fragment sizes was observed from 135bp to 371bp (Table 3).

The SSR markers in our study showed a higher number of alleles than previous analysis of the diversity of sesame accessions conducted with SSR markers (Dixit *et al.*, 2005). This was probably because Ethiopia is considered the origin and centre of diversity of cultivated sesame (Seegeler, 1983). This is further shown by the observed heterozygosity (H_o) that ranged from 0.044 to 0.608, with an average value of 0.232 which is higher than previous study (Dixit *et al.*, 2005). The higher heterozygosity indicates a higher outcrossing rate of Ethiopian sesame populations, which seemed to be line mixtures rather than pure lines. The PIC value of each SSR marker demonstrates the informativeness of the markers. Values of PIC are ranging from 0 to 1 and loci having PIC values near to 1 are more desirable (Mateescu *et al.*, 2005). The average PIC value for all 10 SSR marker loci was 0.634 with a range from 0.393 (GBssr-sa-08) to 0.820 (Si-ssr8920). The highest PIC value of =0.820 was observed for marker Si-ssr8920 (Table 3) and is similar to a previous result found in Korean sesame (Dixit *et al.*, 2005).

TABLE 3. Diversity of the 10 SSR markers in the sesame collection from Ethiopia

Locus SSR	Gen bank accession name	Observed alleles (NA)	Observed heterozygosity (H_o)	Polymorphism information content (PIC)	Fragment size range of alleles (bp)
SSR loci from literature					
GBssr-sa-05	AY838904	13	0.564	0.680	259-362
GBssr-sa-08	AY838905	6	0.084	0.393	135-159
GBssr-sa-09	AY838907	13	0.208	0.800	162-257
GBssr-sa-72	AY838913	11	0.384	0.427	205-278
GBssr-sa-108	AY838915	9	0.080	0.682	184-215
GBssr-sa-123	AY838916	12	0.076	0.747	213-314
GBssr-sa-182	AY838921	16	0.224	0.493	207-315
GBssr-sa-184	AY838922	12	0.044	0.661	154-195
Newly ESTs derived SSR loci					
Si-ssr8910	AY838910	12	0.048	0.637	278-371
Si-ssr8920	AY838920	17	0.608	0.820	209-304
Total		121			
Mean		12.1	0.232	0.634	

Genetic diversity. Genetic diversity measurements of the 50 sesame populations are presented in Table 4. The average numbers of alleles (Na) per locus were 2.4 for landraces and 2.0 for cultivars. The number of alleles ranged from 1.4 to 4.2 for landraces and 1.5 to 2.9 for cultivars. The highest average number of alleles was observed in landrace EW011 (Na = 4.2), followed by BG019 (Na = 3.4) and Acc202433 (Na = 3.3); while landraces Beshbesh and Tejareb (Na = 1.6) and Tejin and Acc07 (Na = 1.4) showed the least allelic richness. The cultivars M80 (Na = 2.9) and Tate (Na = 1.5) showed the highest and lowest allelic richness, respectively.

The heterozygosity for landraces ranged from 0.060 to 0.440. Landrace EW011 showed the highest heterozygosity of 0.440, followed by landraces Acc202433 (Ho=0.420), and J02 and EW009 (Ho=0.380). Landraces GA005 and BG004 (Ho=0.080) showed the least observed heterozygosity, followed by BCS010 (Ho=0.060). High average heterozygosity was observed both in the landraces (0.234) and cultivars (0.193), which was an indication of the existence of high outcrossing in the landraces and cultivars. The proportion of polymorphic loci per marker ranged from 20 to 100% (Table 4).

The average of polymorphic loci was 74.04 for landraces and 63.33% for cultivars. Among the 50 lines evaluated, landrace EW011 was the most diverse with Nei's average gene diversity of 0.658 and Tejin (0.106) was the least diverse. The landraces were more diverse than the cultivars. Nei's average gene diversity for landraces was 0.377 and 0.305 for cultivars (Table 4). However, the differences in all genetic diversity indices between landraces and cultivars was not statistically significant (P<0.05). But significantly (P<0.05) lower mean average number of alleles, mean observed heterozygosity, mean Nei's average gene diversity and percentage of polymorphic loci were observed in cultivars than in landraces. This indicates that landraces were genetically more diverse than the cultivars. Hence, landraces seemed to be rather line mixtures compared to the cultivars.

Genetic differentiation and outcrossing. The fixation index for the cultivars indicated that 26% of the diversity was between cultivars and 74% of the diversity was within, while the Fst value for the landraces was 43% of the variation between and 57.3% of the variation within the landraces. The higher value of Fst in landraces was unexpected as usually greater differentiation is expected between more advanced materials or cultivars (Hamrick and Godt, 1997). The small sample size of 5 individuals used in our study could be one explanations for this result. However, the result may also illustrate that the cultivars were not at all pure lines but still diverse line mixtures. Outcrossing is a very important aspect to be considered in order to establish strategies for plant breeding or landrace management and conservation (Rheenen, 1980).

In this study, we used a rough estimate of outcrossing from observed heterozygosity and Nei's average gene diversity. Different authors reported contradicting results concerning the outcrossing the percentage of sesame (Khidir, 1972; Rheenen, 1980). We also observed a wide range of outcrossing in the landraces (9.1 to 96.4%) and cultivars (15.5 to 77.3%) with means of 45 and 46.4%, respectively (Table 4). This indicated a mixed mating system. In some cases, the value of Ho was higher than the value of He resulted in outcrossing values higher than 100% and those unreasonable results were omitted from Table 4.

The observed heterozygosity exceeded that expected under Hardy-Weinberg Equilibrium in some cases (Table 4). According to Lowe *et al*. (2004) our result can be explained by the presence of heterozygote advantage or the heterogeneity of environments in which the landraces were cultivated.

Cluster analysis. A Neighbour-Joining dendrogram was generated based on MRD values (Fig. 2). Overall, 3 clusters were formed. Cluster I comprised of 12 landraces, 1 each from Gambella and Benishangul-Gumuz regions and 10 of them originated from Amara and Tigray two major sesame growing regions. The two regions are found in the northern part of the country.

TABLE 4. Summary of genetic diversity measures of the 50 sesame populations obtained from Ethiopia

Population	Na^1	H_o^2	H_e^3	P^4	$t_e^5(\%)$	Population	Na^1	H_o^2	H_e^3	P^4	$t_e^5(\%)$
Landraces						Landraces					
BG019	3.4	0.260	0.530	100	32.5	AccBG001	2.3	0.180	0.362	80	33.1
GA005	2.8	0.080	0.480	100	9.1	GA009	2.6	0.300	0.392	70	62
EW011	4.2	0.440	0.658	100	50.2	J01	2.3	0.220	0.376	80	41.4
EW009	3.0	0.380	0.488	90	63.8	Tejahir	1.7	0.160	0.212	40	60.6
WW001	3.0	0.360	0.494	90	57.3	Tajareb	1.6	0.240	0.188	40	*
Shiraro	2.4	0.220	0.400	80	37.9	Hirhir	2.2	0.200	0.392	80	34.2
Niguara	2.8	0.220	0.488	90	29.1	J03	2.6	0.220	0.426	90	34.8
Woldyakobo	2.7	0.360	0.458	80	67.4	G03	2.4	0.200	0.370	80	37
KeySelit	3.2	0.340	0.586	80	40.9	GojamAzene	2.1	0.240	0.318	80	60.6
BCS043	2.1	0.280	0.332	70	72.9	B02	1.8	0.120	0.206	60	41.1
Acc11323	2.1	0.320	0.326	60	96.4	BG004	2.2	0.080	0.336	60	13.5
BM52	2.9	0.300	0.528	100	39.7	AccN5003	2.7	0.200	0.410	80	32.3
Beshbesh	1.6	0.320	0.244	50	*	Acc18	3.3	0.220	0.518	90	27
Gobyo82	2.3	0.300	0.360	60	71.4	KeySelit2	2.1	0.160	0.360	70	28.6
Acc07	1.4	0.260	0.154	30	*	Hirhir 2	2.3	0.100	0.372	80	15.5
BCS010	2.0	0.060	0.302	60	11	BM50	2.4	0.200	0.408	90	32.5
Tejin	1.4	0.160	0.106	20	*	Assosa	2.5	0.200	0.374	80	36.5
Acc212992	2.0	0.140	0.292	60	31.5	BCS022	2.6	0.240	0.460	80	35.3
Dima	1.8	0.140	0.222	40	46.1	Acc09	2.0	0.220	0.292	60	60.4
Acc205	2.2	0.140	0.362	70	24	Acc208672	2.9	0.220	0.446	80	32.7
Acc08	2.6	0.220	0.334	80	49.1	Average	2.4	0.234	0.377	74.04	45
7B	2.4	0.280	0.318	80	78.7	Cultivars					
Acc111502	2.7	0.280	0.406	70	52.6	K74	1.8	0.260	0.298	70	77.3
Abunam	1.8	0.240	0.264	80	83.3	M80	2.9	0.120	0.446	80	15.5
Acc202433	2.8	0.420	0.484	100	76.6	Tate	1.5	0.200	0.172	40	*
Mikadra	2.4	0.200	0.398	80	33.6	Average	2.0	0.193	0.305	63.33	46.4

1 = Average number of alleles; 2= Observed heterozygosity; 3 = Nei's average gene diversity; 4 = Percentage of polymorphic loci at the 5 % level; 5 = Equilibrium outcrossing percentage;
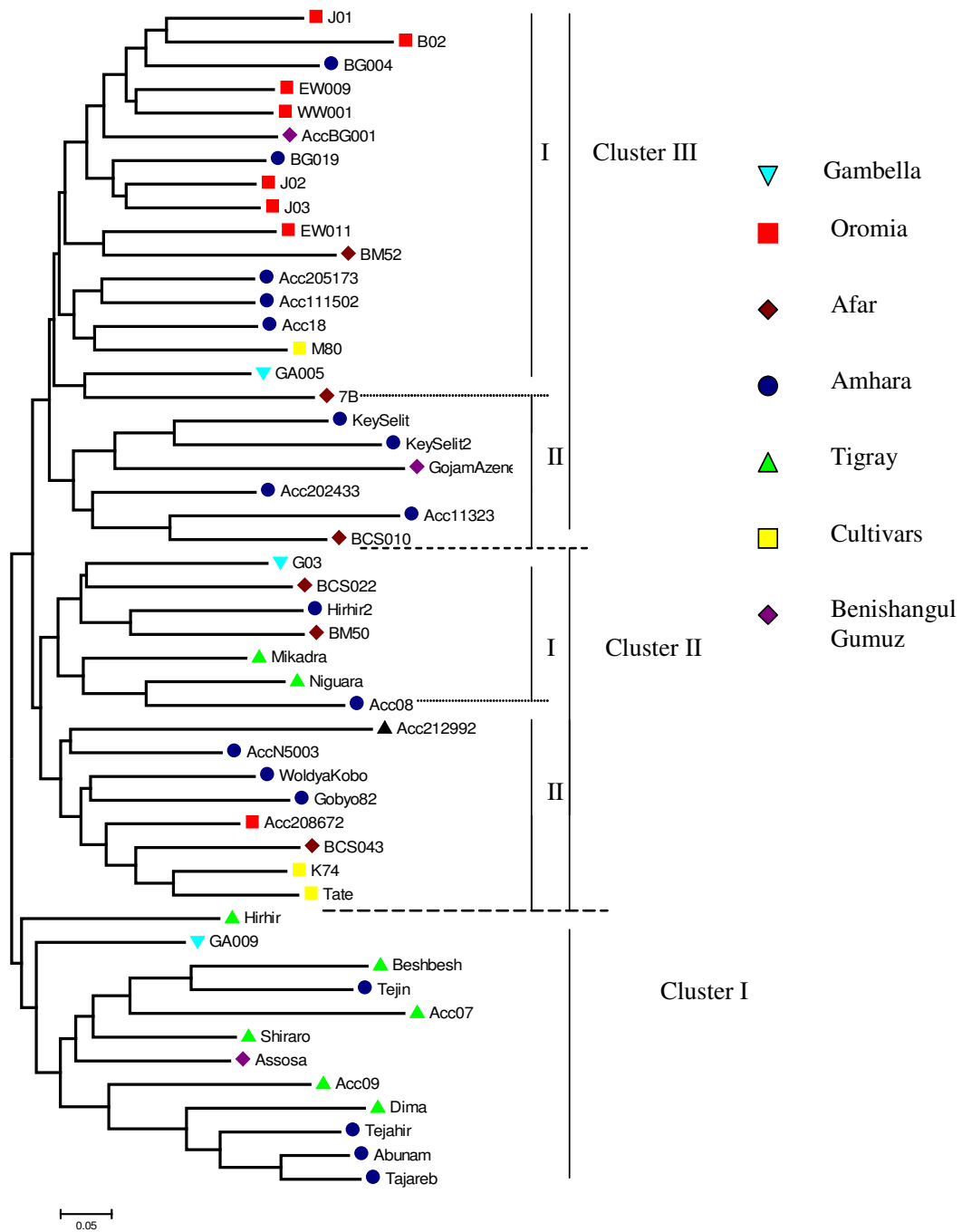* t_e values omitted from the Table

Figure 2. Neighbour-Joining dendrogram illustrating the genetic distance of 50 sesame populations estimated from 10 SSR markers based on MRD.

Amara and Tigray regions are close to each other (Anonymous, 2005), have similar ecological conditions and exchange of seed material expected from farmer to farmer.

Six out of eight landraces from Tigray region were clustered together in Cluster I, indicating the lower genetic diversity of the landraces from this region. The long tradition of sesame farming in this region might have lead to the selection of the landraces by local farmers (Anonymous, 2005), that might have caused lower genetic diversity within sesame landraces from Tigray. Within the Cluster I, two landraces (Hirhir and GA009), appeared to be distinct from all others and can be considered as a separate group.

Cluster II comprised of 13 landraces, 5 of which were collected from the Amara region, 3 from Afar region, 2 from Tigray region, 1 each from Gambella, Oromia and SNNPR regions and 2 cultivars. Cluster II was further grouped in two subclusters (I and II). The first subcluster (I) comprised of 7 landraces and the rest 6 landraces in the second (II) subcluster. Two cultivars were also grouped in the second (II) subcluster of Cluster II.

Cluster III comprised of 22 landraces, 9 of them collected from Amara region, 7 from Oromia region, 3 from Afar region, 2 from Benishangul-Gumuz region, 1 from Gambella region. In this Cluster there was also 1 cultivar. Cluster III was further grouped in two subclusters (I and II). The first (I) subcluster comprised of 16 landraces and 1 cultivar and the remaining 6 landraces were in the II subgroups.

All landraces collected from Oromia region excluding landrace Acc208672 were grouped in subcluster I of Cluster III, which indicated the close genetic relatedness of landraces from the Oromia region. Landraces from Afar region were grouped in Clusters II and III, which indicated the existence of genetic variation among the landraces. The three cultivars were found in Cluster III (M80) and cluster II (K74 and Tate), indicating that two of the cultivars (K74 and Tate) were very similar genetically. The 18 landraces collected from Amara region were distributed over all three different clusters. Three landraces each from Gambella and Benishangul-Gumuz regions were scattered in the dendrogram and found in all three different clusters. This indicated that the materials from the Amara region were not closely related. The same holds true for landraces collected from Gambella and Benishangul-Gumuz regions. The reasons for such genetic diversity of landraces collected from the same region might be heterogeneity of sesame landraces (Kumaresan and Nadarajan, 2003). In general, there was no clear clustering in the Neighbour-Joining dendrogram; this may be due to the presence of a lot of seed exchange between farmers in different regions or, due to the fact that 10 SSR markers were too few to get a clear differentiation between the materials.

Principal coordinates analysis. Principal coordinate analysis of landraces and cultivars of sesame based on simple matching dissimilarity matrix derived from allelic data of 10 SSR markers (Fig. 3) was performed to complement the Neighbour-Joining dendrogram. The entry numbers of the populations were plotted on the two component axis and different colours corresponded to different regions from where the landraces and the cultivars were collected. The first axis explained 9.92% of the variance and the second axis 9.28% of the variance. Three distinct clusters were revealed, 7 landraces in Cluster I, 14 landraces in Cluster II and 17 landraces in Cluster III.

The PCoA showed a clear grouping of landraces colleted from the Northern part of Ethiopia (Amara and Tigray regions) in Cluster I. A clear distinction according to geographical origin was not apparent in Clusters II and III. Cluster II comprised 14 landraces collected from all regions, 6 of them were from Amara, 2 from Afar, 1 each from Benishangul-Gumuz, Gambella, Oromia, SNNPR and Tigray regions and one cultivar (M80). Cluster III comprised of 17 landraces collected from five regions, 5 each from Amara and Oromia region, 3 from Tigray region and 2 each from Afar and Gambella regions. Exchange of seed materials among different sesame growing regions expected that might caused mixed grouping of the landraces. There were 12 lines with no clear clustering.
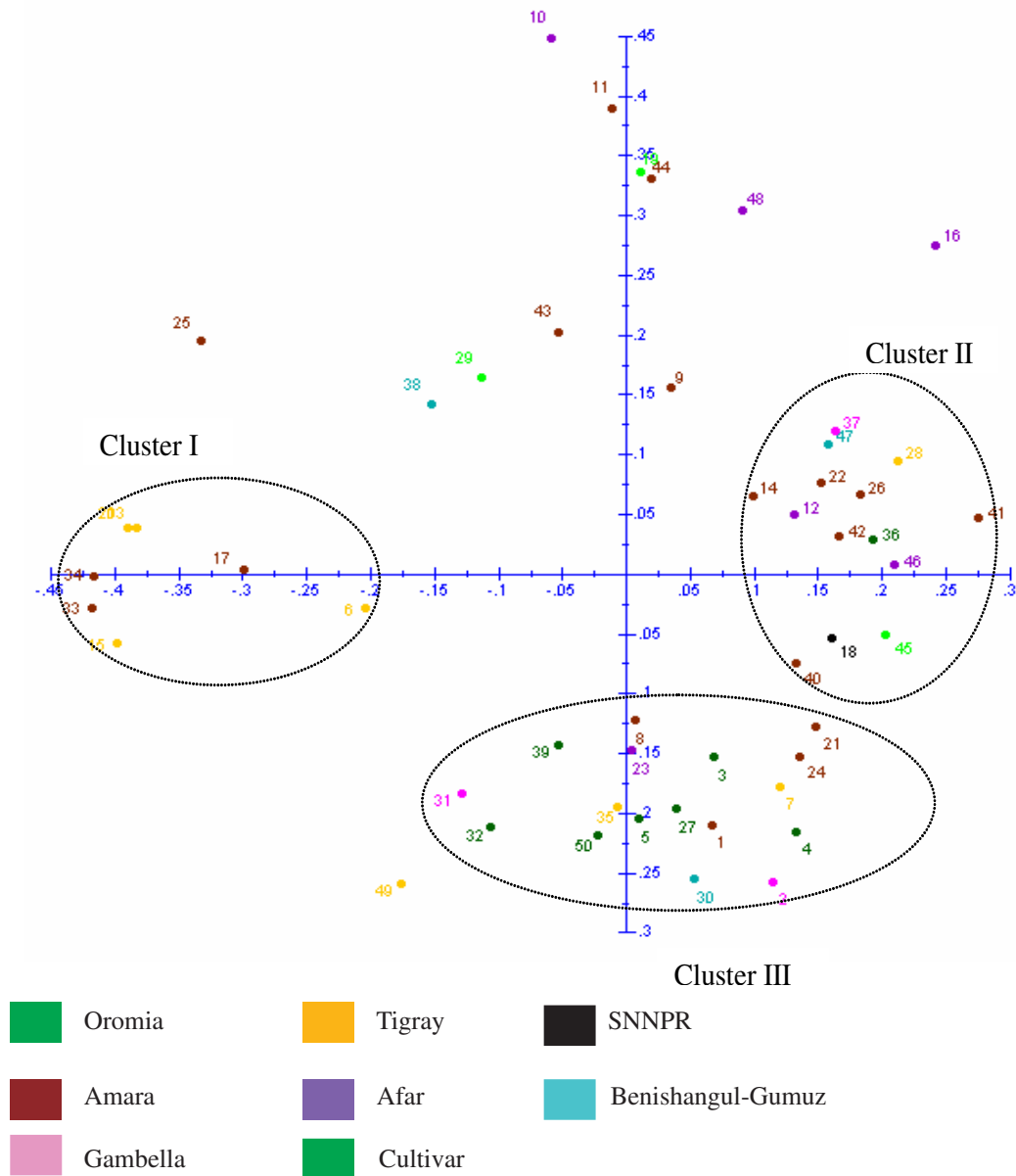
Figure 3. Two dimensional principle co-ordinate analyses based on simple matching dissimilarity index for land races and cultivars of sesame using 10 SSR loci. Different colors correspond to different regions from where landraces and the cultivars are collected. The numbers in the figure correspond to population entry numbers in Table 2.

CONCLUSION

Ethiopian sesame is genetically diverse, a factor that needs to be considered when planning conservation strategies or in breeding programmes. There is a wide range of outcrossing rates both in the landraces and cultivars, which may have contributed to the observed high genetic variability. Therefore, sesame cannot be clearly regarded as autogamous or allogamous, rather as a species with a mixed mating system.

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