# ADDIS ABABA UNIVERSITY

## SCHOOL OF GRADUATE STUDIES

## COLLEGE OF NATURAL SCIENCES

## FACULTY OF LIFE SCIENCE

# GENETIC DIVERSITY OF SESAME (*Sesamumindicum*L.)GERMPLASM COLLECTION AS REVEALED BY ISSR MARKER: IMPLICATION FOR CONSERVATIONAND IMPROVEMENT

**A Thesis submitted to the School of Graduate Studies, Addis Ababa University, in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Applied Genetics)**

**By**

**DAGMAWI TESHOME WOLDESEMBET**

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# Dedication

Tomy beloved mom ASTER HAILEMARIAM ANKETOwithgratitude

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## LIST OF ACRONYMS

| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| AMOVA | Analysis of molecular variance |
| CGIAR | Consultative Group on International Agricultural Research |
| CSA | Central Statistics Authority |
| CTAB | Cetyltrimethyl Ammonium Bromide |
| EDTA | Ethylene Diamine Tetra Acetic acid |
| EIAR | Ethiopian Institute of Agricultural Research |
| FAO | Food and Agriculture Organization |
| GCV | Genotypic Coefficient of Variation |
| GD | Gene diversity |
| IAR | Institute of Agricultural Research |
| IBC | Institute of Biodiversity Conservation |
| IDRC | International Development Research Center |
| ISSR | Inter Simple Sequence Repeats |
| MAS | Marker Assisted Selection |
| Masl | meters above sea level |
| NJ | Neighbor Joining |
| NPL | Number of polymorphic loci |
| PCO | Principal Coordinate Analysis |
| PCR | Polymerase Chain Reaction |
| PCV | Phenotypic Coefficient of Variation |
| PP | Percent Polymorphism |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| RCBP | Rural Capacity Building Project |
| SNP | Single Nucleotide Polymorphism |
| SSR | Simple Sequence Repeats |
| UPGMA | The Un-weighted Pair Group Method with Arithmetic mean |

## *Abstract*

*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. Intersimple sequence repeat (ISSR) marker was used to assess the level of geneticdiversity, genetic structure and genetic distance, and to indirectly estimate the level of gene flowamong populationsof sesame in Ethiopia.A total of 120 (82 Ethiopian and 38 exotic) sesame accessions and six ISSR primers, five of which were dinucleotides (810, 818, 834, 844, 860) and one pentanucleotides (880) 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. A total of 58 clear and reproducible bands were amplified, out of which 44(75.86%) were observed to be polymorphic. The number of polymorphic loci ranged from four for primer-834 to eight for the other primers.Comparative assessment of Ethiopian accessions (considering as a single large population) verses exotic accessions indicated the presence of higher polymorphism among accessions collected from Ethiopia (75.85)than the exotic accessions (65.52). Primers 810, 818 and 860 showed the highest percent polymorphism (88.89%) and the least polymorphic bandswere scored by primer 834 which is only 40.00 %. Grouping Ethiopian accessions into administrative region-based populations and the exotic accessions in to country based populations showed samples from Welega were 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. The average gene diversity relative to the overall population was 0.24 considering accession from different administrative region of Ethiopia and exotic accessions as independent population. 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%). Inter-population genetic distance (D) ranged from 0.031 to 0.165 for the overall population. Among the pairwise population comparisons made within Ethiopian populations,accessions from Welega and Harerge showed the highest genetic distance (0.16) and accessions from Shewa and Harerge show the least genetic distance (0.041). From the exotic accession,samples of South East Asia are distantly related to most of the Ethiopian accessions. Genetic distance between the other pairwise combinations of populations was very low, with the least genetic distance being between samples of Mexico and Tanzania (0.031).UPGMA 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. The second major cluster comprise of accessions from north western part of Ethiopia mainly from Gojam, Benishangul Gumuz and Gonder.In the 3D plot, with the exception of few accessions that come from Welega, Wello and Ilubabore, most of the individual accessions that represent different populations spread all over the 3D space.*

**Key words:**Ethiopia, genetic diversity, ISSR, Sesame (*Sesamum indicum* L.)

# 1. 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 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. On the other hand, India is generally held as the subcontinent where sesame was first domesticated and then spread to other places in the world such as Africa, the Far East, China and Americas along trade routes (Bedigian, 2004).Sesame is probably the most ancient oilseed known and used by man (Weiss, 1983). 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 a warm weather crop and is often grown under marginal or stressed conditions. Weiss (1983) also considered sesame a crop of tropics and subtropics with an altitude normally below 1250m a.s.l. and can adaptup to 1500m a.s.l. Its main distribution is between 25$^{o}$S and 25$^{o}$N.The crop normally requires fairly hot condition with temperature that ranges from 25-27$^{o}$C to encourage rapid germination, initial growth and flower formation. This type of agro-ecological conditions is ideal to give maximum yield (Salehuzzaman and Pasha, 1979; Kostrynsky, 1959).

Sesame can be planted in areas where rainfall distribution is erratic and the specific adaptability of the species has resulted in local varieties extremely well suited to particular conditions. Selection of these varieties with the ability to give high average yield over a fairly wide range of a major agro-ecology is vital in ensuring a profitable return from annual sesame. From West to East Africa sesame is generally grown in those areas too dry to groundnut (400-750mm annual rain) or where there are likely to be local dry periods that would reduce the groundnut yield to unprofitable level (Weiss, 1983). Generally, sesame can grow well within an average rain fall of 500 to 700mm. However, extreme cases like low rainfall distribution up to 300mm and higher rainfall distribution upto 1200mm can be tolerated.

Sesame is an erect and annual herb. The plant can be simple or branched. The leaves are variable in shape and they are opposite in the bottom and alternate above. Flowers are zygomorphic, solitary, occasionally two or three together, axillary, short- pedicelled, borne on the upper part of the stem or branches. The fruit is a deeply grooved capsule (1 to 3 inches in length), erect and oblong. The capsule contains numerous (50 to 100) small ovate seeds. The testa may be smooth or reticulate and could be white, yellow, reddish-brown or black(Ashri, 1998).

Sesame normally is self-pollinated, although insect driven cross pollination is common. Natural cross pollination has been reported to be as low as 10% by Khidir(1972) to over 50% by Rheene(1980). The seeds mature 4 to 6 weeks after fertilization and may vary in colorand the lighter colored seeds are considered of higher quality. One thousand seeds weigh about 2-3g. Sesame is drought-tolerant, this is mainly attributed to an extensive root system. Under optimum condition, the root can grow as branched and becomes fibrous. The crop requires adequate moisture for germination for early growth and reasonable yield. Initiation of flowering is sensitive to photoperiod and varies among varieties. Moisture levels before planting and at flowering have the greatest impact on yield(Bedigian, 2004).

Sesame also has some abiotic constraints. For instance, sesame is extremely intolerant of water-logging, even a one day water logged condition reduce the yield drastically. Moreover, late-season extended rainfall can prolong growth and increases shattering loss. Wind can also cause shattering at harvest and is cited as one of the major reason for the failure of commercial sesame production in the world (Ashri, 1998).

The growth of sesame is indeterminate; and the plant continues to produce leaves, flowers and capsules as long as the weather permits (Bradley J.M. 2002). The oil content of the seed tends to increase with increased photoperiod. Because protein content and oil content are inversely proportional, seed with increased oil content has decreased protein content. In general, the seed may contain up to 60% of very high quality oil so named as queen of oil seed with up to 25% protein (Bedigian *et al.,* 1985;Ashir,1998).

Sesame is an important source of high quality edible oil and protein food for poor farmers of major sesame growing countries such as Sudan, Nigeria, Ethiopia, Uganda, Mexico, Venezuela, India, China, Pakistan, Turkey, and Myanmar who can hardly afford animal fat and protein. Sesame seed is the single readily available source of protein high in sulfur containing monoacids (Bradley, 2002). It is the major cash crop for smallholder farmers and a valuable foreign exchange revenue item for different countries economy. The remaining cakes of sesame are used as a source of crude protein for cattle feed.

To strengthen the research program and broaden the genetic bases in sesame breeding EIAR 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. Among the 221 EIAR sesame collections Daniel Endale (2008) investigated the genetic variability of only 50 landraces using SSR marker. However genetic variability studies, using any marker system,have not yet beenconducted for the IBC collection. To meet the objectives of the national sesame improvement, information on genetic diversity and their relationship within and among collectedaccessions is inevitable.The present study was,therefore, conducted to see the genetic diversity of sesame using ISSR marker.

# 2. LITERATURE REVIEW

## 2.1 Taxonomy of sesame

Sesame is a diploid species with 2n= 26 chromosomes. It belongs to the division spermatophyta, sub division angiospermae, class dicotyledoneae, order tubiflorae family pedaliaceae and genus *Sesamum*. The pedaliaceae family contains 60 species and the *Sesamum* genus contains about 36 species of which 22 species have been indigenous to Africa, including the only cultivated species *Sesamumindicum* L., only fivein Asia, seven in both Africa and Asia, and one species each in Crete and Brazil (Kobayashi *et al.* 1990). There are three cytogenetic groups of which 2n = 26 consist of the cultivated *S. indicum* along with *S. alatum, S. capense, S. schenckii, S. malabaricum*; 2n = 32 consist of *S. prostratum, S. laciniatum, S. angolense, S. angustifolium;* while *S. radiatum, S. occidentale, S. schinzianum* belong to 2n = 64 (Duc, 2010). Mainly due to the difference in chromosomal numbers across the three cyto-taxonomic groups, there is limited cross compatibility among the species. Therefore, it has been difficult to transfer desirable characteristics such as drought tolerance, pest, and resistance to diseases, from wild relatives into cultivated sesame (Carlsson *et al.*, 2008).

## 2.2 Diversity study on sesame

Despite the nutritional value and historic and cultural importance of sesame, the research on sesame has been scarce. For example, no international CGIAR (Consultative Group on International Agricultural Research) agency is mandated to study sesame. Information on the genetic diversity in sesame is limited as well. Sesame diversity centers have been identified as India, China, Central Asia, Near East and Abysinia in classical studies (Laurentin and Karlovsky, 2006). More recently, a high level of variability of morphological characters within different sesame collections was reported. Ercan *et al.* (2002) reported genetic diversity for agro-morphological traits in 52 landraces of sesame originated from Turkey which showed moderate variability. Castro *et al.*(2007) estimated the genetic divergence of 30 morphological and agronomic traits of 108 sesame genotypes by multivariate analysis and they found higher variation for two traits, number of capsules per plant and grain yield. Sileshi Andualem (2008) assessed genetic divergence and character associations among 13 agro - morphological traits of 100 local and exotic sesame genotypes assembled from the EIAR collections. The result

indicated the existence of a wide variability among the genotypes for all the 13 characters studied. The highest PCV and GCV were recorded for oil yield per plant followed by seed yield per plant number of primary branches and distance to first pod respectively. Higher estimates of heritability were recorded for days to flowering, number of primary branches, 1000 seed weight, capsule length days to maturity, and oil content. Duc(2010) in his part evaluated morphology of seventeen sesame germplasms from different origins (El Salvador, Tanzania, Kenya, India, Cambodia and Vietnam) and most of the germplasms from the Cambodian and Vietnamese origin were identified as high yielding

Apart from the visually detectable morphological diversity, genetic variability in sesame has also been studied by biochemical and molecular techniques. Isshiki and Umezaki (1997) studied genetic diversity in 68 sesame cultivars of Japan, Korea and Thailand using isozyme markers and they found that, among seven enzyme system, only isocitrate dehydrogenase exhibited variation. Kim *et al*. (2001) used ISSR marker to analyze the genetic diversity of 75 Korean and exotic sesame accessions. However, based on geographic origin, the dendrogram constructed did not reveal any clear division and structuring. Laurentin and Karlovsky (2006) and Ali*et al*. (2007) respectively studied the genetic relationships among 32 sesame accessions representing five diversity centers and 96 sesame accessions from different parts of the world using AFLP. Both work indicated that the sesame accessions were variable. Bhat *et al* (1999) used RAPD to study 36 Indian and 22 exotic sesame accessions and found greater diversity from the Indian collections as compared to the exotic ones. In another study, which used RAPD marker for ten Sudanese sesame accessions,Abdellatef*et al*. (2008) found low level of genetic similarity in the collected accessions and UPGMA clustering resulted in two major groups. Daniel Endale (2008) used SSR marker to investigate the genetic variability among Ethiopian landraces and found high variation. The variability exists among sesame genotypes at gene level whose effect may or may not be readily visible. Some of these variations may govern important plant processes that lead to disease and pest resistance, high yielding potential that are of significant economic importance.

## 2.3Breeding and agronomy of sesame in Ethiopia

Breeding research to improvethe productivity of sesame in Ethiopia was started in the late 1960s with 72 introduced and fewpure line selection of local germplasm, focusing on introduction

(Tadele, 2005). Ethiopian Institute of Agricultural Research (EIAR) the then Institute of Agricultural Research (IAR) and IDRCinitiated the highland and lowland oil crop projects in 1981 and 1982, respectively. The Lowland Project covered sesame, groundnut, castor and safflower. The project continued with its second phase from 1988 and greatly accelerated the research work on sesame. During this time, both exotic and local sesame lines wereevaluatedfor their disease resistance, yield and yield related traitsat various agroecological zonesof Ethiopia. Out of sesame lines assembled and evaluated at multi locations, 10 lines, some exotic and some landraces, have proved promising and four varieties (T-85, Kelafo 74, S and E) were released during the first phase of the project and the other six (Adi, Mehedo 80, Abasina, Argene, Serkamo and Tate) were evaluated further and released in the second phase of the project (Thomas Development Associates, 1992). Since that time,10 cultivars were recommended for use in Ethiopia. All the cultivars are selected lines from the landraces.

T-85 which is also called Hir Hir as local name and Humera type as market name is appreciated worldwide for its aroma and sweet taste. It has good uniform large white seed and often used as standard in the world market. However, it is susceptible to bacterial blight caused by *Pseudomonas sesame* or *Xanthomonas sesame*. Hence three other varieties, S and E (introductions from Uganda) and Abasena (selected line from landraces) were released which were meant to mitigate the limitation in T-85 as they moderately tolerate the disease (Tadele, 2005).

The productivity of sesame is induced by improved varieties or improved agronomy practices and crop protection. Potential yields are probably as high as 2000 kg/ha which is much higher than the national average 600kg/ha (Wijnands *et al.* 2009). This indicated that, the yield potential of sesame is much higher than the actual yield, as still much damage occurs by pests and diseases, insufficient weed control, too high levels of mono-cropping, inefficient harvesting (shattering) and unrealized genetic potential.

In general, the breeding strategies for sesame are similar to those applicable in other crops and include higher yields, improved plant architecture, length of growing season, resistance to diseases and pests (Ashri, 1998). Specific objectives for sesame breeding vary with the level of

technology and were summarized by Ashri (1998) as follows: (1) high and stable seed yield of good quality under a wide range of environmental conditions, (2) resistance to water logging, drought, salinity, pests, diseases, shattering and other abiotic stresses, (3) increased number of capsules per leaf axil, full seed-set without aborted ovules, and (4) uniform plant type, rapid growth, good adaptation to varying environmental conditions and seasons. These objectives are applicable to all sesame producing countries including Ethiopia.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).

## 2.4Economic importance and distribution

Ethiopia has a total area of 1.13 Million square km with a population of around 80 million (CSA, 2010). Agriculture is currently the most important sector of the economy and the government gives high attention to the development of the agricultural sector and agricultural research with the agricultural lead industrialization policy. Agriculture has always been the backbone of the economy in which 85% of the population is engaged. It supplies 42.2% of the GDP and 90% of the export earnings as well as supplying over 90% of the raw materials for the agro-industries (CSA, 2008). The most important exportable agricultural input in the country is coffee, oilseeds, hide and skin, flower and khat.

Oilseeds are among the important agricultural commodities widely grown in Ethiopia. Oilseeds are cultivated by 30% of the agricultural holdings on 7% (780,915.89 Ha) of the total agricultural land (CSA, 2010). They are the third important crop in acreage after cereals and pulses.Major oilseeds are sesame seed, groundnuts, rape seed, niger seeds, linseed, sunflower, cottonseed, soy beans and others.The majority of oilseeds, mentioned above are cultivated traditionally by millions of smaller land holdings throughout the country except sesame seeds where commercial farms with relatively large land holdings and relatively modern agricultural practices predominate, mainly in Humera and Metema regions(Tadele, 2005).

According to Wijnands *et al.* (2009) the exports of oilseeds from Ethiopia is expanding and total exports are growing worldwide. Next to hides and skins, oil seeds are the best-performing commodities in Ethiopian exports. The bulk of the harvest (more than 50%), are sesame and niger seed which dominate largely the oil seeds category.

Among the important oil crops grown in Ethiopia, sesame seed commands a unique position chiefly on account of the fact that it is highly adapted to arid and semi-arid low land environment with fairly good yields. Viewed in this context, sesame is a major oil crop in North-west Tigray (Humera and the suroundings), North Gonder (Metemma and the surroundings) and Oromia (East Wellega). In these areas the crop is grown alone unlike other places like Pawe, Gambella and Melka Werer where sesame is grown in association with cereals (Daniel Endale, 2008).

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. In 2008, Ethiopia exported sesame to more than twenty countries andChina, Israel, Turkey, and MiddleEastcountries are the major importer of Ethiopian sesame (Wijnands *et al*., 2009). As most of the sesame farms in Ethiopia do not use chemical fertilizers and other chemical inputs, the potential to sell under organic labeling by acquiring appropriate certification is enormous. A very small edible oil producing agro-industries are in service in the country that use sesame seeds.The Ethiopian whitish Humera type is known for its taste (sweet) in the world market henceit is exported to the confectionary market where white seeded types are demanded by the consumers (Wijnands *et al*., 2009).

Sesame is grown in more than sixty countries in the world. According to FAO (2008) over 7.42 million hectares of sesame were harvested worldwide, which come about 3.54 million tons of produce. Ethiopia is ranked $6^{th}$ in sesame production with 164,000 tons (5%) of production per year. Sudan, Uganda and Ethiopia have the lions share in Africa, covering 57% of the production which accounts around 17% of the world. The five major exporters of sesame seeds are India (317,015 tones), Ethiopia (139,653toness), Sudan (105,464 tones), Myanmar (41653 tones) and Nigeria (79,861 tones). Though Ethiopia is the second exporter of sesame in the world, it is not among the top 20 countries in unit price and the country fetch only 951$ per tones of sesame (FAO, 2007). In recent years, sesame has had a low ranking in the world production of edible oil

crops. The low ranking of sesame among oil crops may be attributed to several factors including low seed yield and strong competition from other oil crops such as soybean, sunflower, peanut. Despite the tendency of decrease in total world sesame production among the oil seed production in Ethiopia sesame is higher in both area in hectare and total production in quintal. It reaches 315 thousands hectare in area coverage and 2.61 million quintal in production (CSA, 2010). The production of sesame shows an increasing trend. In 2009/2010 cropping season, its production increased both in area (13.62%) and production (20.21%) as compared to the previous season (2008/2009).

## 2.5Genetic markers for diversity analysis

A number of markers exist that can be used as tool to discern genetic variability within among populations. These markers fall into one of the three broad classes: those based on visually assessable traits (morphological and agronomic traits), those based on gene product (biochemical markers), and those relying on a DNA assay (molecular markers). Genetic markers are widely used by breeders and conservationists to study genetic diversity. Each marker system has its own advantages and disadvantages in terms of ease of use and/or the degree of information provided.

### 2.5.1 Morphological markers

The earliest studies of genetic characterization and divergence were based on morphological markers such as qualitative and quantitative traits (Arriel *et al.,* 2007; Cruz *et al.,* 2004). These markers are inexpensive and simple to score as they are based on distinct phenotypes such as plant color, plant height, seed characteristics, etc. The apparent disadvantage of such markers is that in studies of genetic diversity the expression of the phenotypes is highly influenced by environmental conditions. Dispensable traits in studies of genetic diversity are relatively invariant ones, highly influenced by the environment or redundant for being correlated to other traits. In other words, those that contribute most to the divergence must be weakly correlated (Arriel *et al.,* 2007). In a study, these traits are expected to contribute with exclusive information and their joint action to be complementary to the description of the study genotypes (Bedigian et al. 1986). Lack of adequate genome coverage because of limitation of the number of markers and problems of dominance can also be mentioned as a weakness of morphological markers (Brown, 1978). Furthermore, the expression of these characters which are influenced by the

environment may require that plants be grown to a suitable stage before certain characters can be scored.

### 2.5.2 Biochemical markers

These are also termed isozyme/allozyme markers or simply protein markers. Isozymes represent different molecular forms of an enzyme system with the same catalytic functions. They are variants of an enzyme that can be differentiated by electrophoretic mobility. They originate through amino acid alterations which cause changes in net charge or conformation of an enzyme and hence in electrophoretic mobility. The electrophoretic verification of such amino acid alterations provide a means of monitoring changes in the nucleotide sequence of the respective coding gene. They are distinguished mostly by horizontal gel electrophoresis that separates proteins primarily on the basis of charge and size. The main advantages of protein markers are their codominant inheritance and the technical simplicity and low cost of the assay. Disadvantages include the restricted number of suitable allozyme loci in the genome, the requirement of fresh tissue, and sometimes limited variation (Weeden, 1989;Weising *et al*., 2005).

### 2.5.3 Molecular markers

Molecular markers are identifiable DNA sequence, found at specific locations of the genome, and transmitted from one generation to the next. Molecular or DNA markers are relatively recent but most robust tools for genetic diversity analysis. They complemented most of the shortcomings of morphological and biochemical markers. When compared with other markers systems, DNA based diversity estimates were shown to reflect the actual differences between genotypes, relatively simple to detect, abundant throughout the genome, completely independent of environmental conditions and can be detected at any stage of plant development (Barrett and Kidwell, 1998; O' Neill *et al*., 2003).

Based on the method of analysis two basic types of DNA marker systems are available: those that rely on hybridization between a probe and homologous DNA segment such as restriction fragment length polymorphism (RFLPs) and those that use the polymerase chain reaction (PCR) to exponentially amplify genome segments between arbitrary or specific oligonucleotide priming sites such as random amplified polymorphic DNA (RAPD)(William,s *et al*., 1990), inter-simple

sequence repeat (ISSR)(Zietkiewicz,*et al.,* 1994), amplified fragment length polymorphism (AFLP)(Rafalski,*et al.,* 1996) and simple sequence repeat (SSR)(Ciofi,*et al.,* 1998). 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. Each of these markers systems have their own strengths and weaknesses that is why one has to choose the kind of markers that best suits his/her crop and population. 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).Some of the major and commonly used molecular markers are presented briefly below

***2.5.3.1 Restriction Fragment Length Polymorphism (RFLP)***

RFLP is the most widely used hybridization-based molecular marker. RFLP analysis has been used in the detection of genetic diversity, selection for traits of agronomic importance, and segregation analyses of progenies (Brettshneider, 1998). The analysis involves digestion of complex genomic DNA into small DNA fragments using restriction enzyme such as restriction endonucleases that recognize and cut specific short sequences of DNA followed by separation of these fragments by gel electrophoresis. They differentiate genotypes based on mutations in endonuclease restriction sites across the genome in addition to genome rearrangements. However, the low level of DNA polymorphism exhibited by RFLP markers, time consumption, laborious, expensive and its requirement of radioactively labeled probes limited its application (Weising *et al*., 2005).

PCR is a molecular biology technique for enzymatically replicating (amplifying) small quantities of DNA without using a living organism. It is used to amplify a short well-defined part of a DNA strand from a single gene or just a part of a gene.PCR based markers such as RAPD, ISSR, AFLP and SSR are superior to RFLP in that they show high diversity even in autogamous crops

with a narrow genetic base like soybean and wheat (Russel *et al*., 1997). These markers are currently widely employed in diversity and mapping studies (Milbourne *et al*., 1998).

#### *2.5.3.2 Random Amplified Polymorphic DNA (RAPD)*

Random amplified polymorphic DNA (RAPD) markers were first described in 1990.It was the first and by far the simplest PCR based molecular marker technique developed. RAPDs are DNA fragments amplified by the PCR using short synthetic primers (generally 10 bp) of random sequence. These oligonucleotides serve asboth forward and reverse primer, and are usually able to amplify fragments from 1–10 genomic sites simultaneously (Spooner and Vicente 2005). Amplified fragments, usually within the 0.5–5 kb size range, are separated by agarose gel electrophoresis, and polymorphisms are detected, after ethidium bromide staining, as the presence or absence of bands of particular sizes. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites.Since RAPD technique requires no sequence information for primer design and no probe development it is technically simple, fast and relatively cheap (Williams *et al*., 1990). However its dominant natureand less reproducibility are mentioned as its weakness.

#### *2.5.3.3 Amplified Fragment Length Polymorphism (AFLP)*

AFLP technique combines the power of RFLP with the flexibility of PCR-based technology by ligating primerrecognition sequences (adaptors) to the restricted DNA. AFLP analysis involves restriction digestion of genomic DNA (about 500 ng) with a combination of rare cutter and frequent cutter restriction enzymes. Double-stranded oligonucleotide adaptors are then designed in such a way that the initial restriction site is not restored after ligation. Such adaptors are ligated to both ends of the fragments to provide known sequences for PCR amplification (Spooner and Vicente 2005). The strengths of AFLPs lie in their high genomic abundance, considerable reproducibility, the generation of many informative bands per reaction, their wide range of applications, and the fact that no sequence data for primer construction are required (Rafalski *et al.,* 1996). The drawback of this techniqueisrequirement of high quality DNA. In addition, it is technically demanding, dominant marker, and there is a possibility of co-migration of non-homologes fragments belonging to different loci.

### *2.5.3.4 Simple Sequence Repeat (SSR)*

SSR markers also called microsatellite markers or sequence tagged microsatellite sites-STMS are one of the widely used PCR based molecular markers in the study of genetic diversity and mapping of agriculturally important traits. SSRs are short DNA sequences, usually two to five bases long, repeated variable number of times in tandem. Compared to other markers, SSRs exhibit uniform genome coverage, high levels of polymorphism and are co-dominant. They are evenly distributed and widely dispersed throughout eukaryotic genomes and are often highly polymorphic due to variation in the number of repeat units. Microsatellites may even be used across species and genus boundaries. Microsatellites are not limited to the nuclear genome. They occur in chloroplast as well as in mitochondrial genome. The high information content of the genetic data yielded by microsatellite loci makes these markers one of the molecular tools of choice for population and biodiversity studies (Ciofi *et al.,* 1998). One of the main drawbacks of microsatellites is that high development costs are involved if adequate primer sequencesfor the species of interest are unavailable, making them difficult to apply to unstudied groups(Spooner and Vicente, 2005).

### *2.5.3.5 Inter Simple Sequence Repeats (ISSR)*

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). In other case it is amplification of regions in the genome that lie between microsatellites using a simple sequence repeat (SSR) and additional nucleotides as primers (Zietkiewicz *et al.*, 1994). ISSR is a RAPD-like technique that shares the simplicity of RAPD markers but uses longer PCR primers (15-30mers); hence, it is more reproducible than the original RAPD method. It has relatively low startupcosts and easy to use. ISSR markers have been used successfully in a number of recent studies of genetic diversity in plants including sesame(Godwin *et al.,* 1997, Blair *et al.*, 1999, Huang and Sun 2000, Kim *et al.,* 2002 ), and has the added advantage of permitting convenient development of SSR markers from amplified ISSRs (Van der Nest *et al.,* 2000).

For aiding the breeding program in sesame it is imperative to study genetic diversity of the existing sesame germplasms through systematic evaluation and characterization and make the information available to the researchers. The conservation and sustainable use of sesame

genotypes mainly depends on proper characterization of the sesame genetic resources. ISSR marker, in addition to its suitability to genetic diversity study, is highly polymorphic, reproducible,and cost effective and requires no prior information of the sequence.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; Pomper *et al.,* 2003;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.

# 3. OBJECTIVES

## 3.1 General objective

- The general objective of the study was to investigate the genetic variability among the Ethiopian sesame germplasm collections and some exotic accessions using inter simple sequence repeats marker.

## 3.2 Specific objective

- To determine the level and pattern of distribution of variations among the overall collections of sesame.
- To identify populations and regions with higher diversity for sesame improvement and conservation.
- To compare inter-population variability within Ethiopian population
- To compare inter-population variability between Ethiopian and exotic accession.

# 4 MATERIALS AND METHODS

## 4.1 Experimental materials

This laboratory experiment was involve characterization of the accessions using inter simple sequence repeat (ISSR) markers. 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 Fig 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 on station field plots. The accessions were grown in 2 row plots of 4 m length.

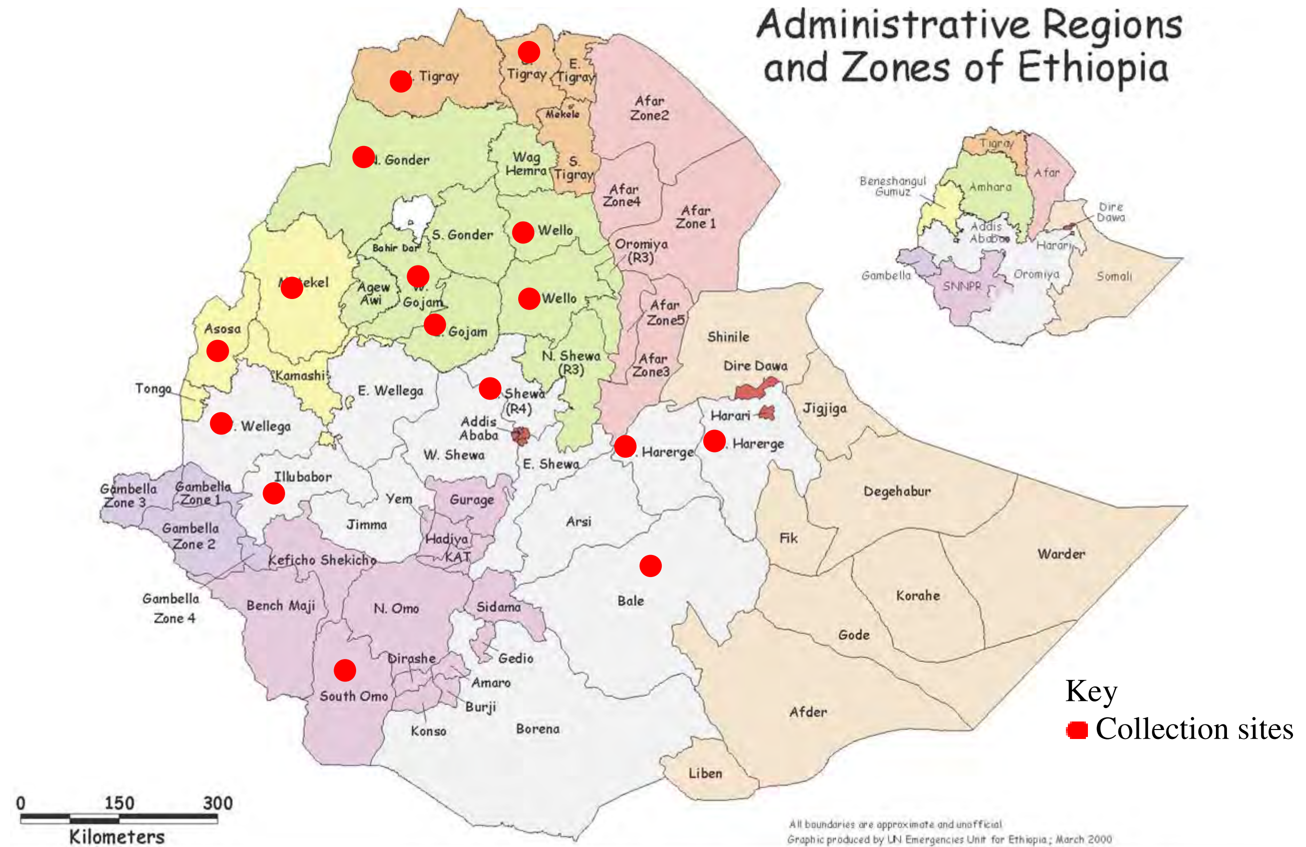



Figure 1. Former administrative region of Ethiopia showing where the sesame accessions used in this study were collected. (*Source: Map of the world)*

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

| No | Accession # | Altitude | Former Administrative Region/ Exotic |
|---|---|---|---|
| 1 | 111501 | NA | Gamo Gofa |
| 2 | 208888 | NA | Gamo Gofa |
| 3 | 208889 | 1050 | Gamo Gofa |
| 4 | 212993 | 1290 | Gamo Gofa |
| 5 | 212994 | 1270 | Gamo Gofa |
| 6 | 212995 | 1290 | Gamo Gofa |
| 7 | 214976 | NA | Gamo Gofa |
| 8 | 111502 | NA | Gojam |
| 9 | 111503 | NA | Gojam |
| 10 | 111513 | NA | Gojam |
| 11 | 211921 | 1600 | Gojam |
| 12 | 230260 | NA | Gojam |
| 13 | 205173 | NA | Gonder |
| 14 | 235769 | 900 | Gonder |
| 15 | 235903 | 1150 | Gonder |
| 16 | 241341 | 600 | Gonder |
| 17 | 241342 | 600 | Gonder |
| 18 | 241334 | 750 | Gonder |
| 19 | 241344 | 980 | Gonder |
| 20 | 241346 | 1890 | Gonder |
| 21 | 241347 | 1700 | Gonder |
| 22 | 202512 | 1600 | Harerge |
| 23 | 208670 | 1830 | Harerge |
| 24 | 208671 | 1900 | Harerge |
| 25 | 208672 | 1350 | Harerge |
| 26 | 208673 | 1840 | Harerge |
| 27 | 228816 | 1500 | Harerge |
| 28 | 223340 | NA | Harerge |
| 29 | 207956 | NA | Illubabor |
| 30 | 207957 | NA | Illubabor |
| 31 | 216733 | 600 | Illubabor |
| 32 | 222876 | NA | Illubabor |
| 33 | 202285 | 1490 | Shewa |
| 34 | 202286 | 1490 | Shewa |
| 35 | 202374 | 1395 | Shewa |
| 36 | 203099 | 1240 | Shewa |
| 37 | 212536 | 1580 | Shewa |

| No | Accession # | Altitude | Former Administrative Region/ Exotic |
|---|---|---|---|
| 38 | 212537 | NA | Shewa |
| 39 | 212540 | 1600 | Shewa |
| 40 | 234015 | 1090 | Tigray |
| 41 | 210871 | NA | Eritrea |
| 42 | 214202 | NA | Eritrea |
| 43 | 235405 | 1650 | Tigray |
| 44 | 237523 | 900 | Tigray |
| 45 | 238269 | 820 | Tigray |
| 46 | 238270 | 710 | Tigray |
| 47 | 238271 | 710 | Tigray |
| 48 | 238272 | 710 | Tigray |
| 49 | 241305 | 1200 | Tigray |
| 50 | 241311 | 1120 | Tigray |
| 51 | 111505 | 1440 | Welega |
| 52 | 202517 | 1420 | Welega |
| 53 | 202518 | 1360 | Welega |
| 54 | 207953 | 1300 | Welega |
| 55 | 207954 | 1200 | Welega |
| 56 | 208752 | NA | Welega |
| 57 | 237994 | 1290 | Welega |
| 58 | 215816 | 1435 | Welega |
| 59 | 212632 | 1460 | Welo |
| 60 | 202318 | 1525 | Welo |
| 61 | 202336 | 1290 | Welo |
| 62 | 202340 | 1555 | Welo |
| 63 | 202345 | 1565 | Welo |
| 64 | 202353 | 1640 | Welo |
| 65 | 202370 | 1590 | Welo |
| 66 | 215260 | NA | Welo |
| 67 | 203631 | NA | Exotic |
| 68 | 203633 | NA | Exotic |
| 69 | 203634 | NA | Exotic |
| 70 | 203637 | NA | Exotic |
| 71 | 203638 | NA | Exotic |
| 72 | 227862 | NA | Exotic |
| 73 | 227864 | NA | Exotic |
| 74 | 227865 | NA | Exotic |
| 75 | 227866 | NA | Exotic |

| No | Accession # | Altitude | Former Administrative Region/ Exotic |
|---|---|---|---|
| 76 | 227867 | NA | Exotic |
| 77 | 227873 | NA | Exotic |
| 78 | 227874 | NA | Exotic |
| 79 | 227875 | NA | Exotic |
| 80 | 227876 | NA | Exotic |
| 81 | 227879 | NA | Exotic |
| 82 | 227903 | NA | Exotic |
| 83 | 227904 | NA | Exotic |
| 84 | 227906 | NA | Exotic |
| 85 | 227907 | NA | Exotic |
| 86 | 227908 | NA | Exotic |
| 87 | 227933 | NA | Exotic |
| 88 | 227934 | NA | Exotic |
| 89 | 231397 | NA | Exotic |
| 90 | 231399 | NA | Exotic |
| 91 | 227855 | NA | Exotic |
| 92 | 231406 | NA | Exotic |
| 93 | 231407 | NA | Exotic |
| 94 | 231408 | NA | Exotic |
| 95 | 231409 | NA | Exotic |
| 96 | 231410 | 700 | Exotic |
| 97 | 205194 | 700 | Exotic |
| 98 | 205195 | 800 | Exotic |

| No | Accession # | Altitude | Former Administrative Region/ Exotic |
|---|---|---|---|
| 99 | 205191 | 900 | Exotic |
| 100 | 205192 | 1100 | Exotic |
| 101 | 203595 | 760 | Exotic |
| 102 | 203597 | NA | Exotic |
| 103 | 203599 | 700 | Exotic |
| 104 | 203600 | 750 | Exotic |
| 105 | 203627 | NA | Exotic |
| 106 | Abasena | 1050 | Released |
| 107 | E | NA | Released |
| 108 | Hir Hir or T-85 | NA | Released |
| 109 | Mehedo | NA | Released |
| 110 | Adi | 1050 | Released |
| 111 | Acc-BG-006 | 1290 | Benishangulgumuz |
| 112 | Acc-BG-002 | 1270 | Benishangulgumuz |
| 113 | Acc-BG-001 | 1290 | Benishangulgumuz |
| 114 | Acc-BG-003 | 1050 | Benishangulgumuz |
| 115 | Acc-BG-010 | NA | Benishangulgumuz |
| 116 | Acc-BG-012(2) | 1050 | Benishangulgumuz |
| 117 | Acc-BG-003(2) | NA | Benishangulgumuz |
| 118 | Acc-BG-019(1) | 1600 | Benishangulgumuz |
| 119 | New EW-004 | NA | Bako |
| 120 | New (EW-015) | NA | Bako |

### 4.2 Tissue harvest and DNA extraction

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).

### 4.3Test gel and electrophoresis

An agarose gel (100ml 1XTBE buffer and 0.98g agarose, boiled in a micro-oven for 3 minutes and poured on to gel making apparatus-tray with comb) was prepared for test gel electrophoresis. genomic DNA (2 μl)samples with 6μl 2x loading dye was loaded on to the gel and electrophoresed at constant voltage of 80V for 45 minutes. Electrophoresis was conducted in 1X TBE buffer using Agagael Midi Wide (Biometra, Biomed. Analytik GmbH) gel tank. The gel was stained for 30 min with 50 μl ethidium bromide (10mg/ml) after well mixed with 450 ml distilled water. Then test gel was de-stained for 30 minutes in 450ml of distilled water alone using stain-destainer apparatus. Gel picture was taken under UV transilluminator byBiodocAnalyse 2.0 with digital canon camera. The second extraction of most and the first extraction of some samples were selected for further PCR amplification based on the result of the test gel (Fig 2 as an example). Selection of the extracts was based on DNA quantity (band intensity) and quality (absence or presence of minimum smear).

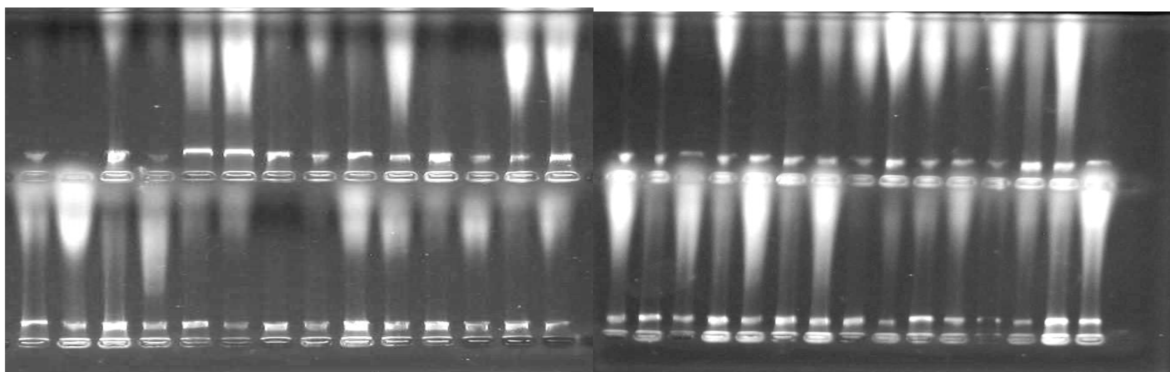


Figure 2: Test gel of diluted DNA samples of selected extracts among 120 sesame accessions

### 4.4Primer selection and optimization

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 Colombia (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 (with 1:5 dilution) 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 2shows the list of primers used and tested, their annealing temperature with respective sequences and other properties.

Table 2:- List of primers, annealing temperature, primer sequence, amplification pattern and repeat motives used for optimization. All the primers are high performance liquid chromatography purified and designated as 'H'.

| Primer | Annealing temperature | Primer Sequence[1] | Amplification pattern | Repeat motives |
|---|---|---|---|---|
| 809-H | $45^0$C | AGAGAGAGAGAGAGAGG | Not reproducible | dinucleotide |
| 810- H | $45^0$C | GAGAGAGAGAGAGAGAT | Reproducible | dinucleotide |
| 811-H | $45^0$C | GAGAGAGAGAGAGAGAC | Not reproducible | dinucleotide |
| 814-H | $45^0$C | CTCTCTCTCTCTCTCTA | Not reproducible | dinucleotide |
| 818-H | $48^0$C | CACACA CAC ACA CAC AG | Reproducible | dinucleotide |
| 834-H | $45^0$C | AGAGAGAGAGAGAGAGYT | Reproducible | dinucleotide |
| 843-H | $45^0$C | CTCTCTCTCTCTCTCTRA | Not reproducible | dinucleotide |
| 844-H | $45^0$C | GAGAGAGAGAGAGAGAYT | Reproducible | dinucleotide |
| 845-H | $45^0$C | CTCTCTCTCTCTCTCTRG | Not reproducible | dinucleotide |
| 860-H | $45^0$C | TGTGTGTGTGTGTGTGRA | Reproducible | dinucleotide |
| 873-H | $45^0$C | GACAGACAGACAGACA | Reproducible not polymorphic | dinucleotide |
| 880-H | $48^0$C | GGAGAGGAGAGGAGA | Reproducible | Pentanucleotide |

*Source: Primer kit 900 (UBC 900);* [1] *Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T)*

### 4.5PCR and gel electrophoresis

The polymerase chain reaction was conducted in Biometra 2000 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1µl template DNA, 14.0µl $H_20$, 5.0µl dNTP (1.25mM), 2.5µl Taq buffer (10xThermopol reaction buffer), 2.0µl $MgCl_2$ (2mM), 0.3µl primer (20pmol/µl) and 0.2µl Taq Polymerase (5u/µl).

The amplification program wasset as 4 minutes preheating and initial denaturation at $94^0$C, followed by 40cycles of 15 seconds at $94^0$C, 1 minute primer annealing at ($45^0$C/ $48^0$C) based on primers used, 1 minuteand 30second extension at $72^0$C and the final extension for 7 minutes at $72^0$C. The PCR products were stored at $4^0$C until loading on gel for electrophoresis. The lid temperature was held at $105^0$C.The PCR products were stored at 4 $^o$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 8µl amplification product of each sample with 2µl loading dye (6 times concentrated) was loaded on gel. DNA marker 1k bp (molecular ladder) was used to estimate molecular weight and size of the fragments. Electrophoreses was done for 2 hours at constant voltage of 100V. The DNA was stained for 30 minutes with (10mg/ml) ethidum bromide (EtBr) which was mixed with 450 ml distilled water and distainedfor 30 minutes with 450ml of distilled water.

**4.6Data management and 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 version1.32 software (Yeh *et al.*, 1999) was used to calculate genetic diversity for each population as number

of polymorphic loci and percent polymorphism. 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, 2000) 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, 2000). The Neighbor Joining (NJ) method (Saitou and Nei1987; 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).

To further examine the patterns of variation among individual samples on 3D, a principal coordinated analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer *et al.,*2001). The first three axes were used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer *et al.,* 2001; statistica soft, Inc.2001).

# 5.RESULT

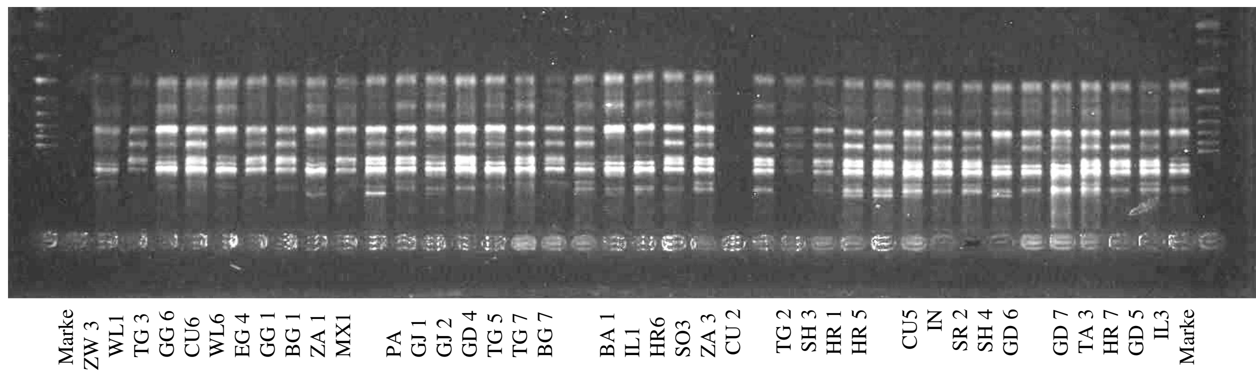
## 5.1 Banding patterns of the ISSR primers used

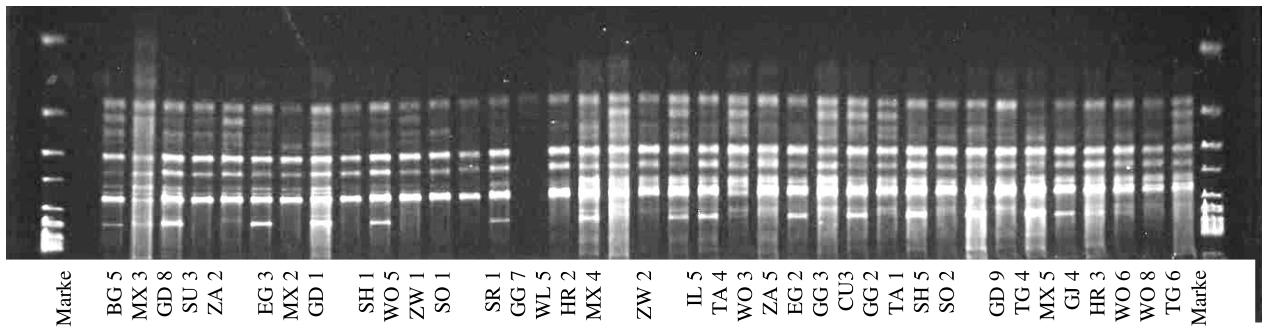
Out of the twelve primers tested initially, sixprimers (five di-nucleotide and one penta-nucleotide)thatgave relatively clear banding patternwere selected and used in this study (Table 3). The molecular weight of thebands amplified using the primers were in the range of 450 bp to 4000 bp. A totalof 58 clear bandswere scored, from 82 Ethiopian and 38 exotic sesame accessions.Out of the total 58 ISSR fragments44 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. Figure3 shows the amplification pattern of primer 844.The other primers gel picturesused in this study along with non-polymorphic primers that failed during optimization are presented in appendix 2, 3 and 4.

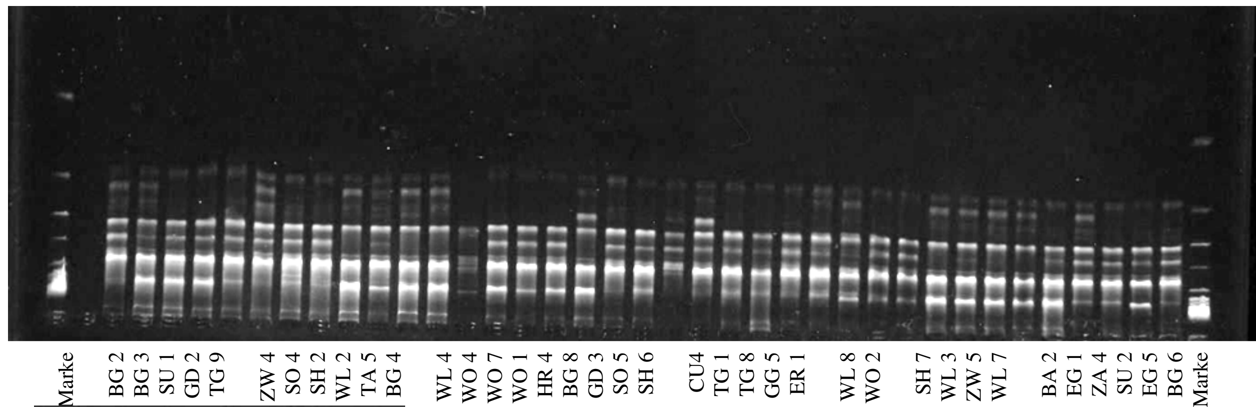
Table 3. Banding patterns generated using the six primers, their repeat motifs, amplification patterns and number of scored bands.

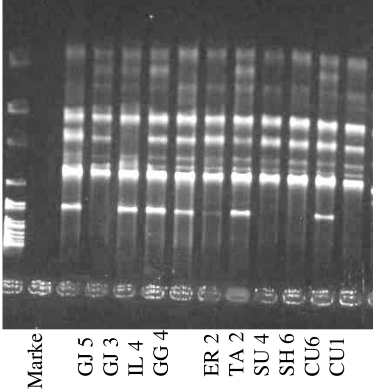
| Primers | Repeat motif[1] | Amplification pattern | Number of scored bands |
|---|---|---|---|
| 810-H | $(GA)_8T$ | Good | 9 |
| 818-H | (CA)8G | Good | 9 |
| 834-H | $(AG)_8YT$ | Good | 10 |
| 844-H | (CT)8RC | Good | 10 |
| 860-H | (TG)8RA | Good | 9 |
| 880-H | (GGAGA)3 | Good | 11 |
| | Total | | 58 |

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




Key- BG1-BG8-Benishangul Gumuz,GD1-GD9-Gonder, GG1-GG7-Gamo Goffa, GJ1-GJ5- Gojam, Hr1-Hr7- Harerge, Il1-Il2-Illibabore,SH1-SH7-Shewa, TG1-Tg11- Tigray, WO1-WO8-Welo, CU1-CU5-cultivated, W1-W9- Welega, EG1-EG5-Egypt, MX1-MX5- Mexico, SO1-SO5-Somalia, SU1-SU4-Sudan, TA1-TA5-Tanzania, ZA1-ZA5-Zambia, ZW1-ZW5-Zimbabwe.

Figure 3: ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 844-H.

## 5.2 Polymorphism based on ISSR analysis

In all populations or individuals the number of polymorphic loci ranges from four for primer-834 to eight forall 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 4). Among the exotic accessions(considering samples from different country as independent population)accessions from Sudan (27.95%) were the least polymorphicandthe 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 %. The rest primers namely, 844 and 880 showed 80.00 and 72.73 per cent polymorphism, respectively(Table5).

## 5.3 Genetic diversity

Among the sesame accessions evaluated the highest percentage polymorphic loci was obtained for samples from Welega (*P* = 58.62%), followed by samples from Tigray (*P* =50.00%) and Shewa (48.28%), while samples from Sudan (27.95%) and Gojam (25.86%) showed the least percent polymorphic loci. Likewise,grouping Ethiopian accessions in to administrative region based population and exotic accessions in to country based population showed that samplesfromWelega 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) werethe least diverse. The average gene diversity relative tothe 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 4). The extent of gene differentiation relative to the total Ethiopian population 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 4).

Table4.Number of scorable bands (NSB), 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±SD | I±SD | $G_{ST}$ | Nm |
|---|---|---|---|---|---|---|
| Benishangul Gumuz | 25 | 43.1 | 0.18 ± 0.22 | 0.26 ± 0.31 | | |
| Gamo Gofa | 24 | 41.38 | 0.17 ± 0.22 | 0.25 ± 0.31 | | |
| Gojam | 15 | 25.86 | 0.1 ± 0.18 | 0.15 ± 0.26 | | |
| Gonder | 26 | 44.83 | 0.19 ± 0.22 | 0.27 ± 0.31 | | |
| Harerge | 22 | 37.93 | 0.14 ± 0.2 | 0.21 ± 0.29 | | |
| Illubabor | 22 | 37.93 | 0.17 ± 0.23 | 0.25 ± 0.32 | | |
| Shewa | 28 | 48.28 | 0.2 ± 0.21 | 0.28 ± 0.31 | | |
| Tigray | 29 | 50 | 0.2 ± 0.21 | 0.27 ± 0.3 | | |
| Welega | 34 | 58.62 | 0.26 ± 0.23 | 0.37 ± 0.32 | | |
| Welo | 23 | 39.66 | 0.17 ± 0.22 | 0.24 ± 0.31 | | |
| Cultivated | 29 | 50 | 0.22 ± 0.23 | 0. 31 ± 0.32 | | |
| **Ethiopian Average** | **25.18** | **43.41** | **0.18** | **0.26** | | |
| **Ethiopia Total** | **44** | **75.86** | **0.24 ± 0.19** | **0.37 ± 0.27** | **0.25** | **1.49** |
| Egypt | 21 | 36.21 | 0.14 ± 0.21 | 0.21 ± 0.30 | | |
| Mexico | 22 | 37.93 | 0.14 ± 0.20 | 0.21 ± 0.28 | | |
| Somalia | 23 | 39.66 | 0.18 ± 0.23 | 0.26 ± 0.32 | | |
| S. E. Asia | 25 | 43.1 | 0.19 ± 0.23 | 0.27 ± 0.32 | | |
| Sudan | 16 | 27.95 | 0.12 ± 0.20 | 0.17 ± 0.29 | | |
| Tanzania | 22 | 37.93 | 0.15 ± 0.21 | 0.23 ± 0.30 | | |
| Zambia | 24 | 41.38 | 0.18 ± 0.22 | 0.25 ± 0.31 | | |
| Zimbabwe | 24 | 41.38 | 0.18 ± 0.22 | 0.25 ± 0.31 | | |
| **Exotic average** | **22.12** | **38.1925** | **0.16** | **0.23125** | | |
| **Exotic Total** | **38** | **65.52** | **0.23 ± 0.20** | **0.34 ± 0.28** | **0.29** | **1.19** |
| **Average population** | **23.89** | **41.21** | | | | |
| **Total Population** | **44** | **75.86** | **0.24 ± 0.19** | **0.37 ± 0.27** | **0.28** | **1.28** |

Table 5. number of Scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP) genetic diversity (GD) Shanon Index (I), for each primer

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

### 5.4 Analysis of molecular variance

Analysis of molecular variance was carried out on the overall ISSR data score of sesame accession without and with grouping (Table 6 and table 7). AMOVAwithout 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 5.5). The variations was found to be highly significant at (P=0.00, 1000 permutations).

Table 6. Analysis of Molecular Variance (AMOVA) of sesame accessions in Ethiopia without grouping.

| Source of variation | Sum of Square | 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 7. 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 | 2.51660 | 94.08632 | | 0.00 |
| **Total** | **302.667** | **2.67477** | | | |

**5.5Genetic Similarity**

Inter-population genetic distance (D*)* ranged from 0.031 to 0.165 for the total 19 population(Table 8).Administrative region based population classification of the Ethiopian sesame accessions revealed inter population genetic distance ranging from 0.041to 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 9).Among the pairwise population comparisons made within Ethiopian population samples from Welega and Harerge showed the highest genetic distance (0.160) andsamples 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 wasverylow with the least genetic distance between samples of Mexico and Tanzania (0.031).

Table 8. 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 | 0.0950 | 0.0666 | 0.0872 | 0.0865 | 0.0595 | 0.1650 | 0.0444 | 0.0571 | 0.1188 | 0.0669 | 0.1119 | 0.0590 | 0.0856 | 0.0779 | 0.0777 | 0.0766 | 0.0835 | **** |

Table 9. 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BG | **** | 0.9377 | 0.9539 | 0.9073 | 0.9384 | 0.9117 | 0.9111 | 0.9302 | 0.9412 | 0.9142 | 0.9253 |
| CU | 0.0643 | **** | 0.9319 | 0.9229 | 0.9161 | 0.9050 | 0.9088 | 0.9261 | 0.9326 | 0.9088 | 0.9067 |
| GD | 0.0472 | 0.0705 | **** | 0.9497 | 0.9329 | 0.9336 | 0.9214 | 0.9405 | 0.9384 | 0.9067 | 0.9372 |
| GG | 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 | **** |

Key- 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

## 5.6 Clustering analysis

UPGMA and neighbor joining analysis was used to construct dendrogram for 19 populations and 120 individuals based on 58 PCR bands amplified by five di-nucleotides (810, 818, 834,844 and 860) and one penta nucleotides (880) ISSR primers as shown in Figure 5 and appendix 5. The dendrogram derived from neighbor-joining analysis of the whole ISSR data set that includes 82 Ethiopian sesame accessions and 38 exotic accessions showed two distinct clusters (cluster I and cluster II) and sub-clusters within the secondmajorcluster (cluster $I^1$ and cluster $II^1$). With the exception of the South East Asian accessions (India, Pakistan and Srilanka) which were outliersand the Mexico accessions which are clustered in cluster II sub division I in the denderogram other exotic accessions did not show a clear grouping i.e. they grouped along with the Ethiopian ones rather than forming their own sub cluster. Few accessions from Benishangul Gumuz, Tigray, Welega and Wello were tended to form their own cluster while the rest accessionsspread all over the tree.

UPGMA analysisof Ethiopian sesame populations revealed two major groups and three outliers (Cultivated, Welega and Illubabore). The first major cluster again forked into twosub 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 somesesame germplasm might have been transported from Welo to Gamo Gofa along with human(Figure 4). The second major cluster comprise of Gojam, Benishangul Gumuz and Gonder populations which embodies the north western parts of the country.

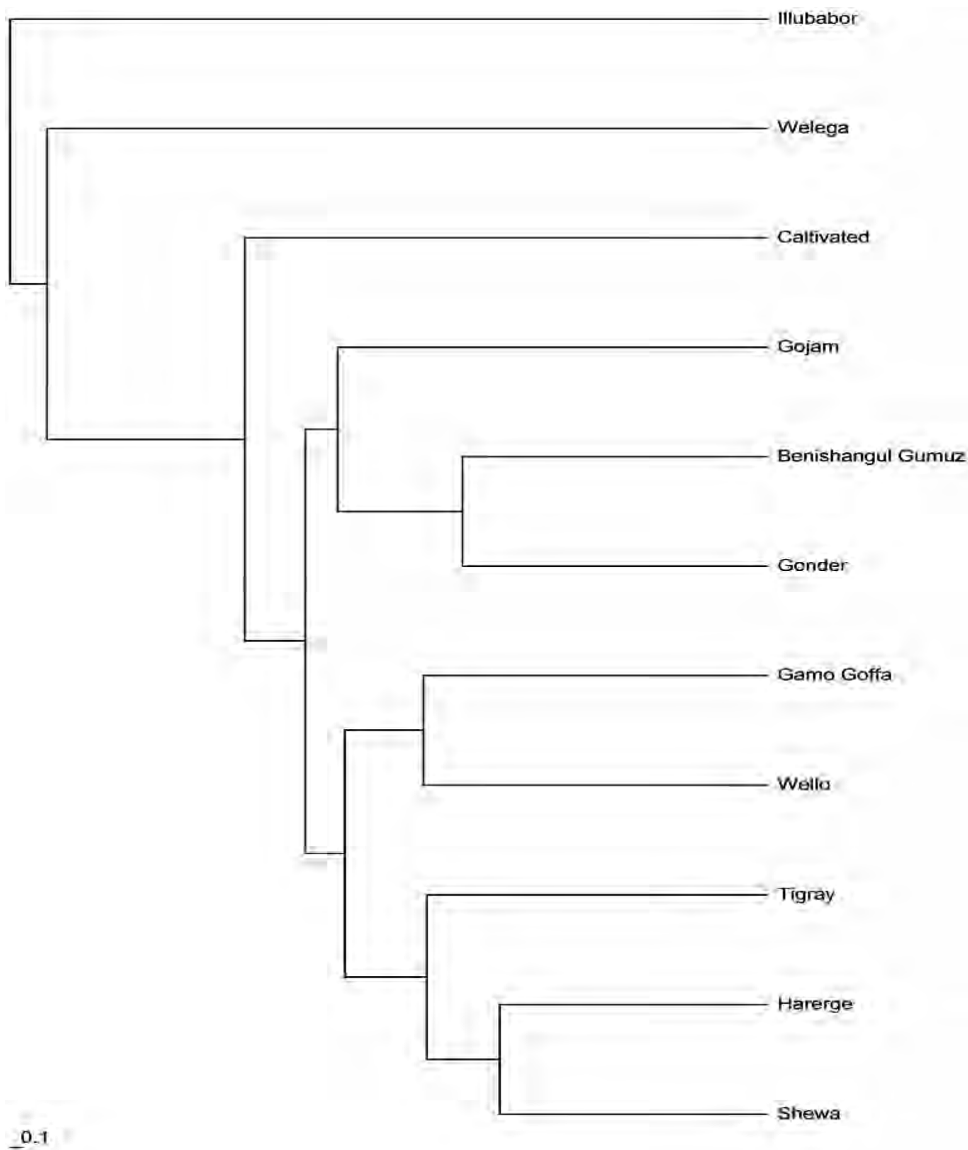
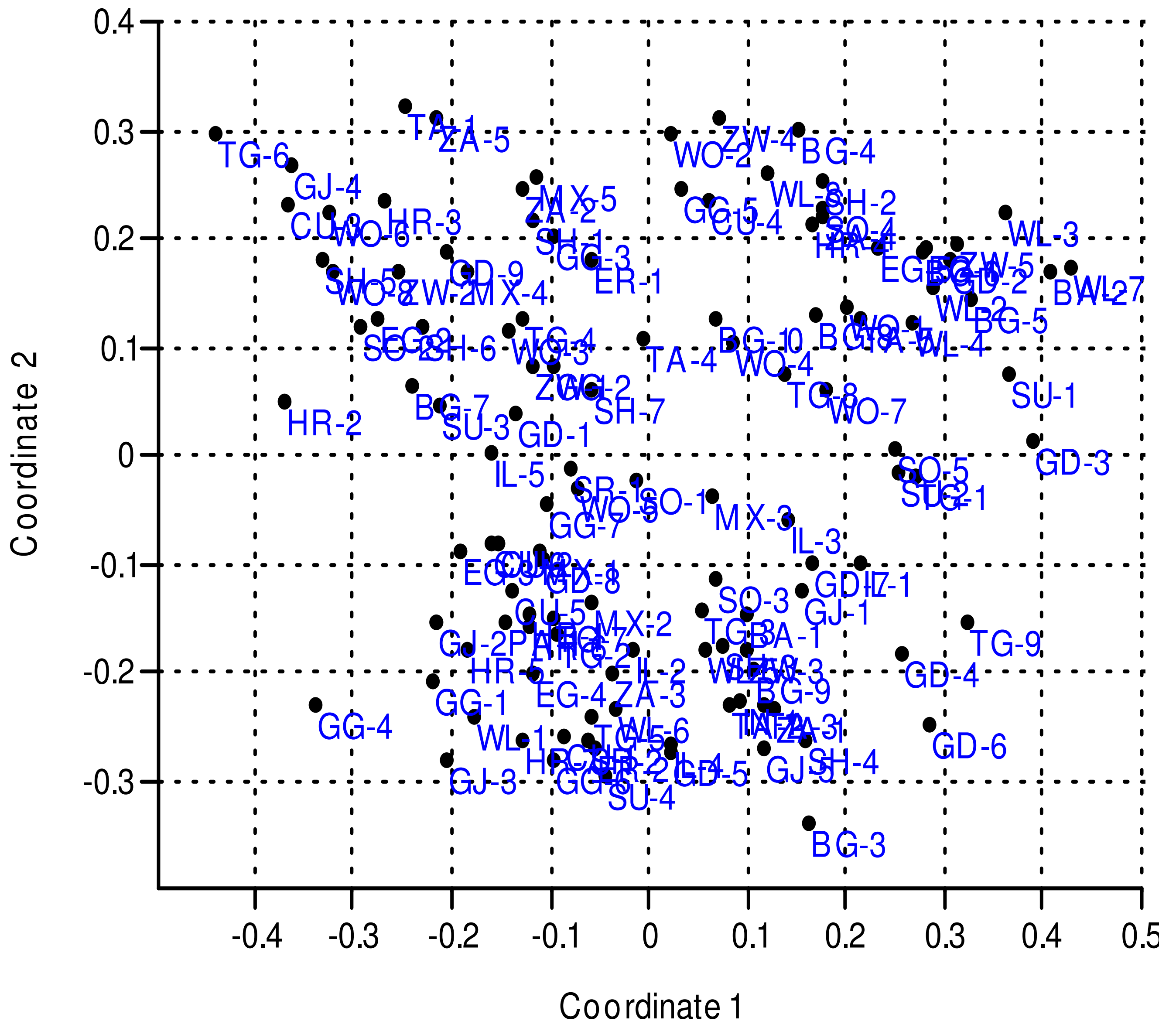


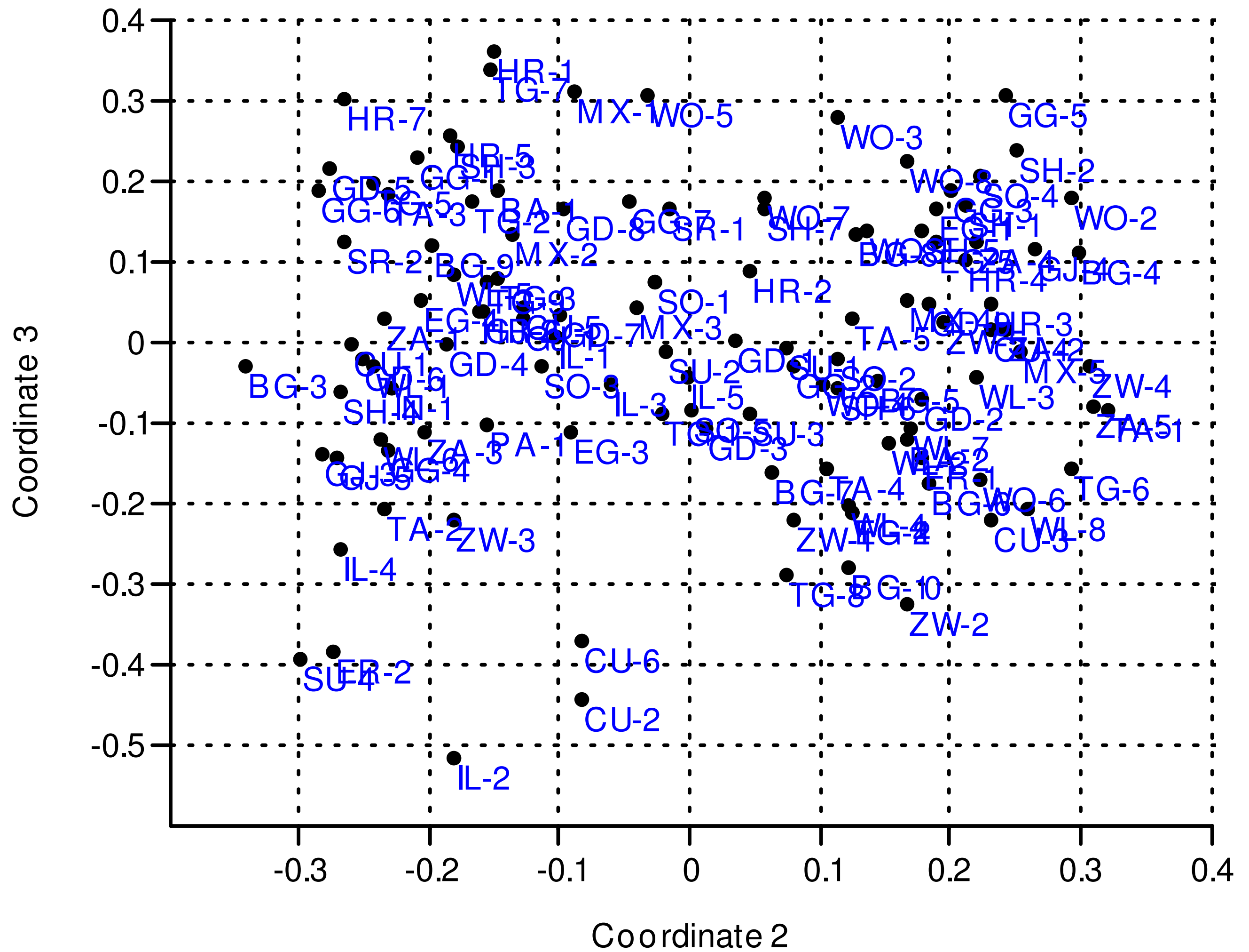

Figure 4. NJ based dendrogram for 11Ethiopian sesame populations using 6 ISSR (5 di and1 penta nucleotide) primers.

Figure5: Neighbor-joining analysis of 120 individuals based on 58 PCR bands amplified by five dinucleotide (810,818,834, 844 and 860) and one pentanucleotide (880) primers. The neighbor joining algorithm is based on Jaccard's coefficients obtained after pair wise comparison of the presence-absence fingerprint.Key- BG1-BG8-Benishangul Gumuz,GD1-GD9-Gonder, GG1-GG7-Gamo Goffa, GJ1-GJ5- Gojam, Hr1-Hr7- Harerge, Il1-Il2-Illibabore,SH1-SH7-Shewa, TG1-Tg11-Tigray, WO1-WO8-Welo, CU1-CU5-cultivated, W1-W9- Welega,EG1-EG5-Egypt, MX1-MX5-Mexico, SO1-SO5-Somalia, SU1-SU4-Sudan, TA1-TA5-Tanzania, ZA1-ZA5-Zambia, ZW1-ZW5-Zimbabwe.

## 5.7 PCO analysis

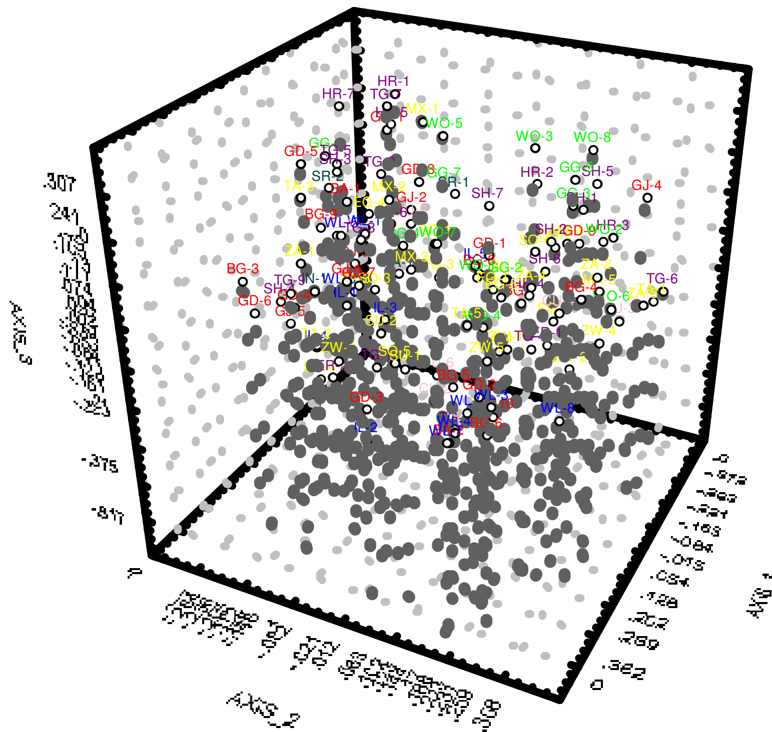
All the data obtained using six ISSR primers were used in PCO analysis using Jaccard's coefficients of similarity. The first three coordinates of the PCO having eigenvalues of 4.85, 4.27 and 3.73 with variance of 7.77%, 6.84% and 5.99% respectively used to show the grouping of individuals using two and three coordinates (Figure 6 and Figure 7) . With the exception of few accessions from Welega, Wello and Ilubabore,most of the individual accessions that represent different populationsspread all over in 3D space. Using two coordinates (Figure 6) almost similar pattern was observed like that of three coordinates.






Key- BG1-BG8-Benishangul Gumuz,GD1-GD9-Gonder, GG1-GG7-Gamo Goffa, GJ1-GJ5-Gojam, Hr1-Hr7- Harerge, Il1-Il2-Illibabore,SH1-SH7-Shewa, TG1-Tg11- Tigray, WO1-WO8-Welo, CU1-CU5-cultivated, W1-W9- Welega, EG1-EG5-Egypt, MX1-MX5- Mexico, SO1-SO5-Somalia, SU1-SU4-Sudan, TA1-TA5-Tanzania, ZA1-ZA5-Zambia, ZW1-ZW5-Zimbabwe.

Figure 6: Two dimensional representation of principal coordinate analysis of genetic relationships among 120 individuals of 82 Ethiopian and 38 exotic accessions of Ethiopia.




Key-

BG1-BG8-Benishangul Gumuz,GD1-GD9-Gonder, GJ1-GJ5- Gojam, SH1-SH7-Shewa, Hr1-Hr7- Harerge, TG1-Tg11- Tigray,WO1-WO8-Welo, GG1-GG7-Gamo Goffa, Il1-Il2-Illibabore,W1-W9-Welega, CU1-CU5-cultivated, EG1-EG5-Egypt, MX1-MX5- Mexico, SO1-SO5-Somalia, SU1-SU4-Sudan, TA1-TA5-Tanzania, ZA1-ZA5-Zambia, ZW1-ZW5-Zimbabwe.

Figure 7: Three dimensional representation of principal coordinate analysis of genetic relationships among 120 individuals of 82 Ethiopian and 38 exotic accessions of Ethiopia.

# 6. DISCUSSION

## 6.1Genetic diversityand 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 observed to be very useful in detecting genetic diversity and population structure of Tef (Assefa, 2003), Coffee (Aga, 2005; Tesfaye, 2006), Lentils (Fikru, 2006), Rice (Gezahegne, 2007; Mitiku, 2011) and sesame (Admas, 2011) collected from different parts of Ethiopia. In addition to the advantages (inexpensive, easy to generate), ISSRs are powerful in detecting polymorphismswith high reproducibility.ISSR can detect even more polymorphism than RFLPs in maize (Kantety *et 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 dictate 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.* 2006; 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* (2006) 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 fourteen reliable ISSR primers and found 33% polymorphismfrom 79 amplification products among the 75 sesame accessions. This is low when it is compared with this results present polymorphism (75.86%).Admas (2011) also used 4ISSR primers for detecting the genetic diversity of 60 individuals ofsesame which were collected from north western Ethiopia and found an overall genetic diversity of 0.34. The four primers used by Admas were used in this study with additional two primers and with the exception of 834 primers 810, 844 and 880 showed higher number of polymorphic loci.The genetic diversity of this study is by far lowas compared to Admas which may be due to his new germplasm collection as compared to the late IBC collections or location of the sample collection (north western Ethiopia). RAPD marker was also used by Abdellatef *etal* (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 (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 Andualem (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 programme 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 areasmoderate to higher genetic diversity is revealed by the

accessionsfrom 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.

### 6.2 Genetic distanceand relationship among populations

Genetic distance is a measure of the allelic substitutions per locus that have occurred during the separate evolution of two populations or species. Smaller genetic distances indicate a close genetic relationship where as large genetic distances indicate a more distant genetic relationship. Crosses between distantly related individuals are expected to give better offspring than those between closely related genotypes. Therefore, prior knowledge of the genetic distance between genotypes or accessions is important in designing breeding program.

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 Ilubabore, Harergea, Gamo Goffa, Gojam and Wello showed moderate to high genetic distance related to their respective pair wise comparison. Samples from Benishangul Gimuz, 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 north west.Even though 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.

### 6.3. Genetic structureand patterns of distribution

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 studythere 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 todifferences within groups (Laurentinand Karlovsky 2006).In another study,Daniel Endale (2008), using SSR marker, also found41.2% of the total genetic variation between population and 58.8 % within populationfor 50 sesame landraces of Ethiopia. Similarly, the result of this study is also in agreement with the above AFLP andSSR 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.* 2006). 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 explainedin linewith the above argument.Geneflow is the exchange or movement of gametes, individuals,and populationson a geographic scale (Joseph and Bruce 1993).Gene flow, in conjunction with other evolutionaryforces, 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 betweenpopulations is sufficient to prevent substantial differentiation between thosepopulations(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 $Nm >$ 1there 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 showed 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. 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 becameapparent from the cluster analysis(UPGMA and Nj) and both the 3D and 2D PCO analysis of this study.

# 7. CONCLUSION

Among the important oil crops grown in Ethiopia, sesame seed commands a unique position chiefly on account of the fact that it is highly adapted to arid and semi-arid low land environment with fairly good yields. In Ethiopia the bulk of sesame seed, more than 90% of the production, is directed to export. Even though there is a firm belief with Indian researchers that there region is the center of origin for cultivated sesame, with other authors diversity centers have been identified as India, China, Central Asia, Near East and Abyssinia(Laurentin and Karlovsky 2006). The present study was conducted with the main objective of assessing the extent of genetic diversity among some Ethiopian and exotic sesame accessions collected by the Institute of Biodiversity conservation, Addis Ababa Ethiopia using Inter Simple Sequence Repeat marker.

This study showed that bulk sampling strategy for DNA analysis and ISSR marker are important for genetic diversity study in sesame. Bulk sampling approach was chosen because it is economical, rapid andpermits representation of the vast accession by optimum number of plants.The study also showed, among the sesame accessions held by IBC, in comparison Ethiopian accessions are more diverse than the exotic ones.

Separate analysis of the Ethiopian accession revealedthere are places with higher genetic diversity which will be valuable for the future collection, conservation and improvement strategies.Samples from Welega,Tigray and Shewa showedrelatively high level of genetic diversity;hence these regions should be the main focus for future collection and population improvement based on selection, whereas samples from Harerge and Shewa showed lower genetic diversity. The availability of accession from these regions is limited. This suggests that lower diversity areas need to be well represented by further collection to enrich the germplasm gene pool.Breeders should consider divergent populations of Illubabor, Harergea, GamoGoffa, Gojam and Welofor higher heterosis effect since they are distantly related to their respective pair wise comparison.

Results from gene flow estimates and the clustering and PCO analysis for the Ethiopian and some exotic sesame accessions revealed that there was a 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 sever if an administrative region is represented by small sample as in the case of IBC.

The analysis of molecular variance for the accessions studied showed that the highest proportion of genetic variation was attributed to within population than among population. It is also highlysignificant. This confirms the result of the Nm and Gst that says there was a high level of gene flow and low level of genetic differentiation.Based on the molecular data, the Ethiopian accessions were clustered into two major groups with some outliers and the first majorcluster again forked in to two su- clusters.

# 8. RECOMMENDATIONS

- Analysis of genetic diversity in crop species using more than one methods helps to better understand the levels of genetic variation and the genetic structure of populations when compared to the results obtained using only one method. Morphological analysis and analysis with co-dominant markers system, like microsatellites, needs to beconducted to better understand and estimate the gene flow, the size of apopulation and levels of inbreeding.Hence, it is highly recommended to carry out the same study with SSR with increased sample number for better representation from other continents. Moreover morphological analysis should be executed to see the accessions performance to disease and traits like yield, yield components and oil analysis.

- As compared to other countries claiming to be the center of diversity for sesame the accession presented in Ethiopian Gene-Bank is very limited hence there is a need forfurther collection of accession to better representsa population/administrative regions by as many collections as possible.In line with this study, prior consideration should be given for administrative regions that show higher genetic diversity in further collection and conservation strategies since they are expected to contribute more accessions than with low diversity administrative regions. On the other hand, apart from targeting areas of high genetic diversity the limited collections from areas of low genetic diversity should be supplemented with additional collections mission so as to get unique landraces confined to certain agro-ecology.

- The Ethiopian region have been considered as the center of diversity for wild sesame. One thing that several sesame researchers agreed upon is this fact. Even though the current accessions in IBC could not show wild sesame,future collection mission should becarried out to include wild and weedy relatives of sesamebefore they get lost irreversibly.The wild relative could be the best sources to transfer traits of agronomic importance and hence further study should be executed to see if some of this wild are compatible for wide hybridization.

- From the very nature of the crop, which is grown in the lowlands of Ethiopia, characterized by poor infrastructure, harsh environment and poor output from research endeavors,there is a limited interest to engage in sesame research among the research community. Seeing the use of the crop for the country's economy, this has tobe changedand the government should design better strategy to develop variety of high export market value with improved marketing system.The lack of knowledge and technology in sesame should be circumvented.

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# 10. APPENDICES

Appendix 1: Pasport data of sesame accessions that were obtained from IBC

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 210871 | Sesamum | indicum | Eritrea | | | | | | | LT | 3 | Eritrea collection |
| 2 | 214202 | Sesamum | indicum | Eritrea | | | | | | | LT | 3 | Eritrea collection |
| 3 | 111501 | Sesamum | sp | Gamo Gofa | SNNP | SEMEN OMO | GOFA ZURIA | | | | LT | 3 | |
| 4 | 208888 | Sesamum | indicum | Gamo Gofa | SNNP | SEMEN OMO | KUCHA | 06-25-00-N | 37-05-00-E | | LT | 4 | |
| 5 | 208889 | Sesamum | indicum | Gamo Gofa | SNNP | SEMEN OMO | ZALA UBAMALE | 06-20-00-N | 37-00-00-E | 1050 | LT | 3 | |
| 6 | 212993 | Sesamum | indicum | Gamo Gofa | SNNP | SEMEN OMO | BOREDA ABAYA | 37-45-00-N | 06-21-00-E | 1290 | LT | 3 | Collected by Brook Abebe & Mohammed Hassen |
| 7 | 212994 | Sesamum | indicum | Gamo Gofa | SNNP | BENCH MAJI | DIRASHE special | 37-31-00-N | 05-55-00-E | 1270 | LT | 4 | Collected by Brook Abebe & Mohammed Hassen |
| 8 | 212995 | Sesamum | indicum | Gamo Gofa | SNNP | SEMEN OMO | GOFA ZURIA | 36-56-00-N | 06-21-00-E | 1290 | LT | 4 | Collected by Brook Abebe & Mohammed Hassen |
| 9 | 214976 | Sesamum | indicum | Gamo Gofa | SNNP | SEMEN OMO | KUCHA | | | | LT | 4 | |
| 10 | 111502 | Sesamum | sp | Gojam | Amara | MIRAB GOJAM | JABI TEHNAN | | | | LT | 4 | |
| 11 | 111503 | Sesamum | sp | Gojam | Amara | MISRAK GOJAM | HULET EJ ENESE | | | | LT | 4 | |
| 12 | 111513 | Sesamum | sp | Gojam | | | | | | | LT | 4 | Origenal collection sheet is missing |

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 211921 | Sesamum | indicum | Gojam | Amara | MISRAK GOJAM | DEJEN | 10-10-00-N | 38-20-00-E | 1600 | LT | 5 | |
| 14 | 230260 | Sesamum | sp | Gojam | Benishangul & Gumuz | METEKEL | DANGUR | | | | LT | 3 | |
| 15 | 205173 | Sesamum | sp | Gonder | Amara | SEMEN GONDAR | DEBARK | | | | LT | 4 | Collected by Hagos W/Gebriel |
| 16 | 235769 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | METEMA | 12-46-00-N | 15-10-00-E | 900 | LT | 5 | Collected by Brhane G/Mariam |
| 17 | 235903 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | LAY ARMACHO | 12-45-00-N | 37-33-00-E | 1150 | LT | 4 | Collected by Brhane G/Mariam |
| 18 | 241341 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | QUARA | | | 600 | LT | 5 | |
| 19 | 241342 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | METEMA | | | 600 | LT | 5 | |
| 20 | 241334 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | METEMA | | | 750 | LT | 5 | |
| 21 | 241344 | Sesamum | indicum | Gonder | Amara | SEMEN GONDAR | CHILGA | | | 980 | LT | 5 | |
| 22 | 241346 | Sesamum | indicum | Gonder | Amara | DEBUB GONDAR | EBENAT | | | 1890 | LT | 5 | |
| 23 | 241347 | Sesamum | indicum | Gonder | Amara | SEMEN GONDA | BELESA | | | 1700 | LT | 5 | |
| 24 | 202512 | Sesamum | sp | Harerge | Oromiya | MISRAK HARERGE | BABILE | | | 1600 | LT | 4 | Source IAR/ Holetta |

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 208670 | Sesamum | indicum | Harerge | Oromiya | MIRAB HARERGE | DAROLEBU | 08-30-00-N | 40-12-00-E | 1830 | LT | 3 | Collected by Dr. Seegeler and Abebe Demisse |
| 26 | 208671 | Sesamum | indicum | Harerge | Oromiya | MIRAB HARERGE | HABRO | 08-50-00-N | 40-25-00-E | 1900 | LT | 3 | |
| 27 | 208672 | Sesamum | indicum | Harerge | Oromiya | MISRAK HARERGE | BABILE | 09-10-00-N | 42-20-00-E | 1350 | LT | 3 | Collected by Dr. Seegeler and Abebe Demisse |
| 28 | 208673 | Sesamum | indicum | Harerge | Oromiya | MISRAK HARERGE | KURFA CHELE | 09-20-00-N | 41-54-00-E | 1840 | LT | 4 | Collected by Dr. Seegeler and Abebe Demisse |
| 29 | 228816 | Sesamum | indicum | Harerge | Oromiya | MISRAK HARERGE | BABILE | | | 1500 | LT | 4 | |
| 30 | 223340 | Sesamum | indicum | Harerge | Oromiya | MIRAB HARERGE | GUBA KORICHA | | | | LT | 4 | |
| 31 | 207956 | Sesamum | indicum | Illubabor | Gambella | ZONE 1 | GAMBELA | 08-15-00-N | 34-30-00-E | | LT | 3 | Collected by Seegeler and Abebe Demissie |
| 32 | 207957 | Sesamum | indicum | Illubabor | Gambella | ZONE 1 | GAMBELA | 08-15-00-N | 34-30-00-E | | LT | 3 | Collected by Seegeler and Abebe Demissie |
| 33 | 216733 | Sesamum | indicum | Illubabor | Gambella | ZONE 2 | ABOBO | 07-56-00-N | 34-41-00-E | 600 | LT | 6 | |
| 34 | 222876 | Sesamum | indicum | Illubabor | Gambella | ZONE 1 | GAMBEL | | | | LT | 4 | |

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | 202285 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | MAFUDMEZEZO MOJANA | 09-55-00-N | 39-51-00-E | 1490 | LT | 4 | Collected by Dott. Giorghis, Getinet & Adugna |
| 36 | 202286 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | KEWET | 09-58-00-N | 39-51-00-E | 1490 | LT | 4 | Collected by Dott. Giorghis, Getinet & Adugna |
| 37 | 202374 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | EFRATANA GIDIM | 10-12-00-N | 39-59-00-E | 1395 | LT | 4 | Collected by Dott. Giorghis, Getinet & Adugna |
| 38 | 203099 | Sesamum | indicum | Shewa | SNNP | GURAGE | GORO | | | 1240 | LT | 4 | Collected by Fassil Kibebew & Hirut Kebede |
| 39 | 212536 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | LAY BETNA TACH BET | 09-57-00-N | 38-54-00-E | 1580 | LT | 3 | Collected by Abebe Demissie and Yohannes Tadess |
| 40 | 212537 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | LAY BETNA TACH BET | | | | LT | 4 | Collected by Abebe Demissie and Yohannes Tadess |
| 41 | 212540 | Sesamum | indicum | Shewa | Amara | SEMEN SHEWA | WEREMO WAJETUNA MID | 10-10-00-N | 38-58-00-E | 1600 | LT | 3 | Collected by Abebe Demissie and Yohannes Tadess |
| 42 | 234014 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | TAHTAY ADIYABO | | | 1090 | LT | 3 | |
| 43 | 234015 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | TAHTAY ADIYABO | | | 1090 | LT | 4 | |
| 44 | 235405 | Sesamum | indicum | Tigray | Tigray | DEBUBAWI | RAYAAZEBO | 12-31-00-N | 39-42-00-E | 1650 | LT | 5 | Collected by Berhane G/Mariam |

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 45 | 237523 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | TSELEMTI | 11-57-00-N | 38-41-00-E | 900 | LT | 5 | Collected by Berhane G/Mariam |
| 46 | 238269 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | KAFTA HUMERA | 14-02-00-N | 36-05-00-E | 820 | LT | 5 | |
| 47 | 238270 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | KAFTA HUMERA | 14-02-00-N | 36-05-00-E | 710 | LT | 5 | |
| 48 | 238271 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | KAFTA HUMERA | 14-02-00-N | 36-05-00-E | 710 | LT | 5 | |
| 49 | 238272 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | KAFTA HUMERA | 14-02-00-N | 36-05-00-E | 710 | LT | 4 | |
| 50 | 241305 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | TAHTAY ADIYABO | | | 1200 | LT | 5 | |
| 51 | 241311 | Sesamum | indicum | Tigray | Tigray | MIRABAWI | TAHTAY ADIYABO | | | 1120 | LT | 4 | |
| 52 | 111505 | Sesamum | sp | Welega | | | | 09-28-00-N | 36-31-00-E | 1440 | LT | 3 | |
| 53 | 215816 | Sesamum | indicum | Welega | Oromiya | MISRAK WELLEGA | DIGA LEKA | 08-15-00-N | 34-35-0-E | 1435 | LT | 4 | Collected By Englse & Tadesse Dadi |
| 54 | 202517 | Sesamum | sp | Welega | Oromiya | MISRAK WELLEGA | GIDA KIREMU | | | 1420 | LT | 4 | Source IAR/ Holetta |
| 55 | 202518 | Sesamum | sp | Welega | Oromiya | MISRAK WELLEGA | DIGA LEKA | | | 1360 | LT | 4 | Source IAR/ Holetta |

| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56 | 207953 | Sesamum | indicum | Welega | Oromiya | MIRAB WELLEGA | GIMBI | 09-04-00-N | 35-57-00-E | 1300 | LT | 3 | Collected by Seegeler and Abebe Demissie |
| 57 | 207954 | Sesamum | indicum | Welega | Oromiya | MISRAK WELLEGA | DIGA LEKA | 09-01-00-N | 36-10-00-E | 1200 | LT | 3 | Collected by Seegeler and Abebe Demissie |
| 58 | 208752 | Sesamum | indicum | Welega | Oromiya | MIRAB WELLEGA | SAYO | | | | LT | 3 | Collected by Ir. J. Engels and Tadesse Dadi |
| 59 | 237994 | Sesamum | sp | Welega | Oromiya | MIRAB WELLEGA | GIMBI | | | 1290 | LT | 5 | Collected by Dibaba Dammesa & Samson Gashu |
| 60 | 202318 | Sesamum | indicum | Welo | Amara | DEBUB WELLO | KALU | | | 1525 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |
| 61 | 202336 | Sesamum | indicum | Welo | Amara | DEBUB WELLO | WEREBABU | | | 1290 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |
| 62 | 202340 | Sesamum | indicum | Welo | Amara | DEBUB WELLO | TEHULEDERE | | | 1555 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |
| 63 | 202345 | Sesamum | indicum | Welo | Amara | SEMEN WELLO | HABRU | | | 1565 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |
| 64 | 202353 | Sesamum | indicum | Welo | Amara | SEMEN WELLO | GUBA LAFTO | | | 1640 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |

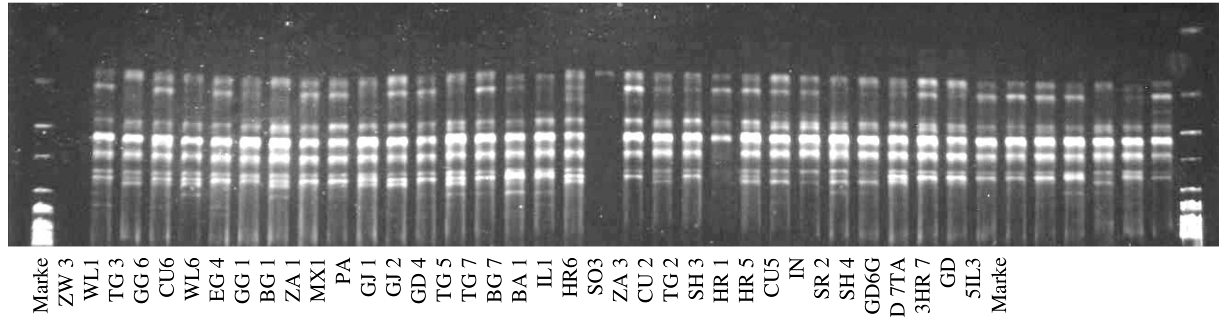
| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 65 | 202370 | Sesamum | indicum | Welo | Amara | SEMEN WELLO | KOBO | | | 1590 | LT | 3 | Collected by Dott. Giorghis, Getinet & Adugna |
| 66 | 212632 | Sesamum | indicum | Welo | Amara | DEBUB WELLO | AMBASEL | 11-25-00-N | 39-37-00-E | 1460 | LT | 4 | Collected by Mahteme H.Giorgis & Asheber Tesha |
| 67 | 215260 | Sesamum | indicum | Welo | Amara | SEMEN WELLO | GIDAN | | | | LT | 4 | Collected by Abebe Demissie |
| 68 | 231408 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Tanzania |
| 69 | 231410 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Tanzania |
| 70 | 203631 | Sesamum | sp | Not Known | | | | 12-11-00-S | 26-23-00-E | | LT | 4 | Donation from ZAMBIA |
| 71 | 203633 | Sesamum | sp | Not Known | | | | 13-15-00-S | 25-15-00-E | | LT | 5 | Donation from ZAMBIA |
| 72 | 203634 | Sesamum | indicum | Not Known | | | | 13-50-00-S | 23-55-00-E | | LT | 3 | Donation from ZAMBIA |
| 73 | 203637 | Sesamum | sp | Not Known | | | | 12-11-00-S | 26-23-00-E | | LT | 3 | Donation from ZAMBIA |
| 74 | 203638 | Sesamum | sp | Not Known | | | | 12-11-00-S | 26-25-00-E | | LT | 3 | Donation from ZAMBIA |
| 75 | 227862 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Mexico |
| 76 | 227864 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Mexico |
| 77 | 227865 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Mexico |

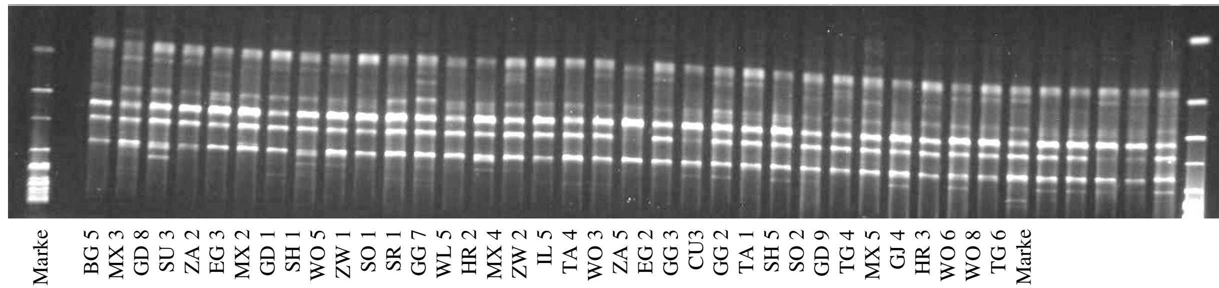
| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | 227866 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Mexico |
| 79 | 227867 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Mexico |
| 80 | 227873 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Egypt |
| 81 | 227874 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Egypt |
| 82 | 227875 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Egypt |
| 83 | 227876 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Egypt |
| 84 | 227879 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Egypt |
| 85 | 227903 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Somalia |
| 86 | 227904 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Somalia |
| 87 | 227906 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Somalia |
| 88 | 227907 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Somalia |
| 89 | 227908 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Somalia |
| 90 | 227934 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Sirilanka |
| 91 | 231397 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From INDIA |
| 92 | 231399 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From INDIA |
| 93 | 227933 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Sirilanka |
| 94 | 227855 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Pakistan |

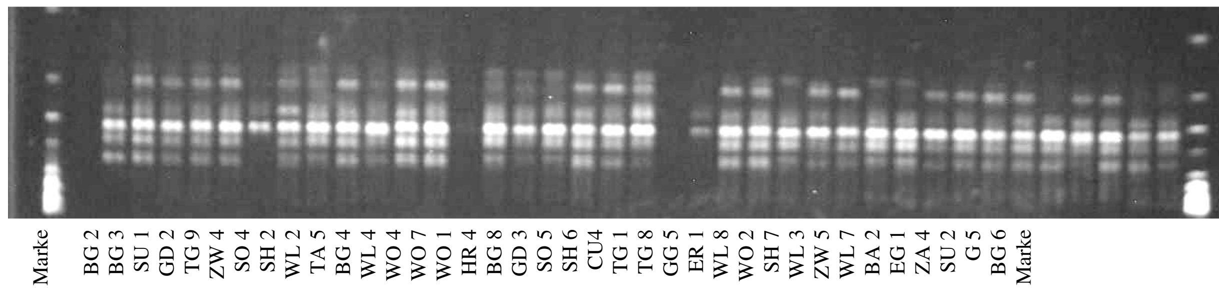
| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 95 | 231406 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Tanzania |
| 96 | 231407 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Tanzania |
| 97 | 231409 | Sesamum | indicum | Not Known | | | | | | | LT | 5 | Donation From Tanzania |
| 98 | 205194 | Sesamum | indicum | Not Known | | | | 12-29-00-N | 22-31-00-E | 700 | LT | 4 | IBPGR(Donation) |
| 99 | 205195 | Sesamum | indicum | Not Known | | | | 12-29-00-N | 22-31-00-E | 700 | LT | 4 | IBPGR(Donation) |
| 100 | 205191 | Sesamum | indicum | Not Known | | | | 13-20-00-N | 22-53-00-E | 800 | LT | 4 | IBPGR(Donation) |
| 101 | 205192 | Sesamum | indicum | Not Known | | | | 13-10-00-N | 22-49-00-E | 900 | LT | 3 | IBPGR(Donation) |
| 102 | 203595 | Sesamum | indicum | Not Known | | | | 20-15-00-S | 31-03-00-E | 1100 | LT | 3 | Donation from ZIMBABWE |
| 103 | 203597 | Sesamum | sp | Not Known | | | | 20-33-00-S | 30-42-00-E | 760 | LT | 4 | Donation from ZIMBABWE |
| 104 | 203599 | Sesamum | sp | Not Known | | | | | | | LT | 3 | Donation from ZIMBABWE |
| 105 | 203600 | Sesamum | indicum | Not Known | | | | 20-18-00-S | 32-49-00-E | 700 | LT | 4 | Donation from ZIMBABWE |
| 106 | 203627 | Sesamum | sp | Not Known | | | | 18-27-00-S | 32-58-00-E | 750 | LT | 3 | Donation from ZIMBABWE |
| 107 | Abasina | Sesamum | indicum | | | | | | | | | | Cultivated |

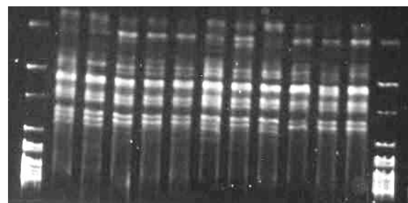
| No | Accession Number | Genus name | Species Name | Former Administrative Region | Region/ State/Province | Zone | Woreda /District | Latitude | Longitude | Altitude | Status | No of available Subsamples | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 108 | E | Sesamum | sp | | | | | | | | | | Cultivated |
| 109 | Hir Hir or T- 85 | Sesamum | sp | | | | | | | | | | Cultivated |
| 110 | Mehedo | Sesamum | sp | | | | | | | | | | Cultivated |
| 111 | Adi | Sesamum | sp | | | | | | | | | | Cultivated |
| 112 | Acc-BG-006 | | Benishangul gumuz | | | | | | | 1290 | | | |
| 113 | Acc-BG-002 | | Benishangul gumuz | | | | | | | 1270 | | | |
| 114 | Acc-BG-001 | | Benishangul gumuz | | | | | | | 1290 | | | |
| 115 | Acc-BG-003 | | Benishangul gumuz | | | | | | | 1050 | | | |
| 116 | Acc-BG-010 | | Benishangul gumuz | | | | | | | NA | | | |
| 117 | Acc-BG-012(2) | | Benishangul gumuz | | | | | | | 1050 | | | |
| 118 | Acc-BG-003(2) | | Benishangul gumuz | | | | | | | NA | | | |
| 119 | Acc-BG-019(1) | | Benishangul gumuz | | | | | | | 1600 | | | |
| 120 | New EW-004 | | Bako | | | | | | | NA | | | |

Appendix 2 ISSR fingerprint generated by primer 810,818,834,860 and 880



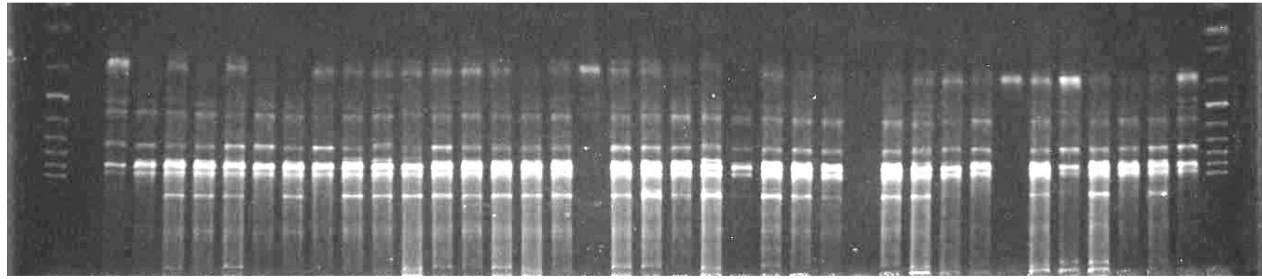



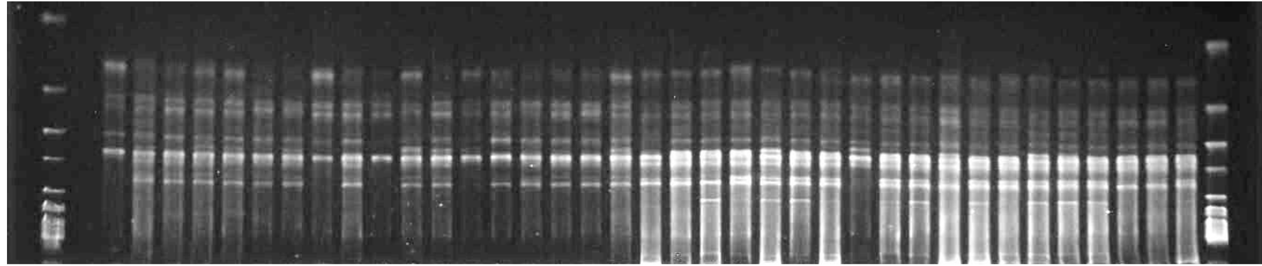



ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 810-H.

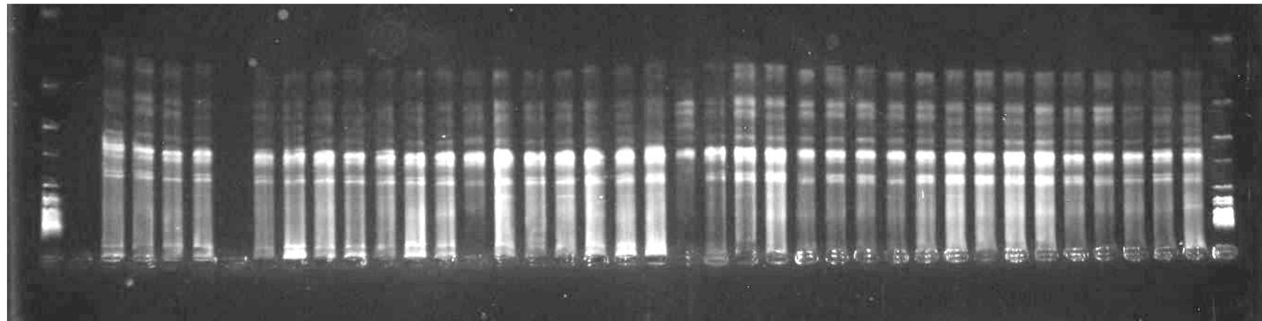
Appendix 2 continued



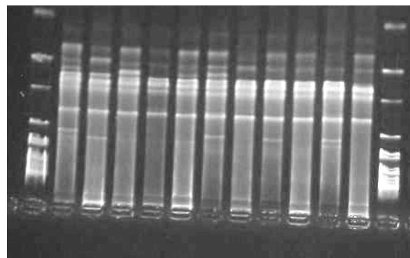
Marke ZW 3 WL1 TG 3 GG 6 CU6 WL6 EG 4 GG 1 BG 1 ZA 1 MX1 PA GJ 1 GJ 2 GD 4 TG 5 TG 7 BG 7 BA 1 IL1 HR6 SO3 ZA 3 CU 2 TG 2 SH 3 HR 1 HR 5 CU5 IN SR 2 SH 4 GD6 GD 7 TA 3 HR 7 GD 5 IL3 Marke



Marke BG 5 MX 3 GD 8 SU 3 ZA 2 EG 3 MX 2 GD 1 SH 1 WO 5 ZW 1 SO 1 SR 1 GG 7 WL 5 HR 2 MX 4 ZW 2 IL 5 TA 4 WO 3 ZA 5 EG 2 GG 3 CU3 GG 2 TA 1 SH 5 SO 2 GD 9 TG 4 MX 5 GJ 4 HR 3 WO 6 WO 8 TG 6 Marke

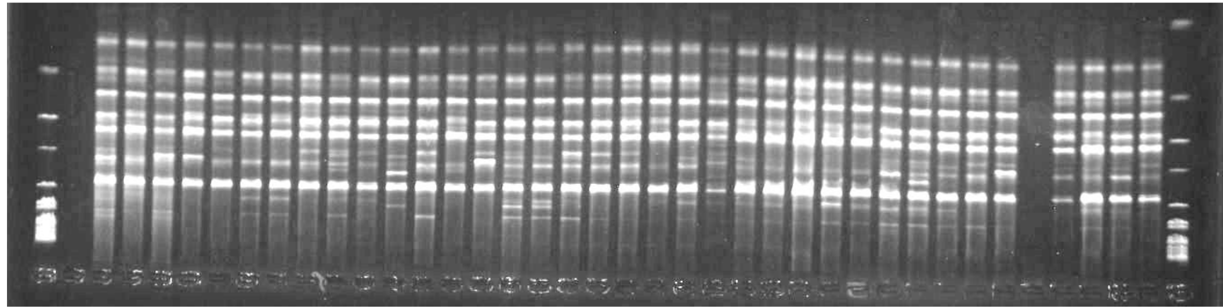


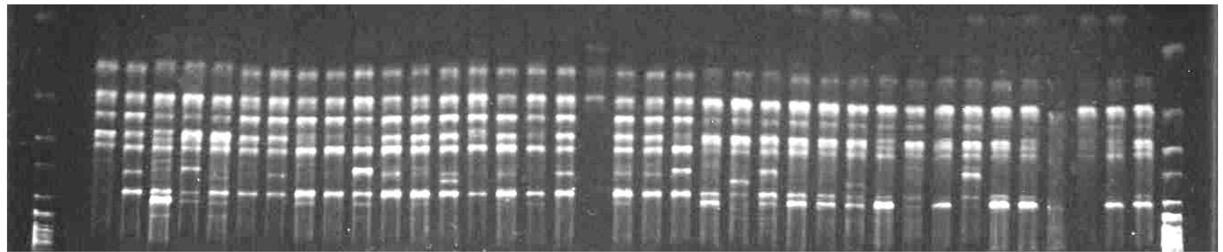
Marke BG 2 BG 3 SU 1 GD 2 TG 9 ZW 4 SO 4 SH 2 WL 2 TA 5 BG 4 WL 4 WO 4 WO 7 WO 1 HR 4 BG 8 GD 3 SO 5 SH 6 CU4 TG 1 TG 8 GG 5 ER 1 WL 8 WO 2 SH 7 WL 3 ZW 5 WL 7 BA 2 EG 1 ZA 4 SU 2 EG 5BG 6 Marke

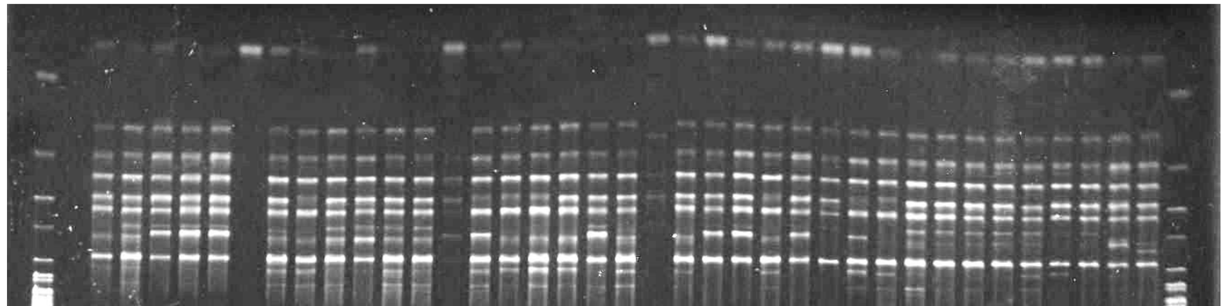


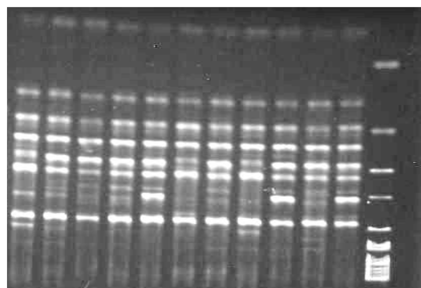
ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 818-H.

Appendix 2 continued



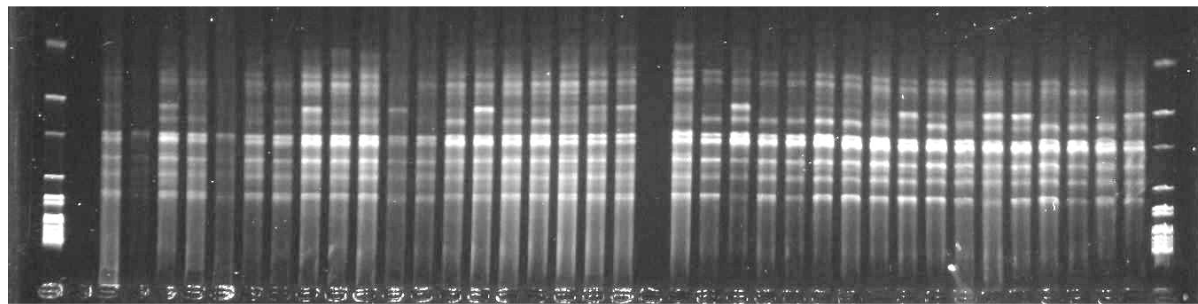



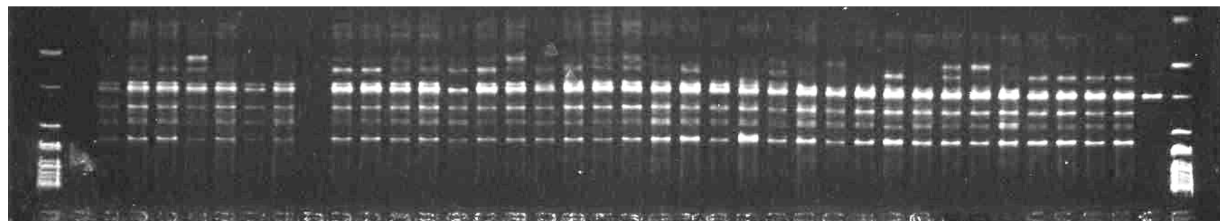



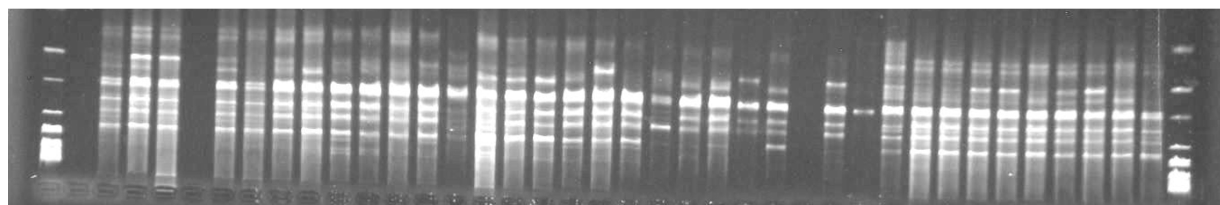



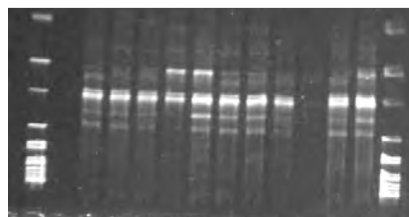
ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 834-H.

Appendix 2 continued



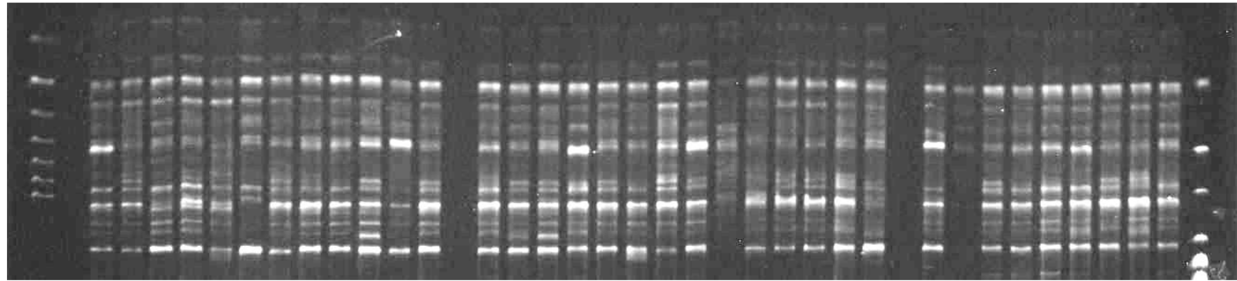



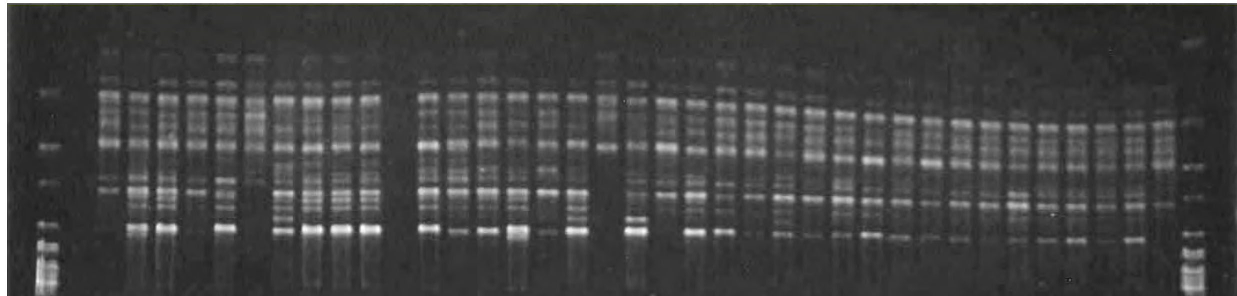



ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 860-H.

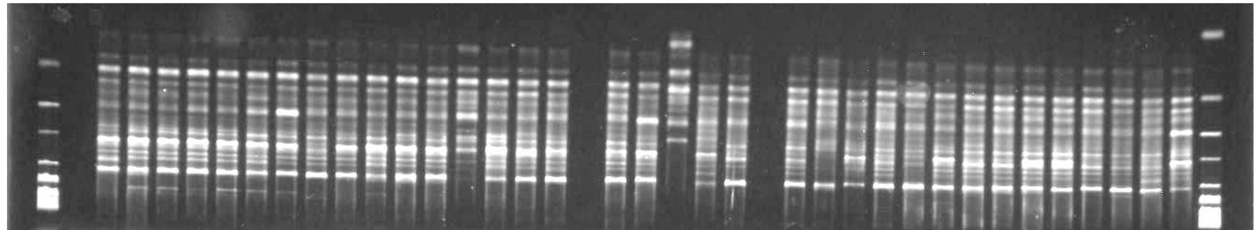
Appendix 2 continued



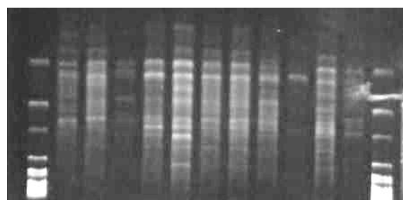
Marke ZW 3 WL1 TG 3 GG 6 CU6 WL6 EG 4 GG 1 BG 1 ZA 1 MX1 PA GJ 1 GJ 2 GD 4 TG 5 TG 7 BG 7 BA 1 IL1 HR6 SO3 ZA 3 CU 2 TG 2 SH 3 HR 1 HR 5 CU5 IN SR 2 SH 4 GD6G D 7TA 3HR 7 GD 5IL3 Marke



Marke BG 5 MX 3 GD 8 SU 3 ZA 2 EG 3 MX 2 GD 1 SH 1 WO 5 ZW 1 SO 1 SR 1 GG 7 WL 5 HR 2 MX 4 ZW 2 IL 5 TA 4 WO 3 ZA 5 EG 2 GG 3 CU3 GG 2 TA 1 SH 5 SO 2 GD 9 TG 4 MX 5 GJ 4 HR 3 WO 6 WO 8 TG 6 Marke

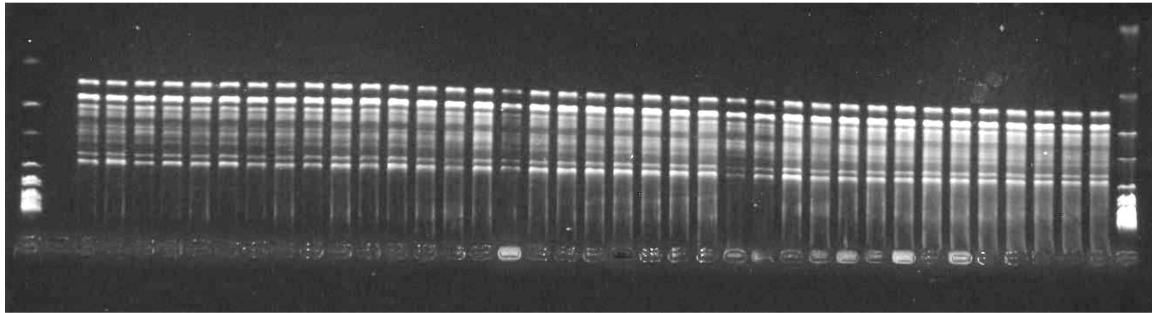


Marke BG 2 BG 3 SU 1 GD 2 TG 9 ZW 4 SO 4 SH 2 WL 2 TA 5 BG 4 WL 4 WO 4 WO 7 WO 1 HR 4 BG 8 GD 3 SO 5 SH 6 CU4 TG 1 TG 8 GG 5 ER 1 WL 8 WO 2 SH 7 WL 3 ZW 5 WL 7 BA 2 EG 1 ZA 4 SU 2 G 5 BG 6 Marke



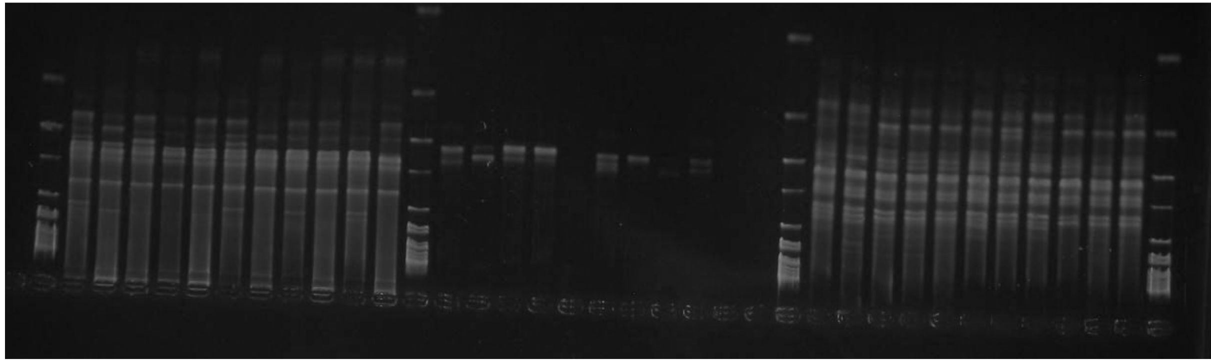
ISSR fingerprint generated from 120 individuals of 82 Ethiopian and 38 exotic accessions using primer 880-H.

Appendix 3 non polymorphic primer

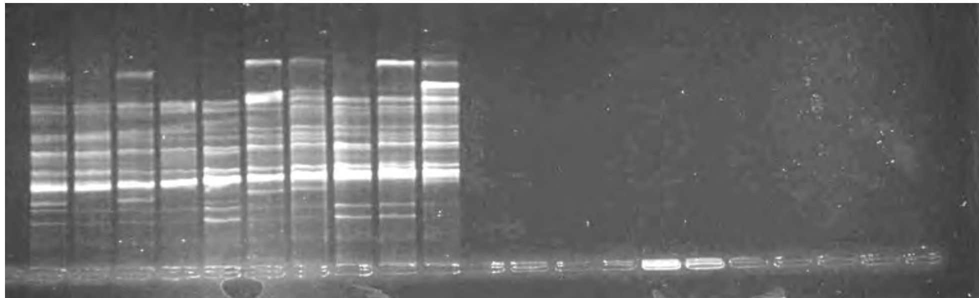


Primer 873 that is not polymorphic

Appendix 4 comparison of primers



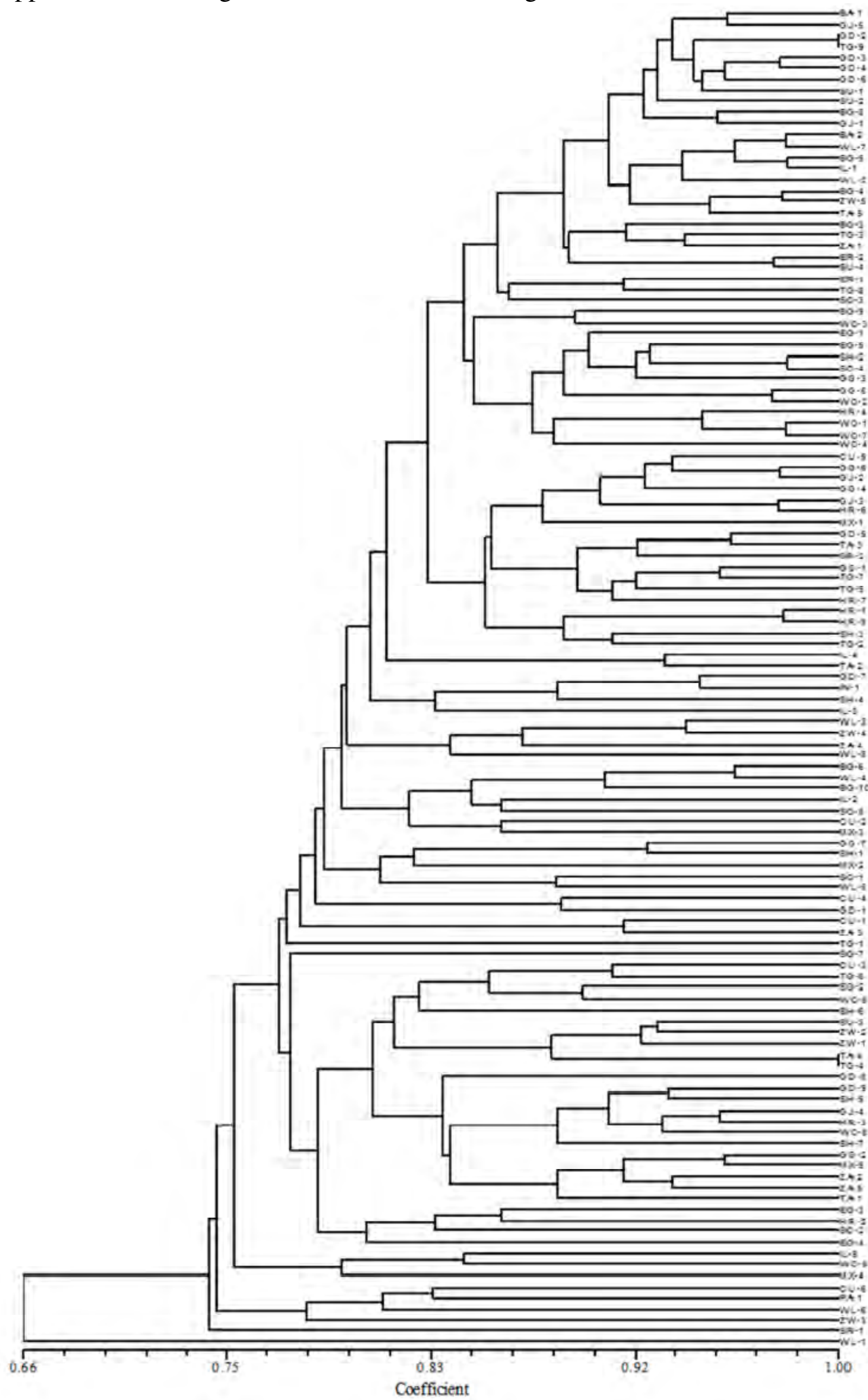
Comparison of primer 810,814 and 818 respectively for their polymorphism and reproducibility



Comparison of primer 843 and 873 respectively for their reproducibility

Comparison of primer 809 and 810 respectively for their reproducibility

Appendix 5: Dendrogramof 120 individuals using UPGMA



**Dendrogramof 120 individuals based on 58 PCR bands amplified with five dinucleotide and one pentanucleotide) primers derived by UPGMA.** **Key- BG1-BG8-Benishangul Gumuz,GD1-GD9-Gonder, GG1-GG7-Gamo Goffa, GJ1-GJ5- Gojam, Hr1-Hr7- Harerge, Il1-Il2-Illibabore,SH1-SH7-Shewa, TG1-Tg11-Tigray, WO1-WO8-Welo, CU1-CU5-cultivated, W1-W9- Welega,EG1-EG5-Egypt, MX1-MX5- Mexico, SO1-SO5-Somalia, SU1-SU4-Sudan, TA1-TA5-Tanzania, ZA1-ZA5-Zambia, ZW1-ZW5-Zimbabwe**