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Genetic Diversity Study of *Lepidium sativum* Populations from Ethiopia using Morphological Characters and ISSR Markers

A Thesis Submitted to the School of Graduate Studies, Addis Ababa University, in Partial Fulfillment of the Requirements for Degree of Master of Science in Biology

(*Applied Genetics*)

By

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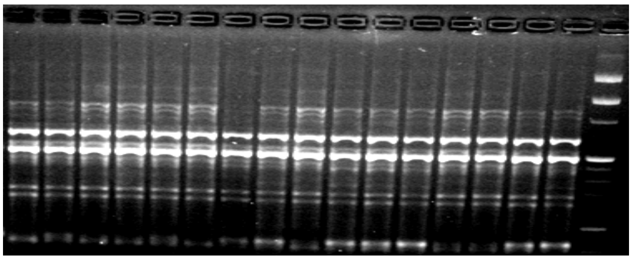

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Declaration

I, the undersigned declare that this thesis is my original work and that all sources of material used for the thesis have been correctly acknowledge.

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Table of Contents page

LIST OF TABLES **Page**

LIST OF FIGURES **Page**

LIST OF ABBREVIATIONS

1000SW	Thousand seed weight
2D	Two dimensions
3D	Three dimensions
A	Amhara
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
CT	Correction Term
CV	Coefficient of Variation
DF	Days to Flowering
DG	Days to Germination
DM	Days to Maturity
GC	Garden Cress
H	Genetic Diversity
HARC	Holeta Agricultural Research Center
HT	Total genetic diversity
I	Shannon information index
IBC	Institute of Biodiversity Conservation
IL	Inter node Length
ISSR	Inter Simple Sequence Repeat

L1B	Length of Primary Branch
LL	Leaf Length
LS	*Lepidium sativum*
MAX	Maximum
MIN	Minimum
N1B	Number of Primary Branch
N2B	Number of Secondary Branch
NJ	Neighbor-Joining
NPL	Number of Polymorphic Loci
NSB	Number of Scorable Bands
PCO	Principal Coordinates Analysis
PH	Plant Height
PN	Pod Number
PP	Percent Polymorphism
RAPD	Random Amplified Polymorphic DNA
RFLP	Restricted Fragment Length Polymorphism
RSS	Raw Sum Square
SO	Somali
SN	Seed Number
SS	Sum Square
SSR	Simple Sequence Repeats

T	Tigray
UBC	University of British Colombia
UPGMA	Unweighted Pair Group Method with Arithmetic mean
Un	Unknown
WHO	World Health Organization

ABSTRACT

Lepidium sativum *L. (*family Brassicaceae), *is an underutilized medicinal plant with worldwide distribution. In Ethiopia*, Lepidium sativum occurs *in all regions and agro-ecologies at different altitudinal ranges. The study was conducted to assess the genetic diversity of* Lepidium sativum *populations from Ethiopia using both morphological and ISSR markers. A total of 86* L. sativum *genotypes were planted in Complete Randomized Block Design with two replications at Holeta Agricultural Research Center during 2011/2012 cropping season. Ten plants were tagged for morphological data collection and fourteen quantitative traits were recorded. The ISSR fingerprint analysis was conducted at the Genetics Laboratory of Addis Ababa University, Ethiopia. Analysis of variance was computed for 12 morphological traits and significant variation within and between* L. sativum *populations was recorded. Yield data indicated that accession 205162 (2324.08 kg/ha) and 90020 (2008.46 kg/ha) from Amhara; accession 234828 (1983.4kg/ha) from Oromia observed to give higher yield. The seed color based bulk analysis of oil content also indicated that Brown gave the highest (25%) oil content. Molecular data generated from ISSR bands recorded was used for computing gene diversity, percent polymorphism and Shannon diversity index and AMOVA. Moreover, the ISSR data was used to construct UPGMA, Nj trees and PCO plot using Jaccard's coefficient. Tigray and Amhara populations showed higher gene diversity (*0.24*) and Shannon information index (*0.35*). Both Nj, UPGMA and PCO analysis showed very week grouping among individuals collected from the same regions. Generally, Tigray and Amhara regions showed moderate to high diversity in both morphological and ISSR analysis. High variation was recorded for number of seeds/plant, number of pod/plant, number of secondary branch/plant, and plant height, which indicates the presence of ample variability for* L. sativum *improvement in Ethiopia. Different geographical regions of Ethiopia, showed different levels of variation; thus conservation priority should be given for those regions that have genetic diversity. This result also indicated the presence of genetic diversity that can be exploited to improve the productivity of* L. sativum *in Ethiopia.*

Key words: Genetic diversity, ISSR, *Lepidium sativum*, Morphology

1. INTRODUCTION

The genus *Lepidium L.* comprises about 150 species distributed worldwide. In tropical Africa, only nine species are found. The genus *Lepidium* belongs to the family Brassicaceae (Zhan *et al*., 2009). The garden cress, *Lepidium sativum* L., a fast growing annual herb is native to Egypt and West Asia (Zhan *et al*., 2009).

The garden cress is grown in India, Europe, and US and it is an underutilized crop. The edible whole seed is known to have health promoting properties. Hence, it is assumed that these seeds can be a functional food (USDA, 2011).The seed contains 12% protein, 4% fat and 74.3% dietary fiber and exhibited desirable functional properties such as dispersibility, gelling ability, stability and forms homogenous mild alkine suspension and is comparable to proprietary dietary fiber (Gokavi *et al.,* 2004).

There was a study that demonstrated the protective action of *L. sativum* against carcinogenic compounds and growth inhibition of *Pseudomonas aeruginosa*, a bacterial strain with a potent antibiotic resistance (Kassie *et al.,* 2002; Walikhan and Khatoon, 2008; Gupta *et al., 2010*). *L. sativum* is recommended in the treatment of hypertension, diabetes and renal disease (Gupta *et al., 2010*).

Salinity adversely reduces the overall productivity of plants including crops by inducing numerous abnormal morphological, physiological and biochemical changes that cause delayed germination, high seedling mortality, poor crop stand, stunted growth and lower yields. *L. sativum* has been evaluated and proved to be moderately salt tolerant at germination and seedling growth stage (Muhammad and Hussain, 2010).

Various concentrations of salt had a highly significant effect upon the survival percentage, plant height, number of branches, shoot fresh and dry weight, and root fresh and dry weight and root moisture contents. Number of leaves also varied significantly. However, leaf length and shoot moisture contents exhibited non-significant differences. Differences among the species for all the parameters under consideration were also highly significant. Some findings suggested that

Lepidium species are tolerant to moderate salinity and might be tried on saline soils to obtain some biomass (Muhammed and Husian, 2010).

In Ethiopia, *Lepidium sativum* occurs in all regions and agroecology at different altitudinal range. It is not cultivated widely; instead it is cultivated with teff field and available in all local markets. It is not cultivated in large amount as other crops. The main purpose of its cultivation in Ethiopia is to use as a medicinal plant. It is used for human abdominal ache and diarrhea. Moreover, *L. sativum* is also used to treat skin diseases and other internal problems in livestock.

Despite of its medicinal use, there was no any genetic diversity study on Ethiopian *Lepidium sativum,* particularly using molecular markers. Very few studies have been carried out using morphological markers outside Ethiopia. Hence, this study is proposed to investigate the genetic diversity and population structure of *L. sativum* populations collected from Ethiopia. Variation was studied using morphological and molecular markers. This will give the overall genetic variability, patterns of distribution and population structure which will be very critical to design sustainable conservation and use strategy.

2. LITERATURE REVIEW

2.1. Origin and distribution

The exact origin of *Lepidium sativum L.* is not known. However, it is believed to have originated in the highland of Ethiopia and central and south west Asia, then spread to the rest part of the world. This species is commonly cultivated in SW Asia (perhaps Persia) from where it, spread many centuries ago, to W. Europe, as shown by the philosophical trace of its names in different Indo-European languages (Muhammed and Husian, 2010).

In English it is named as common cress, garden cress, pepperwort, town cress; Arabic-Habb-er-reshad, habrasiot, half, hurf, harfulabaz, jarir, reschad; French-Anitor, cresson alenois, cresson des jardins, cresson de terre, nasitar, wasitor, nasit art, passerage cultive; German-Gartenkresse, kresse, pfefferkraut; Italian- Lepidio, nasturzio; Portuguese- Mastruco; Spanish- Berro de jardin, malgica, mastuezo; Chinese- Jia du xing cai (Jaferi,1973; Al-jozi *et al.*, 1999; Oudhia, 2003; USDA,2011). *Lepidium sativum* is fetto in Amharic, Shimfa- Tigrigna, Shimp- Kaffa and Shufu in Harer (Adere language) (Amare Getahun, 1976).

Lepidium sativum is distributed worldwide (USDA, 2011). Fetto is commonly grown in Ethiopia as a garden plant or as a cultivated crop in teff fields. It is generally found in any market, though usually in small quantities (Amare Getahun, 1976). Garden cress predominantly grows in temperate region and can grow in all elevation throughout the year, but best crop is obtained in winter season in highland area (Kruk and Benech, 1998; USDA, 2011).

2.2. Biology of *Lepidium sativum*

Lepidium sativum is fast growing (30-60cm) annual herb. Leaves are entire (upper-sessile and lower- petiolate); flowers- white, small and long racemes, fruits- small pods, obviate, two seeds per pod; seeds- brownish red and slimy when soaked in water, seed shape-elliptic (Arkroyd *et al.*, 1966; Jafri, 1973; Gokavi *et al.*, 2004; Zhan *et al*., 2009). This species reproduces sexually pollen. It is both self and cross pollinating plant. Insects are well known for cross pollination (Quirós and Cárdenas, 1998). Glucosinolate contents of *L. sativum* seeds revealed the isolation and identification of glucotropaeolin and 2- Phenyl ethyl glucosinolate while the study of the glucosinolate contents of the fresh herb reveals the presence of 2- ethyl butyl glucosinolate, methyl glucosinolate, butyl glucosinolate and glucotropaeolin (Radwan *et al.*, 2007)

The seeds of *Lepidium sativum* are known source of essential oil. The oil constitutes 22.7% (Moser *et al.*, 2009); 23.08% Dolya *et al*. (1976). Generally *L. sativum* seed contains it has 20-25% oil content (Datta *et al*., 2011).

2.3. Medicinal uses of *Lepidium sativum*

Medicinal plants are excellent sources of unknown chemical substances for therapeutic effects (Nadkarni, 1954; Chopra *et al.*, 1986; Rao, 2004). *Lepidium sativum* seeds contain flavonoids, coumarins, sulphur, glycosides, triterpenes, sterols and various imidazole alkaloids (Radwan *et al.*, 2007; Agarwal and Verma, 2011; Datta *et al.*, 2011). Ethno-medicinal uses of *L. sativum* leaves as salad, cooked with vegetables, curries and also used as fodder for cattle (Vohora and Khan, 1977; Kiple and Ornelas, 2000; Moser *et al.*, 2009; Patel *et al.*, 2009; Rehman *et al.*, 2010). The leaves are stimulant, diuretic, used in scorbutic disease and hepatic complaints (Maghrani *et al.*, 2005; Ahmed *et al.*, 2009; Raval and Pandya, 2009).

Seeds are given along with green tea or milk before breakfast to relief backache pain. It is also used for animal diarrhea (Jan *et al.*, 2008; Alkhargy and Gilani, 2010). Gastrointestinal disorders are treated by herbal products with *L. sativum* seeds, which consist of abdominal pain, dysentery and worms (Jan *et al.*, 2008; Gupta *et al.*, 2010). For snake and dog bit treatment the whole plant or root is used (Yahya *et al.*, 1994; Kassie *et al.*, 2002; Oudhia, 2003). It prevents postnatal complication, for indigestion and constipation, for nephroprotective and curative activity (Maghrani *et al.*, 2005; Gupta *et al*, 2010; Seth *et al.*, 2010). The seeds are also used to treat bacterial and fungal infection (Uphof, 1959; Arkroyd *et al.*, 1960; Chopra *et al.*, 1986; Ahmed, 1999; Jouad *et al.*, 2001; Ateyyat *et al.*, 2009; Diwakar *et al.*, 2010; Gupta *et al*, 2010; Rehman *et al*., 2010).

Siengenthaler (1960) cited in Amare Getahun (1976) reported that fetto ripe, dried seeds are ground into fine flour and mixed with water to use as a drench for stomach disorders of livestock. The paste is made of the seed flour and water and used on chapped lips, sunburn, and other skin diseases. The flour is mixed with honey and taken as a treatment for amoeba. The paste is rubbed on to the skin to repel mosquitoes and other insects. In times past, the paste was rubbed on to the skin by warriors for warmth during cold nights. For stomach cramps, fetto, tukur- asmud (*Nigellac sativa* L. Ranunculaceae) and salt are ground together to make a thick dough and allowed to ferment for seven days. One tea spoon of this is taken once a day for stomach cramps (Amare Getahun, 1976).

2.4. Genetic diversity study and application of different markers

Biological markers could have many different types but mainly classified into morphological and biochemical and molecular types. The morphological (classical or visible, agronomic traits) markers are phenotypic traits, while; biochemical markers are isozymes, and are based on gene product. The DNA (molecular) markers which reveal sites of variation in DNA is marker with better resolution of diversity. Each type of marker system has its own advantages and disadvantages. The type of marker is selected based on the species types, availability and cost benefit analysis (Winter and Kahl, 1995; Semagn *et al.,* 2006).

2.3.1. Genetic diversity study by morphological markers

Morphological markers are usually visually described phenotypic characters such as flower color, seed shape, growth habits or pigmentation (Winter and Kahl, 1995). Morphological markers are phenotypic markers with distinguishable trait that have evident to human eyes (Bagali *et al.,* 2010).

Morphological characterization does not require expensive technology, but large tracts of land are often required for these experiments, making it possibly more expensive than molecular markers assessment. These traits are often susceptible to phenotypic plasticity conversely; this allows assessment of diversity in the presence of environmental variation (Mondini *et al.,* 2009).

Although phenotypic traits cannot be reliable measures of genetic differences because of the influence of the environment on gene expression, it is appropriate to mention salient traditional approaches employed for assessment of variability (Singh *et al.,* 2010).

Phenotypic variation is positively associated with genetic diversity, but it is also dependent on environmental factors as well as on the interaction between genotypes and environment (Moose and Mumm, 2008). Morphological characters may be unstable due to environmental influences; therefore methods to assess and detect genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits (Muthusamy *et al.,* 2008).

2.3.2. Genetic diversity study by using isozyme markers

Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining (Winter and Kahl, 1995). Biochemical analysis is based on the separation of proteins in to specific banding patterns. It is a fast method which requires only small amount of biological material; however only a limited number of enzymes are available and thus, the resolution of diversity is limited (Mondini *et al.*, 2009).

The number of polymorphic enzymatic systems available is limited and the enzymatic loci present only a small and not random part of the genome (the expressed part), therefore the observed variability may not be representative of the entire genome, although it allows large numbers of samples to be analyzed, comparisons of samples from different species, loci, and laboratories are problematic, since they are affected by extraction methodology, plant tissue, and plant storage (Mondini *et al.,* 2009).

2.3.3. Molecular markers

DNA markers are the most widely used types of markers predominantly due to their abundance. They arise from different classes of DNA mutations such as substitution mutation, point mutation, rearrangements, insertion and deletion; or errors in replication of tandomely repeated DNA (Paterson, 1996a). Molecular markers are selectively neutral because they are usually located in non-coding regions of DNA (Collard *et al*., 2005). It is a sequence of DNA or a gene, which is situated on a chromosome (Schulmann, 2007). They are fragments of DNA sequence that are associated to a part of the genome carrying genes responsible for a trait (Bagali *et al*., 2010).

The common types of dominant DNA markers that are used for genetic diversity study are Random Amplified Length Polymorphism (RAPD), Inter Simple Sequence Repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP), DNA amplification fingerprinting (DAF) and arbitrarily primed polymerase chain reaction (AP-PCR). Whereas the co-dominant markers includes RFLP, SSR, sequence characterized amplified region(SCAR), cleaved amplified polymorphic sequence(CAPS), expressed sequence tag (EST) and single nucleotide polymorphism(SNP)(De Vicente and Fulton, 2003).

DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). Molecular markers are commonly used to characterize genetic diversity within or between populations or groups of individuals because they typically detect high levels of polymorphism (Dalirsefat *et al.,* 2009). Overall DNA marker system can be classified as non- PCR based and PCR based markers.

2.4. Non- PCR based molecular markers

2.4.1. Restricted Fragment Length Polymorphism (RFLP)

Restricted fragment length polymorphism (RFLP) is a variation in the DNA sequence of a genome that can be detected by breaking the DNA in to pieces with restriction enzymes and analyzing the size of the resulting fragments by gel electrophoresis. RFLP was first developed by Grodzicker *et al.* (1974) as cited in (Maheswaran, 2004). It was first developed cheap method with wide application (Kochert, 1994; Bhat, 1999; Srivastava and Mishra, 2009).

RFLP is non PCR based co-dominant markers that can detect DNA polymorphism by hybridizing chemically labeled DNA probe to a southern blot of DNA digested by restriction endo nuclease, resulting in differential DNA fragment profile. RFLP are bands that correspond to DNA fragments, usually within the range of 2-10 Kb, that have resulted from the digestion of genomic DNA with restriction enzymes (Young *et al.,* 1992; Weising *et al.,* 1995).

2.5. PCR- based molecular markers

PCR is a molecular biology technique for enzymatically replicating small quantities of DNA without using a living organism (Weising *et al.,* 1995). PCR was the major breakthrough for molecular markers in that for the first time, any genomic region could be amplified and analyzed in many individuals without the requirement for cloning and isolating large amounts of ultrapure genomic DNA (Schlotterer, 2004).

PCR based approach uses only small quantities of DNA, avoid DNA blotting and use of radioactivity and are amenable to automation (Nagaraju *et al.,* 2001). PCR technology has emerging which promise precision, economy and speed (Vos *et al.,* 1995). The advent of PCR technology has offered efficient marker systems for diagnosis of genetic diversity in large scale studies (Saiki *et al.,* 1988). This technology has led to the development of a number of simple and quick techniques such as RAPD, ISSR and AFLP (Najaraju *et al.,* 2001). Most of the PCR based markers with other novel approaches have been extensively utilized in genetic diversity assessment (Montes *et al.,* 2009).

2.5.1. Random Amplified Polymorphic DNA (RAPD)

RAPD were the first PCR- based molecular markers to be employed in genetic variation analysis (Welsh and McClelland, 1990; Schierwater and Ender, 1993). It is a type of PCR reaction, but the segments of DNA that are amplified are random. This marker is first introduced by Williams *et al.* (1990). RAPD markers are useful DNA based method for initial assessment of genetic variation, especially the assessment of genetic diversity in plant species (Song, 2005). Fedlu Hassen *et al.* (2007) used RAPD to study genetic variability of five indigenous Ethiopian cattle breeds.

Because of its simplicity, RAPD technology is being extensively used in genetic variability analysis of various plant and animal species (Penner, 1996; Bardacki, 2001; Nagaraju *et al.,* 2001; Binneck *et al.,* 2002; Arya *et al.,* 2011; Beiranvand *et al.,* 2011). To study the genetic diversity of rare species; random amplified polymorphic DNA is useful (Williams, *et al.,* 1990).

2.5.2. Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism is a PCR-based tool used in genetics research, DNA finger printing. It uses restriction enzymes to cut genomic DNA, followed by legation of adaptors to the sticky ends of the restriction fragments (Srivastava and Mishra, 2009). It is finger print of any DNA regardless of its sources and without any prior knowledge of DNA sequence, and used to distinguish closely related individuals in sub species level (Althoff *et al.,* 2007). It utilizes fragments of DNA amplified using directed primers from restriction digested genomic DNA (Vos *et al.,* 1995; Gaudeul *et al.,* 2000). AFLP gives high level of resolution to allow delineation of complex genetic structures, to differentiate individuals in a population gene flow experiments and also to register plant varieties (Misra *et al.,* 2010). AFLP was first developed by Vos *et al.* (1995). This marker also used by Mesfin Bogale *et al.* (2006) in characterization of *Fusarium oxysporum* isolates from Ethiopia.

AFLP technique is more useful in detecting genetic diversity of rare and endangered species (Krauss, 1999). In this technique current diversity status was also demonstrated for a number of trees and shrub species (Barker *et al.,* 1999). The AFLP is useful in genetic of large number of polymorphic markers (Song, 2005).

2.5.3. Inter- Simple Sequence Repeat (ISSR)

ISSR are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions (Joshi *et al.,* 2000; Bussell *et al.,* 2005). ISSR marker was first developed by Zietkiewicz *et al.* (1994).

This marker system combines the high reliability and variability of SSR markers and the speed and ease of RAPD markers (Souframanien and Gopalakrishna, 2004). ISSR involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in opposite direction. This marker is quick, easy to apply, highly reproducible, polymorphous, low quantity of template DNA required and randomly distributed throughout the genome, no prior information about genomic sequence is required (Bornet and Branchard, 2001; Buhulikar *et al.,* 2004; Coetes and Byrne, 2005).

Since the ISSR technique can produce a much large number of fragments per assay unit, it is a more powerful fingerprint tool than SSR; RFLP and RAPD marker system (Marsh and Ayres, 2002). ISSR markers are also more reliable and reproducible than RAPDs because of the higher annealing temperature contributed by longer primers (Zietkiewicz *et al.,* 1994; Domyati *et al.,* 1996; Powell *et al.,* 1996; Fang and Roose, 1997; Kojima *et al.,* 1998; Moreno *et al.,* 1998; Denduangboripant *et al.,* 2010).

This technique is useful to endangered, rare and less commercially important species, which are generally little studied for their genetic structure (Song, 2005). So, ISSR markers have been widely used for detecting genetic diversity of rare and endangered plant species (Marsh and Ayres, 2002). Therefore, ISSR is mainly used in many respects such as the study of genetic diversity in many plant species (Brantestem *et al.,* 2004).

Edossa Fikru *et al.* (2010) used ISSR marker on comparative study of morphological and molecular diversity in Ethiopian lentil (*Lens culinaris* Medikus) landraces. Dagmawit Chombe

Tobiaw and Endashaw Bekele (2011) used ISSR marker in analysis of genetic diversity among cultivated enset (*Ensete ventricosum)* populations from Essera and Kefficho, South Western part of Ethiopia. Solomon Balami (2007) studied genetic diversity analysis of the Wild *Coffea arabic*a L. populations from Harenna Forest, Bale Mountains of Ethiopia, Using Inter Simple Sequence Repeats (ISSR) Marker.

ISSR markers target divergence in regions containing dispersed repetitive DNA and can rapidly differentiate closely related individuals, it is especially useful in detecting clonal variation and fingerprinting of related individuals. It has great potential in the study of natural populations for addressing questions ranging from conservation biology to molecular ecology and systematic (Bornet and Branchard, 2001; Song, 2005; Mudibu *et al.,* 2011).

2.5.4. Simple Sequence Repeats (SSR)

Microsatellites are short nucleotide tandem repeats of a motif, usually one to six bases (Morgante and Olivieri, 1993; Bhat, 1999; Kylin, 2010). Microsatellite repeats are sets repeated sequences found within eukaryotic genomes (Bell and Ecker, 1994; Gupta and Varshey, 2000; Ghislain *et al.,* 2004). This marker system was first developed by Akkaya *et al.* (1992). The genus *Lepidium* showed high genetic diversity by using SSR marker. Both *Lepidium verginicum* and *Lepidium meyenii* was studied using SSR marker and had showed variability at each locus (Hasan *et al.,* 2010). Successful cross-species amplification highlights the possibility of using these markers for genetic studies in other closely related species of *Lepidium* (Bon *et al.,* 2005)

Mesfin Bogalle *et al.* (2006) used the SSR marker to study on characterization of *Fusarium oxysporum* isolates from Ethiopia. Gemechu Keneni *et al.* (2011) also used this marker to study genetic diversity and population structure of Ethiopian Chickpea (*Cicer arietinum* L.) germplasm accessions from different geographical origins.

The use of molecular markers for genetic diversity studies in crop species has increased worldwide in the last decade although it is still limited in developing countries (McCouch *et al.,* 1997; Garcia *et al.,* 2004; Collard *et al.,* 2005; FAO, 2010c).

In general, there is no one and best system of DNA marker used to study in genetic diversity and knowing the pros and cons of each DNA marker is advantageous before starting the project

(Hartl and Jones, 2005; Mondini *et al.,* 2009). There is no single molecular approach for many of the problems facing gene bank managers, and many techniques component each other, but some techniques are clearly more appropriate than others for some specific applications. No marker is superior to all others for a wide range of applications (Spooner *et al.,* 2005). The most appropriate genetic marker will depend on the specific application, the presumed level of polymorphism, the presence of sufficient technical facilities, know-how, time constraints and financial limitations (Spooner *et al.*, 2005; Muchugi *et al.,* 2008).

Ideally, the DNA marker can have co-dominance inheritance, easy assay, fast assay, frequent occurrence in the genome, high reproducibility, high polymorphic nature; selective neutral behavior (Bagali *et al.,* 2010; Eagles *et al.*, 2011). It is extremely difficult to find a molecular marker which would meet all the above criteria. Depending on the type of study to be undertaken a marker system can be identified that would fulfill at least a few of the above criteria (Weising *et al.*, 1995; Karp *et al.,* 1996; Garcia-mas *et al.,* 2000; Uptmoor *et al.*, 2003). Molecular markers vary in techniques and cost, so no ideal marker system that can be defined for all molecular genetic studies (Muchugi *et al.,* 2008).

Awareness of the level of genetic diversity and the proper management of genetic resources are important issues in the modern scenario (Farooq and Azam, 2002; Somasundaram and Balaise, 2007). Using different markers is more reliable than only using one type of marker to quantify the genetic diversity (Leal *et al.*, 2010).

3. OBJECTIVES OF THE STUDY

3.1. General objective

- This research aimed at studying genetic diversity and population structure of *Lepidium sativum* populations collected from Ethiopia using morphological and ISSR markers.

3.2. Specific objectives

- To reveal patterns of diversity and populations structure of *Lepidium sativum* from Ethiopia.
- Identify *Lepidium sativum* populations that deserve conservation attention.

4. MATERIALS AND METHODS

4.1. Description of the study area used for morphological evaluation

The present study was conducted at Holeta Agricultural Research Center (HARC). HARC is located 40 Km South West of Addis Ababa with 09^0 04'N, 38^o 29'E. Its altitude is 2400 meter above sea level (m.a.s.l) and receives annual rainfall of 1037.70 mm. The average minimum temperature of HARC is 5.19^0C, while the average maximum temperature is 23.4^0C. The dominant soil types are Vertisols and Nullisols (HARC).

4.2. Experimental materials

Eighty four accessions of *Lepidium sativum* species were received from Institute of Biodiversity Conservation (IBC), and two accessions were bought from Sholla market Addis Ababa; a total of 86 accessions were planted for morphological variability study at Holeta Agricultural Research Center (HARC) during the main cropping season of 2011. The two accessions that were bought from Sholla market were used as a control only.

Table 1. List of *L. sativum* accessions, altitude and regions of collection used in the study and their respective symbol used in molecular analysis result *(Source: IBC database).*

No.	**Accession No.**	**Altitude**	**Regions**	**region**	**Accession symbol**
1	90018	2500	Amhara	Wello	A1
2	208030	*Uk	Amhara	Gonder	A2
3	90004	2400	Amhara	Gonder	A3
4	205162	*Uk	Amhara	Gonder	A4
5	229799	2550	Amhara	Gojam	A5
6	215713	2580	Amhara	Wello	A6
7	229205	2690	Amhara	Shewa	A7
8	241777	2110	Amhara	Wello	A8
9	214243	*Uk	Amhara	Gonder	A9
10	215714	2200	Amhara	Wello	A10
11	212628	1500	Amhara	Wello	A11
12	235892	2270	Amhara	Gonder	A12
13	229200	2150	Amhara	Shewa	A13
14	207542	*Uk	Amhara	Gonder	A14
15	229203	2620	Amhara	Shewa	A15
16	229201	2310	Amhara	Shewa	A16
17	229798	2250	Amhara	Gojam	A17
18	229204	2580	Amhara	Shewa	A18

No.	**Accession No.**	**Altitude**	**Regions**	**region**	**Accession symbol**
19	229202	2600	Amhara	Shewa	A19
20	205162	2820	Amhara	Gonder	A20
21	90020	2000	Amhara	Shewa	A21
22	229199	2220	Amhara	Shewa	A22
23	208769	1900	Oromia	Wollega	O1
24	208667	1900	Oromia	Hererrege	O2
25	212852	*Uk	Oromia	Balle	O3
26	90002	*Uk	Oromia	Harerrge	O4
27	215808	1950	Oromia	Wollega	O5
28	212853	*Uk	Oromia	Balle	O6
29	215807	1720	Oromia	Wollega	O7
30	208666	*Uk	Oromia	Harerrge	O8
31	208693	*Uk	Oromia	Harerrge	O9
32	230830	2450	Oromia	Harerrge	O10
33	230831	2050	Oromia	Harerrge	O11
34	237991	2150	Oromia	Balle	O12
35	230524	*Uk	Oromia	Harerrge	O13
36	90023	*Uk	Oromia	Keffa	O14
37	90021	2620	Oromia	Shewa	O15
38	90005	*Uk	Oromia	Harrerge	O16

No.	Accession No.	Altitude	Regions	region	Accession symbol
39	216885	1570	Oromia	Arsi	O17
40	208669	2180	Oromia	Harerrege	O18
41	90022	1480	Oromia	Wollega	O19
42	234828	*Uk	Oromia	Wollega	O20
43	216886	1570	Oromia	Arsi	O21
44	216816	*Uk	Oromia	Harerrge	O22
45	219961	*Uk	Tigray	Tigray	T1
46	219960	1940	Tigray	Tigray	T2
47	233984	1860	Tigray	Tigray	T3
48	219959	*Uk	Tigray	Tigray	T4
49	233981	2140	Tigray	Tigray	T5
50	233986	2080	Tigray	Tigray	T6
51	207910	*Uk	Tigray	Tigray	T7
52	237512	1900	Tigray	Tigray	T8
53	242609	*Uk	Tigray	Tigray	T9
54	234355	2090	Tigray	Tigray	T10
55	207991	*Uk	Tigray	Tigray	T11
56	233983	1950	Tigray	Tigray	T12
57	233982	2210	Tigray	Tigray	T13

No.	Accession No.	Altitude	Regions	region	Accession symbol
58	219958	2130	Tigray	Tigray	T14
59	233370	*Uk	Tigray	Tigray	T15
60	233679	*Uk	Tigray	Tigray	T16
61	240396	1950	SNNPR	Keffa	SN1
62	242916	2200	SNNPR	Keffa	SN2
63	214660	*Uk	SNNPR	Sidamo	SN3
64	202116	*Uk	SNNPR	Keffa	SN4
65	225799	2650	SNNPR	Gammo goffa	SN5
66	205141	1900	SNNPR	Shewa	SN6
67	240578	*Uk	SNNPR	Gammo goffa	SN7
68	240397	*Uk	SNNPR	Keffa	SN8
69	90016	*Uk	SNNPR	Gammo goffa	SN9
70	225725	*Uk	SNNPR	Gammo goffa	SN10
71	240808	1950	SNNPR	Gammo goffa	SN11
72	90009	*Uk	Unknown	Unknown	UN1
73	90014	*Uk	Unknown	Unknown	UN2
74	90012	*Uk	Unknown	Unknown	UN3
75	90017	*Uk	Unknown	Unknown	UN4
76	90010	*Uk	Unknown	Unknown	UN5

77	240579	*Uk	Unknown	Unknown	UN6
78	90008	*Uk	Unknown	Unknown	UN7
79	80001	*Uk	Unknown	Unknown	UN8
80	80002	*Uk	Unknown	Unknown	UN9
81	216815	*Uk	Somali	Somali	SO1
82	230829	*Uk	Somali	Somali	SO2
83	231210	1910	Somali	Somali	SO3
84	230523	*Uk	Somali	Somali	SO4
85	90007	*Uk	Somali	Somali	SO5
86	90006	*Uk	Oromia	Harerrge	O23

**Uk= Unknown*

4.3. Field experimental Design

The field experiment was laid out using Randomized Complete Block Design (RCBD) with two replications. A total of 9m x 9m area was used to plant the 86 accessions. Each accession was represented by a single plot per replication with 20 cm gap between accessions. Each plot had 20 plants and the spacing between plants of each plot was 5 cm. To study the quantitative data five individual plants were tagged within each plot randomly, hence a total of ten plants per accession were tagged from both replications for later morphological evaluation.

4.4. Oil content analysis procedures

Non- destructive method was used to analyze the oil content of *Lepidium sativum*. This method has the following procedures:

- Pure seed samples of 25 g were kept in oven at 130^0 C for 30minute. The purpose of this method is to remove moisture content and the only thing that left is the oil.
- Seeds were placed in diskette to cool and to avoid moisture absorption
- 22 gm were taken from the 25 g sample and placed in test tube
- Put the test tube in sample analyzer
- Carbon dioxide container was used to cool the sample analyzer
- The electronic analyzer was used to determine the oil content.
- Each sample was analyzed three times and the mean of three was used as the oil content of that sample.

4.5. Morphological characters scored

Fourteen agro-morphological quantitative traits were recorded for the 86 accessions planted at HARC during the main cropping season of year 2011. Germplasm collected from Somali Regional State were excluded from few agronomic analyses because of poor performance under field condition and lack of important data. Thus, Accessions 216815, 230829, 231210, 230523 and 90007 from Somali were excluded from regional comparison.

Table 2. Quantitative morphological traits of *Lepidium sativum* recorded (IPGRI, 2005).

Variable	**Data recording criteria**
Days to germination	The number of days from planting until 50% seedling emergence.
Days to flowering	Number of days from the date of sowing to the date at which 50% of the plants in the plot showed blooming on about 50% of their flower buds.
Days to maturity	The number of days from the date of sowing to the stage when 90% of plants have reached their physiological maturity.
Plant height	The height of plant in each plot measured in cm from the ground surface to the top of the main stem at maturity.
Number of primary branch/plant	The average number of primary branches/ plant.
Number of secondary branch/ plant	The average number of secondary branch formed on primary branch/plant.
Length of primary branch/ plant	The average length of randomly selected 3 primary branches per plant.
Number of seeds/ plant	The average numbers of seed taken from randomly selected 5 plants that were tagged to take the whole quantitative data.
1000 seed weight	The weight in g of 500 seeds sampled from each plot and multiplied by two.
Number of pods/plant	The average number of pods taken from randomly selected 5 plants per plot.
Variable	**Data recording criteria**

Inter node length	Measured as the average of 3 internodes distances on the same stalk, from the same plant.
Oil content	The proportion of oil in the seed to the total oven dried seed weight as measured by nuclear magnetic resonance spectroscope (NMRS).
Leaf length	The length of 5 randomly selected leaves/ plant
Yield	Yield of accessions in kg/ha

Thus, data was analyzed by using M STATC soft ware. The average values of each morphological traits and the coefficient of variation including the other statistical parameters like mean comparison and correlation was tested. Correlation is calculated as:

$$r = \frac{SS_{XY}}{\sqrt{(SS_{XX})(SS_{YY})}}$$

Where: - SSXY-is sum squares of X and Y scores

SSXX- Is sum square of the X score

SSYY- is sum square of the Y score

4.6. Analysis using ISSR markers

4.6.1. Tissue harvest and DNA extraction

The experiment was designed to characterize these accessions using inter simple sequence repeat (ISSR) markers. Young leaves were collected separately from 5 randomly selected individual plants per accession after four weeks of planting and dried in silica gel (Table 2). Approximately equal amounts of the dried leaf samples were bulked for each accession and ground with pestle and mortar. Total genomic DNA was isolated from about 0.4g of the pulverized leaf sample using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique as described by Borsch *et al*. (2003) (Appendix 7).

4.6.2. Primer selection and optimization

The ISSR marker assay was conducted at Genetics Laboratory of the Microbial, Cellular and Molecular Biology Program Unit, College of Natural Sciences, Addis Ababa University, Addis Ababa. A total of 10 primers, obtained from the Genetic Research Laboratory (Primer kit UBC 900) and primers used by Kim *et al*., (2002) were used for the initial testing of primers variability and reproducibility. One individual was selected from each population to screen the primers with 1:5 dilutions. A total of 4 polymorphic and reproducible ISSR primers (812, 834, 873 and 880) were selected after testing and screening. Table 3 shows the list of primers used and tested, their annealing temperature with respective sequences and other properties.

Table 3. List of primers, annealing temperature, primer sequence, amplification quality and repeat motives used for optimization

Primer	**Annealing temperature**	**Primer sequence**	**Amplification quality**	**Repeat motives**
810	45^{o}C	GAGAGAGAGAGAGAGAT	Monomorphic	Dinucleotide
812	45^{o}C	GAGAGAGAGAGAGAGAA	Polymorphic, reproducible	Dinucleotide
818	48^{o}C	CACACACACACACACAAG	Monomorphic	Dinucleotide
824	48^{o}C	TCTCTCTCTCTCTCTCG	Monomorphic	Dinucleotide
834	45^{o}C	AGAGAGAGAGAGAGAGYT	Polymorphic, reproducible	Dinucleotide
844	45^{o}C	GAGAGAGAGAGAGAGAYT	No banding	Dinucleotide
872	38^{o}C	GATAGATAGATAGATA	No banding	Tetra-nucleotide
873	45^{o}C	GACAGACAGACAGACA	Polymorphic, reproducible	Tetra-nucleotide
878	45^{o}C	GGATGGATGGATGGAT	No banding	Tetra-nucleotide
880	45^{o}C	GGAGAGGAGAGGAGA	Polymorphic, reproducible	Penta-nucleotide

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

4.6.3. PCR and gel electrophoresis

The polymerase chain reaction was conducted in Biometra 2003 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1µl template DNA, 13.45µl H_20, 5.60µl dNTP (1.25mM), 2.6µl Taq buffer (10XH buffer S), 1.25µl $MgCl_2$ (50mM), 0.6µl primer (20pmol/µl) and 0.5µl Taq Polymerase (3u/µl). The amplification program was 4 minutes preheating and initial denaturation at 94^0C, then 40 x 15 seconds at 94^0C, 1 minute primer annealing at (45^0C/ 48^0C) based on primers used, 1.30 minutes extension at 72^0C and the final extension for 7 minutes at 72^0C. The PCR reactions were stored at 4^0C until loading 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 100 bp was used to estimate molecular weight and size of the fragments. The electrophoreses were done for 3 hours at constant voltage of 100V. The DNA was stained with 10mg/ml Ethidum Bromide which were mixed with 250 ml distilled water for 30 minutes and washed with distilled water for 30 minutes.

4.6.4. Statistical analysis

ISSR profiles/bands were scored visually for each individual accession from the gel photograph. Accession 90006 from Oromia region was excluded in molecular analysis because of improper leave quality. The bands were recorded as discrete characters, presence ‘1’ or absence ‘0’ and ‘?’ for missing data. 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, percent polymorphism, Gene diversity (H) and Shannon diversity index (I). 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 numbers 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 generate phenogram using NTSYS- pc version 2.02 (Rohlf, 2000). The Neighbor Joining (NJ) method (Saitou and Nei 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek *et al.*, 1999).

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. RESULTS

5.1. Variability in morphological and agronomic characters

5.1.1. ANOVA analysis

Analysis of variance revealed high significant variation among the accessions for all the traits studied ($P< 0.01$). There was a wide range of expression across the accessions for all the traits, including a 35.5 days range in maturity, 1450.0 range in seed number and 725.0 ranges in pod number (Table 4).

Table 4. Minimum, mean and maximum values of morphological agronomic characters of *Lepidium sativum.*

Traits	**Min.**	**Mean**	**Max.**
DG	4.00	5.03	6.00
DF	54.50	74.73	92.00
DM	111.00	127.78	145.50
PH	38.60	59.26	76.30
N1B	6.00	12.41	18.10
N2B	5.00	10.32	30.00
L1B	5.50	10.77	21.20
LL	4.00	5.47	6.00
IL	2.50	3.53	4.90
SN	150.00	379.42	1600.00
PN	75.00	189.71	800.00
1000 swt.	1.53	2.22	2.91

Key: DG-Days to Germination, DF-Days to Flowering, DM-Date to Maturity, PH- Plant Height, N1B-Number of Primary Branch, N2B- Number of Secondary Branch, L1B-Length of Primary Branch, LL-Leaf Length, IL-Inter Node Length, SN- Seed Number, PN- Pod Number,1000Swt- One thousand Seed Weight. All have cm as a unit except for 1000swt (g) and for SN and PN no unit; DG, DF and DM in days.

The variance analysis performed on 12 quantitative morphological characters showed that the differences between accessions were statistically significant at the level of 0.01($p<0.01$) (Table 5). Coefficients of variation ranged between 3.02 – 85.95%. Pod number, length of primary branch, number of secondary branch and seed number reached 76.18, 38.93%, 85.95%, and 76.18% of variation, respectively. Few traits such as the days to 50% germination (3.02%) and days to maturity (9.01%) presented less than 10% of variation, (Table 5).

Table 5. ANOVA table for the morphological traits of *L. sativum*

Traits	**Source V**	**DF**	**SS**	**MS**	**CV %**
DG	Replication	1	0.023	0.023	3.02
	Accession	85	13.628	0.160 *	
	Error	85	1.977	0.023	
DF	Replication	1	42.006	42.006	10.47
	Accession	85	31935.610	375.713*	
	Error	85	5056.494	59.488	
DM	Replication	1	502.535	502.535	9.01
	Accession	85	58931.791	693.315*	
	Error	85	10862.465	127.794	
PH	Replication	1	586.083	586.083	13.25
	Accession	85	24266.903	285.493*	
	Error	85	5040.442	59.299	
N1B	Replication	1	0.145	0.145	13.37
	Accession	85	1704.340	20.051*	
	Error	85	222.355	2.616	
N2B	Replication	1	808.022	808.022	85.95
	Accession	85	8777.519	103.265*	
	Error	85	5545.738	65.244	

L1B	Replication	1	81.228	81.228	38.93
	Accession	85	3867.354	45.498*	
	Error	85	1173.582	13.807	
LL	Replication	1	1.140	1.140	10.06
	Accession	85	134.698	1.585*	
	Error	85	23.860	0.281	
IL	Replication	1	7.535	7.535	14.51
	Accession	85	84.613	0.995*	
	Error	85	21.536	0.253	
SN	Replication	1	2.768	2.768	76.18
	Accession	85	10444206.782	122873.021*	
	Error	85	5879225.724	69167.361	
SW	Replication	1	0.117	0.117	17.68
	Accession	85	37.264	0.438*	
	Error	85	12.354	0.145	
PN	Replication	1	5755.959	5755.959	85.89
	Accession	85	3192826.192	37562.661*	
	Error	85	1977349.541	23262.936	

**High significant at 0.01*

The mean comparison of 86 accessions is presented in table 6. Accession 229201 has the highest mean score (7.0) and accession 90008 was the least mean score (4.0) in days to germination. For days to flowering, the highest mean score was for accession 219959 (92) and the minimum was accession 90008 (54.5). Accession 219959 was highest and accessions 212852, 90008 and 216885 were the least (111.5) in days to maturity.

Accession 205162 was highest (76.3) in height mean score whereas accession 207911(39cm) was the least. Number of secondary branch was highest (18.1) for accession 90014 and lowest for accession 215714 (6). The mean number of secondary branches was maximum (30) for accessions 90014 and 233983 and the minimum for accession 215714 (1). Length of primary branch was highest for accession 205162(21.2cm) and the minimum was accession 229199 (1cm). Internode length has maximum mean score in accession 216885 (4.9) and the minimum was accession 229199 (0.9) (Table 6).

Accessions 80002 (1326.4), 229203 (2.9) and 80002 (663.5) had the highest mean score for seed number, seed weight and pod number, respectively.

Table 6. Mean comparison of 86 accessions for all the traits studied

Accessions	**DG**	**DF**	**DM**	**PH**	**N1B**	**N2B**
205162	5.0^{D}	65^{H-R}	116.5^{A-E}	76.3^{A}	13^{B-V}	29.9^{A}
208030	5.0^{D}	78.5^{A-O}	133.5^{A-E}	60.1^{A-P}	10.7^{N-Y}	24.2^{A-C}
208693	5.0^{D}	67.5^{D-Q}	119^{A-E}	68.7^{A-H}	11.4^{I-Y}	3.8^{C-E}
208769	5.0^{D}	67^{E-Q}	118.5^{A-E}	65.8^{A-J}	9.9^{Q-Z}	9^{A-E}
208666	5.0^{D}	61^{L-R}	112.5^{C-E}	57.8^{A-Q}	8.8^{V-Z}	6.8^{B-E}
215714	5.0^{D}	58^{P-R}	115.5^{B-E}	42.8^{N-Q}	6^{Z}	3^{C-E}
215713	5.0^{D}	81^{A-L}	126.5^{A-E}	40.1^{P-Q}	7.5^{Y-Z}	2.5^{D-E}
215807	5.0^{D}	80^{A-M}	125^{A-E}	47.5^{I-Q}	11.2^{K-Y}	7.1^{B-E}
215808	5.0^{D}	71.5^{B-Q}	120^{A-E}	64.9^{A-K}	11.3^{J-Y}	18.8^{A-E}
90023	5.0^{D}	68.5^{D-Q}	124.5^{A-E}	68.8^{A-H}	11^{L-Y}	13.5^{A-E}
205141	5.0^{D}	67^{E-Q}	119.5^{A-E}	71.6^{A-D}	11.6^{G-Y}	22.9^{A-D}
205163	5.0^{D}	66^{F-R}	117^{A-E}	64.8^{A-L}	11.6^{G-Y}	7.4^{B-E}
208669	5.0^{D}	61.5^{K-R}	112.5^{C-E}	58.6^{A-Q}	8.1^{X-Z}	5.7^{B-E}
225799	5.0^{D}	61.5^{K-R}	115.5^{B-E}	71.9^{A-D}	9.9^{Q-Z}	20.2^{A-E}
225725	5.0^{D}	61.5^{K-R}	115.5^{B-E}	68.5^{A-H}	9.5^{T-Z}	14.9^{A-E}
229203	5.0^{D}	83.5^{A-H}	130.5^{A-E}	50.4^{F-Q}	12^{F-X}	6.9^{B-E}
229201	7.0^{A}	-	-	-	-	-
90014	5.0^{D}	65.5^{G-R}	142^{A-C}	59.3^{A-P}	18.1^{A}	30^{A}
207910	5.0^{D}	81^{A-L}	139^{A-E}	65.8^{A-J}	15.2^{A-L}	17.5^{A-E}
208667	5.0^{D}	69.5^{C-Q}	125^{A-E}	55.9^{B-Q}	10.6^{O-Y}	12^{A-E}
230829	5.0^{D}	58.5^{O-R}	112^{D-E}	65.4^{A-K}	10.3^{O-Y}	18.5^{A-E}
230831	5.0^{D}	68.5^{D-Q}	115^{B-E}	60.8^{A-O}	9.4^{T-Z}	10.3^{A-E}
230830	5.0^{D}	62.5I-^{R}	115.5^{B-E}	63^{A-N}	10.5^{O-Y}	12.1^{A-E}

230523	5.0^{D}	61.5^{K-R}	112.5^{C-E}	64.6^{A-L}	9.9^{Q-Z}	11.2^{A-E}
90018	5.0^{D}	81.5^{A-K}	136^{A-E}	53.7^{B-Q}	13.8^{B-S}	9.9^{A-E}
202116	5.0^{D}	68^{D-Q}	118.5^{A-E}	72.9^{A-B}	11.3^{J-Y}	15.6^{A-E}
231210	5.0^{D}	58^{P-R}	112^{D-E}	65.4^{A-K}	9.7^{S-Z}	14.6^{A-E}
233370	5.0^{D}	85.5^{A-G}	140^{A-E}	50.4^{F-Q}	14.4^{A-O}	12.9^{A-E}
233986	5.0^{D}	85^{A-H}	142.5^{A-B}	60.5^{A-O}	16.2^{A-F}	13.3^{A-E}
233982	5.0^{D}	82.5^{A-I}	140.5^{A-E}	58.3^{A-Q}	14.2^{A-P}	15.9^{A-E}
233984	5.0^{D}	86.5^{A-E}	141.5^{A-D}	53.9^{B-Q}	15.6^{A-I}	13.4^{A-E}
90022	5.5^{C}	74.5^{A-Q}	127.5^{A-E}	71.4^{A-D}	12.8^{D-V}	13.6^{A-E}
207542	5.0^{D}	79^{A-N}	135^{A-E}	61.9^{A-N}	12.8^{D-V}	6.3^{B-E}
233679	5.0^{D}	85^{A-H}	139.5^{A-E}	53.2^{B-Q}	13.8^{B-S}	9.6^{A-E}
235892	5.0^{D}	87.5^{A-D}	138^{A-E}	62.6^{A-N}	14.1^{A-Q}	5.5^{B-E}
234355	5.0^{D}	90^{A-B}	142^{A-C}	59.6^{A-P}	16.6^{A-D}	2.7^{D-E}
233983	5.0^{D}	85.5^{A-G}	140.5^{A-E}	71.6^{A-D}	17.2^{AB}	30^{A}
233981	5.0^{D}	79^{A-N}	128.5^{A-E}	66.9^{A-I}	15.1^{A-L}	13.3^{A-E}
90004	5.0^{D}	81^{A-L}	139.5^{A-E}	63.6^{A-M}	14^{A-R}	12.7^{A-E}
207911	6.0^{B}	90^{A-B}	140^{A-E}	39^{Q}	14.2^{A-P}	-
237991	5.0^{D}	85^{A-H}	141^{A-E}	53.6^{B-Q}	15.3^{A-K}	8.6^{B-E}
237512	5.0^{D}	86^{A-F}	140.5^{A-E}	49.6^{G-Q}	14.9^{A-N}	8.4^{B-E}
234828	5.0^{D}	89.5^{A-C}	142.5^{A-B}	64.9^{A-K}	17.2^{A-B}	25.1^{A-B}
219958	5.0^{D}	87.5^{A-D}	141.5^{A-D}	59.4^{A-P}	17.1^{A-C}	12.6^{A-E}
219959	5.0^{D}	92^{A}	145.5^{A}	59.5^{A-P}	16.3^{A-E}	3.4^{C-E}
90007	5.0^{D}	81^{A-L}	135.5^{A-E}	65^{A-K}	13.6^{B-T}	7^{B-E}
230524	5.0^{D}	61^{L-R}	113^{B-E}	65.4^{A-K}	10.2^{O-Z}	7.6^{B-E}

240808	5.0^{D}	59.5^{N-R}	115^{B-E}	58.6^{A-Q}	10^{P-Z}	4.8^{B-E}
219960	5.0^{D}	89^{A-C}	139^{A-E}	47.1^{I-Q}	13.6^{B-T}	1.1^{E}
90010	5.0^{D}	75^{A-P}	119^{A-E}	60^{A-P}	11.3^{J-Y}	5.1^{B-E}
240579	5.0^{D}	73^{A-Q}	123.5^{A-E}	45.5^{K-Q}	9.8^{R-Z}	6.3^{B-E}
240397	5.0^{D}	59^{N-R}	112.5^{C-E}	69.8^{A-G}	9.7^{S-Z}	8.6^{B-E}
219961	5.0^{D}	89.5^{A-C}	140.5^{A-E}	53.5^{B-Q}	15.7^{A-H}	5^{B-E}
90012	5.0^{D}	84^{A-H}	135.5^{A-E}	60.2^{A-P}	14^{A-R}	3^{C-E}
212853	5.0^{D}	89.5^{A-C}	140^{A-E}	43.4^{M-Q}	13.1^{B-U}	1.6^{E}
90017	5.0^{D}	76.5^{A-P}	128^{A-E}	62.8^{A-N}	13.4^{B-U}	1.2^{E}
241777	5.0^{D}	80^{A-M}	131.5^{A-E}	55.2^{B-Q}	12.9^{C-V}	1.2^{E}
212852	5.0^{D}	62^{J-R}	111.5^{E}	61.1^{A-O}	9.9^{Q-Z}	7.3^{B-E}
90016	5.0^{D}	72^{A-Q}	123.5^{A-E}	50.8^{E-Q}	10.8^{M-Y}	5^{B-E}
80001	5.0^{D}	85.5^{A-G}	140^{A-E}	59.7^{A-P}	15.8^{A-G}	7.6^{B-E}
242916	5.0^{D}	57^{P-R}	114.5^{B-E}	62.8^{A-N}	9.6^{S-Z}	9.3^{A-E}
212628	5.0^{D}	67.5^{D-Q}	120.5^{A-E}	66.9^{A-I}	12^{F-X}	5.5^{B-E}
242609	5.0^{D}	89^{A-C}	138.5^{A-E}	45.6^{J-Q}	14.3^{A-O}	-
90021	5.0^{D}	76^{A-P}	135^{A-E}	70.4^{A-F}	13.6^{B-T}	14^{A-E}
214660	5.0^{D}	66.5^{E-Q}	117.5^{A-E}	41.2^{O-Q}	8.4^{W-Z}	3.2^{C-E}
214243	5.0^{D}	58^{P-R}	122^{A-E}	49.7^{G-Q}	9.6^{S-Z}	6.9^{B-E}
229205	5.0^{D}	80^{A-M}	133^{A-E}	61.2^{A-O}	15^{A-M}	4.4^{B-E}
90002	5.0^{D}	59^{N-R}	114.5^{B-E}	52.5^{C-Q}	10^{P-Z}	9.3^{A-E}
90006	5.0^{D}	66^{F-R}	117.5^{A-E}	70.7^{A-E}	12.8^{D-V}	11.9^{A-E}
229798	5.0^{D}	74.5^{A-Q}	124.5^{A-E}	72.3^{A-C}	14.3^{A-O}	12.7^{A-E}
208030	7.9^{C-Q}	4.5^{A-B}	3.4^{C-O}	323^{C-G}	2.2^{A-G}	162^{D-F}

Acce.	DG	DF	DM	PH	N1B	N2B
229202	5.0^{D}	85.5^{A-G}	140^{A-E}	49^{H-Q}	11.7^{G-Y}	2.2^{D-E}
229204	5.0^{D}	84^{A-H}	139.5^{A-E}	44.6^{L-Q}	12.3^{E-X}	-
229799	5.0^{D}	87.5^{A-D}	135.5^{A-E}	51.7^{D-Q}	12.6^{D-W}	-
229200	5.0^{D}	90.5^{A-B}	140.5^{A-E}	38.6^{Q}	11.8^{G-X}	1.2^{E}
216886	5.0^{D}	82^{A-J}	128.5^{A-E}	45.8^{J-Q}	10.5^{O-Y}	-
216816	5.0^{D}	76^{A-P}	129^{A-E}	52.1^{C-Q}	9.3^{U-Z}	1^{E}
216815	5.0^{D}	71.5^{B-Q}	119.5^{A-E}	71.8^{A-D}	11.5^{H-Y}	8^{B-E}
229199	5.0^{D}	46^{R}	68.5^{F}	17.1^{R}	-	-
90020	5.0^{D}	81.5^{A-K}	139.5^{A-E}	65.7^{A-K}	15.5^{A-J}	17.2^{A-E}
90005	5.0^{D}	66^{F-R}	112.5^{C-E}	71^{A-E}	11.8^{G-X}	10.8^{A-E}
90009	5.0^{D}	81.5^{A-K}	133^{A-E}	59.8^{A-P}	14.3^{A-O}	7.5^{B-E}
90008	4.0^{E}	54.5^{Q-R}	111.5^{E}	56.8^{A-Q}	10.4^{O-Y}	11^{A-E}
240396	5.0^{D}	83.5^{A-H}	139.5^{A-E}	57.7^{A-Q}	13.6^{B-T}	5^{B-E}
240578	5.0^{D}	67.5^{D-Q}	119^{A-E}	60.1^{A-P}	10.3^{O-Y}	5.3^{B-E}
216885	5.0^{D}	60^{M-R}	111.5^{E}	69.2^{A-H}	10.5^{O-Y}	13.5^{A-E}
80002	5.0^{D}	83.5^{A-H}	127.5^{A-E}	68.7^{A-H}	17.1^{A-C}	20.3^{A-E}
LSD	0.3996	20.32	29.79	20.29	4.262	21.28

NB: the same letters show no significant

Accessions	**L1B**	**LL**	**IL**	**SN**	**SW**	**PN**
208693	12.8^{A-M}	6^{A}	3.7^{A-O}	303^{C-G}	2.8^{A-E}	152^{D-F}
208769	13.5^{A-L}	6^{A}	3.3^{C-O}	294.4^{C-G}	2.8^{A-E}	147.5^{D-F}
208666	9^{B-Q}	6^{A}	3.3^{C-O}	181.7^{D-G}	2.3^{A-G}	91^{D-F}
215714	6.7^{D-Q}	6^{A}	3.1^{G-O}	73.5^{F-G}	1.7^{F-H}	70^{D-F}
215713	3.9^{L-Q}	5.5^{A-B}	2.7^{M-O}	195.5^{D-G}	1.8^{E-H}	98^{D-F}
215807	7.9^{C-Q}	5.5^{A-B}	3^{G-O}	434.7^{C-G}	2.4^{A-G}	217.5^{D-F}
215808	16.4^{A-D}	5.5^{A-B}	3.5^{B-O}	449.9^{C-G}	2.8^{A-D}	225.5^{C-F}
90023	18.7^{A-B}	6^{A}	3.9^{A-N}	475.2^{C-G}	2.5^{A-G}	238^{C-F}
205141	15.4^{A-F}	6^{A}	4^{A-M}	517.6^{C-G}	2.6^{A-F}	259^{C-F}
205163	14^{A-K}	6^{A}	3.8^{A-O}	417.2^{C-G}	2.6^{A-F}	209^{D-F}
208669	8.1^{C-Q}	6^{A}	3.6^{A-O}	87.2^{F-G}	2.4^{A-G}	44^{E-F}
225799	21.1^{A}	6^{A}	3.8^{A-N}	276.5^{C-G}	2.8^{A-B}	139^{D-F}
225725	15.6^{A-F}	6^{A}	4.1^{A-J}	240.1^{D-G}	2.6^{A-F}	120.5^{D-F}
229203	5.5^{G-Q}	5^{A-C}	3.6^{A-O}	324.5^{C-G}	2.9^{A}	162.5^{D-F}
229201	-	-	-	-	-	-
90014	10.3^{B-P}	4.5^{B-C}	3.9^{A-M}	309.2^{C-G}	1.8^{C-H}	154.5^{D-F}
207910	11.1^{B-O}	5^{A-C}	3.9^{A-N}	614.1^{B-G}	2.1^{A-G}	307.5^{A-F}
208667	9.2^{B-Q}	5.5^{A-B}	3.2^{D-O}	109^{F-G}	2.4^{A-G}	55^{E-F}
230829	12.6^{A-N}	6^{A}	4.2^{A-H}	272.5^{C-G}	2^{A-G}	136.5^{D-F}
230831	9.8^{B-P}	6^{A}	3.6^{A-O}	203.5^{D-G}	2.3^{A-G}	102^{D-F}
230830	13.5^{A-L}	6^{A}	3.8^{A-O}	131.2^{F-G}	2.4^{A-G}	66^{E-F}
230523	10.5^{B-P}	6^{A}	4^{A-L}	217.4^{D-G}	2.2^{A-G}	109.5^{D-F}
90018	8.6^{C-Q}	5^{A-C}	3.8^{A-O}	283^{C-G}	2.2^{A-G}	92^{D-F}

202116	15.1^{A-G}	6^{A}	4^{A-K}	310.1^{C-G}	2.8^{A-C}	155.5^{D-F}
231210	10.9^{B-O}	6^{A}	4^{A-K}	147.2^{E-G}	2.1^{A-G}	74^{D-F}
233370	4.1^{L-Q}	5A-C	2.7^{L-O}	471.7^{C-G}	2.3^{A-G}	236.5^{C-F}
233986	10.6^{B-P}	4.5^{B-C}	3.5^{C-O}	482.8^{C-G}	2^{A-G}	242^{C-F}
233982	8.7^{C-Q}	5^{A-C}	3.7^{A-O}	326.3^{C-G}	2.2^{A-G}	163.5^{D-F}
233984	7.8^{C-Q}	4.5^{B-C}	3.1^{G-O}	547.6^{C-G}	1.9^{A-G}	274^{B-F}
90022	16^{A-E}	5.5^{A-B}	4.2^{A-I}	449.3^{C-G}	2.5^{A-H}	225.5^{C-F}
207542	8.8^{C-Q}	5^{A-B}	3.8^{A-O}	547.1^{C-G}	2.1^{A-G}	274^{B-F}
233679	6.1^{F-Q}	5^{A-C}	3.2^{F-O}	709.4^{A-F}	2.2^{A-G}	355^{A-F}
235892	4.6^{I-Q}	5^{A-C}	4^{A-L}	270.9^{C-G}	1.8^{B-H}	136^{D-F}
234355	3.8^{L-Q}	4.5^{B-C}	3.5^{C-O}	523.5^{C-G}	2.2^{A-G}	262^{B-F}
233983	14.7^{A-H}	4.5^{B-C}	3.6^{A-O}	728.6^{A-F}	2.2^{A-G}	364.5^{A-F}
233981	14.7^{A-H}	4.5^{B-C}	3.3^{C-O}	599.3^{B-G}	1.8^{D-G}	300^{A-F}
90004	11.2^{B-O}	5^{A-C}	3.4^{C-O}	411.9^{C-G}	1.6^{F-H}	206^{D-F}
207911	2.8^{O-Q}	5^{A-C}	2.8^{K-O}	171.3^{D-G}	1.6^{F-H}	86^{D-F}
237991	5.4^{G-Q}	5^{A-C}	3^{G-O}	481.5^{C-G}	2.2^{A-G}	241.5^{C-F}
237512	5.5^{G-Q}	5^{A-C}	3.1^{F-O}	597.2^{B-G}	2.1^{A-G}	299^{B-F}
234828	10.1^{B-P}	4^{C}	3.4^{C-O}	938.5^{A-C}	2.1^{A-G}	470^{A-D}
219958	9.7^{B-Q}	4.5^{B-C}	3.5^{C-O}	665.1^{A-G}	2^{A-G}	333^{A-F}
219959	4.7^{I-Q}	4.5^{B-C}	3.2^{F-O}	494^{C-G}	2^{A-G}	247.5^{C-F}
90007	10.8^{B-O}	5^{A-C}	3.8^{A-O}	414.3^{C-G}	2.2^{A-G}	207.5^{D-F}
230524	9.9^{B-P}	6^{A}	3.8^{A-O}	130.7^{F-G}	2.3^{A-G}	66^{E-F}
240808	8.1^{C-Q}	6^{A}	3.4^{C-O}	97.7^{F-G}	2.4^{A-G}	49.5^{E-F}
219960	3.4^{M-Q}	4.5^{B-C}	2.8^{J-O}	137.8^{F-G}	1.7^{F-H}	69^{D-F}

90010	11.3^{B-O}	6^{A}	3.5^{C-O}	222.9^{D-G}	2.3^{A-G}	111.5^{D-F}
240579	7.6^{C-Q}	5^{A-C}	2.9^{H-O}	135.4^{F-G}	2.2^{A-G}	68^{E-F}
240397	14.8^{A-H}	6^{A}	4^{A-L}	151.1^{E-G}	2.3^{A-G}	76^{D-F}
219961	4.3^{K-Q}	5^{A-C}	3.6^{B-O}	350.5^{C-G}	1.9^{A-G}	175.5^{D-F}
90012	6.6^{E-Q}	5^{A-C}	3.1^{G-O}	209.1^{D-G}	2.3^{A-G}	105^{D-F}
212853	2.7^{O-Q}	5^{A-C}	3.1^{G-O}	229.8^{D-G}	1.9^{B-H}	115.5^{D-F}
90017	6.5^{E-Q}	5.5^{A-B}	3^{G-O}	173.6^{D-G}	2^{A-G}	87.5^{D-F}
241777	6.7^{D-Q}	5.5^{A-B}	3^{G-O}	174.4^{D-G}	1.9^{A-H}	87.5^{D-F}
212852	9.7^{B-Q}	5.5^{A-B}	4.4^{A-F}	130.8^{F-G}	2.5^{A-G}	65.5^{E-F}
90016	7.9^{C-Q}	6^{A}	3.2^{E-O}	160.8^{E-G}	2.1^{A-G}	81^{D-F}
80001	9^{B-Q}	5.5^{A-B}	3.9^{A-N}	389.6^{C-G}	2.1^{A-G}	195.5^{D-F}
242916	14.1^{A-J}	5.5^{A-B}	3.6^{A-O}	265.1^{C-G}	2.6^{A-F}	133^{D-F}
212628	12.9^{A-M}	6^{A}	3.6^{B-O}	195.2^{D-G}	2.2^{A-G}	48^{E-F}
242609	2.5^{O-Q}	5^{A-C}	3^{G-O}	248.4^{C-G}	2.2^{A-G}	125^{D-F}
90021	15.4^{A-F}	4.5^{B-C}	4.5^{A-D}	662^{A-G}	2.6^{A-F}	331.5^{A-F}
214660	5.3^{H-Q}	6^{A}	2.5^{O}	128.5^{F-G}	2.1^{A-G}	64.5^{E-F}
214243	9.7^{B-Q}	6^{A}	3.6^{A-O}	122.3^{F-G}	2.5^{A-G}	61.5^{E-F}
229205	6.7^{D-Q}	5.5^{A-B}	3.5^{B-O}	364^{C-G}	2.1^{A-G}	182.5^{D-F}
90002	10.9^{B-O}	5.5^{A-B}	3.9^{A-N}	109.3^{F-G}	2.2^{A-G}	55^{E-F}
90006	14.3^{A-I}	6^{A}	4.5^{A-E}	423.1^{C-G}	2.3^{A-G}	212^{D-F}
229798	15.6^{A-F}	6^{A}	4.3^{A-G}	488.2^{C-G}	1.8^{D-H}	237^{C-F}
229202	4.5^{J-Q}	5.5^{A-B}	2.6^{N-O}	226.3^{D-G}	1.5^{G-H}	113.5^{D-F}
229204	3.5^{M-Q}	5^{A-C}	3^{G-O}	84.5^{F-G}	2.1^{A-G}	42.5^{E-F}
229799	1.7^{O-Q}	5^{A-C}	3.1^{G-O}	66.1^{F-G}	2.2^{A-G}	33.5^{E-F}

229200	2.9^{N-Q}	4.5^{B-C}	2.6^{N-O}	1246.5^{A-B}	2^{A-G}	623.5^{A-C}
216886	3.9^{L-Q}	5^{A-C}	2.8^{I-O}	137.8^{F-G}	2.4^{A-G}	69^{D-F}
216816	4^{L-Q}	5^{A-C}	2.8^{K-O}	-	-	-
216815	17.1^{A-C}	6^{A}	4^{A-L}	397.4^{C-G}	2.3^{A-G}	192^{D-F}
229199	1^{P-Q}	2^{D}	0.9^{P}	71.5^{F-G}	0.9^{H-I}	36^{E-F}
90020	14.1^{A-J}	4.5^{B-C}	3.7^{A-O}	861.4^{A-D}	2.3^{A-G}	431^{A-E}
90005	14.2^{A-J}	6^{A}	4.8^{A-B}	310.5^{C-G}	2.1^{A-G}	155.5^{D-F}
90009	8.6^{C-Q}	5^{A-C}	3.4^{C-O}	344.3^{C-G}	1.8^{B-H}	172.5^{D-F}
90008	14.5^{A-H}	6^{A}	4.1^{A-J}	207.7^{D-G}	2.3^{A-G}	104.5^{D-F}
240396	5.4^{G-Q}	5^{A-C}	3.2^{D-O}	304.3^{C-G}	2.2^{A-G}	152.5^{D-F}
240578	9.7^{B-Q}	6^{A}	3.4^{C-O}	238.2^{D-G}	2.4^{A-G}	119.5^{D-F}
216885	14.3^{A-I}	6^{A}	4.9^{A}	309.8^{C-G}	2.5^{A-G}	84.5^{D-F}
80002	16.2^{A-E}	5^{A-C}	3.9^{A-N}	1326.4^{A}	2.5^{A-G}	663.5^{A-B}
205162	21.2^{A}	6^{A}	4.6^{A-C}	834.9^{A-E}	2.8^{A-E}	418^{A-E}
LSD	9.791	1.397	1.325	693.0	1.003	401.9

Key: A- The highest mean scores

Table 7. Correlation table for 12 agronomic traits studied on *L. sativum* populations

Traits	DG	DF	DM	PH	N1B	N2B	L1B	LL	IL	SN	SW	PN
DG	*											
DF	-0.23*	*										
DM	0.13*	0.91*	*									
PH	-0.47*	0.19**	0.34*	*								
N1B	-0.33*	0.69*	0.69*	0.48*	*							
N2B	-0.17**	-0.02***	0.07***	0.56*	0.33*	*						
L1B	-0.28*	-0.19**	-0.05***	0.78*	0.17*	0.71*	*					
LL	-0.50*	0.13***	0.32*	0.58*	0.04***	0.09*	0.403*	*				
IL	-0.47*	0.13***	0.28*	0.84*	0.38*	0.52*	0.684*	0.59*	*			
SN	-0.14**	0.34*	0.30*	0.35*	0.53*	0.54*	0.412*	-0.11***	0.27*	*		
SW	-0.40*	0.18**	0.28*	0.54*	0.23*	0.25*	0.450*	0.53*	0.51*	0.16**	*	
PN	-0.13**	0.26*	0.30*	0.34*	0.57*	0.57*	0.386*	-0.15***	0.28*	0.87*	0.12***	*

highly significant ** significant * non significant*

The highest positive correlation was observed in days to flowering and days to maturity (0.91) followed by plant height and internode length (0.84). Whereas the highest negative correlation were observed in days to germination with plant height and internode length (-0.47) (Table 7). Days to germination was only positively correlated with days to maturity, whereas negatively correlated with all traits even though it showed highly significant and significant. Days to flowering was positively correlated with all traits except number of primary and secondary branches. Days to maturity positively correlated with all traits, but not with number of primary branches. Non significant correlation observed between days to maturity with number of primary and secondary branches. Plant height, number of primary and secondary branches and length of primary branches correlated positively with all traits. Seed number and pod number not showed correlation with leaf length (Table 7).

5.1.2. Agronomic trait variation within and among regions of collection

Mean value calculated for each trait per region showed variation among the traits (Table 8, 9 and 10). The mean germination days were in Amhara (5.10), Oromia (5.04), Tigray (5.07) and SNNPR (5.00), respectively. Average flowering days were 77.40, 70.58, 86.65, and 65.27 in Amhara, Oromia, Tigray and SNNPR, respectively. Maturation days had 130.50 in Amhara, 122.45 days in Oromia, 140.07 in Tigray and 119.54 days in SNNPR. The plant height of the four regions, Amhara, Oromia, Tigray and SNNPR, were 56.56, 61.01, 56.97 and 62.32 cm, respectively (Table 8).

Table 8. Minimum, mean and maximum values of days to germination, flowering, maturity and plant height in accessions of *L. sativum* populations from four regions in Ethiopia.

Reg.	**DG**			**DF**			**DM**			**PH**		
	Min.	mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
AMH	5.0	5.10	6.0	58.0	77.40	90.5	111.0	130.5	140.5	38.6	56.56	76.3
ORO	5.0	5.04	6.0	59.0	70.58	89.5	111.5	122.45	142.5	43.4	61.01	71.4
TIG	5.0	5.07	6.0	79.0	86.65	92.0	128.5	140.07	145.5	39.0	56.97	71.6
SNN	5.0	5.0	5.0	57.0	65.27	83.5	112.5	119.54	139.5	41.2	62.32	72.9

Key: AMH- Amhara, ORO- Oromia, TIG- Tigray, SNN- South Nations, Reg-region, DG- Days of germination, DF- Days of flowering, DM-Days of maturity and PH- Plant height.

The average number of primary branches 12.20, 11.30, 15.49 and 10.43; whereas the number of average secondary branches were 9.02, 10.04, 11.61 and 10.63 for Amhara, Oromia, Tigray and SNNPR, respectively. The average length of primary branches was 10.12 for Amhara, 11.53cm for Oromia, 9.31 for Tigray and 12.49 for SNNPR. Leaf average length was 5.45, 5.56, 5.15 and 5.82cm for Amhara, Oromia, Tigray and SNNPR accessions, respectively (Table 9).

Table 9. Minimum, mean and maximum values of number of primary and secondary branch, length of primary branches and leaf length of *L. sativum* populations of Amhara, Oromia, Tigray and SNNPR regions.

Reg.	**N1B**			**N2B**			**L1B**			**LL**		
	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
AMH	6.0	12.20	15.5	5.0	9.02	25.0	5.5	10.12	21	4.0	5.45	6.0
ORO	8.1	11.30	17.2	5.0	10.04	25.1	8.0	11.53	18.7	4.0	5.56	6.0
TIG	13.6	15.49	17.3	5.0	11.61	30.0	8.0	9.31	16.6	5.0	5.15	6.0
SNN	8.4	10.43	13.6	5.0	10.63	22.9	8.0	12.49	21.2	5.0	5.82	6.0

Key: AMH- Amhara, ORO- Oromia, TIG- Tigray, SNN- South Nations, Reg-Region, N1B- Number of Primary Branch, N2B- Number of Secondary Branch, L1B- Length of Primary Branch and LL- Leaf Length.

The mean internodes length among the regions was 3.44 cm in Amhara, 3.68 in Oromia, 3.34 in Tigray and 3.59 in SNNPR. The highest mean variation was observed in number of seeds; that was 406.70, 320.26, 455.07 and 252.36 in Amhara, Oromia, Tigray and SNNPR, respectively. Pod number and thousand seed weight of the four regions respectively were 203.35 and 2.12 g; 160.13 and 2.38 g; 227.54 and 1.99 g; 126.18 and 2.45 g (Table 10).

The range of the four regions in 12 agronomic traits were 0.1 for days to germination, 21.38 days to flowering, 20.53 days to maturation, 5.76 cm in plant height, 5.06 in number of primary branch, 2.59 in number of secondary branch, 3.18cm in length of primary branch, 0.67 cm in leaf length, 0.34 cm in inter node length, 202.71 in seed number, 101.36 in pod number and 0.46 in thousand seed weight. The highest range was observed for seed number while the least was for days to germination.

Table 10. Minimum, mean and maximum values of inter node length, seed number, pod number and thousand seed weight of *L. sativum* populations collected from four regions of Ethiopia.

Reg.	**IL**			**SN**			**PN**			**1000swt.**		
	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
AMH	2.6	3.44	4.6	150	406.7	1600	75	203.35	800	1.53	2.12	2.88
ORO	2.8	3.68	4.9	150	320.26	940	75	160.13	470	1.88	2.38	2.91
TIG	2.8	3.34	3.9	150	455.07	730	75	227.54	365	1.65	1.99	2.24
SNN	2.5	3.59	4.2	150	252.36	518	75	126.18	259	2.06	2.45	2.83

Key: AMH- Amhara, ORO- Oromia,TIG- Tigray,SNN- South nations, Reg-region, IL- Inter node length, SN- seed number, PN- pod number,1000 Swt.- one thousand seed weight.

In Amhara high coefficient of variation was observed in seed and pod number i.e. 86%; whereas the lowest variation was 5% of germination day. The other agro morphological traits recline with this range (Table 11).

In Oromia regional state the highest coefficients of variation were scored in seed number and pod number i.e. 62%, whereas the lowest variation (3%) was in days of germination (Table 11).

In Tigray regional state the highest variation was observed in number of secondary branch which was 65%. In contrast to this the lowest variation was in days of maturity (2%).The other agronomic traits lie between this range (Table 11).

In SNNPR the highest variation was observed in the number of secondary branch which was 62%. But, the lowest coefficient of variation was 0% of germination day. The other agronomic traits were in between these traits (Table 11).

Table 11. Coefficient of variation of agronomic traits among regions of collections

	Coefficient of variation (CV %) by regions			
Traits	Amhara	Oromia	Tigray	SNNPR
DG	5%	3%	5%	0%
DF	12%	13%	4%	11%
DM	6%	8%	2%	6%
PH	18%	14%	15%	16%
N1B	19%	18%	7%	13%
N2B	69%	49%	65%	62%
L1B	36%	28%	26%	35%
LL	11%	10%	7%	6%
IL	15%	16%	10%	13%
SN	86%	62%	41%	42%
PN	86%	62%	41%	42%
1000swt	16%	9%	10%	10%

Key: DG- Days to germination, DF- Days to flowering, DM-Days to maturity and PH- Plant height,N1B- Number of primary branch, N2B- Number of secondary branch, L1B- Length of primary branch and LL- Leaf length, IL- Inter node length, SN- Seed number, PN- Pod number, 1000 SW- Thousand seed weight, CV- coefficient of variation.

The highest yield was recorded from accession 205162 (2324.08 kg/ha) followed by accession 90020 (2008.46 kg/ha) from Amhara and accession 234828 (1983.4 kg/ha) from Oromia regions.

The overall average yield performance for populations collected from Tigray (946.40kg/ha) and Amhara (874.60 kg/ha) were higher than that of Oromia (765.40 kg/ha) and SNNPR (608.60 kg/ha) regions (Table 12).

Table 12. Average yield performance of *L. sativum* populations collected from four regions of Ethiopia

Regions	**Yield**
Amhara	874.60 kg/ha
Oromia	765.40 kg/ha
Tigray	946.40 kg/ha
SNNPR	608.60 kg/ha

Key: ha- Hectares, SNNPR- South Nation's Nationalities People Region

Oil content analysis

There was variation in oil content of *Lepidium sativum* depending on the types of seed color; the light yellow seed color had the lowest (20.67%), where as the black seed color medium (23%) and the brown seed color the highest (25%) oil content. The highest overall oil content was observed for samples collected from Northern part of Ethiopia with 24.2%, and the least oil content by Southern Ethiopia with 21.4 % (Table 13).

Table 13. Mean oil content value of seeds of different seed colors of *L. sativum* collected from four regions of Ethiopia.

Geographical region		**Seed colors**		**Average**
	Light yellow	**brown**	**black**	
Southern Ethiopia	19.2%	23%	22%	21.4%
Northern Ethiopia	21.5%	27%	24%	24.2%
Eastern Ethiopia	20%	26%	23%	23%
Western Ethiopia	22%	24%	23%	23%
Average	20.67%	25%	23%	22.9%

NB: Northern Ethiopia = Amhara and Tigray regions, Southern Ethiopia = South Region, Eastern and Western Ethiopia = Oromia region.

5.2. ISSR Primers and their banding patterns

Out of the ten primers tested initially, four (two di-nucleotide, one tetra nucleotide and one penta-nucleotide) gave relatively clear reproducible banding pattern. These four primers (812, 834, 873, and 880) were selected and used in this study (Table 14). The molecular weight of the bands amplified using the primers were in the range of 100 bp to 800bp.

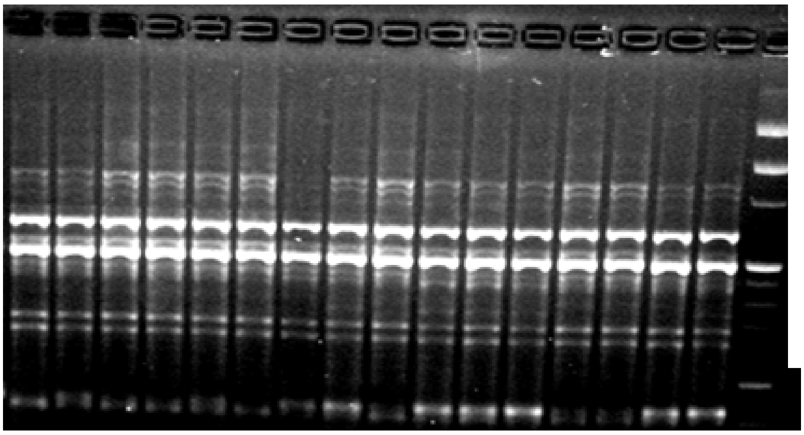
A total of 53 clear and scorable bands were recorded from 85 *Lepidium sativum* accessions representing six populations; out of which 43 were found to be polymorphic. The least polymorphic bands (9) were scored from primers 812 and 834; whereas primer 880 had ten polymorphic bands. The remaining one primer (873) showed the highest polymorphic bands (fifteen). Average number of bands and polymorphic bands per primer were 13.25 and 10.75, respectively. Figure 1 shows the amplification pattern of primer 873. The other primers gel pictures used in this study are presented in Appendix 1, 2, 3 and 4.

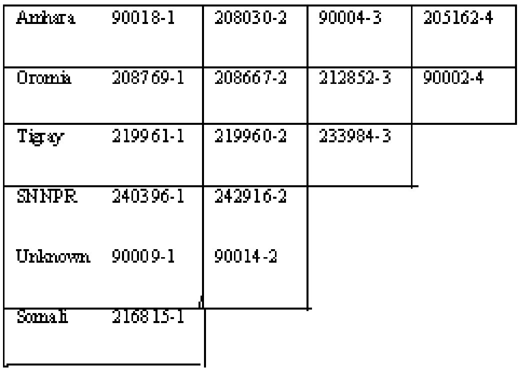
Overall, none of the primers showed 100 percent polymorphism. However, primer 873 was with the highest percent polymorphism (93.75%). The least polymorphic bands were scored for primer 812 (64.29%); while primers 834 and 880 showed 81.82 % and 83.33% polymorphism, respectively.

Table 14. Banding patterns generated using the four selected primers, their repeat motifs, amplification patterns and number of scored bands

Primers	Repeat motif	Amplification quality	Number of scored bands
812	(GA)8A	good	14
834	(AG)8YT	good	11
873	(GACA)4	good	16
880	(GGAGA)3	good	12
Total			53

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





Amhara	90018-1	208030-2	90004-3	205162-4
Oromia	208769-1	208667-2	212852-3	90002-4
Tigray	219961-1	219960-2	233984-3	
SNNPR	240396-1	242916-2		
Unknown	90009-1	90014-2		
Somali	216815-1			

Figure 1. ISSR fingerprint generated from 16 individual accessions using primer 873.*key: Lanes 1-4 from Amhara, 5-8 from Oromia, 9-11 from Tigray, 12-13 from SNNPR, 14-15 from unknown and sample number 16 s from Somali. The 17 is DNA marker. The accession numbers corresponding to the lanes are as shown above.*

5.2.2. Genetic diversity as revealed by percent polymorphism, shannon and gene diversity values

Of the total 53 loci scored, 81.13% (43) were observed to be polymorphic. From all the populations studied, Amhara and Tigray were (66.04%), Oromia (50.94%), SNNPR (47.17%) and Somali (45.28%) percent polymorphic. Amhara and Tigray showed more percent polymorphism; while the least polymorphism was detected in population from Somali region. No unique bands were observed for either the accessions or the populations.

Among the *L .sativum* accessions evaluated using ISSR markers, samples from Tigray and Amhara exhibited the highest gene diversity (H = 0.24), whereas samples from Oromia had (H = 0.17), from SNNPR (H = 0.18) and Somali (H= 0.18) gene diversity values. The average gene diversity for the total population (H_T) was 0.27 (Table 16).

Primer 873 showed highest gene and Shannon diversity (0.36 and 0.53, respectively) and primer 812 was the least (0.20 and 0.31, gene and Shannon diversity, respectively) (Table 18).

Table 15. Number of scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H), Shanon Index information (I) of 85 *L. sativum* accessions based on all primers used.

Primers	**NSB**	**NPL**	**PP**	**H±SD**	**I±SD**
812	14	9	64.29	0.20±0.20	0.31±0.28
834	11	9	81.82	0.24±0.15	0.38±0.22
873	16	15	93.75	0.36±0.13	0.53±0.18
880	12	10	83.33	0.25±0.15	0.39±0.22
Over all	53	43	81.13	0.27±0.17	0.41±0.24

Table 16. The number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H) and Shannon information index (I) among the five regions of Ethiopia.

Populations	**NPL**	**PP**	**H±SD**	**I±SD**
Amhara	35	66.04	0.24±0.19	0.35±0.28
Oromia	27	50.94	0.17±0.19	0.26±0.28
Tigray	35	66.04	0.24±0.19	0.35±0.27
SNNPR	25	47.17	0.18±0.21	0.27±0.30
Somali	24	45.28	0.18±0.21	0.26±0.30
Total	182	343.39	1.27±1.2	1.87±1.72

5.2.3. Analysis of molecular variance

Analysis of molecular variance was carried out on the overall ISSR data score of *L. sativum* accessions without grouping by region or geographic location (Table 20). AMOVA revealed high percentage of variation (94 %) that is attributed to within population variation while the remaining variation is due to among population variation (6 %). The variation was found to be highly significant at (P = 0.00). The result shows that there is high gene flow or seed flow among population in different region; this resulted in low genetic variation and differentiation among population.

Table 17. Analysis of Molecular Variance (AMOVA) of *L. sativum* accessions in Ethiopia without grouping.

Source of variation	Sum of squares	Variance components	Percentage of variation	Fixation	P
Among populations	4.122	0.02834	6.00	0.06	0.00
Within population	34.765	0.44387	94.00		
Total	38.888	0.47221	100		

5.2.4. Clustering analysis

UPGMA and Neighbor Joining tree construction methods was used to construct dendrogram for six populations and 85 individuals based on 53 PCR bands amplified by two di-nucleotides (812 and 834), one penta nucleotides (880) and one tetra nucleotide (873). The dendrogram derived from neighbor-joining analysis of the whole ISSR data with 85 *Lepidium sativum* accessions showed four distinct clusters and two sub-clusters within each major cluster (Fig. 4). Most of the individual accessions collected from the same region tend to spread all over the tree without forming their own grouping. The wider distribution of *L. sativum* accession all over the tree shows the low divergence among populations from different localities. UPGMA analysis based on regions of collection of *L. sativum* revealed three major groups. The first cluster contains Oromia, Amhara and Tigray; while the second cluster contains SNNPR and individual from unknown origins. The final major cluster contains the Somali group. However UPGMA with individual accessions showed intermixing of individuals to different groups, except in two groups where individuals from Oromia clustered together (Fig. 2 and Fig. 3).

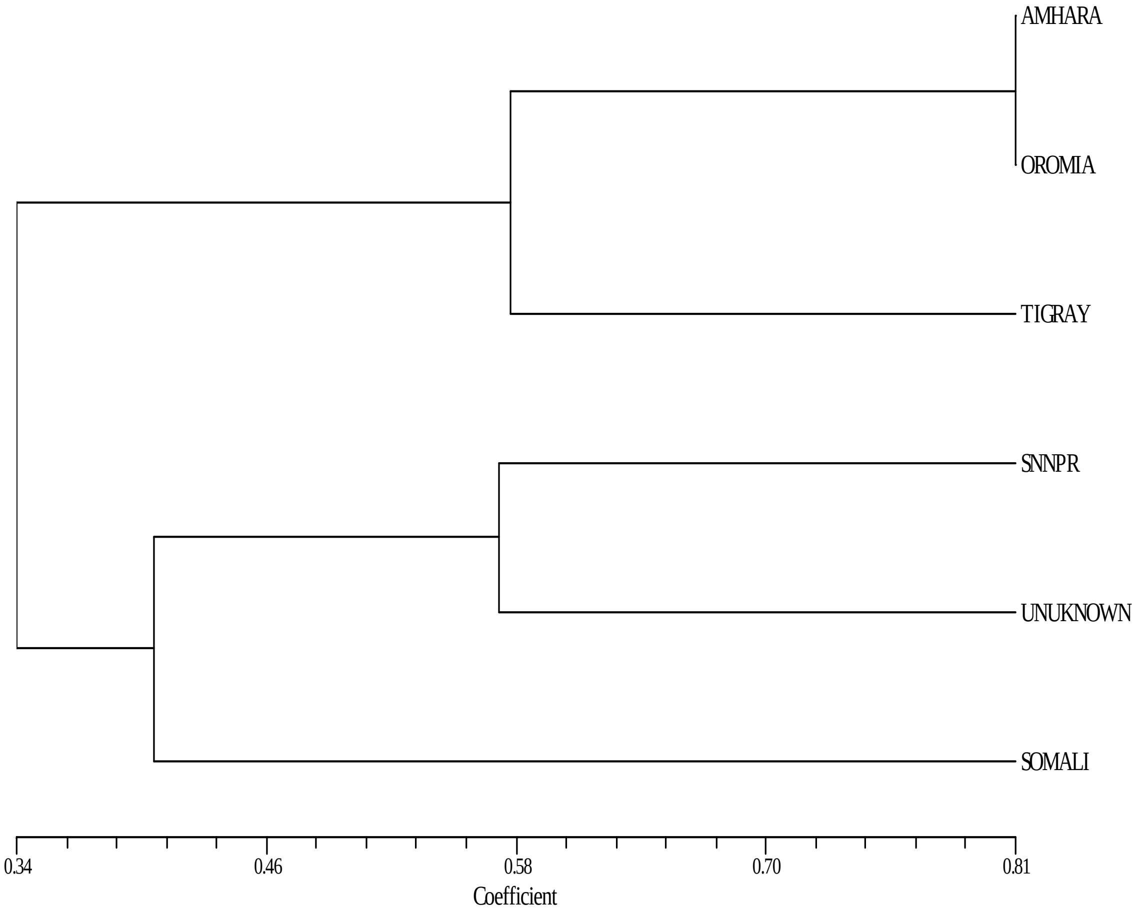


Figure 2. UPGMA based dendrogram for 6 *L. sativum* populations using 4 ISSR (2 di, 1 penta and 1 tetra nucleotide) primers.

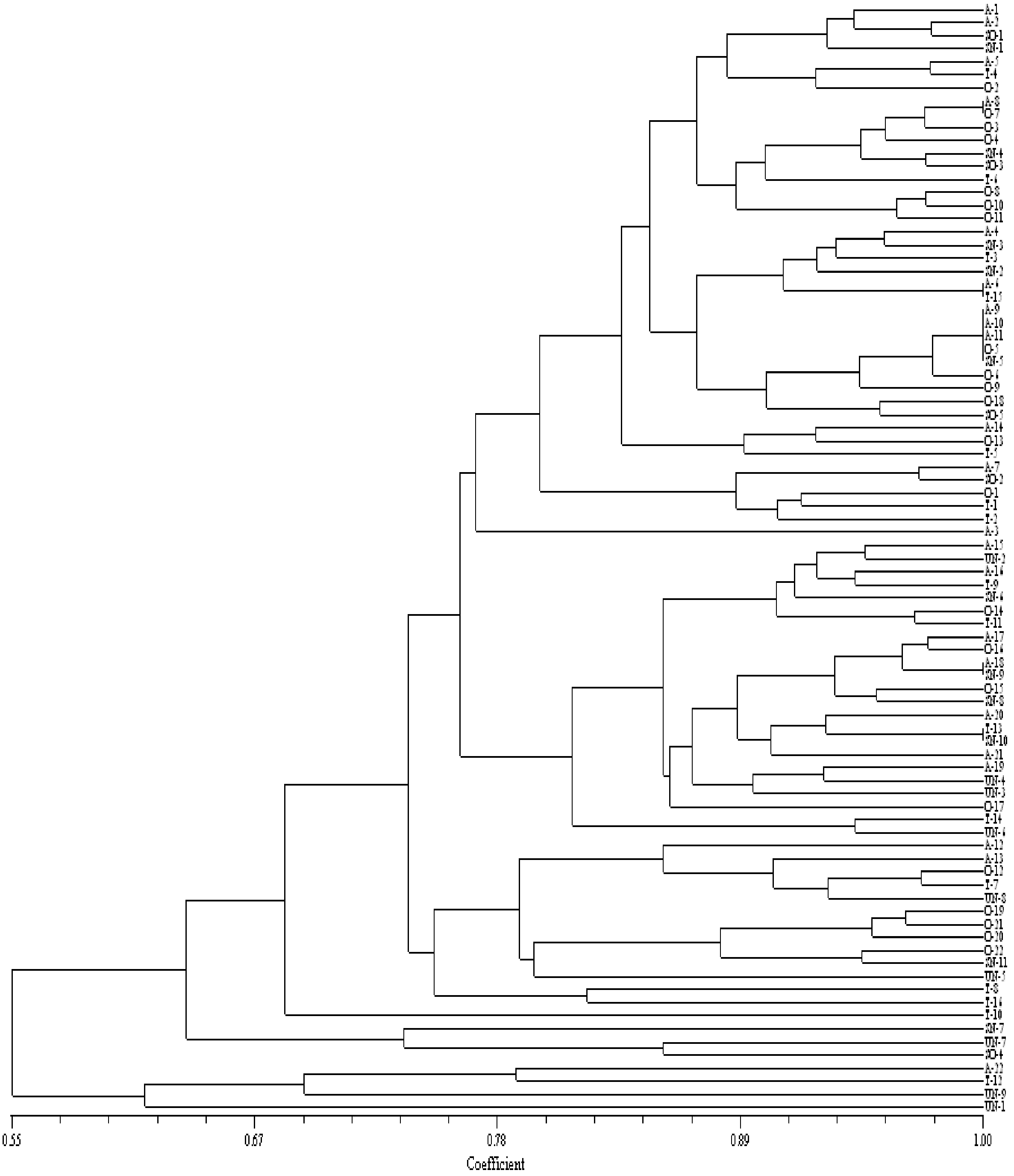


Figure 3. UPGMA based dendrogram for 85 *L. sativum* individuals using 4 ISSR (2 di, 1 penta and 1 tetra nucleotide) primers. *Key: A = Amhara, O = Oromia, T = Tigray, SN = South Nations, UN = Unknown and SO = Somali. See table 1 for accessions symbols.*

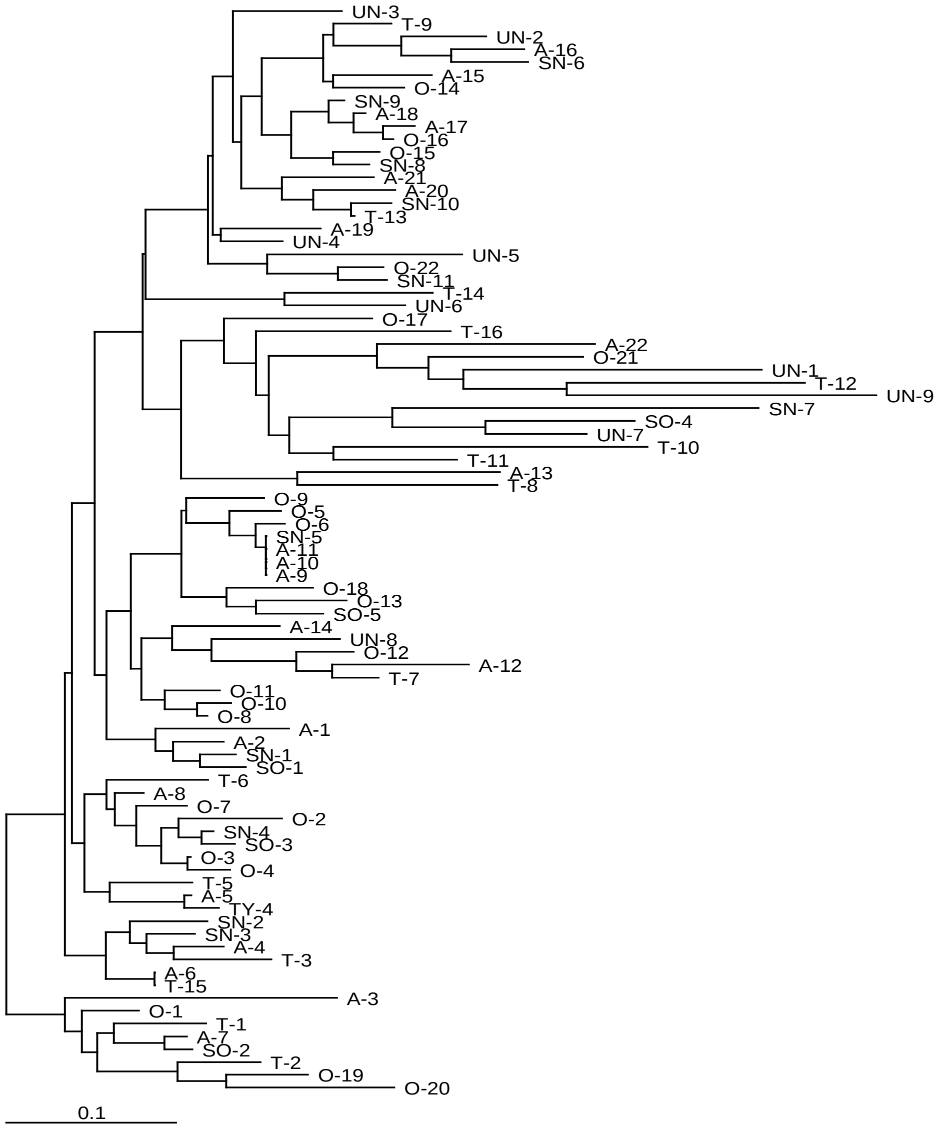


Figure 4. Neighbor-joining analysis of 85 individuals based on 53 PCR bands amplified by two dinucleotide (812 and 834); one tetranucleotide (873); and one pentanucleotide (880) primers. The neighbor joining algorithm is based on Jaccard's coefficient. *Key: A-Amhara, O-Oromia, T-Tigray, SN-South Nations, UN-Unknown and SO-Somali. See table 1 for accessions symbols.*

5.2.5. PCO analysis

All the data obtained using the four ISSR primers were used in PCO analysis using Jaccard's coefficients of similarity. The first three coordinates of the PCO having Eigen values of 4.83, 4.55 and 1.63 with variance of 18.28%, 17.26% and 6.20%, respectively were used to show the grouping of individuals using two and three coordinates (Fig. 5 and Fig. 6) . In 3D most of the individual accessions that represent different populations spread all over the plot. Using two coordinates (Fig. 5) almost similar result was observed like that of three coordinates. Overall, no clear grouping was observed among individuals collected from different locality.

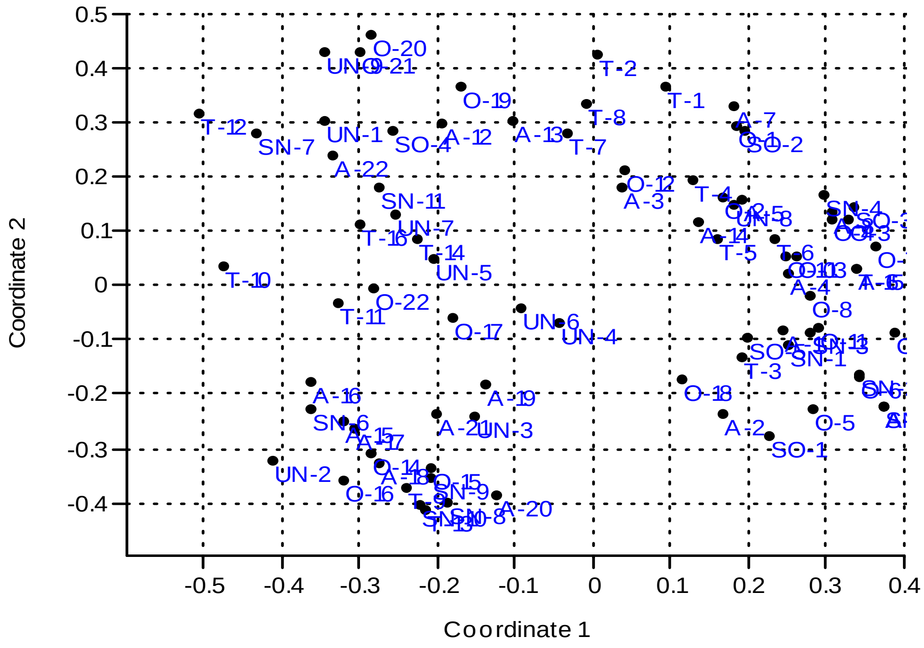


Figure 5. Two dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of *L. sativum* accessions using ISSR data. *Key: A-Amhara, O-Oromia, T-Tigray, SN-South Nations, UN-Unknown and SO-Somali. See table 1 for accessions symbols.*

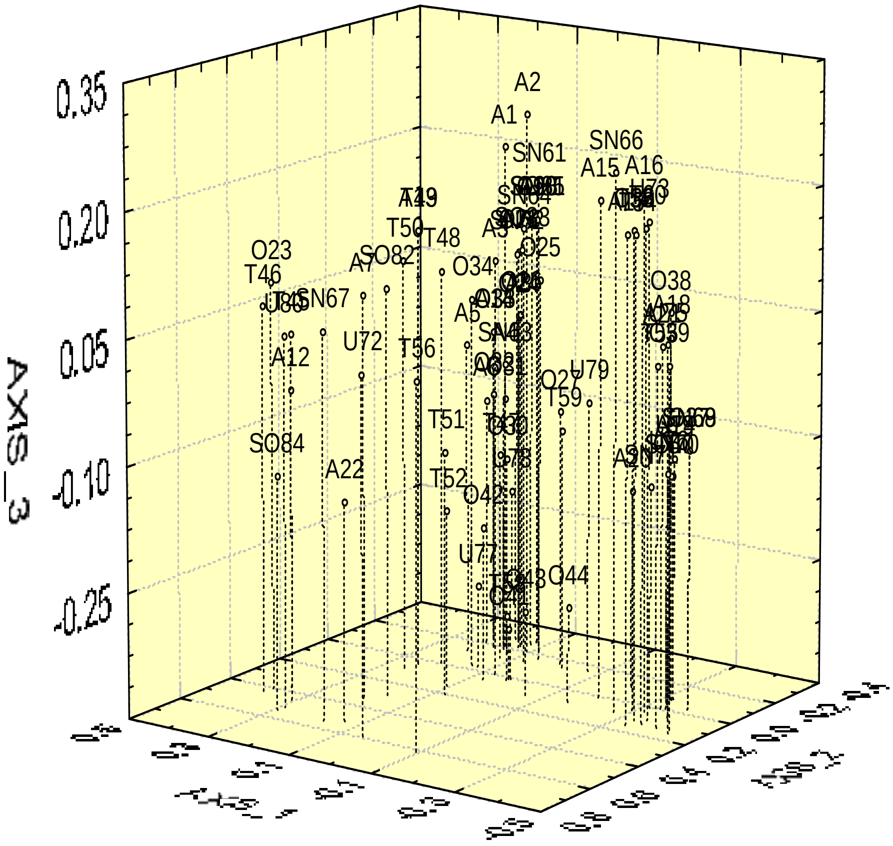


Figure 6. Three dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of *L. sativum* accessions. *Key: A-Amhara, O-Oromia, T-Tigray, SN-South Nations, UN-Unknown, and SO-Somali. See table 1 for accessions symbols.*

6. DISCUSSIONS

6.1. Morphological variation and implications for improvement

Performance of a long term breeding program usually needs to obtain some information about the amount and nature of genetic variation and correlation between traits in order to practice selection and hybridization for a breeding program (Salamati *et al*., 2011).

In the present study, 86 *Lepidium sativum* accessions collected from different administrative regions of Ethiopia were used to assess morphological variation. The presence of high significant morphological variation between the accessions indicates the existence of high degree of phenotypic diversity implying great potential of the species for improvement programs.

From this result, the number of secondary branch per plant and the number of pods per plant showed the highest variation (85.95% and 85.89%, respectively) and followed by seed number per plant (76.18%). The least variation was observed in germination days (3.02%). In the current study, germination took from 4-6 days only. This is in concurrence with Morris *et al.* (2011) study which reported 4-6 germination days for *L. sativum.*

In the present study, the maximum and minimum plant heights were 76.3 and 38cm, respectively with an average value of 59.26cm. Another study on height of *L. sativum* showed that it can grow up to 50 cm (Adam *et al.*, 2010) which is close to the average value of the present study. Zhan *et al*. (2009) also reported that the height of *L. sativum* plant was 80 cm, which correspond to the maximum height observed in this study. However, Shehzad *et al.* (2011) studied and found maximum (118.8 cm) and minimum (107.4 cm) *L. sativum* heights. This could be explained by optimum growing condition for *Lepidium* and the use of inputs since the height of the plant can be significantly affected by adding different inputs such as compost, fertilizers or other nutrients (Tafaghodinia, 2010).

The present study showed the number of primary branch ranged from 6-18.1. Similarly, Shehzad *et al.* (2011) also reported that the number of branches per plant ranged from 10.75-16.25. The present study showed high range in the number of seed per plant (150-1600). According to Shehzad *et al.* (2011) the number of seeds per plant ranged from 540-1030 which is within the limits of the present study. Higher thousand seed weight of *Lepidium* (1.53-2.91g) was observed in the present study, which is bigger than that of Shehzad *et al.* (2011) work.

The present study, also grouped the morphological agronomic traits based on the regions of collection. This study showed less variation of germination days among the regions. However, the flowering and maturity days of *L. sativum* showed high variation. The maximum was recorded in Tigray with 86 and 140.47 and the minimum in SNNPR with 65.25 and 119.54 days of flowering and maturity, respectively. This study showed that accessions from Tigray and Amhara matured late and this is important for highland area for crop improvement, whereas SNNPR and Oromia had early matured accessions and this is important for lowland areas and are of potential to produce drought tolerant varieties. In terms of the average plant height, the maximum was recorded in SNNPR (62.32 cm) and Oromia (61.01 cm); whereas the minimum was from Tigray (56.97 cm) and Amhara (56.56 cm).

The maximum numbers of primary branches were recorded in Tigray (15.49) and Amhara (12.20); whereas the minimum was in genotypes from Oromia (11.30) and SNNPR (10.43). The maximum numbers of secondary branches were recorded in Tigray accessions (11.61) and the minimum in Amhara accessions (9.02). The length of primary branch was highest in SNNPR (12.49 cm), followed by Oromia (11.53), the other minimum length of primary branch were recorded in Amhara (10.12 cm) and Tigray (9.31 cm). Shehzad *et al.* (2011) also reported that the number of branches per plant ranged from 10.75-16.25 without grouping in to regions.

Number of seeds per plant was maximum in Tigray (455.07) and Amhara (406.7), but minimum in Oromia (320.26) and SNNPR (252.36). The number of seeds per plant showed one of the highest variations among regions. Likewise pod number was maximum in Tigray (227.54) and Amhara (203.35), however minimum in Oromia (160.13) and SNNPR (126.18).

The highest yield was recorded from accessions 205162 (2324.08 kg/ha), and accession 90020 (2008.46 kg/ha) from Amhara and accession 234828 (1983.40 kg/ha) from Oromia region. These accessions should be further evaluated for their yield at multi locations. The average maximum yield per region was recorded for genotypes from Tigray with 946.40 kg/ha and followed by genotype from Amhara with 874.60 kg/ha; while Oromia and SNNPR showed the least yield performance per hectare with 765.40 kg/ha and 608.60 kg/ha, respectively. Based on regional average, accessions from Amhara and Tigray had high yield, when compared to Oromia and SNNPR. So these areas need high priority in breeding and conservation program. Moreover, the observed variation in different regions hint into the possibility of getting individual with better seed yield with appropriate maturity time for target location.

The average maximum thousand seed weight was recorded in SNNPR (2.45 g) and Oromia (2.38 g), whereas the minimum in Amhara (2.12 g) and Tigray (1.99 g). Target trait selection for higher seed size should go for SNNPR and Oromia.

For the morphological traits studied, accessions from Amhara and Tigray were more similar in average values of the quantitative traits; similarly Oromia and SNNPR were similar in terms of their agronomical property. In the present study, days to flowering and days to maturity showed maximum correlation (0.91), followed by internodes length and plant height (0.84) and length of primary branches and plant height (0.78). Similarly, Shehzad *et al.* (2011) reported that plant height, number of branches per plant and number of seed per plant show higher correlation. In contrast to Shehzad *et al.* (2011) result, the present study did not show direct concurrence between plant heights with the number of seed per plant.

In the present study, the light yellowish seed color had the least % oil content (20.67%). The black and brown seed colors had 23% and 25% oil content, respectively. The seed oil content of *L. sativum* also studied by Moser, *et al*. (2009) and Dolya *et al*. (1976) were found to be 22.7% and 23.08%, respectively. Datta *et al*. (2011) reported that *L. sativum* seed contains 20-25% which is yellowish and semidrying oil. Therefore the result of the present study correlates with previous works which ranges 20.67-25%. Similar to the present study, previous studies on other oil crops have show some correlation between seed color and seed oil content. Turiho-Habwe (1992) studied the oil content of sunflower and found from 10 - 30%, where the white seeded sunflower varieties showed the lowest oil content, followed by the striped and black varieties. In another study, Jing *et al.* (2008) showed that the average oil content of white kernels was significantly higher than that of yellow kernel of sunflower. Similarly, the present study showed seed oil contents variation with seed coat color and light yellow seeds coat had less oil content. In sunflowers, the correlation was due to the fact that the oil content QTL is tightly linked with the seed color gene on chromosome 6 (Jing *et al.,* 2008). Anthocyanin and melanin are the most important pigments in testa and their content was responsible for the variation in seed color ranging from orange to black yellow in rapeseed (*Brassica napus* L.) with 36.2% to 45.5 % (Xue- Kun *et al.*, 2003).

6.2. Molecular diversity and its implications for improvement and conservation

In the present study, ISSR was used for the first time to assess genetic variation of *Lepidium sativum* populations from Ethiopia. This method provides an alternative choice to other system for obtaining highly reproducible markers without any necessity for prior sequence information for various genetic analyses. Because of the abundant and rapidly evolving SSR regions, ISSR amplification has the potential of illuminating much larger number of polymorphic fragments per primer than any other marker system used such as RFLP or microsatellites. ISSRs are regions that recline within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers, since they reveal variation with-in unique regions of the genome at several loci simultaneously. Several property of microsatellite such as high variability among taxa, ubiquitous occurrence and high copy number in eukaryotic genome make ISSRs extremely useful marker for variability analysis (Morgante *et al.*, 2002).

In this study, bulk sampling approach was chosen, since 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. Gilbert *et al.* (1999) also reported that pooling of DNA from individuals within accessions is the most appropriate strategy for assessing large quantities of plant material and concluded that 2-3 pools of five genotypes is sufficient to represent the genetic variability within and between accessions in the lupin and similar collections. Edossa Fikru *et al*, (2010) 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. Dagmawi Teshome (2011) also used bulked sample in germplasm diversity study of sesame populations, and found moderate genetic diversity of both Ethiopian and exotic populations.

The present study showed that out of 53 loci generated by four primers two di, one penta and one tetra; 43 of them were polymorphic with 81.13% polymorphism. In regions based analysis, Amhara and Tigray showed higher percent polymorphism (66.04%); while, SNNPR and Somali showed least polymorphism with 47.17% and 45.28%, respectively. The same patterns of diversity were observed with gene diversity and Shannon index.Generally, *L. sativum* populations from Amhara and Tigray showed higher diversity than the other regions.

Edossa Fikru *et al*. (2010) studied the morphological and molecular diversity of Ethiopian lentil (*Lens culinaris* Medikus) using four ISSR primers and found 59.57% polymorphism with higher percent variation attributed with in populations (56.28%). Gezahegne Girma *et al*. (2009) studied wild and cultivated rice species of Ethiopia using six ISSR primers and reported 38.3% and 28.3% polymorphism of wild and cultivar rice species, respectively. Moreover, higher proportion of genetic diversity was observed within populations of rice (Gezahegne Girma *et al*., 2009). Hence, the present study showed higher percent polymorphism and higher proportion of diversity within population of *L. Sativum* comparable with that of Edossa Fikru *et al*. (2010) and Gezahegne Girma *et al*. (2009).

In general Amhara and Tigray had good genetic diversity than Oromia, SNNPR and Somali. But this has to be further studied using proper sampling strategy and multilocation comparison.

AMOVA analysis resulted in high genetic diversity within population (94%) and very low genetic diversity among population (6%). This could be due to high seed exchange among different regions and markets which could lead to intermix of populations between regions. Unlike other landraces of cultivated plants, *Lepidium sativum* in Ethiopia is not restricted to a given area rather it is wildly exchanged among local community and markets. This shows that there is very high gene flow between populations and regions. Jiang *et al.* (2012) who studied on the genetic diversity of *Chimonanthus grammatus* populations by using ISSR marker showed that there was 73.6% within population variation whereas the rest 26.4% was due to among population variation. As compared to the present study, there was less gene flow. Jiang *et al.*, (2012) recommended that gene flow, genetic drift and evolutionary history might have important influence on genetic structure and diversity of a given population.

Lepidium sativum is both self and cross pollinated plant (Quirós and Cárdenas, *1998*). Hence, the proportion of genetic variation is dependent on the type of pollination that the species undergoes. If the species has large proportion of cross pollination, then we expect high genetic variation within population and less divergence among populations. In addition to pollination, behavior of insects; market exchange could facilitate gene flow among regions which could result in higher percent variation within population and less genetic structure. This is also supported with the spread of individual accessions on UPGMA, NJ and PCO graphs.

Dendrogram of the present study by using UPGMA of jaccard’s coefficient of similarity showed Amhara and Oromia populations of *L. sativum* were closely related. Based on this study, the samples with unknown origins could probably been collected from Southern part of Ethiopia since they closely clustered with the SNNPR population. The Somali population had its own lineage far from the other populations and diverted as anoutlies. 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 whereas 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.

Genetic diversity of plant populations is largely influenced by factors such as reproduction system, genetic drift, evolutionary history and life history (Loveless and Hamrick 1984). In broad-spectrum, outcrossing species have higher levels of genetic diversity than selfing and clonal plants (Rossetto *et al.*, 1995).

7. CONCLUSIONS

Lepidium sativum is believed to have originated in South West Asia and Ethiopia and also distributed worldwide. *L. sativum* is culturally believed to have medicinal value among local people. Because of insufficient amount of modern medicine, most of the Ethiopia population relies on traditional medicine. In Ethiopia, it is not commonly cultivated using separate farm plot rather planted along teff field and backyard as boarder crop. It is planted for the purpose of medicine for human and cattle. The present study was conducted with the main objective of assessing the extent of genetic diversity among Ethiopian accessions of *L. sativum* provided by from the Institute of Biodiversity Conservation (IBC) using Inter Simple Sequence Repeat marker and morphological markers.

This study showed that, ISSR and morphological markers are important markers for genetic diversity study in *L. sativum* accessions. Few accessions are observed to be moderately diverse than the other accessions.

Samples from Tigray and Amhara regions show relatively high level of genetic diversity whereas samples from Oromia, SNNPR and Somali regions showed lower genetic diversity. Moreover, the morphological traits analysis also showed that individuals from Amhara and Tigray regions had more yield than from Oromia and SNNPR regions.

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 highly significant. This confirms that there was a high level of gene flow and low level of genetic differentiation. Based on the UPGMA data, the Amhara, Tigray and Oromia accessions were clustered in to one group, where as the SNNPR and the Unknowns to the other cluster. Samples from Somali formed a distinct cluster and showing that it is distantly related to accessions from the entire regions.

8. RECOMMENDATIONS

Analysis of genetic diversity in crop species using more than one method helps to better understand the levels of genetic variation and the genetic structure of populations. No single method is adequate for assessing genetic variation in germplasm collections, because different methods of sampling genetic variation allows sampling at different levels and differ in their power of genetic resolution as well as the quality of information content.

The result of this study reveals that the *Lepidium sativum* germplasm analyzed are genetically closely related; regardless of their origin from divers' geographical locations. However, generating more information on the genetic diversity using both ISSR and morphological markers is an important parameter in the future efforts of *Lepidium sativum* genetic resources conservation and sustainable utilization.

- More collection is needed to increase the number of *Lepidium sativum* germplasm in the gene bank.
- Germplasms from Amhara and Tigray regions should be targeted for yield improvement work by breeders.
- Germplasms from Oromia and SNNPR (early maturing) and Tigray and Amhara (late maturing) should be targeted to screen variety for low and high rainfall area.
- Collection of wild relatives *Lepidium*spp. (*Lepidium africanum*, *Lepidium verginicum*, *Lepidium armoracia* and *Lepidium keniense),* should be carried out to serve as supplementary to *L. sativum* germplasm.
- Study on the relationship between oil content and seed color should be carried out.
- Analysis should be carried out using microsatellite and chloroplast markers to determine and evaluate gene flow, systematics and phylogeography of *Lepidium sativum* in Ethiopia.

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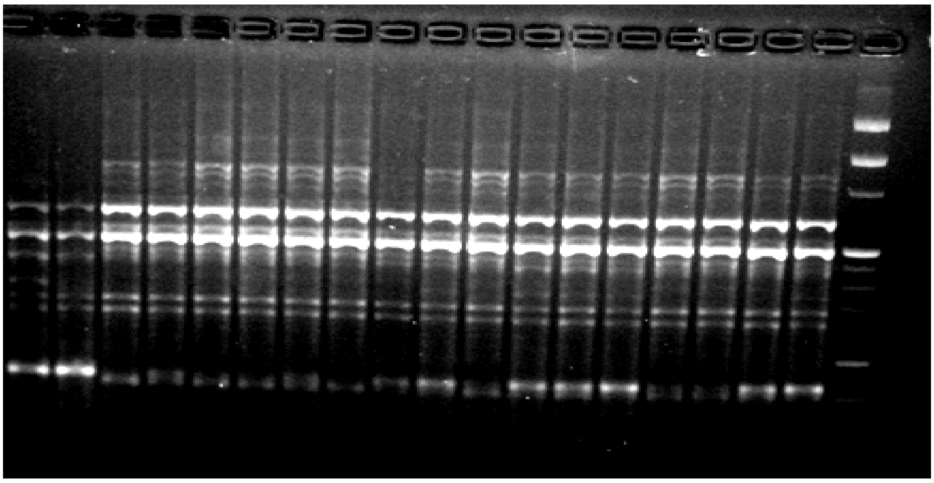
10. APPENDICES



Appendix: 1: Figure 7. ISSR fingerprint generated from 18 individual accessions using primer 873. *Key: the first nine were from Amhara, and then the next nine from Oromia, the last one is marker.*

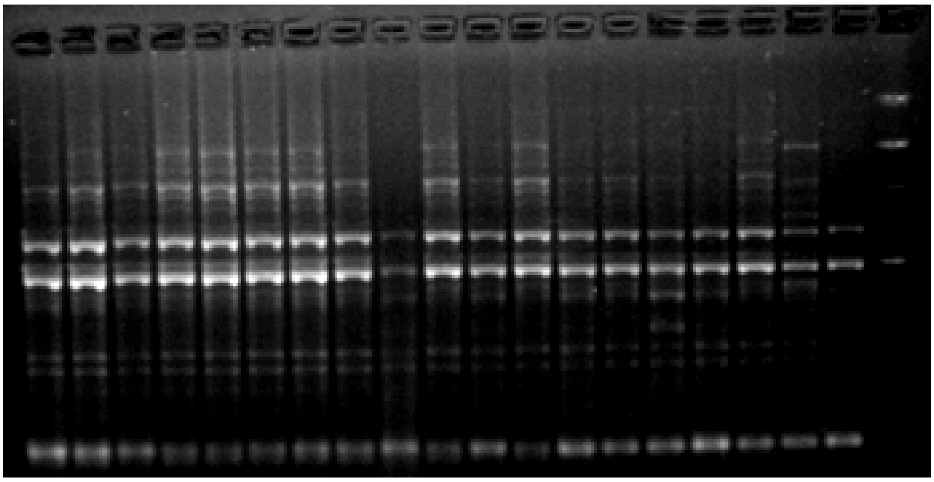
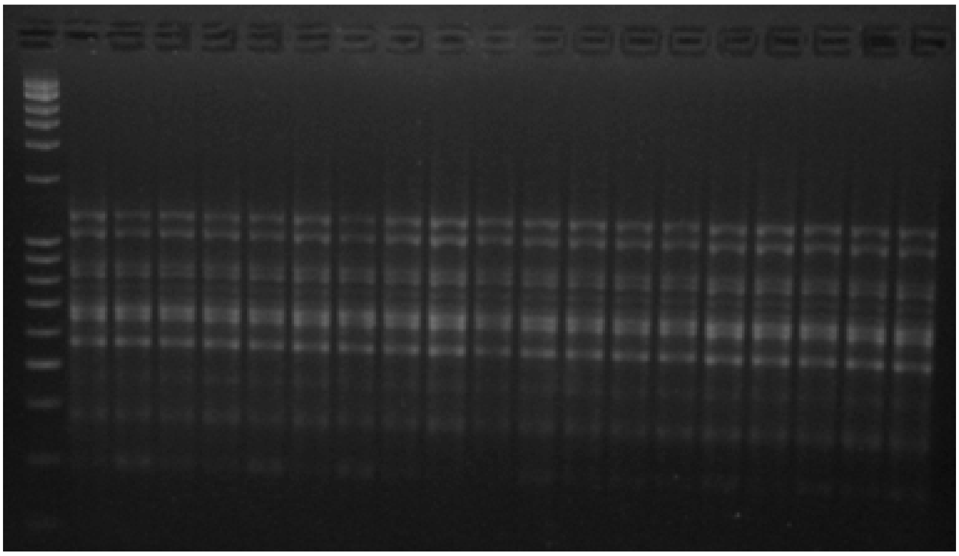


Figure 8. ISSR fingerprint generated from 19 individual accessions using primer 873.*key*: *the first seven from Tigray, then the next six were from SNNPR, and then followed by unknown with 4 samples and Somali with the last two. The final fragment is DNA marker.*



Appendix: 2 Figure 9. ISSR fingerprint generated from 19 individual accessions using primer 880. *Key: the first is DNA marker, then the next ten were from Amhara, and then the last nine from Oromia.*

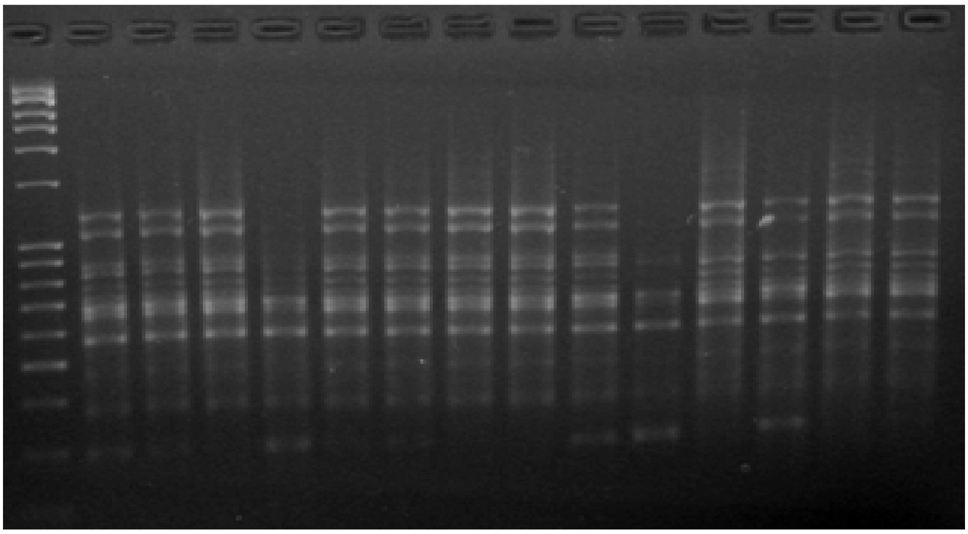
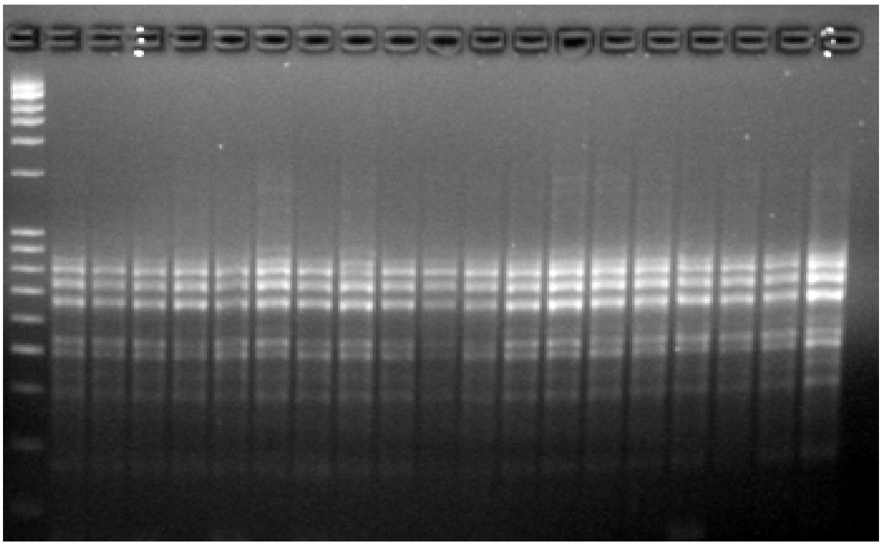


Figure 10. ISSR fingerprint generated from 14 individual accessions using primer 880. *Key*: *the first is DNA marker, the next four from Tigray, then the next four were from SNNPR, and then followed by unknown with 4 samples and Somali with the last two.*



Appendix: 3 Figure 11. ISSR fingerprint generated from 19 individual accessions using primer 834. *Key: the first is DNA marker, then the next ten were from Amhara, and then the last nine from Oromia.*

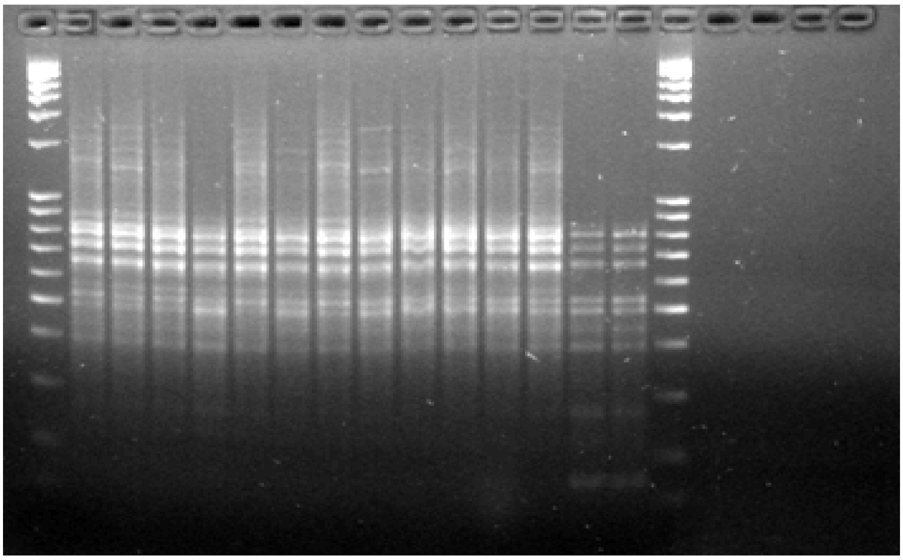
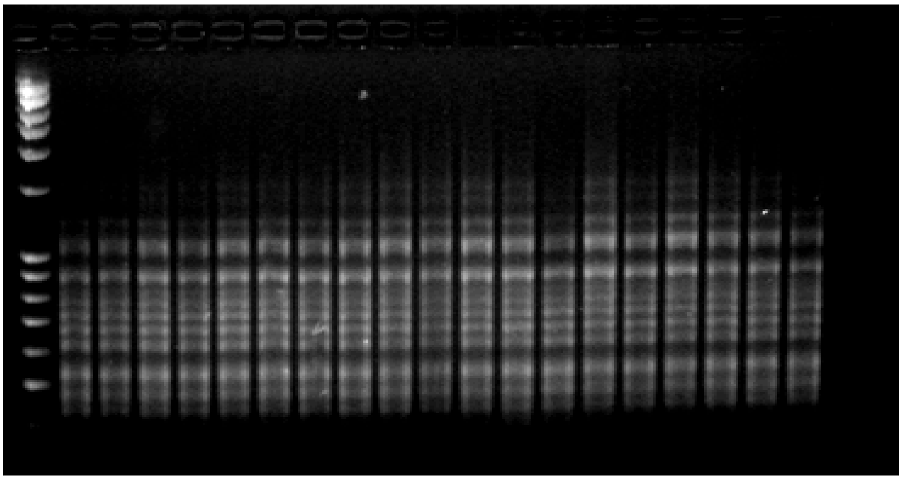


Figure 12. ISSR fingerprint generated from 14 individual accessions using primer 834. *Key*: *the first and the last is DNA marker, the next four from Tigray, then the next four were from SNNPR, and then followed by unknown with 4 samples and Somali with the last two.*



Appendix: 4 Figure 13. ISSR fingerprint generated from 19 individual accessions using primer 812. *Key: the first is DNA marker, then the next ten were from Amhara, and then the last nine from Oromia.*

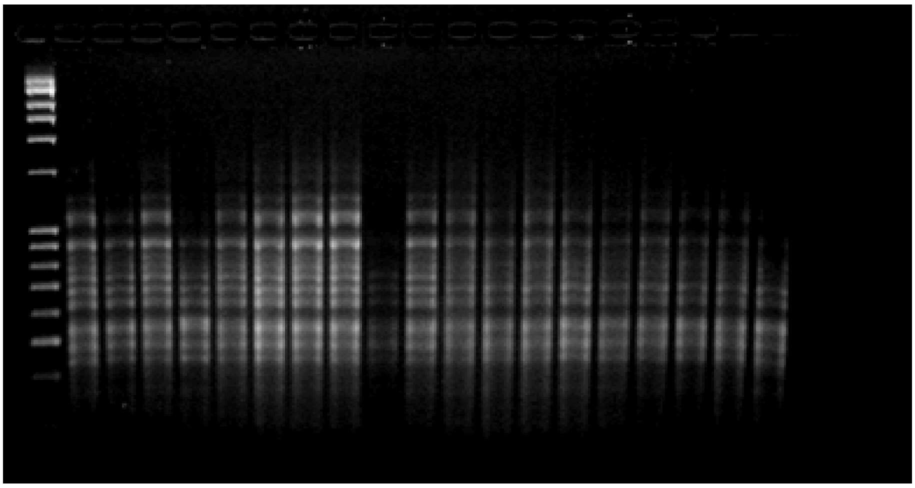


Figure 14. ISSR fingerprint generated from 19 individual accessions using primer 812. *Key*: *the first is DNA marker, then the next seven from Tigray, then the next six were from SNNPR, and then followed by unknown with 4 samples and Somali with the last two.*

Appendix: 5 Figures 15. Pictures took from the field study to record agro- morphological traits.

Appendix 6: Table 18.Passport data of *Lepidium sativum* population took from IBC.

Altitude	Accession Number	Former administrative region	Region/State/Province	Latitude	Longitude
2400	90004	gonder	Amara	11-36-00-N	38-34-00-E
	90001	Bale	Oromiya		
	90003	Bale	Oromiya		
	90005	Harerge	Oromiya		
	90006	Harerge	Oromiya		
	90007	Gojam	Benishangul and Gumuz	10-40-00-N	38-56-00-E
	90016	Gamo Gofa	SNNP		
2000	90020	Welo	Amara	11-05-00-N	39-42-00-E
2520	90018	Welo	Amara	10-50-00-N	38-30-00-E
2560	90019	Welo	Amara	11-32-00-N	39-56-00-E
1480	90022	Welega	Oromiya	08-52-00-N	35-04-00-E
2620	90021	Shewa	Oromiya	09-04-00-N	36-36-00-E
	90023	Kefa	Oromiya		
	202116	Kefa	SNNP		
1900	205141	Shewa	SNNP		
1900	208667	Harerge	Oromiya	08-56-00-N	40-36-00-E
1900	208769	Welega	Oromiya		
2180	208669	Harerge	Oromiya	09-10-00-N	41-32-00-E
2270	235892	gonder	Amara	13-38-00-N	37-14-00-E
2820	205163	Gonder	Amara		
	205162	Gonder	Amara		
	207542	Gonder	Amara		
	207910	Tigray	Tigray		
	207911	Tigray	Tigray		

Altitude	Accession Number	Former administrative region	Region/State/Province	Latitude	Longitude
	208030	Gonder	Amhara	12-40-00-N	37-23-00-E
	208666	Harerge	Oromiya	09-10-00-N	40-10-00-E
	208668	Harerge	Oromiya	09-30-00-N	41-50-00-E
	208693	Harerge	Oromiya	09-18-00-N	42-23-00-E
1500	212629	Shewa	Amhara	10-12-00-N	39-60-00-E
2400	212628	Welo	Amhara	11-26-00-N	39-25-00-E
2430	208832	Bale	Oromiya	07-20-00-N	40-10-00-E
2920	212627	Welo	Amhara	11-21-00-N	39-43-00-E
	211055	Shewa	Oromiya		
	214243	Gonder	Amhara		
	234828	Welega	Oromiya		
1950	215808	Welega	Oromiya	08-32-00-N	34-46-00-E
2200	215714	Welo	Amhara	11-20-00-N	39-47-00-E
2580	215713	Welo	Amhara	11-20-00-N	39-47-00-E
	212852	Bale	Oromiya		
	212853	Bale	Oromiya		
	214660	Sidamo	SNNP		
	215314	Gojam	Amara		
1570	216885	Arsi	Oromiya		
1570	216886	Arsi	Oromiya		
1700	219962	Tigray	Tigray	14-03-00-N	38-41-00-E
1940	219960	Tigray	Tigray	14-04-00-N	38-15-00-E
2130	219958	Tigray	Tigray	14-08-00-N	38-47-00-E
2340	216884	Arsi	Oromiya		
	216815	Harerge	Somali		

Altitude	Accession Number	Former administrative region	Region/State/Province	Latitude	Longitude
	216816	Harerge	Oromiya		
	219959	Tigray	Tigray		
	219961	Tigray	Tigray		
2220	229199	Shewa	Amhara		
2250	229798	Gojam	Amhara	11-06-00-N	37-49-00-E
2310	229201	Shewa	Amhara		
2550	229799	Gojam	Amhara		
2580	229204	Shewa	Amhara		
2600	229202	Shewa	Amhara		
2620	229203	Shewa	Amhara		
2650	225799	Gamo Gofa	SNNP	06-04-00-N	37-17-00-E
2690	229205	Shewa	Amara		
	225725	Gamo Gofa	SNNP		
2150	229200	Shewa	Amhara		
1910	231210	Harerge	Somali	09-30-00-N	42-37-00-E
2050	230831	Harerge	Oromiya	09-01-00-N	41-58-00-E
2450	230830	Harerge	Oromiya	09-29-00-N	42-13-00-E
	230523	Harerge	Somali		
	230524	Harerge	Oromiya		
	230829	Harerge	Somali		
	233370	Eritrea			
	233679	Eritrea			
1860	233984	Tigray	Tigray	14-05-00-N	38-58-00-E
1910	233985	Tigray	Tigray	14-14-00-N	38-09-00-E
1950	233983	Tigray	Tigray	14-07-00-N	38-57-00-E

Altitude	Accession Number	Former administrative region	Region/State/Province	Latitude	Longitude
2080	233986	Tigray	Tigray	14-14-00-N	38-39-00-E
2090	234355	Tigray	Tigray	14-13-00-N	39-15-00-E
2140	233981	Tigray	Tigray	14-12-00-N	39-18-00-E
2210	233982	Tigray	Tigray	14-12-00-N	39-21-00-E
1900	237512	Tigray	Tigray	14-11-00-N	38-52-00-E
2430	237991	Bale	Oromiya		
2150	238273	Tigray	Tigray	14-02-00-N	38-04-00-E
1950	240396	Kefa	SNNP	07-13-64-N	36-15-59-E
1950	240808	Gamo gofa	SNNP		
2050	240809	Sidamo	SNNP		
	240578	Gamo Gofa	SNNP		
	240810	Gamo gofa	SNNP		
2320	240811	Gamo gofa	SNNP		
2110	241777	Welo	Amara	11-48-00-N	39-37-00-E
	242609	Tigray	Tigray	14-29-00-N	39-28-00-E
	240397	Kefa	SNNP		
2200	242916	Kefa	SNNP	07-09-67-N	35-49-85-E
1720	215807	Welega	Oromiya	08-34-00-N	34-51-00-E
	90002	Bale	Oromiya		
	90008	Not Known			
	90009	Not Known			
	90010	Not Known			
	90011	Not Known			
	90012	Not Known			
	90013	Not Known			

Altitude	Accession Number	Former administrative region	Region/State/Province	Latitude	Longitude
	90014	Not Known			
	90015	Not Known			
	90017	Not Known			
	240579	Not known			

APPENDIX 7.DNA extraction protocol

1. Introduction

This protocol describes a modified CTAB method for the isolation of DNA from plant material. A maximum of 24 samples can be isolated at once using this protocol.

2. Materials

CTAB - 3 x 700 µl per sample

ß-Mercapto-Ethanol

Chloroform

Isopropanol - 100 %, 4°C

Ethanol - 100 % and 70% in sterile ddH_2O

TE - 1x, p.a. grade

NH_4Ac - 7.5 M Solution, sterile

NaAc - 3 M Solution, sterile

Water bath - 65°C

Centrifuge - 4°C

Cut 1000 µl tips - Sterile

3. Procedure

1. Poor CTAB solution (700 µl per sample) in a 15ml-tube and add 0.2 vol % Mercapto-ethanol (use fume hood!). Mercapto-ethanol is stored at 4°C.
2. Aliquot CTAB in 1,5 ml Eppendorf-caps and warm in water bath up to 65°C.
3. Weigh in 100 mg fresh leave material (50mg dry material) per sample. Pulverise thoroughly using a clean mortar and pestle. For fresh material add liquid nitrogen or quartz sand for dry material. First grind down slightly, then more powerful (cells have to be crashed). Use safety goggles!
4. Transfer the powder into an Eppendorf cap with warm CTAB solution immediately (use a new, clean spatula for each sample)
5. Add 700 µl of warm CTAB solution to the powdered sample (open the caps carefully), dissolve the powder and incubate the sample for 30 minutes at 65°C.
6. Centrifuge for 5 minutes at 15000 rpm.
7. Transfer the supernatant (only clear liquid) in a new Eppendorf-cap. Use blue pipette tips which are cut.
8. Add new CTAB solution (700 µl) to the tissue pellet and stir slightly with a new 1000 µl pipette tip, incubate 30 min at 65°C. Step 6 and 7 are repeated. The same is carried out for a third extraction. Each fraction proceeds with step 9 and is treated separately.
9. Add 600 µl Chloroform to the cap with supernatant and shake carefully a few times upside down. This chloroform step should be carried out immediately.

10. Shake the samples thoroughly by turning inversing the Eppendorf caps for approximately 5 minutes. (longer incubation is possible)
11. Centrifuge for 5 min at 15000 rpm.
12. Transfer the supernatant (only clear liquid) in a new Eppendorf-cap. Use blue pipette tips which are cut. Work carefully; do not transfer suspended matter (normally the Chloroform is covered by a thin layer of fine sediment material). Chloroform has to be disposed of in a special waste bottle.
13. Repeat the chloroform extraction (step 9-12) to make sure that all impurities are removed, then proceed with step 14.
14. Add cooled Iso-propanol (4°C), approximately $^2/_3$ of the solution volume. Shake carefully by inversing the Eppendorf cap. In most cases DNA becomes visible as white threads. Freeze for more than 2 h at -20°C. (BREAK POSSIBLE)
15. Centrifuge 10 min at 15000 rpm.
16. Aspirate liquid using yellow tips (without touching pellet!). If pellet is solid enough the larger part of the liquid may be poured out. (Alternatively add TE and proceed with Qiagen kit)
17. Add 200 µl Ethanol 70 % to the pellet. Rinse the inner cap surface by turning the cap.
18. Centrifuge for 10 min at 15000 rpm in a cooled centrifuge.
19. Aspirate Ethanol using yellow tips. Dry the DNA-pellet at room temperature. (Usually 15 min are sufficient; after drying no liquid drops are to be seen)
20. Dissolve pellet in 100 µl TE (1x, p.a. grade) and store at 4°C. (BREAK POSSIBLE)

21. Add cooled 7.5 M NH_4Ac-solution (4°C, half of the solution volume). Mix carefully.

22. Add cool Ethanol 100 % (double of the solution volume). Mix carefully. Freeze for more than 2 h at -20°C. (BREAK POSSIBLE)

23. Centrifuge 30 min at 15000 rpm. Aspirate fluid carefully.

24. Add 200 µl Ethanol 70%. Rinse the inner cap surface by turning the cap.

25. Centrifuge 10 min. at 15000 rpm. Aspirate liquid and dry pellet at room temperature. Dissolve the pellet in 100 µl TE (1x, p.a. grade)

26. Repeat steps 21 to 24 with 3 M Na-AC-solution (4°C, half the volume) then proceed with step 28.

27. Centrifuge 10 min. at 15000 rpm. Aspirate liquid and dry pellet at room temperature. Dissolve the pellet in 100 µl TE (1x, p.a. grade)

- Cleaning the mortar and pestle:
    - Rinse the mortar and pestle with water
    - Clean the mortar and pestle in a 1:10 Klorix-bath for 24 hours
    - Rinse with ddH_2O

Autoclave the mortar and pestle wrapped in aluminium foil at 134°C