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Morphological and Molecular Diversity in the Ethiopian lentil (*Lensculinaris medikus*) Landrace Accessions and their comparison with some Exotic Genotypes

By
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DEDICATION

This thesis work is dedicated to my mother.

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LIST OF ACRONYMS

AAT	Aspartate Aminotransferase
Acc	Accession
Adh	Alcohol Dehydrogenase
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of variance
ARs	Administrative regions.
Biom	Biomass
CACC	Central Agricultural Census commission
CE	Central Ethiopia (Shewa)
CFIA	Canadian Food Inspection Agency
CTAB	Cetyltrimethylammoniumbromide
DTF	Days to flowering
DTM	Days to maturity
DZARC	Debre-Ziet Agricultural Research Center
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FAOSTAT	FAO Statistical Database
GRs	Geographical regions.
HI	Harvest index
HSW	Hundred seed weight
IBC	Institute of Biodiversity Conservation
ICARDA	International Center for Agricultural Research in the Dry Areas
IPGRI	International Plant Genetic Resources Institute
ISSR/inter-SSR	Inter-Simple Sequence Repeat
LAP	Leucine Aminopeptidase
Me	Malic Enzyme
NEE	Northeastern Ethiopia (Wello and Tigray)
NEW	Northwestern Ethiopia (Gojam and Gonder)
NP	Number of pods per plant

NPB	Number of primary branch per plant
NS	Number of seeds per plant
NSB	Number of secondary branch per plant
PCR	Polymerase Chan Reaction
PCV and GCV	Phenotypic and Genotypic Coefficients of Variation, respectively
Pgd	6-phosphogluconate dehydrogenase
Pgm	Phosphoglucomutase
PH	Plant height
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Polymorphism
SAHN	Sequential, Agglomerative, Hierarchical and Nested clustering methods
SARC	Sinana Agricultural Research Center
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SEE	Southeastern Ethiopia (Arsi and Bale)
SNPs	Single Neucleotide Polymorphisms
SSR	Simple Sequence Repeat
SY	Seed yield per plant.
TBE	Tris Borate EDTA
TE	Tris EDTA
UPGMA	Unweighted Pair Group Arithmetic Mean

ABSTRACT

Seventy landrace accessions and eleven genotypes were used for morphological and ISSR diversity study. The plant materials were grown in a simple lattice design of two replications for morphological diversity study at Sinana Agricultural Research Center. Ten plants were selected randomly from each accession/genotype and marked before flowering. Morphological data were recorded from all selected plants on eleven traits. DNA was extracted from a bulk sample of five plants using a triple CTAB extraction technique. Molecular data were generated using four ISSR primers. ANOVA of morphological data indicated significant differences among the plant materials. Estimates of phenotypic and genotypic coefficients of variation showed wider variability within and between the landrace populations and between landraces and exotic genotypes for different morphological characters. Morphological dissimilarity between the populations ranged from 0.0519 to 0.73. Clustering analysis based on morphological dissimilarity matrix revealed three groups with distinctive morphological traits. The wider phenotypic variability observed encourages improvement activities between the lentils. Molecular analysis, on the other hand, showed that exotic genotypes were more diverse compared to the landraces. Genetic diversity within the total landrace population (H_T) was 0.1734. High AR-based genetic diversity (0.1395 – 0.1039) was observed for samples from Gonder, Shewa and Wello. High within GR diversity was obtained for samples from NEE and the least for those from SEE. Exotic genotypes were more distantly related with samples from Tigray, Gojam and Shewa than to others. The genetic distances between populations of Ethiopian lentil landraces ranged from 0.2284 – 0.012. Samples from CE were more distantly related to samples of SEE, while the distance between other GRs was relatively low. Estimates of population differentiation and gene flow were moderately high for ARs. On the other hand, GR-based population differentiation was low, whereas among GR gene flow was high relative to AR-based population differentiation. AMOVA revealed higher within population variation than among population. Cluster analysis based on Dice's similarity coefficient revealed three groups with distinct molecular profiles both with and without exotic genotypes. There were highly significant ($p < 0.001$) correlations, $r = 0.45$ and $r = 0.26$, between morphological and molecular distance matrices, respectively, of the entire plant materials and landraces separately, implying the validity of both methods for diversity study in lentils. Observation of low within AR/population diversity invites further collection activities, while enriching populations with high genetic diversity.

1. INTRODUCTION

Lentil belongs to the genus *Lens* of the *Viceae* tribe in the *Legumnosae (Fabaceae)* family, commonly known as the legume family. The genus consists of the species: *Lens culinaris* Medikus, its progenitor *L. orientalis* (Bioss.) Hand.-Maz., *L. nigricans* (m. Bieb.) Grand., *L. ervoides* (Bring.) Grand., *L. odemensis* Landiz., *L. lamottei* Czfranova, and *L. tomentosus* Landz. (Ladizinsky *et al.*, 1984; van Oss *et al.*, 1987). The cultivated lentil, *L. culinaris spp culinaris*, has two varietal types: small seeded (*microsperma*) and large seeded (*macrosperma*). The haploid genome size of the cultivated lentil is 4063 Mbp (Arumuganathan and Earle, 1991). Wild *Lens* species are represented by *L. culinaris* spp. *orientalis*, *L. odemensis*, *L. nigrican* and *L. ervoides*. All members of *Lens* are self-pollinating diploids (2n = 2x = 14; Sharma *et al.*, 1995). All *Lens* species are annual herbaceous plants.

The center of origin of *L. culinaris* is the Near East and the species was first domesticated in the Fertile Crescent around 700 BC (Zohary, 1972). Lentil first spread to the Nile from the Near East, to Central Europe and then to the Indian Subcontinent and the Mediterranean Basin by the end of the Bronze Age (Cubero, 1981; 1984). Lentil was one of the crops to be cultivated, with archeological evidence dating from the early Stone Age (Brookfield, 2001; CFIA, 2003).

Lentil is an important pulse crop grown widely throughout the Indian Subcontinent, Middle East, Northern Africa, Southern Europe, North and South America, Australia (Ford and Taylor, 2003) and West Asia (Erskine, 1997). The major lentil producing regions are Asia (58 % of the area) and the West Asia-North Africa region (37 % of the acreage of developing countries). Lentil ranks seventh among grain legumes and is grown on over 3.5 million hectares in over 48 countries with a total production of over 3 million metric tons (Erskine, 1998; CGIAR, 2005).

In Ethiopia, lentil is predominantly grown for human consumption as a rich source of protein (23-24 %) (Addise and Asfaw, 1993) and, therefore, corrects important amino acid deficiencies of cereals when used in mixture with tef, wheat and barley. It is a cash crop fetching the highest price in domestic market compared to all other food legumes and cereals (Geletu *et al.*, 1996). It is also an important export crop, although the varietal types (*microsperma* or small seeded) grown in Ethiopia are not much preferred in international market. The crop is generally grown in

rotation with cereals to break cereal disease cycles and fix atmospheric nitrogen, thus reducing the demand of other cereal crops for nitrogen fertilizers.

Ethiopia is one of the major lentil producing countries in the world and the first in Africa (FAOSTAT, 2006). During 2001/02, lentil was grown on 60,120 hectares with a total production of 384.1 x 10^3 Quintals. However, its average seed yield has remained very low, 0.64 t/ha (CACC, 2002). The major reasons are the susceptibility of the landraces to diseases, the inherently low yield potential of the landraces and poor management practices (Geletu and Yadeta, 1994). The former two problems necessitated introduction of lentil germplasms from abroad in order to improve production and productivity. Accordingly, ten improved varieties of exotic origin were released for production in Ethiopia. However, in the long run, these released varieties of narrow genetic base might lead to replacement of the indigenous landraces ultimately resulting in genetic erosion. Furthermore, because of the aforementioned problems, farmers are eager to replace lentil with more renumerative crops and hence exacerbating the threat of genetic erosion. This might occur in the near future because Ethiopian farmers are being encouraged or "urged" by the government to practice market- and profit-oriented agriculture.

Local populations of traditional cultivars provide a valuable resource for plant breeding as well as for the preservation of genetic diversity (Kölliker *et al.*, 2003), irrespective of the problems mentioned in Ethiopian lentil landraces. The exploration, evaluation, and conservation *in situ* and *ex situ* of genetic diversity in natural populations is imperative to guarantee sustainable development (Nevo, 1998). Therefore, the enormous merits of the landraces coupled with the threat of genetic erosion mentioned above indicates the importance of diversity study in the landraces, which is important to know the level of variability within the available collections under conservation and to plan future conservation practices of major priority. Genetic variability study can be achieved by using morphological traits, molecular markers or both. Physiological markers are seldom used in diversity study since they are largely influenced by environmental conditions. Among the available alternatives, morphological traits and inter-simple sequence repeat (ISSR) marker were found to be suitable for lentil variability study.

Variability study based on morphological traits is easy, fast and least cost method, irrespective of the large environmental influences. In addition, to date, Ethiopian lentil landrace accessions are not exhaustively characterized using morphological traits. This might be due to their susceptibility to various diseases, which may cause complete death of the crop plant during heavy infestations. On the other hand, their susceptibility to diseases suggests the use of other alternative methods (molecular markers) to characterize the available genetic diversity.

There are numerous DNA-based molecular marker systems that can be used for genetic diversity assessment. Some of the most commonly used marker systems are restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers, each having its own advantages and disadvantages. Inter-simple sequence repeat (ISSR) marker, a recently developed DNA-based molecular marker, in addition to its suitability to genetic diversity study, is specific as compared to RAPD, highly polymorphic, reproducible, cost effective and requires no prior information of the sequence (Bornet *et al.*, 2002). These facts suggest that ISSR could be an unbiased tool to evaluate the changes of diversity in agronomically important crops (Brantestam *et al.*, 2004). To date, there is no information available on the genetic diversity of Ethiopian lentils using any of the molecular markers with the exception of some studies done in other countries in which very small number of Ethiopian landrace accessions were included.

2. LITERATURE REVIEW

2.1. Diversity: Its Importance and Threat

Knowledge of genetic variation and relationships between accessions or genotypes is important to understand the genetic variability available and its potential use in breeding programs, to estimate any possible loss of genetic diversity, to offer evidence of the evolutionary forces shaping the genotypic diversities, and to choose populations to be given priority for conservation (Thormann *et al.*, 1994).

Over several millennia, local constraints have produced wide diversity within the lentil, resulting in a myriad of landraces (Erskine, 1985). However, because of social and economic changes in the recent past, lentil cultivation has been progressively reduced resulting in genetic erosion, for instance in Italy (Piergiovanni, 2000). According to the author, lentil cultivation is mainly confined to marginal lands, where a small number of local populations are still cultivated, but the survival of the remaining populations is severely threatened because farmers are eager to replace lentil with more remunerative crops.

2.2. Methods of Diversity Assessment

2.2.1. Morphological Method

Morphological traits were among the earliest markers used in germplasm management (Stanton *et al.*, 1994) but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992), which in turn may affect the estimation of genetic relationships. However, if the traits are highly heritable, morphological markers are one of the choices for diversity studies because the inheritance of the marker can be monitored visually (Yoseph, 2005). Despite the problems associated with this method, it continues to play a major role in studying and characterizing germplasm because it is easy, not costy and rapid since it requires no complicated laboratory facilities and procedures.

2.2.2. Molecular Markers

Characterization of genetic resource collections has been greatly facilitated by the availability of a number of molecular marker systems. Different types of molecular markers have been used to

assess the genetic diversity in crop species, but no single technique is universally ideal. Therefore, the choice of the technique depends on the objective of the study, sensitivity level of the marker system, financial constraints, skills and facilities available (Yoseph *et al.*, 2005). The molecular markers best suited for detecting genotypic diversity should be relatively easy and inexpensive to use and should evolve rapidly enough to be variable within populations (Esselman *et al.*, 1999).

In the past, genetic diversity in species has typically been assessed using morphological, physiological and biochemical traits. However, since morphological and physiological traits are subject to environmental influences, emphasis has been shifted to biochemical studies (Moodie *et al.*, 1997). In particular, allozyme analysis has been used to document genetic diversity in a range of different species. However, allozymes may underestimate genetic diversity (Esselman *et al.*, 1999). Recently, more sensitive DNA-based techniques have been developed to detect the genetic variation underlying morphological and phenological variation (Meekins *et al.*, 2001). Microsatellite or simple sequence repeat (SSR), RAPD, and AFLP techniques have all been used to produce molecular markers for studies of genetic variation at the population level (Wolf and Liston, 1998). All of these techniques are useful molecular tools; however, SSR markers require large monetary inputs to optimize protocols and purchase materials and equipment, and RAPD markers may not always show enough variability to be useful and are not necessarily reproducible, although it is quick, simple and efficient technique (Wolf and Liston, 1998). RFLP, apart from its advantage of being highly reproducible, is difficult (in finding approprate probes), time consuming and requires huge investment (Karp *et al.*, 1997). AFLPs have a high multiplex ratio, offering a distinctive advantage when genome coverage is a major issue (Pejic *et al.*, 1998), but limited by the number of steps required to produce results, requirement of additional expenses and the necessity to use probes (Wolf and Liston, 1998). The relative advantages and disadvantages of the most commonly used molecular markers is summarized in Table 1.

Another very recently developed molecular marker is the inter-simple sequence repeat (ISSR) approach. This polymerase chain reaction (PCR)-based technique uses single anonymous primers to produce markers from genomic DNA. Each primer is composed of di-, tri-, tetra- or penta-nucleotide repeats with one of the three additional nucleotides at the 5' or 3' end to serve as an

Table 1. Comparison of the most commonly used molecular markers

Characteristics	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (in μg)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	High
PCR-based	No	Yes	Yes	Yes	Yes
Level of polymorphism	High	Moderate	High	Very high	High
Ease of use	Not easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	High	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

(Extracted from: Yoseph, 2005)

anchore (Gupta *et al.*, 1994) to help prevent strand slippage during amplification (Meekins *et al.*, 2001). Identical repeat sequences with different anchoring sequences can yield different banding patterns (Wolf *et al.*, 1998). These hypervariable nuclear markers, like RAPD data, are analyzed as dominant markers (Wolf and Liston, 1998). In contrast with other molecular techniques, ISSR markers are easy to use, often have three- to five-fold greater variability than RAPDs, and are variable for all plant groups (Meekins *et al.*, 2001). They can be used for systematic, molecular, ecological, evolutionary and crop improvement studies (Tsumura *et al.*, 1996; Fang and Roose, 1997; Hollingsworth *et al.*, 1998; Esselman *et al.*, 1999). Most studies have utilized ISSRs to study genetic diversity of germplasm collections and to differentiate cultivars (Tsumura *et al.*, 1996; Fang and Roose, 1997; Nagaoka and Ogihara, 1997). Popularity of ISSR is now growing; ISSRs are alternative methods to isozymes and RAPDs in terms of population genetic studies (Meekins *et al.*, 2001).

2.2.3. Sampling Strategy for DNA Extraction

The value of molecular biology for monitoring the genetic status of germplasm collections is subject to practical limitations. The large number and variability of accessions held usually dictates the approach that can be employed. A quick, simple but reliable molecular protocol must be combined with an appropriate strategy for handling large sample sizes (Gilbert *et al.*, 1999).

Individual plant and bulk sampling are the two methods of sampling plants for DNA extraction. Analysis based on individual plant sampling requires a huge investment in terms of time, labor and financial resources. Besides, it is hardly possible to analyze large number of germplasm using this sampling technique compared to the bulk approach. However, the technique is advantageous in that it allows the assessment of intra-accession diversity. Bulk analyses, on the contrary, are economic and rapid, and it is possible to estimate the genetic variability between accessions, whereas it is not possible to obtain information about the genetic variability within accessions (Fernández *et al.*, 2002). However, it can be argued that bulk sampling reduces the total genetic diversity present in the total population since populations are collections of individuals within each accession whose genetic variation has been nullified by bulking/pooling. The number of individual plants bulked for the accessions is an important experimental factor whether the bulked analysis revealed the genetic relationship between the accessions. Yang and Quiros (1993) found that bulked samples with 10, 20, 30, 40 and 50 individuals had resulted in the same RAPD profiles as that of the individual plants constituting the bulk sample. Hou *et al.* (2005) used a minimum of 10 individuals for representing each barley accession and indicated that bulk analysis for RAPD and ISSR markers could successfully be used to investigate the genetic diversity in landraces and wild forms of barley. On the other hand, Gilbert *et al.* (1999) reported that pooling of DNA from individuals within accessions is the most appropriate strategy for assessing large quantities of plant material. Band profiles generated from DNA pools containing five individuals were fully representative of all constituent individuals used in the mix, while pools comprising 10 or 20 individuals, however, sometimes failed to contain minor bands that had been present only in the profile of one individual (Gilbert *et al.*, 1999). However, the DNA pooling technique is more costly compared to leaf sample bulking strategy since it requires extraction of DNA from each individual plants of the accession to be represented.

2.3. Diversity Studies in Lentil

2.3.1. Morphological Diversity

A wide range of genetic variability was reported in lentil (Jain *et al.*, 1995; Chakraborty and Haque, 2000) for plant height, number of branches per plant, biological yield, number of pods per plant, pod weight per plant, number of seeds per plant and harvest index. The genotypic and phenotypic variances were high for number of pods per plant followed by plant height (Rajput and Sarwar, 1989). Furthermore, maximum coefficient of variation was observed for seed yield per plant, number of pods and secondary branches per plant (Zaman et al, 1989; Rakesh et al, 1999; Stoilva and Pereira, 1999). The highest magnitude of phenotypic variability was observed for hundred seed weight, number of secondary branches, pods per plant, biological yield per plant and seed yield per plant, whereas days to maturity, days to flowering and plant height showed relatively lower estimates of phenotypic and genotypic variability indicating little opportunity for selection of these traits (Chauhan and Singh, 1998). Lower estimates of coefficients of variation were reported for days to maturity (Thakur and Banjpai, 1993), days to flowering and plant height (Singh and Singh, 1991) in lentil. Nevertheless, Rao and Yadav (1995) observed significant variability for days to maturity, hundred seeds weight, biological yield, harvest index and seed yield.

Geletu *et al.* (1996) reported consistent regional differences among Ethiopian lentil landraces for time to flower and maturity, 100-seed weight, number of seeds/pod and plant height using 156 landrace populations of lentil collected from 10 provinces of Ethiopia for a set of 6 quantitative traits at three sites with contrasting altitude. Furthermore, they observed that lentil of the West Highlands were early maturing and short, that of the North Highlands were large-seeded, whereas lentils from the Central Highlands were the least distinctive group.

2.3.2. Molecular Diversity

On the macro-geographical scale, previous studies showed that lentil germplasm from the Mediterranean Basin is characterized by a higher genetic diversity than the germplasm from South Asia, Ethiopia and the Americas (Erskine *et al.*, 1989, 1998; Echeverrigarary *et al.*, 1998; Ferguson *et al.*, 1998). Little information is available about diversity on the regional scale because only germplasm from a few countries has been studied in detail (Geletu *et al.*, 1996;

Lazaro *et al.*, 2001). Sonnante and Pignone (2001) observed from UPGMA trees (generated by Jaccard's similarity matrices of RAPDs, microsatellite-primed PCR and ISSR markers data) that accessions of Ethiopia differentiated the most from accessions of Italy, Mediterranean region and others.

The presence of allozyme variation in lentil was first reported by Skibinski *et al.* (1984) who assayed a collection of lentil germplasm from ICARDA for polymorphism of aspartate aminotransferase (AAT) and detected two allozymes, *Aat-1*^{F} and *Aat-1*^{S} with gene frequencies of 0.51 and 0.49, respectively. Furthermore, Zamir and Ladizinsky (1984) studied the genetics of allozyme and found monogenic inheritance with codominant expression for 6 isozyme loci, whereas alcohol dehydrogenase (*Adh-1*) and malic enzyme (*Me-1*) showed disturbed segregation in one cross. Erskine and Muehlbauer (1991) made a survey of qualitative genetic variation at 3 morphological trait loci, 17 isozyme loci and a putative isozyme locus (amylase) for 105 lentil (*L. culinaris* Medikus) germplasm accessions from Chile, Greece and Turkey. They reported new alleles for *Lap-1, Me-2, Pgm-c, Pgm-p* and *6-Pgd-c*, and the average polymorphic loci proportion of 0.19 per population, with a range of 0 to 0.42 over populations.

Ford *et al.* (1997) assessed the genetic relationships between 16 accessions and cultivars of lentil in the Australian lentil breeding program using RAPD marker. They reported polymorphism in all lines with a maximum dissimilarity value of 0.36 indicating, according to them, a limited degree of genetic variation. Furthermore, other reports indicate that RAPD marker revealed low level of intra-specific genetic variation in cultivated lentils as compared to most of the wild species (Abo-elwafa *et al.*, 1995; Ahmad *et al.*, 1996; Ferguson *et al.*, 1998).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins showed considerable genetic variation in Italian lentil populations (Piergiovanni and Taranto, 2004), whereas RAPDs and microsatellite-primed PCR markers detected a relatively low genetic variation (Sonnante and Pignone, 2001) in a study comprising most accessions from Italy and some from Mediterranean, Ethiopia and others. Similarly, de la Rosa and Jouve (1992) and Alvarez *et al.* (1997), observed a high genetic diversity in Spanish lentil populations by using isozymes or SDS-PAGE of storage proteins, but a low polymorphism by using RAPDs.

Furthermore, Piergiovanni and Taranto (2004) reported that germplasms from different regions of Italy exhibited strong similarity and were grouped into the same cluster, which is, according to them, in agreement with the results obtained using ISSR markers by Sonnante and Pignone (2001). The authors explained that the similarity was attributable to the influence of agricultural and horticultural practices of one region on the neighboring areas.

2.3.3. Comparison of Morphological and Molecular Markers

Piergiovanni and Taranto (2004) reported that the result from SDS-PAGE analysis agrees with that of the previous agronomic studies in lentil. There were no other additional accessible literatures using the combination of morphological traits and any of the molecular markers in lentil. However, therefore, it is important to review similar works in other crops at least to have a landmark and to compare with the results of lentil. Hence, similar studies conducted in maize and ryegrass are summarized here.

In studies conducted on Ethiopian traditional maize landrace accessions using morphological and molecular markers (AFLP and SSR), there were significant correlation between distance matrices of morphological data and the two marker systems ($r = 0.39$ and $r = 0.43$, respectively for AFLP and SSR; Yoseph *et al.*, 2005) indicating the agreement between morphological data and the two molecular marker systems. Similarly, Bolaric *et al.* (2005) working on 12 phenotypic characters of 22 perennial ryegrasses reported a correlation value of $r = 0.10$ between morphological and RAPD marker. In contrast, Roldan-Ruiz *et al.* (2001) reported a correlation value of $r = -0.06$ between morphological data (using 15 morphological traits) and AFLP marker in 16 ryegrass varieties.

3. OBJECTIVES

The major objectives of this thesis research were to study genetic variability of Ethiopian lentil landrace accessions from the different administrative regions (ARs) separately and in comparison to exotic genotypes using morphological traits and ISSR marker.

The specific objectives were:

1. To study the morphological variability and genetic diversity of Ethiopian lentil landrace accessions compared to the exotic genotypes,
2. To estimate the level of genetic variability and genetic structure within the total landrace population,
3. To compare the level of variability within and between landrace populations (ARs),
4. To compare the level of genetic variability and determine the genetic structure of within and between Ethiopian lentil landrace accessions based on geographical (GR) classification of the ARs,
5. To classify/cluster lentil landrace accessions and exotic genotypes into groups based on both morphological traits and molecular profiles,
6. To assess the level of correlation between phenotypic and genotypic distances.

4. MATERIALS AND METHODS

4.1. Description of the Study Area

The morphological part of the present study was conducted at Sinana Agricultural Research Center (SARC), which is located at about 463 km away from Addis Ababa in Southeastern Ethiopia, Bale Zone. It is 2400 meters above sea level. It has a bimodal rainfall pattern. The major rainy season, called "Bona" ("Meher"), extends from August to December with an average monthly rainfall ranging from 18.1 mm (in December) to 125.9 mm (in September). The other rainy season, called "Ganna" ("Belg"), extends from March to July with average monthly rainfall ranging from 52.7 mm (in July) to 100.4 mm (in April). In the past 16 years, the total annual rainfall distribution ranged from 534.9 mm to 1003.4 mm, while the average annual rainfall was about 752.4 mm. In general, rainfall data of the specific growing season used for this study shows that there was lower amount of rainfall in that particular season as compared to the average of 16 years' data (Appendices 1 and 2). This necessitated supplemental irrigation water, which was done twice before flowering.

The average minimum and maximum annual temperature of the study area were about 9.4 ^{0}C and 21.2 ^{0}C, respectively, in the past 16 years. The average annual minimum temperature varied from 9.0 ^{0}C to 10.4 ^{0}C, whereas the minimum monthly temperature varied from 7.6 ^{0}C (in December) to 10.4 ^{0}C (in May). The average minimum monthly and yearly temperature was 9.4 ^{0}C. The maximum monthly temperature of the specific growing season ranged from 20.2 ^{0}C to 22.1 ^{0}C (Appendices 3 and 4).

4.2. Plant Materials

Seeds (3g) of seventy lentil landrace accessions collected from seven different ARs of Ethiopia were obtained from Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia. The ARs were Arsi, Bale, Gojam, Gonder, Shewa, Tigray and Wello. Each AR was considered as a population and ten samples collected from ten representative districts ("Woreda"s) were selected. In addition, ten released varieties and one genotype (candidate variety) of exotic origin obtained from Debre-Ziet Agricultural Research Center (DZARC; nine released varieties) and SARC (one released and one candidate variety) were included in the study (Tables 2 and 3).

Table 2. Passport data of Ethiopian lentil landrace accessions used in the study (Source: IBC)

Accession Name	Regional State	Zone	Wereda/District	Administrative Region	Latitude	Longitude	Altitude
Acc 36007	Oromiya	Arsi	Dodota Sire	Arsi	8-09-N	39-21-E	2050
Acc 36008	Oromiya	Arsi	Gedeb	Arsi	7-10-N	39-12-E	2440
Acc 36041	Oromiya	Arsi	Merti	Arsi	8-24-N	39-52-E	2040
Acc 36042	Oromiya	Arsi	Chole	Arsi	08-22-N	39-53-E	2520
Acc 36047	Oromiya	Arsi	Sude	Arsi	07-53-N	39-44-E	2520
Acc 36131	Oromiya	Arsi	Robe	Arsi	7-49-N	39-47-E	2480
Acc 216879	Oromiya	Arsi	Bekoji	Arsi	---------	---------	------
Acc 216881	Oromiya	Arsi	Dodota Sire	Arsi	08-07-N	39-27-E	2370
Acc 231239	Oromiya	Arsi	Sherka	Arsi	Aug-35	39-52-	2330
Acc 231240	Oromiya	Arsi	Jeju	Arsi	Aug-37	39-41-	1920
Acc 36029	Oromiya	Bale	Agarfa	Bale	7-18-N	39-58-E	2580
Acc 36033	Oromiya	Bale	Sinana Dinsho	Bale	---------	---------	2620
Acc 36121	Oromiya	Bale	Dodola	Bale	---------	---------	------
Acc 212848	Oromiya	Bale	Goro	Bale	7-00-N	40-28-E	1800
Acc 212851	Oromiya	Bale	Nansebo	Bale	---------	---------	------
Acc 230015	Oromiya	Bale	Kokosa	Bale	---------	---------	2620
Acc 230017	Oromiya	Bale	Agarfa	Bale	7-10-N	34-11-E	------
Acc 230020	Oromiya	Bale	Ginir	Bale	07-08-N	40-36-E	2020
Acc 231243	Oromiya	Bale	Adaba	Bale	---------	---------	------
Acc 237988	Oromiya	Bale	Adaba	Bale	---------	---------	2460
Acc 36026	Amhara	Debub Goder	Este	Gonder	11-34-N	38-45-E	2590
Acc 36065	Amhara	Semen Goder	Dembia	Gonder	---------	---------	------
Acc 36072	Amhara	Debub Goder	Farta	Gonder	11-48-N	35-28-E	3114
Acc 36086	Amhara	Debub Goder	Lay Gayint	Gonder	11-41-N	38-29-E	3120

Table 2. Continued

Accession Name	Regional State	Zone	Wereda/District	Administrative Region	Latitude	Longitude	Altitude
Acc 36146	Amhara	Semen Goder	Debark	Gonder	13-13-N	38-01-E	3220
Acc 207257	Amhara	Semen Goder	Wegera	Gonder	---------	---------	------
Acc 207259	Amhara	Debub Goder	Fogera	Gonder	---------	---------	------
Acc 207266	Amhara	Debub Goder	Kemekem	Gonder	---------	---------	------
Acc 207291	Amhara	Semen Goder	Janamora	Gonder	---------	---------	------
Acc 207305	Amhara	Debub Goder	Simada	Gonder	---------	---------	------
Acc 36024	Amhara	Mirab Gojam	Dega Damot	Gojam	---------	---------	------
Acc 36025	Gul &	Gojam (Metekel)	Wenbera	Gojam	---------	---------	1580
Acc 36027	Amhara	Misirak Gojam	Enarj Enawga	Gojam	10-38-N	38-10-E	2510
Acc 36028	Amhara	Misirak Gojam	Hulet Ej Enese	Gojam	11-4-N	37-051-E	2270
Acc 36069	Amhara	Misirak Gojam	Guzamn	Gojam	10-20-N	37-44-E	2460
Acc 36118	Amhara	Misirak Gojam	Shebel Berenta	Gojam	10-27-N	38-21-E	2420
Acc 212745	Amhara	Misirak Gojam	Enemay	Gojam	38-11-N	10-34-E	2580
Acc 219507	Amhara	Gojam (Agaw Awi)	Banja	Gojam	---------	---------	------
Acc 238978	Amhara	Mirab Gojam	Bahir Dar Zuria	Gojam	11-38-N	37-13-E	1930
Acc 241132	Amhara	Mirab Gojam	Achefer	Gojam	---------	---------	2030
Acc 36001	Oromiya	Semen Shewa	Berehna Aleltu	Shewa	9-50-N	39-13-E	2820
Acc 36003	Amhara	Semen Shewa	Moretna Jiru	Shewa	9-57-N	39-13-E	2820
Acc 36006	Oromiya	Misrak Shewa	Gimgichu	Shewa	8-57-N	39-5-E	2370
Acc 36009	Oromiya	Mirab Shewa	Ejere	Shewa	9-2-N	38-10-E	2270
Acc 36014	Oromiya	Semen Shewa	Wuchalena Jido	Shewa	09-39-N	38-49-E	2695
Acc 36020	Oromiya	Mirab Shewa	Walisona Goro	Shewa	8-39-N	37-54-E	2260
Acc 36048	Oromiya	Semen Shewa	Kembibit	Shewa	09-19-N	39-16-E	2890
Acc 36056	Oromiya	Mirab Shewa	Ambo	Shewa	---------	---------	------

Table 2. Continued

Accession Name	Regional State	Zone	Wereda/District	Administrative Region	Latitude	Longitude	Altitude
Acc 229184	Amhara	Semen Shewa	Lay Betna Tach Bet	Shewa	---------	---------	2720
Acc 236891	Oromiya	Semen Shewa	Girar Jarso	Shewa	39-42-N	38-48-E	2650
Acc 207260	Tigray	Misrakawi	Gulomahda	Tigray	---------	---------	-----
Acc 213254	Tigray	Misrakawi	Wukro	Tigray	---------	---------	-----
Acc 219953	Tigray	Mehakelegnaw	Laelay Maychew	Tigray	---------	---------	-----
Acc 219954	Tigray	Mirabawi	Asegede Tsimbela	Tigray	---------	---------	-----
Acc 219957	Tigray	Mehakelegnaw	Adwa	Tigray	14-09-N	38-56-E	2330
Acc 221719	Tigray	Debubawi	Enderta	Tigray	---------	---------	2500
Acc 223220	Tigray	Misrakawi	Saesi Tsaedaemba	Tigray	14-15-N	39-28-E	2580
Acc 223222	Tigray	Debubawi	Alaje	Tigray	22-43-N	39-32-E	2600
Acc 223223	Tigray	Debubawi	Endamehoni	Tigray	12-42-N	39-32-E	2450
Acc 223224	Tigray	Misrakawi	Ganta Afeshum	Tigray	14-14-N	39-28-E	2360
Acc 36084	Amhara	Semen Wello	Guba Lafto	Wello	---------	---------	------
Acc 36097	Amhara	Semen Wello	Dawuntna Delant	Wello	11-32-N	39-15-E	2200
Acc 36101	Amhara	Semen Wello	Wadla	Wello	---------	--------	2830
Acc 36103	Amhara	Debub Wello	Kutaber	Wello	11-20-E	39-18-E	2620
Acc 36104	Amhara	Debub Wello	Tenta	Wello	11-16-N	39-15-E	2900
Acc 36141	Amhara	Debub Wello	Were Ilu	Wello	10-44-N	39-28-E	2660
Acc 36151	Amhara	Debub Wello	Dessie Zuria	Wello	11-6-N	39-38-E	2260
Acc 36162	Amhara	Debub Wello	Legambo	Wello	---------	---------	3230
Acc 36168	Amhara	Debub Wello	Tehuledere	Wello	---------	---------	------
Acc 207309	Amhara	Debub Wello	Ambasel	Wello	---------	---------	------

Table 3. Exotic lentil genotypes/improved varieties used in the study

Pedigree	Local Name	Origin	Source	Year of release (E.C.)	Type of variety
----------	Chalew	ICARDA	DZARC	1977	Released
----------	EL-142	----------	DZARC	1972	
ILL-60-27	Ada'a	ICARDA	DZARC	1987	
----------	Chekol	ICARDA	DZARC	1986	
ILL-88-3 X ILL-470	Gudo	ICARDA	DZARC	1987	
----------	Teshale	ICARDA	DZARC	1986	
----------	R-186	ICARDA	DZARC	1972	
----------	Alemaya	ICARDA	DZARC	1989	
----------	Alemtena	ICARDA	DZARC	1986	
ILL-46-05 X ILL-25-78	Assano	ICARDA	SARC	1994	
ILL-55-06 X ILL-55-82	FLIP-88-3L	ICARDA	SARC	-------	Candidate

4.3. Experimental Setup and Sampling Procedure

The plant materials were grown in a 9 x 9 simple lattice design in two replications of 0.62 m^2 plot size (2 rows of 1.55 m length spaced at 0.2 m) during "Bona"/"Meher" (August-December) Season. Randomization was done both within and between blocks. Seed rate was 65kg/ha as per the recommendation for the area. No fertilizer was applied.

Five individual plants were selected from each of the two replications and marked just before flowering. Morphological data were collected from all the marked plants. On the other hand, only five of the ten marked plants selected at random from both replications were used for molecular part of the study.

4.4. Morphological Data

Phenotypic data were recorded on days to flowering and maturity, plant height (cm), number of pods and seeds, hundred seed weight (g), seed yield (g), biomass, and number of primary and secondary branches. Harvest index was calculated as the ratio of biomass to seed yield (Appendix 5 for definition of the agro-morphological traits).

4.5. Molecular Data

The molecular part of the present study was conducted in the Genetics Research Laboratory of the Department of Biology, Addis Ababa University, Ethiopia.

4.5.1. Leaf Sampling and DNA Extraction

Young leaf samples were collected separately from five individual plants of each accession and genotype just before flowering and dried in silica gel. Approximately equal amounts of the silica-gel-dried leaf samples from the five plants of each accession and genotype were bulked for DNA extraction. The bulked leaf sample of each accession/genotype was ground in a clean sterile mortar using quartz sand and sterile pestle.

Total genomic DNA was isolated from the ground leaf sample using a modified (2% cetyltrimethylammoniumbromide (CTAB), 1% polyvinylpyrrolidone, 100 mM Tris (pH 8), 20 mM EDTA, 1.4 M NaCl) CTAB method. The isolation procedure was modified by introducing triple CTAB extractions to yield optimal quantities of high-quality DNA from tissues with considerable amounts of secondary compounds that occur in many basal angiosperms (Borsch *et al.*, 2003). This protocol is modified from a miniprep procedure described in Liang and Hilu (1996) by Borsch *et al.* (2003). About 0.2 g of the pulverized bulk sample of each accession were incubated at 65 ^{0}C for 30 min with 700 µl of CTAB. After centrifuging and transferring the supernatant into a clean tube, the same tissue was re-incubated twice with CTAB solution. All three preparations were kept separate. The CTAB solutions were then extracted with chloroform twice, and the DNA was subsequently precipitated with ethanol. After separately re-suspending the pellets from all extraction steps in TE, two cleaning steps were carried out: the first by adding one-half volume 7.5 M ammonium acetate and precipitating with 100 % ethanol, and the second by adding one-half volume 3 M sodium acetate and precipitating with ethanol. Genomic DNA from the second and third extractions was usually clean enough to be directly used for polymerase chain reaction (PCR) amplification.

4.5.2. Test Gel Electrophoresis and Staining

Agarose gel (0.98 %) was prepared for test gel electrophoresis by boiling 0.98g agarose in 100 ml of 1X TBE buffer. Electrophoresis was conducted in 1X TBE buffer using Agagel Midi-Wide

(Biometra®, biomed. Analytik GmbH) gel tank. 2 µl genomic DNA per sample was loaded with 6 µl 2X loading dye. 4 µl of 100 bp DNA ladder (PEQLAB Biotechnologie GmbH) was used on each side of the gel as a marker. Electrophoresis was run at 80 V for about 1hr. The resultant gel was visualized by ethidium bromide staining.

4.5.3. Selection and Dilution of DNA Extract

The third extractions of most and the second extractions of some samples were selected for further PCR amplification based on the results of the test gel (Figure 1 as an example). Selection of the extracts was based on DNA quantity (band intensity) and quality (absence of or presence of minimum smear). The selected genomic DNA samples were diluted with sterile distilled water in 1:5 ratio with the only exception of DNA from sample of Assano, which was diluted in 1:10 ratio. The remaining concentrated genomic DNA was maintained at -20^{0}C. Dilution ratio was estimated based on the intensity of the test gel bands where more intense bands were assumed to reveal high DNA concentration.

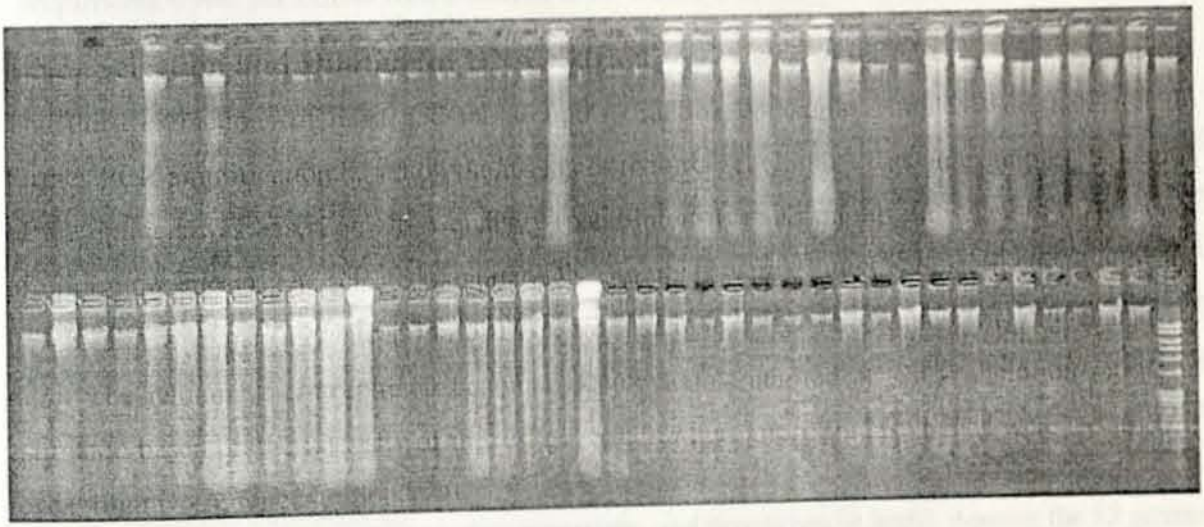

Figure 1. Test gel of diluted genomic DNA samples of the selected extracts of 79 lentil plant materials (with the exception of Acc 221790, Acc 219953 and FLIP-88-3L).

4.5.4. PCR Amplification and Electrophoresis

4.5.4.1. PCR Reaction Components and Profiles

PCR reaction components optimized for ISSR molecular genotyping in coffee (Govers *et al.*, in press) was tested and produced a good banding pattern in lentil (Figures 2-5) and therefore, was adopted. The optimum reaction components were 16.7 µl dH_2O, 250 µM of each dATP, dGTP, dCTP and dTTP, 2.6 µl 10X *Taq* buffer 1 U *Taq* polymerase, 0.23 µM primer and 1 µl of template DNA. The final reaction volume per sample was 26µl (dNTPs, *Taq* buffer, *Taq* polymerase and ISSR primers were purchased from PEQLAB Boitechnologie GmbH, Germany).

Similarly, the PCR amplification profiles of Govers *et al.* (in press) were tested and produced good results in lentil and hence were adopted. The amplification conditions were set as: initial denaturing at 94^0C for 4 minutes followed by 40 cycles of 94^0C for 15 s, $45/48^0$C for 1 min, and 72^0C for 1 min and 30 s and ended with an elongation phase of 72^0C for 7 min. The lid temperature was held at 105 ^{0}C. The amplified PCR products were stored at 4^0C. PCR amplification was performed with Biometra® T3 thermorecycler.

4.5.4.2. Primer Screening

12 ISSR primers (PEQLAB Biotechnologie, Germany) named 810, 812, 814, 818, 824, 834, 835, 836, 844, 873, 880, and 881 were selected based on previous experimental results in lentil (Ford *et al.*, 2003; Kahraman *et al.*, 2004) and other related legume species such as common bean (de la Cruz *et al.*, 2004; Gozález *et al.*, 2005), *Ammopiptanthus* (Ge *et al.*, 2005) and chickpea (Flandez-Galvez *et al.*, 2003). Their sequence, % GC content, melting and annealing temperatures recommended by the producing factory were presented in Table 4. These primers were screened for the amplification of unambiguously visible and polymorphic ISSR bands (ISSR markers) on some representative accessions and genotypes in lentil. Among the 12 primers screened, 812, 818, 835 and 881 produced unambiguously visible polymorhic bands/loci, and hence were used for further ISSR analysis. The representative pictures of each ISSR primer selected are shown in Figures 2-5.

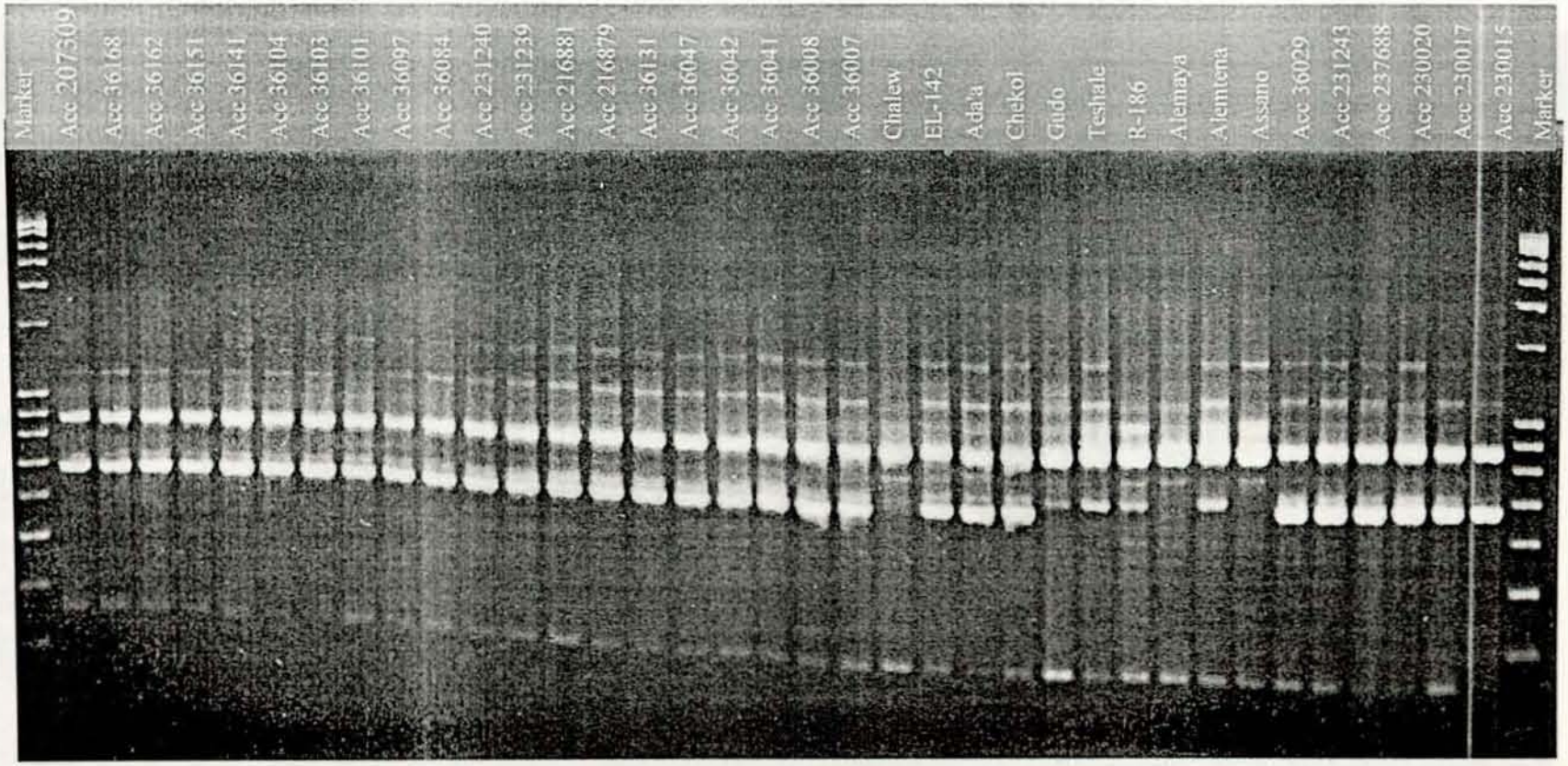

Figure 2. Banding pattern produced by ISSR primer 812 in genetic diversity assessment of lentil landraces and exotic genotypes.

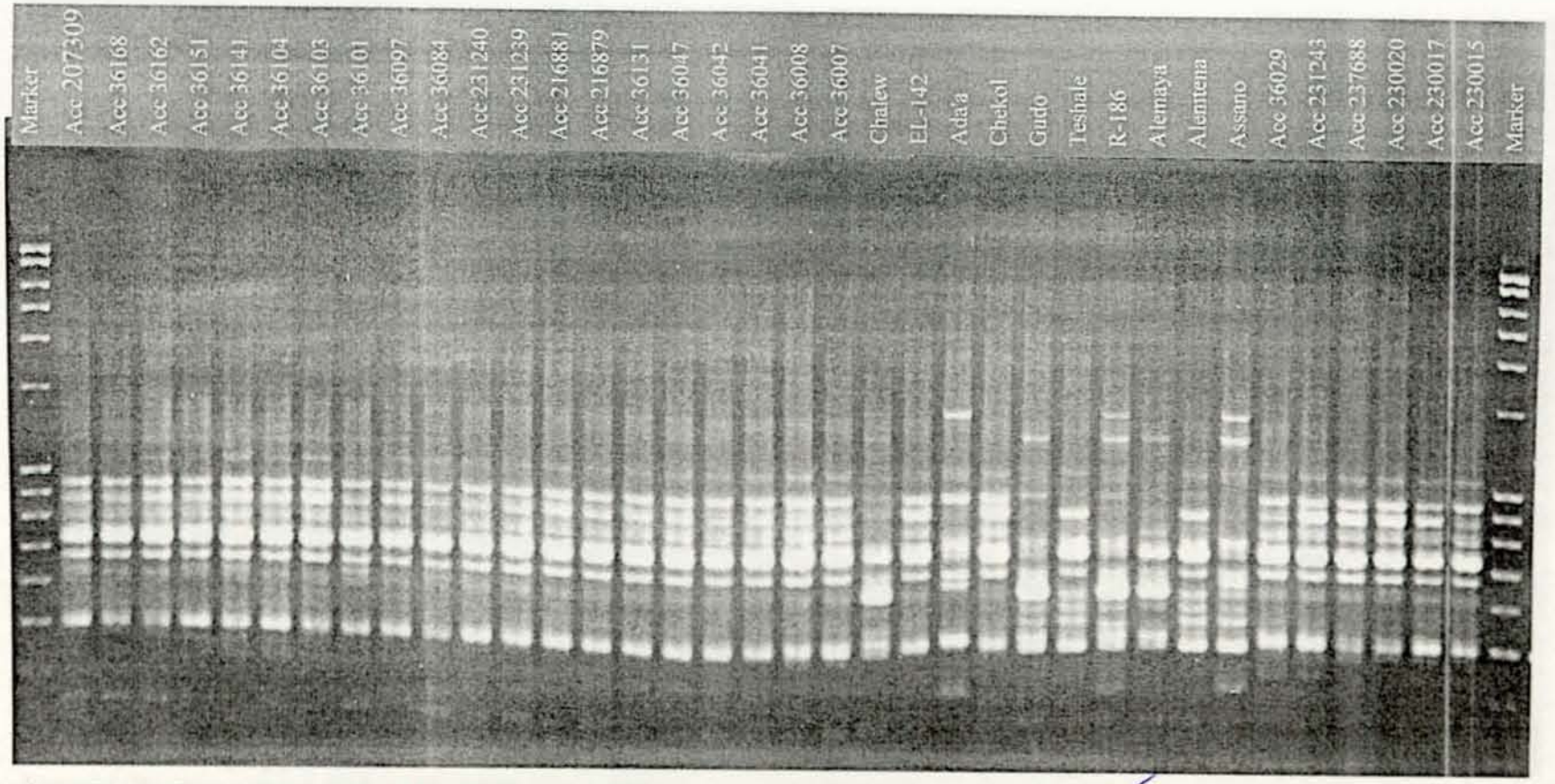

Figure 3. Banding pattern produced by ISSR primer 818 in genetic diversity assessment of lentil landraces and exotic genotypes.

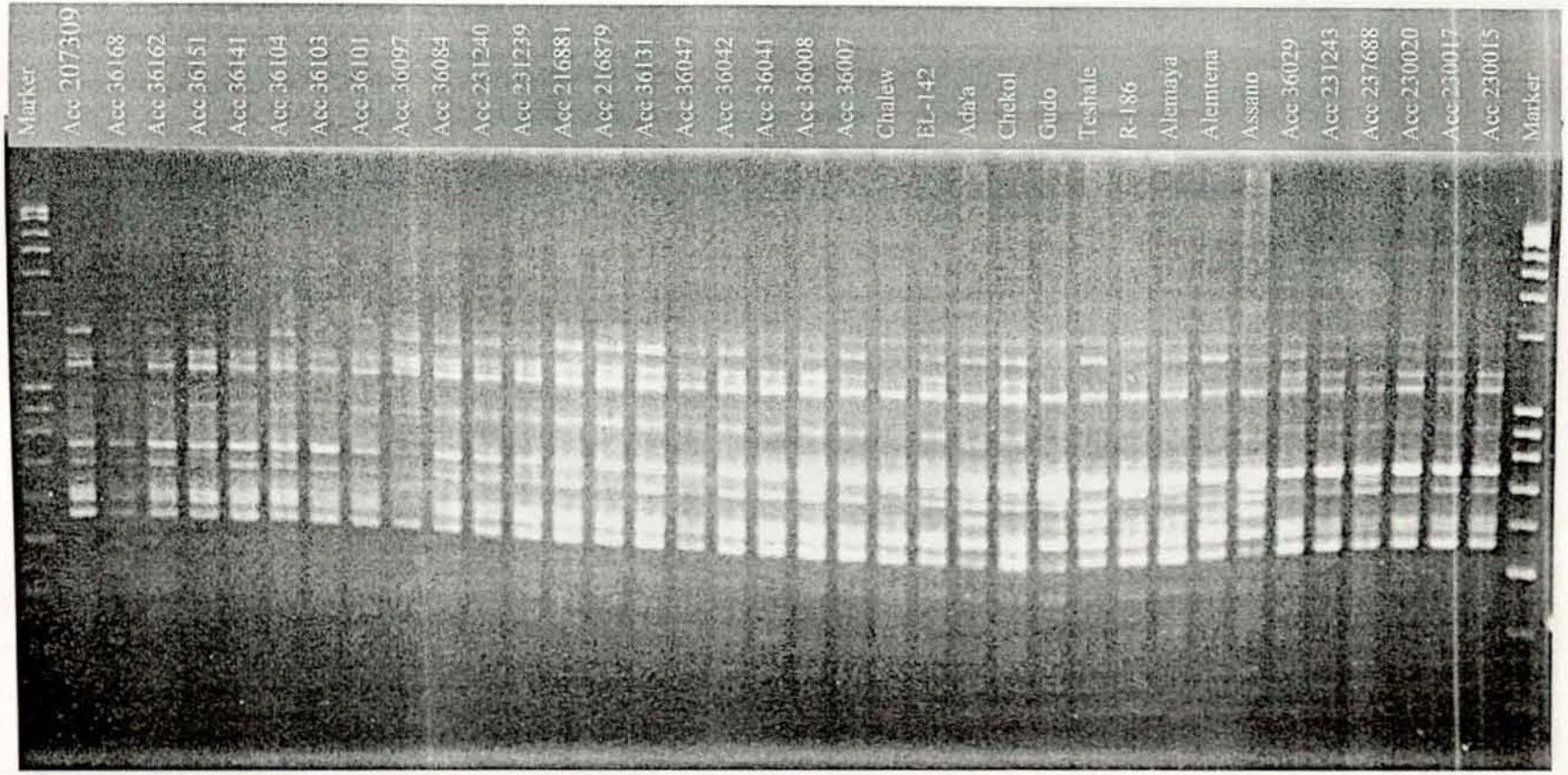

Figure 4. Banding pattern produced by ISSR primer 835 in genetic diversity assessment of lentil landraces and exotic genotypes.

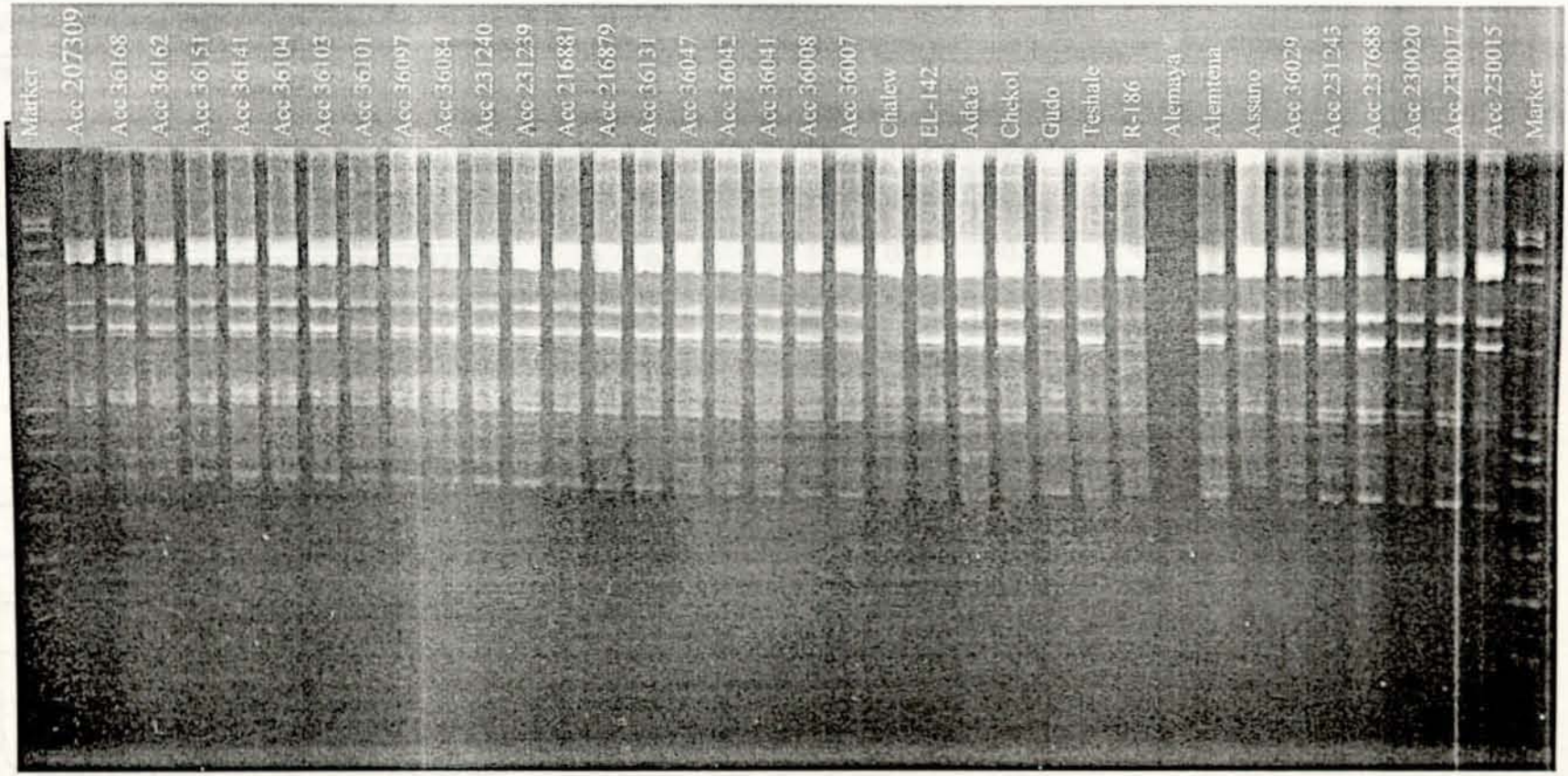

Figure 5. Banding pattern produced by ISSR primer 881 in genetic diversity assessment of lentil landraces and exotic genotypes.

Table 4. List and sequence of ISSR primers screened for molecular study

Primer Name	Primer Sequence 5'→3'	Number of Nucleotides					%GC	Temperature		Successful PCR at [°C]
		A	G	C	T	Total		Melting	Annealing	
810	GAGAGAGAGAGAGAGAT	8	8	0	1	17	0.47	50.4	45	45
812	GAGAGAGAGAGAGAGAA	9	8	0	0	17	0.47	50.4	45	45
814	CTCTCTCTCTCTCTCTA	1	0	8	8	17	0.47	50.4	45	45
818	CACACACACACACACAG	8	1	8	0	17	0.53	52.8	48	48
824	TCTCTCTCTCTCTCTCG	0	1	8	8	17	0.53	52.8	48	48
834	AGAGAGAGAGAGAGAGYT	8	8	0	1	17	0.47	50.4	45	45
835	AGAGAGAGAGAGAGAGYC	8	8	1	0	17	0.53	52.8	48	48
836	AGAGAGAGAGAGAGAGYA	9	8	0	0	17	0.47	50.4	45	45
844	CTCTCTCTCTCTCTCTRC	0	0	9	8	17	0.53	52.8	48	48
873	GACAGACAGACAGACA	8	4	4	0	16	0.50	49.2	45	45
880	GGAGAGGAGAGGAGA	6	9	0	0	15	0.60	50.6	45	45
881	GGGTGGGGTGGGGTG	0	12	0	3	15	0.80	58.8	55	50

Y stands for pyrimidine (C or T)

R stands for purine (A or G)

4.5.4.3. ISSR-PCR Product Gel Electrophoresis

Gel electrophoresis of the ISSR-PCR product was conducted and visualized in a similar way for the test gel but with 1.67 % agarose gel concentration, 9 µl of ISSR-PCR product and 2 µl 6X loading dye while electrophoresis was run at 100 V for nearly 1hr and 40 minutes (Figures 2-5 for representative gel pictures).

4.6. Data Analysis

4.6.1. Morphological Data Analysis

4.6.1.1. Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) was conducted for all metric characters according to Gomez and Gomez (1984) using MSTATC (Freed, 1989) computer software. Furthermore, significance of the variation between treatments due to harvest index was determined by chi-square test using SPSS (SPSS Inc., 1999) computer software.

4.6.1.2. Estimation of Phenotypic and Genotypic Variances

The extent of phenotypic and genotypic variability of the experimental materials in the morphological traits measured in terms of phenotypic and genotypic coefficients of variation (PCV and GCV, respectively) was estimated from the ANOVA table according to Burton and de Vane (1953):

$$\delta^2_g = \frac{\delta^2_p - \delta^2_e}{r},$$

$$\text{PCV} = \frac{\sqrt{\delta^2_p}}{\bar{x}} \times 100, \text{ and}$$

$$\text{GCV} = \frac{\sqrt{\delta^2_g}}{\bar{x}} \times 100, \text{ where, } \sqrt{\delta^2_p} \text{ is the square root of the phenotypic variance, } \sqrt{\delta^2_g} \text{ is the}$$

square root of the genotypic variance and $\bar{x}$ is the average value of character x.

4.6.1.3. Euclidian Distance and Clustering Analysis

Euclidian distance ($E_{ij} = \sqrt{\sum_{k=1}^{n}(x_{ki} - x_{kj})^2}$, where x_{ki} and x_{jk}, respectively, denoted the kth variable on accession/genotype or population i and j, and n represent the number of traits; Rohlf, 2004) between the landrace populations and exotic genotypes were calculated from the log transformed and standardized trait mean values (mean of each trait was subtracted from the data values and the result divided by the maximum value of the trait) over each population using NTSYSpc Version 2.11T (Rohlf, 2004) software. Similarly, the log-transformed and standardized trait mean values over each accession/genotype were used to perform cluster analysis with the same software. To group the plant materials based on morphological dissimilarity, cluster analysis was conducted on the Euclidian distance matrix with Unweighted Pair Group Method based on Arithmetic Averages (UPGMA) procedure of the Sequential, Agglomerative, Hierarchical and Nested (SAHN) clustering methods (Sneath and Sokal, 1973) with NTSYSpc2.1.

4.6.2. Molecular Data Analysis

All of the statistical parameters were analyzed by grouping the experimental materials in four ways. The first analysis considered landrace populations (ARs) and exotic genotypes to compare exotic genotypes against the landrace populations. The second round of analysis compared the entire landraces as a single population against released varieties (exotic genotypes). The third level of analysis considered the landrace populations (ARs) independently to compute the level of genetic variability among and within the populations (ARs). And finally, the fourth analysis was conducted by grouping the landrace accessions into four geographical regions (GRs) based on proximity of the ARs to one another.

4.6.2.1. Scoring Bands

ISSR profiles were scored manually for each individual as discrete characters (presence (1) or absence (0) of the amplified products). Only bands that could be unambiguously scored across all the sampled populations were used in this study.

4.6.2.2. Genetic Diversity Analysis

Like RAPD, ISSRs are usually dominant markers, so only the presence or absence of an allele can be determined. The assumption therefore has to be made that each band position corresponds to a locus with two alleles represented by the presence or absence of a band (Powell *et al.*, 1996) and that each individual is homozygous, a valid assumption for a highly inbreeding species, such as *Lens* species (Ferguson *et al.*, 1998). Genetic diversity parameters were determined for each population and/or group of accessions on those amplification products that could be reliably scored across all the populations.

Genetic diversity measured by the percentage of polymorphic bands (P; the ratio of the number of polymorphic bands to the total number of bands surveyed), mean Nei's gene diversity (H; Nei, 1973, 1978; formula given below), genetic distance (Nei, 1972, 1978; formula given below), coefficient of gene differentiation (Gst = D_{ST}/H_T; Nei, 1973) and gene flow estimates (Nm = [1-Gst]/4Gst; McDermott and McDonald, 1993) were computed with POPGENE ver 1.32 (Yeh *et al.*, 2000) computer software. The assumption of Hardy-Weignberg equilibrium built in softwares for population genetic analysis holds for outcrossing species. However, since lentil is a completely self-pollinated species, all the loci are expected to be homozygous and hence the assumption does not hold for lentil. Therefore, the problem was taken care of by analyzing the ISSR data using the haploid option of POPGENE.

Nei's (1973) gene diversity in the total population is defined as: $H_T = 1 - J_T$, where J_T is the gene identity in the total population. When analyzed into the gene diversities within and between subpopulations, the formula takes the form: $H_T = H_S + D_{ST}$, where H_S and D_{ST}, respectively, are the average gene diversity within subpopulations and among subpopulations.

Nei's (1972) original measure of genetic distance is defined as: $D = -\log_e I$, where D stands for Nie (1972) genetic distance between a randomly mating diploid populations X and Y; I defined as $I = J_{XY} / \sqrt{J_X J_Y}$ is the normalized identity of genes between X and Y; and J_X, J_Y and J_{XY} are the arithmetic means of j_X, j_Y and j_{XY}, respectively, which in turn are the probabilities of identity of two randomly chosen genes in population X, Y and J, respectively. j_X, j_Y and j_{XY}, respectively are defined as $j_X = \sum x_i^2$, $j_Y = \sum y_i^2$, and $j_{XY} = \sum x_i y_i$.

The unbiased estimate of D (Nei, 1978) is given as: $\hat{D} = -\ln[\hat{G}_{XY} / \sqrt{\hat{G}_X \hat{G}_Y}]$, where $\hat{G}_X$ and $\hat{G}_Y$ are the unbiased estimates of J_X and J_Y given above.

4.6.2.3. Genetic Structure

4.6.2.3.1. Analysis of Molecular Variance (AMOVA)

The interpretation of allele (ISSR fragment) frequency data of dominant markers must be approached with caution, because statistical methods are based on assumptions of Hardy-Weinberg equilibrium (Lynch and Milligan, 1994). In this study, the non-parametric analysis of molecular variance (AMOVA), based on the analysis of pairwise genetic distances (Excoffier *et al.*, 1992) was used to estimate variance components for the ISSR phenotypes using Arlequin ver 3.01 (Excoffier *et al.*, 2006) computer software to describe the genetic structure at AR and GR level. This method provides an estimate of the fraction of between population diversity (*FST*) and population specific *FST* indices of Weir and Hill (2002). Variation was partitioned among individuals (within ARs), among ARs, among ARs within GRs, and among GRs. The resulting coefficients of subdivision (for GR-based analysis), *FST*, *FSC*, and *FCT* are analogous to Wright's (1951) F-statistics (Fst). The significance of these F-statistic analogues was tested by 1000 random permutations.

4.6.2.3.2. Clustering Analysis

The level of genetic similarity was determined using Dice's similarity coefficient (Dice, 1945) with NTSYSpc ver 2.1 computer software. A dendrogram was constructed based on the similarity matrix using UPGMA procedure of the SAHN methods (Sneath and Sokal, 1973) with NTSYSpc2.1. The goodness of fit of clustering to the data set was tested using NTSYSpc2.1. In this case, the co-phenetic matrix generated from the UPGMA tree was compared with the similarity matrix from which the tree has been produced.

4.6.3. Correlation of Morphological and Molecular Distances

The relationships between the Euclidian distance matrix based on morphological data and genetic distance matrix (Nei, 1973) obtained with ISSR marker were analyzed using the approach developed by Mantel (1967) with Arlequin ver 3.01 computer software.

5. RESULTS

5.1. Morphological Variability

5.1.1. Analysis of Variance (ANOVA)

Analysis of variance showed highly significant ($p < 0.01$) variation between the lentil plant materials used (landrace accessions and exotic genotypes) for all the traits measured. Furthermore, there was also significant ($p < 0.05$) variation between treatments in plant height (Table 5).

5.1.2. Phenotypic and Genotypic Coefficients of Variation

There was a wide variation between all the lentil materials evaluated and landraces separately with respect to the average of each agro-morphological trait measured. The average morphological performance of the entire plant materials for the measured characters is given in Appendix 6.

Considering the whole experimental plant materials, number of secondary branches per plant, number of seeds per plant, seed yield, biomass, hundred seed weight and number of pods per plant showed very high phenotypic and genotypic variations. Moderately high phenotypic and genotypic variations were obtained for number of primary branches per plant. On the other hand, a relatively smaller PCV and GCV values were obtained for plant height and days to flowering and maturity (Table 6).

Indigenous lentil landraces of Ethiopia expressed better mean values and wider phenotypic variation in important yield parameters such as number of secondary branches per plant, number of pods and seeds per plant as compared to exotic genotypes. Furthermore, they showed wider PCV for biomass and seed yield compared to exotic genotypes. On the contrary, exotic lentil genotypes showed a wider phenotypic variation for hundred seed weight, days to flowering and maturity. Furthermore, exotic genotypes were phenotypically slightly more variable as compared to the landrace accessions for plant height and number of primary branches per plant. There was very narrow phenotypic variation in Ethiopian lentil landrace accessions for days to maturity (Table 7).

Table 5. ANOVA table for the agro-morphological characters measured in Ethiopian lentil landrace accessions and exotic genotypes

Source of Variance	df	Days to Flowering	Days to Maturity	Plant Height	Number of Primary Branches	Number of Secondary Branches
		MS	MS	MS	MS	MS
Replications	1	29.56	25.28	77.06	38.32	1185.09
Treatments						
-Unadjusted	80	33.24**	63.86**	10.70	2.67	20.49
-Adjusted	80	33.73**	66.80**	10.26*	3.00**	19.99**
Blocks within Reps (adj.)	16	11.67	47.44	25.25	6.18	83.41
Error						
-Effective	64	5.54	14.63	6.14	1.38	4.54
-RCB Design	80	6.31	19.70	9.29	2.18	19.73
-Intrablock	64	4.97	12.76	5.30	1.19	3.81
Efficiency of Lattice: Compared with RCBD		113.89	134.66	151.30	158.57	435.03
CV%		3.56	3.44	8.46	15.03	26.92

** Significant at $P < 0.01$

* Significant at $P < 0.05$

Table 5. Continued

Source of Variance	df	Number of Pods/Plant	Number of Seeds/Plant	Hundred Seed Weight (g)	Biomass (g)	Seed Yield /Plant (g)
		MS	MS	MS	MS	MS
Replications	1	41854.64	118190.90	0.01	279.16	59.34
Treatments						
-Unadjusted	80	1610.65	4824.99*	0.52**	10.28	2.50
-Adjusted	80	1630.60**	5392.30**	0.52**	10.27**	2.52**
Blocks within Reps (adj.)	16	4513.40	10581.37	0.06	18.90	5.65
Error						
-Effective	64	432.53	1433.48	0.05	5.69	1.07
-RCB Design	80	1194.97	3090.62	0.05	7.75	1.86
-Intrablock	64	365.37	1217.93	0.05	4.96	0.91
Efficiency of Lattice: Compared with RCBD		276.28	215.60	101.18	136.11	174.46
CV%		19.69	25.38	8.56	28.54	26.56

** Significant at $P < 0.01$

* Significant at $P < 0.05$

Considering the samples from different ARs as an independent population, samples from Gojam showed better phenotypic variation for days to flowering and maturity and hundred seed weight. Samples from Gonder showed wider phenotypic variation for plant height, number of pods per plant, biomass and seed yield. Samples from Tigray, Arsi, Wello and Gonder showed wider and better phenotypic variation for number of secondary branches per plant. Samples from Bale had the highest phenotypic variation for number of primary branches per plant. Samples from Wello, Arsi, Gonder and Bale were characterized by high phenotypic variation for number of secondary branches per plant. Samples from Gonder, Bale and Arsi showed wider phenotypic variation for number of seeds per plant. Samples from Wello, Bale, Gonder and Arsi were characterized by high average values for number of primary and secondary branches, pods, seeds, biomass and seed yield per plant. Samples from Tigray, Gonder and Wello were characterized by higher seed size on average basis (Table 8).

Table 6. Summary statistics of the agro-morphological traits measured in Ethiopian landrace accessions and exotic genotypes (improved varieties)

Traits	Mean	Range	$\sqrt{\delta^2}$	PCV	δ_g^2	$\sqrt{\delta^2_g}$	GCV
Days to flowering	66.10	32.50	4.45	6.74	14.06	3.75	5.68
Days to maturity	111.16	50.00	6.46	5.81	26.11	5.11	4.59
Plant height (cm)	29.28	21.90	3.23	11.02	2.07	1.44	4.90
Number of primary branches	7.81	14.50	1.63	20.86	0.81	0.9	11.52
Number of secondary branches	7.91	29.83	5.23	66.09	7.73	2.78	35.14
Number of pods per plant	105.64	269.20	40.67	38.50	599.27	24.48	23.17
Number of seeds per plant	149.19	419.40	68.32	45.79	1979.36	44.49	29.82
Hundred seed weight (g)	2.60	3.11	0.53	20.52	0.23	0.48	18.64
Biomass (g)	9.28	18.96	3.27	39.11	4.58	2.14	25.59
Seed yield (g) per plant	3.89	9.23	1.59	40.96	0.72	0.85	21.89

5.1.3. Euclidian Distances

The Euclidian distance calculated from the log-transformed and standardized average morphological data of the different AR-based populations of landraces and exotic genotypes is

Table 7. Comparison of lentil landrace accessions with improved varieties/genotypes based on mean, range and PCV of the measured agro-morphological traits

Types of lentils	Statistical parameter	DTF	DTM	PH	NPB	NSB	NP	NS	HSW	Biom	SY
Landraces	Mean	65.50	110.09	29.03	7.89	7.84	108.76	156.11	2.47	8.44	3.90
	Range	17.50	16.00	17.00	14.20	29.50	264.00	402.60	1.84	18.96	9.23
	$\sqrt{\delta^2}$	3.36	2.19	3.01	1.59	5.27	40.48	69.13	0.27	3.39	1.66
	PCV	5.13	1.99	10.38	20.09	67.18	37.22	44.28	11.10	40.22	42.43
Exotic genotypes	Mean	69.92	123.7	30.97	7.12	8.44	82.21	103.56	3.52	14.63	7.04
	Range	28.00	48.00	16.30	6.70	19.83	102	156.00	2.89	9.66	4.63
	$\sqrt{\delta^2}$	7.72	15.87	4.05	1.76	5.15	30.4	42.78	0.92	2.31	1.12
	PCV	11.05	13.41	13.09	24.75	60.97	36.98	41.31	26.07	29.01	29.29

DTF = Days to flowering, DTM = Days to maturity, PH = Plant height, NPB = Number of primary branch per plant, NSB = Number of secondary branch per plant, NP = Number of pods per plant, NS = Number of seeds per plant, HSW = Hundred seed weight, Biom = Biomass, SY = seed yield per plant, $\sqrt{\delta^2}$ = Standard deviation

presented in Table 9. Exotic genotypes were more distantly related to samples from Tigray (0.7300), Gojam (0.5806), Shewa (0.5454) and Arsi (0.5440). Considering the landrace populations separately (Table 10), samples from Tigray were more distantly related to samples of Wello (0.6123) and Bale (0.5385). In general, samples from Tigray followed by those from Wello showed the highest Euclidian distance from all other populations as compared to the distances of other pair-wise population combinations.

5.1.4. Clustering Analysis

A dendrogram generated from the standardized morphological data on Euclidian distance matrix (of both indigenous landrace accessions and exotic genotypes) is presented in Figure 6. The UPGMA cluster analysis classified the entire experimental materials into two major clusters. One of the major clusters (cluster II) comprised most samples from Wello (8), some from Bale (4), some from Arsi (3), Gonder (2) and Gojam (1) in which Chekol was included from exotic genotypes. The other major cluster (cluster I), comprised all the rest of the experimental plant

materials, in which all samples from Tigray and Shewa, 9 exotic genotypes, and most samples from Gojam (9), Gonder (8), Arsi (7) and Bale (6) were included. Cluster I further subdivided into two subclusters. One of the subclusters (subcluster I_2) comprised most of the samples from Tigray (9), Gojam (3), Gonder (3), Arsi (2), exotic genotypes (2) and Bale (1). In the other subcluster (subcluster I_1), most samples from Shewa (6), Gojam (6), exotic genotypes (6) and some from Gonder (4), Bale (4), Arsi (3) and Wello (2) were included. The result indicated that samples from Arsi, and Gonder were found dispersed within the three distinct groups and did not tend to form a distinct cluster or subcluster.

Clustering conducted on the landraces using the standardized morphological data on Euclidian dissimilarity matrix with UPGMA method produced two major clusters. One of the major clusters (cluster II) comprised most samples from Tigray (10), Shewa (10) Gojam (9) and Gonder (3). Wello (8) and some from Bale (4), Gonder (3), and Arsi (1). No samples from Shewa and Tigray were grouped into cluster II. The other major cluster (cluster I) comprised all the rest of the samples: all samples from Tigray and Shewa, most samples from Arsi (9), Gonder (7), Bale (6) and some samples from Wello (2). This major cluster was further subdivided into subclusters. The second subcluster (I_2) comprised most samples from Tigray (8) and some from Shewa (3), Gonder (2), Gojam (2) and Arsi (1). Most samples from Gojam (8), Arsi (8), Shewa (7), Bale (6), Gonder (5) and some from Wello (2) and Tigray (1) formed the other subcluster. Similar grouping was observed for population based clustering analysis (Figure 8).

The correlations between cophenetic values generated from the UPGMA tree and Euclidian distance matrices were $r = 0.81$ and $r = 0.72$, respectively for the entire plant materials and the landraces separately.

5.2. Molecular Diversity

5.2.1. Genetic Diversity

The DNA amplification pattern using ISSR primers in this study was very reproducible across gels based on the results from 80 DNA samples. The four ISSR primers amplified 56 scorable bands. Nine of the total bands scored were unique to exotic lentil genotypes. Primer 812, 818, 835 and 881 produced 14, 14, 13, and 15 bands, respectively.

Table 8. Population based summary statistics of the agro-morphological traits measured in Ethiopian landrace accessions

Population	Statistical parameter	DTF	DTM	PH	NPB	NSB	NP	NSP	HSW	Biom	SY
Arsi	Mean	66.25	110.50	28.22	7.79	8.56	107.86	160.99	2.27	8.01	3.69
	Range	10.00	4.00	10.20	5.60	18.50	157.80	253.40	0.56	12.94	6.37
	$\sqrt{\delta^2}$	2.85	1.10	3.05	1.42	5.45	44.65	74.44	0.14	3.28	1.71
	PCV	4.31	1.00	10.81	18.24	63.66	41.39	46.24	6.33	40.95	46.37
Bale	Mean	64.98	109.20	29.86	8.93	9.90	127.57	189.94	2.35	9.73	4.57
	Range	11.00	5.00	7.80	13.40	19.17	153.20	379.20	0.79	18.23	8.73
	$\sqrt{\delta^2}$	2.79	1.28	2.01	2.73	5.69	46.91	92.25	0.18	4.58	2.19
	PCV	4.30	1.17	6.72	30.59	57.46	36.77	48.57	7.56	47.04	48.06
Gojam	Mean	66.13	111.60	28.67	7.77	6.53	96.63	130.81	2.49	7.30	3.33
	Range	17.50	16.00	12.80	4.60	11.50	60.80	92.60	1.58	4.91	2.76
	$\sqrt{\delta^2}$	5.03	3.89	3.09	1.05	2.81	17.43	23.42	0.39	1.29	0.67
	PCV	7.60	3.48	10.76	13.52	43.09	18.04	17.90	15.54	17.73	20.19
Gonder	Mean	65.84	110.30	29.10	8.03	8.03	111.63	160.19	2.58	8.88	4.16
	Range	13.67	7.00	13.60	4.00	18.50	255.80	401.20	0.92	18.66	8.94
	$\sqrt{\delta^2}$	3.87	1.84	3.20	1.06	4.73	54.22	89.26	0.23	4.37	2.06
	PCV	5.88	1.67	11.01	13.21	58.96	48.58	55.72	9.03	49.22	49.43
Shewa	Mean	64.59	109.40	27.56	7.72	6.89	100.02	139.31	2.47	7.93	3.51
	Range	11.00	8.00	9.40	4.00	8.83	77.80	142.60	0.79	9.56	3.62
	$\sqrt{\delta^2}$	2.92	2.06	2.12	0.96	2.30	22.22	34.82	0.24	2.20	0.92
	PCV	4.53	1.89	7.68	12.42	33.37	22.21	24.99	9.79	27.79	26.33
Tigray	Mean	65.11	110.30	28.35	6.78	4.08	81.88	110.74	2.59	6.25	2.92
	Range	10.00	7.00	10.00	5.40	9.83	72.00	91.80	1.02	5.05	2.42
	$\sqrt{\delta^2}$	2.68	1.66	2.77	1.22	2.00	17.95	26.76	0.29	1.48	0.70
	PCV	4.12	1.50	9.76	18.03	49.13	21.92	24.16	11.04	23.62	23.91
Wello	Mean	65.58	109.30	31.43	8.21	10.92	135.71	200.77	2.52	10.96	5.13
	Range	9.00	6.00	12.00	5.20	27.17	135.80	222.00	0.99	10.13	5.81
	$\sqrt{\delta^2}$	2.86	1.42	3.28	1.28	8.29	39.93	63.07	0.25	3.03	1.61
	PCV	4.36	1.30	10.45	15.63	75.89	29.42	31.41	9.88	27.66	31.39

DTF = Days to flowering, DTM = Days to maturity, PH = Plant height, NPB = Number of primary branch per plant, NSB = Number of secondary branch per plant, NP = Number of pods per plant, NS = Number of seeds per plant, HSW = Hundred seed weight, Biom = Biomass, SY = seed yield per plant;

$\sqrt{\delta^2}$ = Standard deviation

Table 9. Euclidian distance between lentil landrace populations and exotic genotypes

Population	Arsi	Bale	Exotic genotypes	Gojam	Gonder	Shewa	Tigray	Wello
Arsi	0.0000							
Bale	0.1677	0.0000						
Exotic genotypes	0.5440	0.4445	0.0000					
Gojam	0.1552	0.2861	0.5806	0.0000				
Gonder	0.1337	0.1460	0.4369	0.1713	0.0000			
Shewa	0.1199	0.2422	0.5454	0.0519	0.1287	0.0000		
Tigray	0.3731	0.5030	0.7300	0.2284	0.3769	0.2682	0.0000	
Wello	0.2570	0.1135	0.3548	0.3613	0.1960	0.3159	0.5689	0.0000

Table 10. Euclidian distance between lentil landrace populations of Ethiopia

	Arsi	Bale	Gojam	Gonder	Shewa	Tigray	Wello
Arsi	0.0000						
Bale	0.1874	0.0000					
Gojam	0.1725	0.3133	0.0000				
Gonder	0.1661	0.1644	0.1925	0.0000			
Shewa	0.1343	0.2635	0.0576	0.1457	0.0000		
Tigray	0.3974	0.5385	0.2381	0.4029	0.2830	0.0000	
Wello	0.2952	0.1318	0.3982	0.2137	0.3473	0.6123	0.0000

The size of the fragments ranged from 280 to 1400 bp for 812, 350 to 1800 bp (although only 500 to 1500 bp were scorable) for 818, 300 to 2200 bp for 835 (although only 550 to 1500 bp could be scored) and 790 to 3500 bp for 881.

On the other hand, only 47 bands were generated using the four ISSR primers in Ethiopian lentil landrace accessions. Primer 812, 818, 835 and 881 produced 13, 9, 12 and 15 bands, respectively.

No unique bands were observed for the landraces. Furthermore, no population (AR)-specific bands were observed.

5.2.1.1. Polymorphic Loci

Exotic genotypes, when compared to Ethiopian lentil landrace accessions, both as a single and multiple populations, showed more polymorphic (73.21 %) bands. Low numbers of polymorphic loci, ranging from 10 (17.86 %) for Arsi and Tigray to 17 (30.36 %) for samples from Gonder was observed for the landraces as a single population (ARs) compared to exotic genotypes. The total landrace population (considered as a single large population) produced 29 (51.79 %) polymorphic bands/loci as compared to exotic genotypes (Table 11).

On the other hand, when samples from different ARs were considered as independent populations, the highest number of polymorphic loci was obtained for samples from Gonder (17; 30.36 %), followed by samples from Wello (16; 28.57 %) and Shewa (15; 26.79 %), while samples from Arsi and Tigray produced the least number of polymorphic loci (10; 17.86 %; Table 11). There were 29 (51.79 %) polymorphic loci across the landrace populations.

Furthermore, grouping the landrace accessions into geographical regions (GRs) revealed that samples from NEE were more polymorphic (25 bands; 53.97 %), followed by samples from NWE (22 bands; 46.81 %), while samples from CE (15 bands; 31.91 %) and SEE (14 bands; 29.79 %) were the least polymorphic. There were 28 (59.57 %) polymorphic bands across the GRs (Table 11).

5.2.1.2. Nei's Mean Gene Diversity (h)

Comparison of exotic genotypes with the landrace accessions, both as a single population and as multiple populations, revealed the presence of higher between genotype diversity for exotic genotypes ($h = 0.2637$). The mean genetic diversity (Nei, 1973) across all populations (where exotic genotypes and landraces from different ARs were considered as independent populations) was 0.1935, and the average diversity across the two populations of landraces and exotic genotypes (each considered as a separate independent population) was 0.1935 (Table 11).

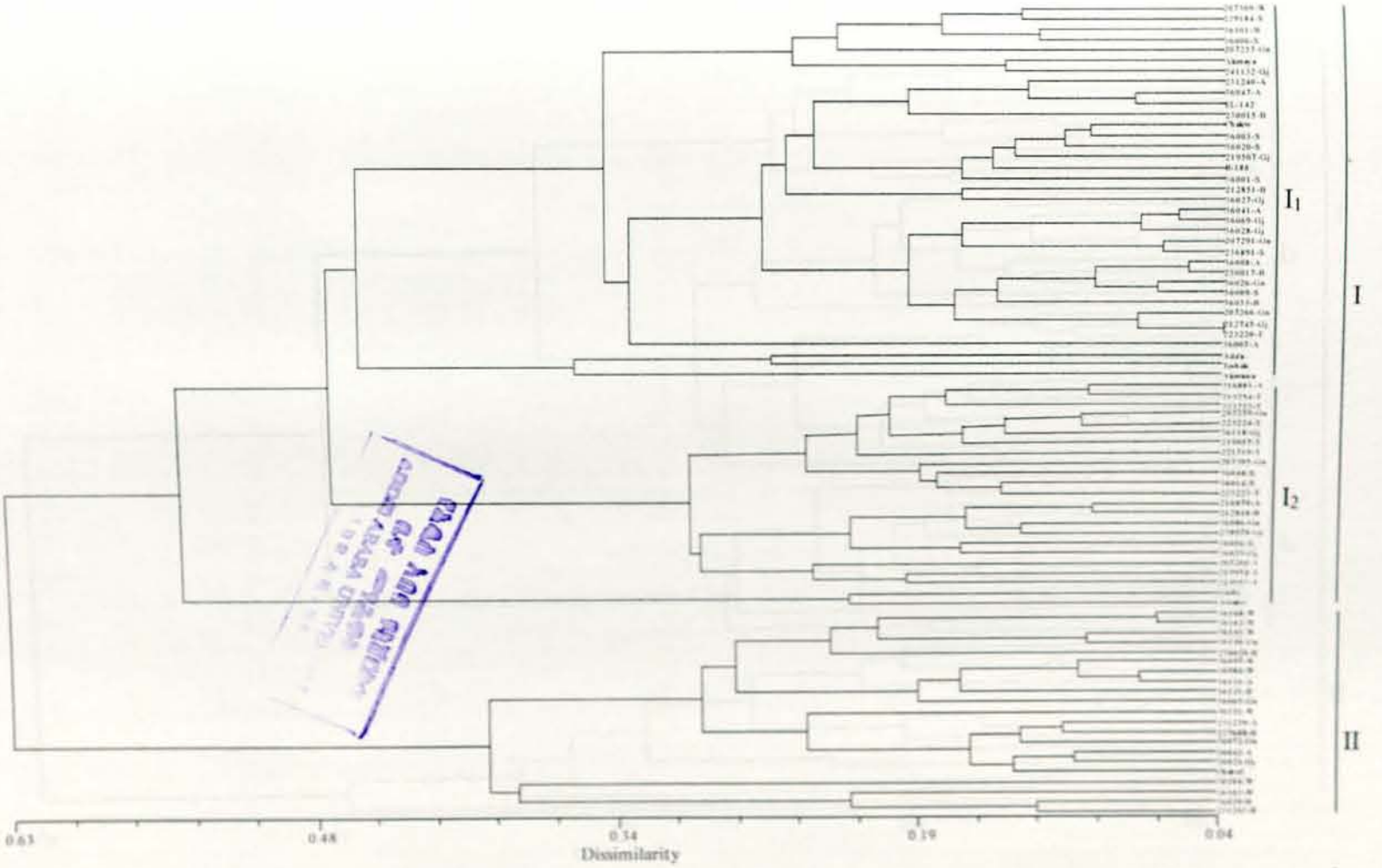

Figure 6. Dendrogram of Ethiopian landrace accessions and exotic genotypes of lentil derived by UPGMA from the Euclidian dissimilarity matrix of morphological data.

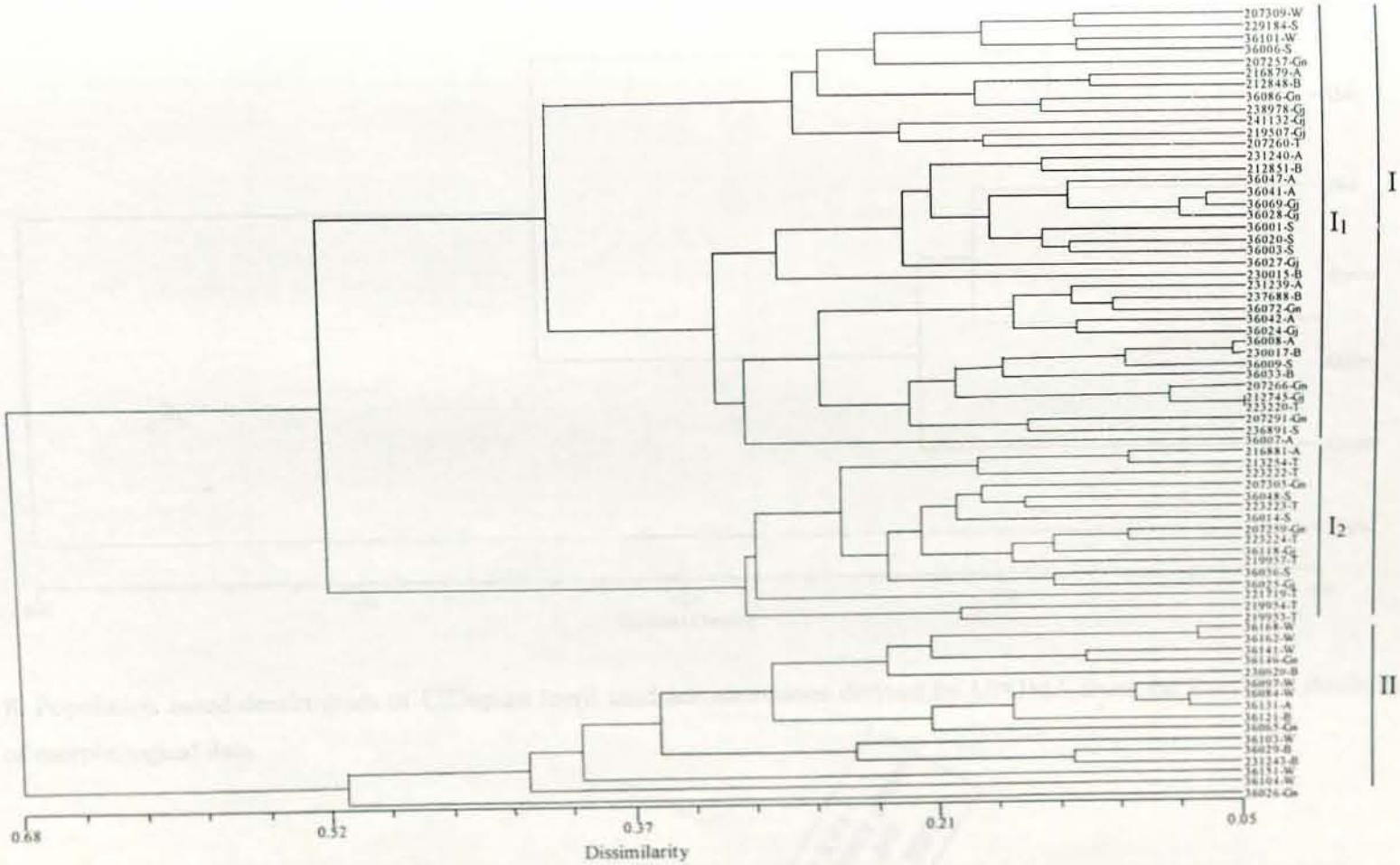

Figure 7. Dendrogram of Ethiopian lentil landrace accessions derived by UPGMA from the Euclidian dissimilarity matrix of morphological data.

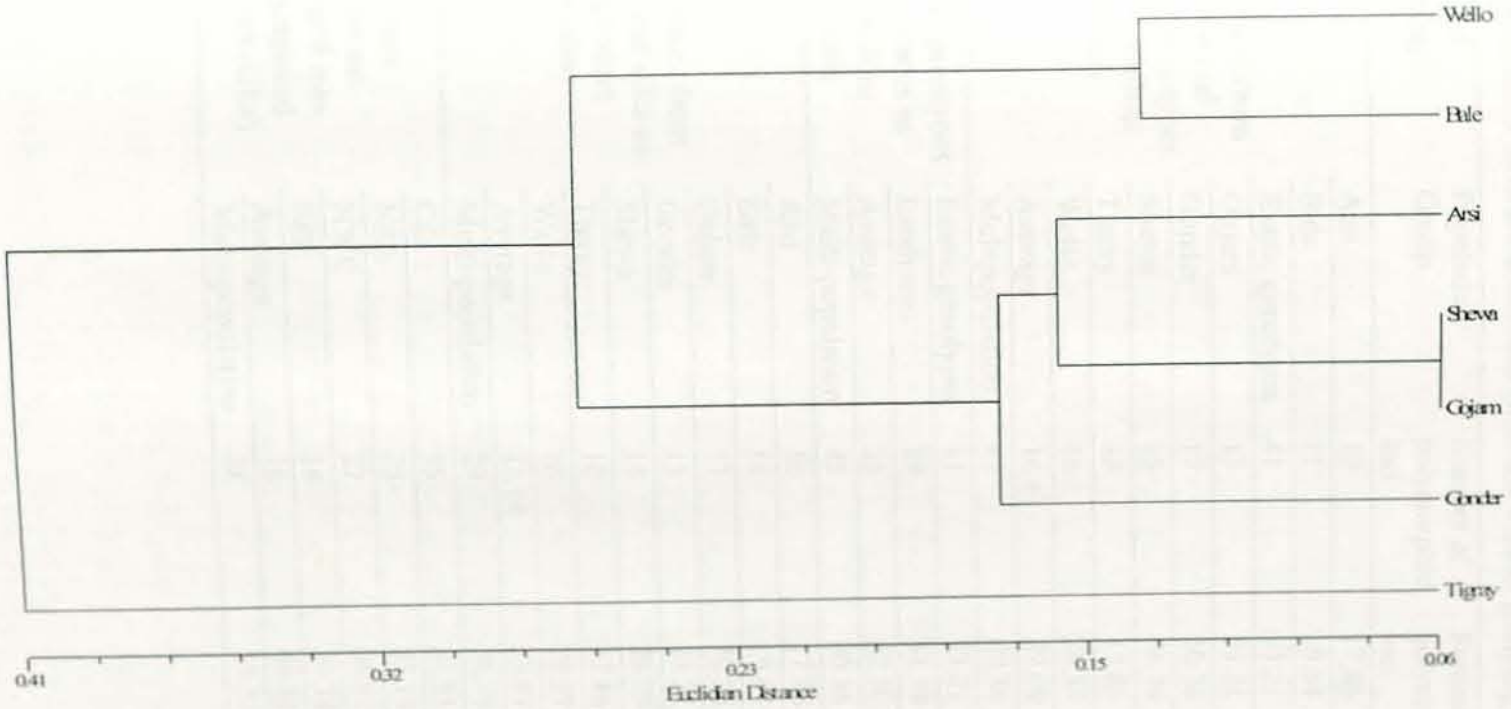

Figure 8. Population based dendrogram of Ethiopian lentil landrace accessions derived by UPGMA from the Euclidian dissimilarity matrix of morphological data.

Table 11. Number and percentage of polymorphic loci, and Nei's mean gene diversity (h) in Ethiopian lentil landrace accessions (with and without grouping) and exotic genotypes

Level of grouping	Population/ Group	Number of polymorphic loci	Percentage of polymorphic loci	h
Landraces from each AR and exotic genotypes as independent population	Arsi	10	17.86	0.0589 (±0.1364)
	Bale	11	19.64	0.0625 (±0.1344)
	Exotic genotypes	41	73.21	0.2637 (±0.1893)
	Gojam	13	23.21	0.0739 (±0.1481)
	Gonder	17	30.36	0.1203 (±0.1920)
	Shewa	15	26.79	0.0972 (±0.1671)
	Tigray	10	17.86	0.0641 (±0.1483)
	Wello	16	28.57	0.0869 (±0.1533)
	Average	16.63	29.69	0.10344
	Multipopulation	45	80.36	0.1935 (±0.1770)
Exotic genotypes vs landraces as independent population	Exotic genotypes	41	73.21	0.2637 (±0.1893)
	Landraces	29	51.79	0.1460 (±0.1921)
	Average	35	62.50	0.20107
	Multi-population	45	80.36	0.1935 (±0.1770)
Landraces from different ARs as independent population	Arsi	10	17.86	0.0702 (±0.1464)
	Bale	11	19.64	0.0745 (±0.1438)
	Gojam	17	30.36	0.0881 (±0.1580)
	Gonder	13	23.21	0.1395 (±0.2027)
	Shewa	15	26.79	0.1158 (±0.1766)
	Tigray	10	17.86	0.0764 (±0.1592)
	Wello	16	28.57	0.1039 (±0.1625)
	Average	13.14	23.47	0.107738
	Multi-population	29	51.79	0.1734 (±0.1984)
Landrace accessions grouped into geographical regions (GRs)	CE	15	31.91	0.1158 (±0.1766)
	NEE	25	53.19	0.1690 (±0.1955)
	NWE	22	46.81	0.1415 (±0.1751)
	SEE	14	29.79	0.0891 (±0.1534)
	Average	19	40.43	0.13776
	Multi-population	28	59.57	0.1734 (±0.1984)

On the other hand, grouping the landrace accessions into AR-based populations showed that samples from Gonder were the most diverse (h = 0.1395) followed by samples from Shewa (h = 0.1158) and Wello (h = 0.1039). Among the landrace populations (ARs) evaluated, samples from Gojam (h = 0.0881), Tigray (h = 0.0764), Bale (h = 0.0745) and Arsi (h = 0.0702) were the least diverse (samples from Arsi being the least of all). Genetic diversity across populations of Ethiopian lentil landrace accessions (the total landrace population) was h = 0.1734. Furthermore, GR-based grouping revealed the highest genetic diversity for samples from NEE (h = 0.1690), which was followed by samples from NWE (h = 0.1415) and CE (h = 0.1158), while those from SEE (h = 0.0891) were the least diverse (Table 11).

5.2.1.3. Genetic Distances (D and $\hat{D}$)

Comparison of exotic genotypes with individual landrace populations (ARs) revealed the highest genetic distance (D = 0.3143) between exotic genotypes and samples from Tigray, followed by samples from Gojjam (0.3138) and Shewa (0.3075). Genetic distance between exotic genotypes and samples from Arsi (0.1876) and Bale (0.1937) was the least. The genetic distances (D) of exotic genotypes from samples of Gonder and Wello, respectively, were 0.2067 and 0.2168 (Table 12). Furthermore, comparison between exotic genotypes and the total landrace population yielded a genetic distance of 0.2072 (Nei's original measure of genetic distance). Nei's unbiased genetic distance ($\hat{D}$) between them was 0.1968 (Table 13).

AR-based population classification of the landrace accessions revealed inter-population genetic distance (D) ranging from 0.0122 to 0.2284. Samples from Wello had the largest genetic distance from samples of Shewa (0.2284), Gojam (0.2284) and Tigray (0.2093). In general, among the pairwise population comparsons made, samples of Shewa, Tigray and Gojam showed the highest genetic distance from samples of Wello, Arsi and Bale. Genetic distance between the other pairwise combinations of populations was very low with the least genetic distance between samples of Tigray and Gojam (0.0122) (Table 14). Comparison based on GRs revealed small genetic distance between samples of different GRs with a single exception of the distance between samples of SEE and CE (0.1756). The least genetic distance (0.0324) obtained was between samples of NWE and CE (Table 15).

Table 12. Nei's original measures of genetic identity and genetic distance in Ethiopian lentil landrace accessions and exotic genotypes

Populations	Wello	Arsi	Exotic genotypes	Bale	Gonder	Shewa	Gojam	Tigray
Wello	****	0.9634	0.8051	0.9356	0.9159	0.8322	0.8430	0.8443
Arsi	0.0373	****	0.8290	0.9700	0.9470	0.8578	0.8642	0.8573
Exotic genotypes	0.2168	0.1876	****	0.8239	0.8132	0.7353	0.7306	0.7303
Bale	0.0666	0.0305	0.1937	****	0.9613	0.8637	0.8682	0.8599
Gonder	0.0878	0.0545	0.2067	0.0395	****	0.9416	0.9479	0.9478
Shewa	0.1836	0.1534	0.3075	0.1466	0.0602	****	0.9798	0.9759
Gojam	0.1708	0.1459	0.3138	0.1414	0.0535	0.0204	****	0.9899
Tigray	0.1693	0.1539	0.3143	0.1510	0.0537	0.0243	0.0101	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 13. Nei's original and unbiased (in bracket) measures of genetic identity and genetic distance between Ethiopian lentil landrace accessions and exotic genotypes

Types of plant materials	Ethiopian lentil landrace accessions	Exotic lentil genotypes (released varieties)
Ethiopian lentil landrace accessions	****	0.8128 (0.8213)
Exotic lentil genotypes (released varieties)	0.2072 (0.1968)	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

5.2.1.4. Genetic Differentiation and Gene Flow

When the landrace accessions from different ARs were considered as independent populations, the average gene diversity (Nei, 1978) among ARs as measured by Dst (Ht–Hs = 0.0793) was slightly lower than the average gene diversity within ARs (Hs = 0.0952). The average gene diversity relative to the total population (Ht; between and within ARs) was 0.1745. The extent of

Table 14. Nei's original measures of genetic identity and genetic distance in Ethiopian lentil landrace populations

Population	Arsi	Bale	Gojam	Gonder	Shewa	Tigray	Wello
Arsi	****	0.9638	0.8360	0.9361	0.8278	0.8278	0.9557
Bale	0.0369	****	0.8407	0.9534	0.8348	0.8309	0.9220
Gojam	0.1791	0.1735	****	0.9370	0.9755	0.9878	0.8094
Gonder	0.0661	0.0478	0.0651	****	0.9292	0.9369	0.8978
Shewa	0.1890	0.1805	0.0248	0.0735	****	0.9709	0.7958
Tigray	0.1889	0.1853	0.0122	0.0652	0.0295	****	0.8112
Wello	0.0454	0.0813	0.2115	0.1078	0.2284	0.2093	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 15. Nei's original measures of genetic identity and genetic distance in Ethiopian lentil landrace accessions in GR-based grouping

Groups	CE	NEE	NWE	SEE
CE	****	0.9327	0.9681	0.8389
NEE	0.0697	****	0.9732	0.9397
NWE	0.0324	0.0272	****	0.9133
SEE	0.1756	0.0622	0.0907	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).
NEE=Northeastern Ethiopia (Wello and Tigray); SEE=Southeastern Ethiopia (Arsi and Bale); CE=Central Ethiopia (Shewa); NWE=Northwestern Ethiopia (Gojam and Gonder).

gene differentiation relative to the total population as given by Gst (Gst = Dst/Ht) was 0.4548. The extent of gene flow given by Nm (Nm = 0.5(1-Gst)/Gst) within the total population of Ethiopian lentil landrace accessions (or among ARs) was 0.5995 (Table 16).

In addition, grouping the landrace accessions into GRs revealed a higher within GR gene diversity (Hsg = 0.1289) than among GR (Dstg = 0.0479). The total gene diversity with reference to the total GR was Ht = 0.1768. The extent of gene differentiation within the total GRs was Gstg = 0.2713, while the extent of gene flow among the GRs (Gstg) was 1.3432 (Table 16).

Table 16. Coefficient of gene differentiation in Ethiopian lentil landrace accessions with and without grouping into GR

Analysis for	Ht/Ht_g[1]	Hs/Ds_g[2]	Dst/Dst_g[3]	Gst/Gst_g[4]	Nm/Nm_g[5]
Indigenous landraces and exotic genotypes	0.2802 (± 0.0359)	0.2049 (± 0.0209)	0.0753	xxxxxx	xxxxxx
Administrative regions	0.1745 (±0.0395)	0.0952 (±0.0130)	0.0793	0.4548	0.5995
Geographical regions	0.1768 (±0.0387)	0.1289 (±0.0193)	0.0479	0.2713	1.3432

Numbers in parenthesis are standard deviations.

Ht = gene diversity in the total landrace population (among and within ARs); Dst = average gene diversity between Ars; Hs = average gene diversity within Ars; [1]Dst_g = represents the average gene diversity in the total group; [2]Ht_g = represents Dst in the context of the subgroups; [3]Hs_g = represents Hs in the context of the groups; [4]Gst_g = represents Gst in the context of the groups; [5]Nm_g = represents Nm in the context of the groups.

5.2.2. Genetic Structure

5.2.2.1. Analysis of Molecular Variance

AMOVA analysis conducted on the landrace populations without grouping indicated the presence of highly significant ($p < 0.0001$, 1023 permutations) variation among ARs. Genetic variation within population (56.28%) was higher than that among population (43.72%) (Table 17). Wright's (1951) gene fixation index (*FST* = 0.43721), which is equivalent to Nei's (1978) coefficient of gene differentiation relative to the total population, was highly significant ($p < 0.001$, 1023 permutations). Population specific *FST*s were also highly significant ($p < 0.001$, 1023 permutations) with the highest *FST* value for samples from Arsi (0.45081). Population specific *FST* values above the average *FST* value were also obtained for samples from Tigray (0.44874), Bale (0.44822) and Gojam (0.43994). Population specific *FST* values below the average *FST* were obtained for samples from Wello (0.43166), Shewa (0.42934) and Gonder (0.41175) being the least (Table 18).

Populations of the landrace accessions were grouped into GRs in the assumption that there was gene flow (represented by seed movement in the case of lentil) as a result of many social, economical, and other factors. Such grouping revealed highly significant variation ($p<0.001$, 1000 permutations) among GRs, among ARs within GRs and within GRs. The highest proportion of genetic variance was attributed to within GR variation (55.91 %). The variation among ARs within geographical regions was also high (39.47 %), whereas the variation among geographical regions was low (4.63 %) (Table 17). F-statistic values (Wright's fixation indices: *FSC*, *FST* and *FCT* which are equivalent to Nei's, 1973 coefficients of gene differentiation) were highly significant ($p < 0.0000$, 1023 permutations) for among GRs (*FCT*=0.04625, 1023 random permutations), within GRs (*FST* = 0.44093; p = 0.0000, 1023 permutations) and among ARs within GRs (*FSC* = 0.41381; p = 0.0000, 1023 permutations). F-statistic value for within GRs was by far higher than that for among GRs (Table 18).

Table 17. Analysis of molecular variance (AMOVA) for Ethiopian lentil landrace populations with and without grouping to GRs

	AR-based landrace populations			Landrace populations grouped into GRs			
	Source of variation			Source of variation			
	Among ARs	Within ARs	Total	Among GRs	Among ARs within GRs	Within GRs	Total
d.f.	6	63	69	3	3	63	69
Sum of squares	127.19	152.30	279.49	68.74	58.45	152.3	279.49
Variance components	1.88	2.42	4.30	0.20	1.71	2.42	4.32
Percentage of variation	43.72	56.28		4.63	39.47	55.91	
P-value	0.0000			0.0083	0.0000	0.0000	

P-values are the probabilities of having a more extreme variance component than the observed values by chance alone. Probabilities were calculated by 1000 random permutations across populations and the significance values were obtained at 1023 random permutations.

Table 18. AR- and GR-specific *FST* indices for Ethiopian lentil landraces

Levels of grouping	Population /Group	Type of indices	Value of F-stat	P-value
Landrace accessions from each AR[1] as independent population	Wello	FST	0.43166	0.00000
	Arsi	FST	0.45081	
	Bale	FST	0.44822	
	Gonder	FST	0.41175	
	Shewa	FST	0.42934	
	Gojam	FST	0.43994	
	Tigray	FST	0.44874	
	Across ARs	FST	0.43721	
Landrace accessions grouped into GR[2]s	Among ARs within GRs	FSC	0.41381	0.00000
	Within GR	FST	0.44093	0.00000
	Among GRs	FCT	0.04625	0.00827

AR[1] = administrative region; GR[2] = geographical region

5.2.2.2. Clustering Analysis

The dendrogram generated from the whole ISSR data (including exotic genotypes and landrace accessions) using the similarity coefficient of Dice (1945) produced two major clusters at 0.76 similarity level. Of the 10 exotic genotypes included in the study, 7 were grouped in to one cluster (cluster II), while the rest 3 (EL-142, Chekol and Ada'a) were grouped into the other major cluster (cluster I) along with indigenous landrace accessions. The first major cluster (cluster I) was further subdivided into two major subclusters at the similarity coefficient level of 0.84, leaving Ada'a as an outlier. Most of the samples from Wello, Arsi, Bale and Gonder were clustered into one subcluster (I_1), while most of the samples from Tigray, Gojam and Shewa were grouped into the other subcluster (I_2; Figure 9).

A separate clustering analysis for Ethiopian lentil landrace accessions based on Dice' (1945) similarity coefficient produced two major clusters at the genetic similarity coefficient level of 0.85. Most of the samples from Wello, Gonder, Bale and Arsi were grouped into the first major

cluster (cluster I), while most of the samples from Shewa, Gojam and Tigray were grouped into the second major cluster (cluster II). Cluster I further subdivided into two subclusters: subcluster I_1 included most samples from Wello subclsuter I_2 was constituted of most samples from Arsi and Bale. However, samples from Gonder were dispersed within the major clusters and subcluster s and did not form a distinct sort of grouping (Figure 10). Similar grouping was observed for population based clustering analysis (Figure 1).

The correlations between cophenetic values generated from the UPGMA tree and Dice's similarity matrices (the tree matrices) were r = 0.93 and r = 0.82, respectively for the entire plant materials and the landraces separately.

5.2.3. Comparison of Morphological and Molecular Data

In order to compare the extent of agreement between dendrograms derived from morphology and ISSR markers, a distance matrix was constructed for each assay and compared using the Mantel (1967) matrix correspondence test.

When the distance matrices generated from all lentil plant materials (both landraces and exotic genotypes) were compared, there was a positive and highly significant ($p < 0.001$, 1000 random permutations) correlation ($r = 0.42$) between the Euclidian and genetic distance matrices. The average Euclidian distance (0.38) with reference to the total plant materials was greater than the average molecular dissimilarity (0.18; Table 19).

On the other hand, a separate Mantel test analysis conducted on dissimilarity matrices generated from morphological and ISSR data of Ethiopian lentil landrace accessions, revealed a positive and highly significant ($p < 0.001$, 1000 permutations) correlation ($r = 0.26$) between the two genetic matrices (Tables 19). The average morphology-based genetic distance (0.50) was greater than the average dissimilarity generated from ISSR data (0.13; Table 19).

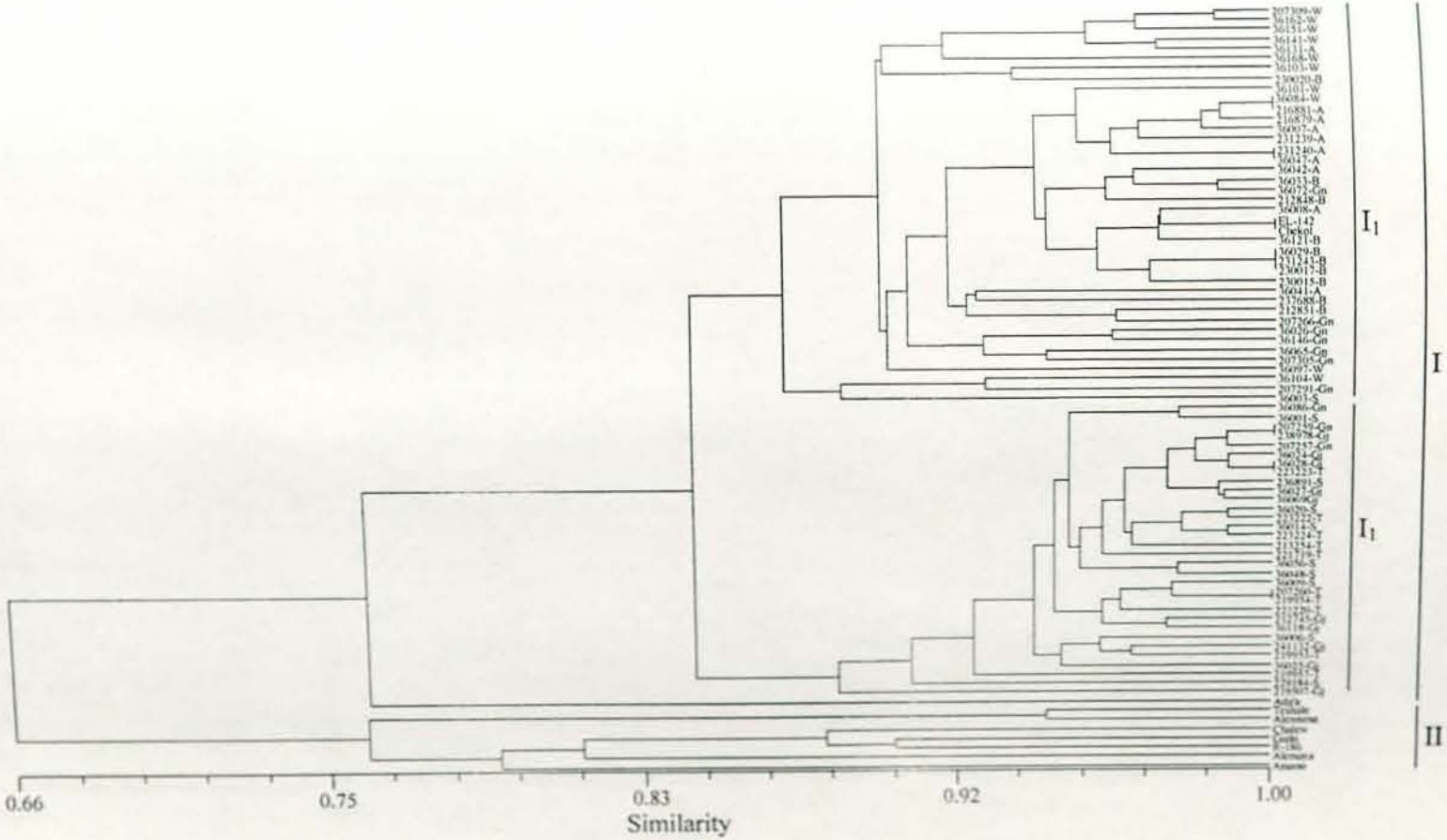

Figure 9. ISSR-based dendrogram of Ethiopian lentil landrace accessions and exotic genotypes derived by UPGMA from Dice's (1945) similarity coefficient.

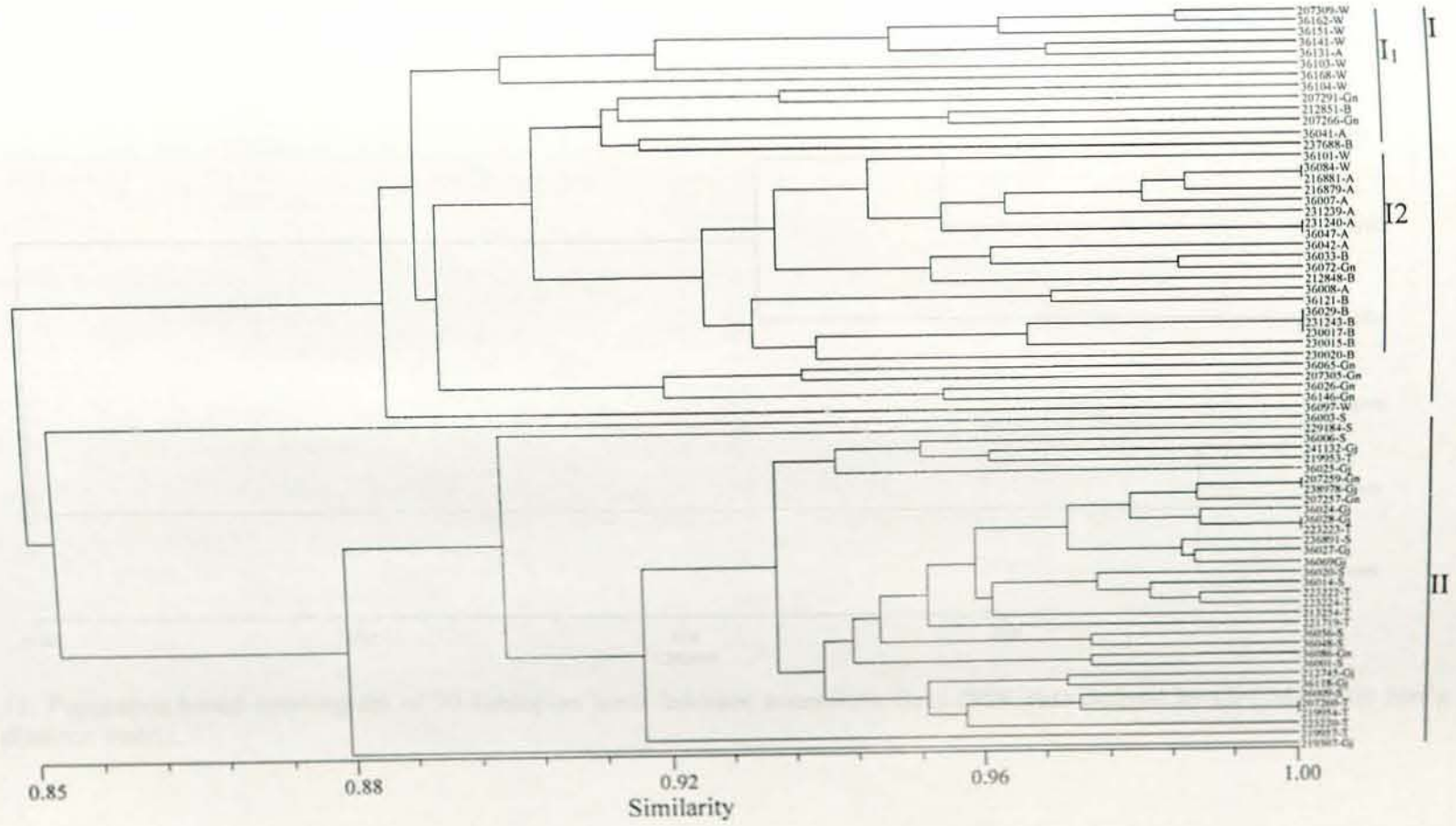

Figure 10. ISSR-based dendrogram of 70 Ethiopian lentil landrace accessions derived by UPGMA from Dice's (1945) similarity coefficient.

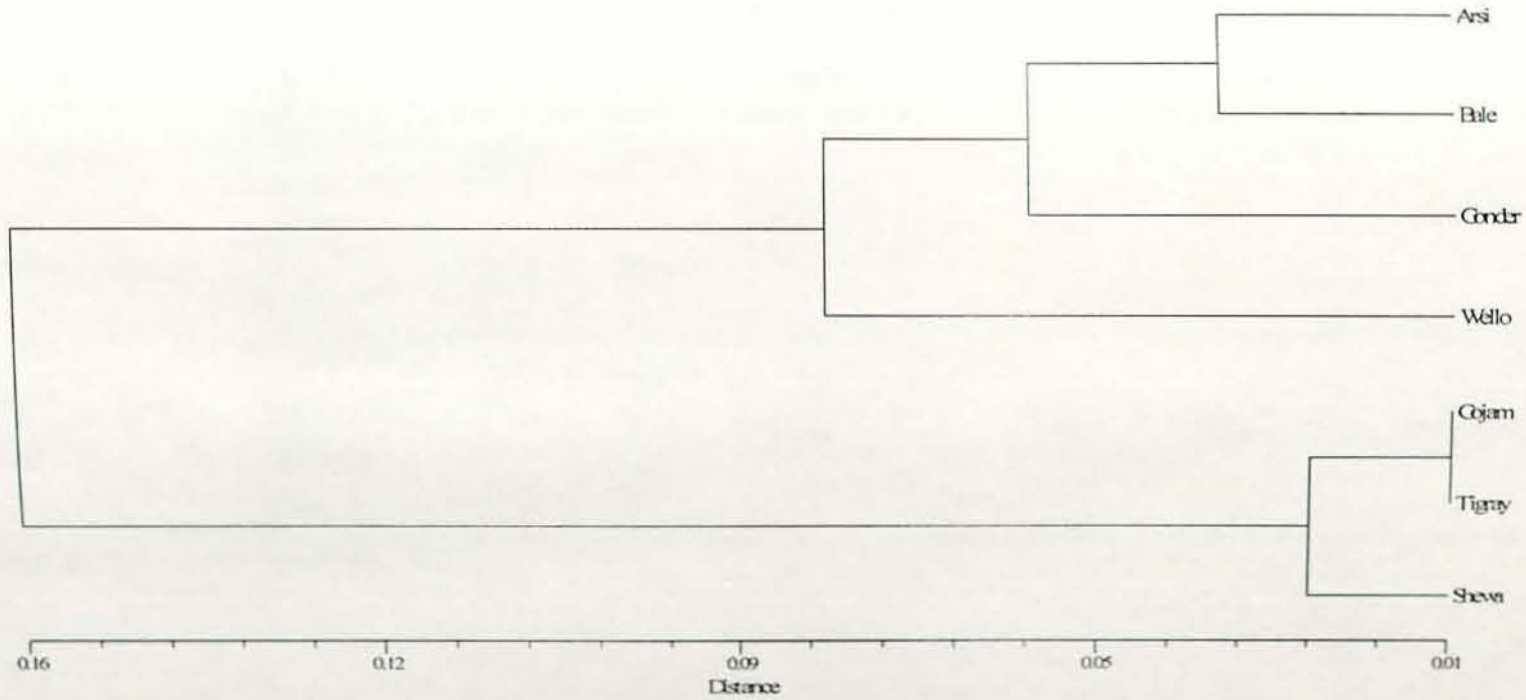

Figure 11. Population based dendrogram of 70 Ethiopian lentil landrace accessions from ISSR data derived by UPGMA from Nei's (1972) distance matrix.

Table 19. Correlation analysis between morphological and ISSR (molecular) marker dissimilarity matrices using Mantel test

Lentil plant materials for clustering	Types of matrix compared	Mean value	Sum of squares	Raw mantel statistic (Z-value)	Sum of products	Correlation coefficient (r)	P-value
All experimental materials (landraces and exotic genotypes)	Morphological distance (X1)	0.38	106.55	239.47	30.35	0.42	0.00000
	Molecular distance (Y)	0.18	49.49				
Landraces accessions separately	Morphological distance (X1)	0.50	140.90	159.65	8.90	0.26	0.00000
	Molecular distance (Y)	0.13	8.23				

Mantel test at 1000 random permutations.

6. DISCUSSION

6.1. Morphological Variability

In this study, seventy lentil landrace accessions collected from seven different regional sites of Ethiopia and eleven exotic genotypes were used. The variability within all the experimental plant materials, landraces separately and between landraces and exotic genotypes were assessed.

The wider variation in the measured agronomic traits between all the lentil plant materials, within the landraces, and between the landraces and exotic genotypes based on average and range of the measured traits has got a good implication in improvement programs.

6.1.1. Analysis of Variance

The highly significant variation between the lentil materials for the agro-morphological traits measured indicates the presence of high degree of phenotypic diversity between the lentil materials evaluated and implies the availability of great potential for improvement. Similar results were reported by Yadav (1995) for days to maturity, hundred seeds weight, biological yield, harvest index and seed yield.

6.1.2. Phenotypic and Genotypic Coefficients of Variation

Very wide PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) were observed for number of secondary branches per plant, number of seeds per plant, seed yield, biomass and number of pods per plant, which agrees with the findings of Jain *et al.* (1995) Chakraborty and Haque (2000) in other lentil materials. On the other hand, narrow PCV and GCV were observed for days to flowering and maturity, and plant height, for the entire experimental plant materials, which agrees with the findings of Thakur and Banjpai (1993) for days to maturity, Singh and Singh (1991) for days to flowering and plant height and disagrees with the findings of Jain *et al.* (1995), and Chakraborty and Haque (2000) for plant height. The results of the present study, therefore, indicate the possibility of improving specific trait(s) by intercrossing the lentil materials evaluated.

Indigenous lentil landraces of Ethiopia expressed better mean values and wider phenotypic variation in important yield parameters such as number of secondary branches per plant, number

of pods and seeds per plant compared to exotic genotypes, whereas exotic genotypes showed a wider phenotypic variation for hundred seed weight, days to flowering and maturity as compared to the landraces. Indigenous landraces showed very narrow PCV and GCV for days to flowering and maturity. This result indicates that the landraces have important traits distinct from exotic genotypes. This implies the need to undertake further collections to enrich the gene pool. Furthermore, the result suggests the possibility of improving the aforementioned traits of the landraces or improved varieties by intercrossing them.

When samples of landraces from different ARs were compared in separate analysis, they expressed wider phenotypic variability for different types of traits with the exception of days to flowering and maturity, and plant height. Geletu *et al.* (1996) reported consistent regional differences among Ethiopian lentil landraces for time to flower and maturity, 100-seed weight, number of seeds/pod and plant height in 156 Ethiopian landrace populations of lentil. In the present study, samples from Wello, Bale, Gonder and Arsi were characterized by high number of primary and secondary branches, pods, seeds, biomass and seed yield per plant. Consistent with previous report (Geletu *et al.*, 1996), samples from Tigray, Gonder and Wello were characterized by larger (relatively) seed size on average basis, whereas samples from Shewa and Gojam were the least distinctive group. The result suggests the possibility and high potential for improvement through intra- or inter-population breeding activities. Furthermore, the presence of different types of characters in different populations (ARs) suggests the need for further collection of the landrace accessions for conservation of more wider variability (*ex-situ* gene-pool enrichment) before possible replacement by "improved varieties" of narrow genetic base, which can be considered as a type of genetic erosion.

6.1.3. Euclidian Distance

In general, exotic genotypes were more distantly related to the different landrce populations than do the landrace populations to one another. Similary, the landraces showed a range of pairwise population dissimilarities, but generally, samples from Tigray and Wello showed the highest Euclidian distance from all other populations. Such results have higher importance in choosing populations for improvement activities since distantly related individuals or populations are expected to be more heterotic.

6.1.4. Clustering Analysis

A dendrogram generated from the standardized morphological data on Euclidian distance matrix with UPGMA cluster analysis classified the entire experimental materials into two major clusters. One of the major clusters (cluster II) comprised most samples from Wello and Bale, and some samples from Arsi, Gonder and Gojam in which Chekol was included from exotic genotypes. Accessions of Wello were characterized with above average to higher mean values for all the characters. Therefore, this cluster identified mainly accessions from Wello and some from other ARs with similar morphological characters. The other major cluster (cluster I), comprised all samples from Tigray and Shewa, most exotic genotypes, samples from Gojam, Gonder, Arsi and Bale suggesting the morphological similarity of most of the accessions/genotypes. Further subdivision of cluster I into two subclusters mainly identified sumples of Tigray and few samples of Gonder, Arsi, Bale and exotic genotypes in one group (subcluster I_2). The other subcluster (subcluster I_1) identified most exotic genotypes and samples of Shewa and Gojam and few samples of Gonder, Bale, Arsi and Wello. Samples from Arsi, and Gonder were found dispersed within the three distinct groups and did not tend to form a distinct cluster or subcluster.

A similar but separate clustering analysis conducted on Ethiopian lentil landraces also produced two major clusters for Ethiopian lentil landraces. One of the major clusters (cluster II) comprised most samples from Wello and some samples from other ARs. Thus, this cluster identified most samples of Wello. The other major cluster (cluster I) was formed by all samples from Tigray and Shewa, most samples from Arsi, Gonder and Bale. This major cluster was further subdivided into two subclusters. The second subcluster (I_2) identified most samples from Tigray, while most samples from Gojam, Arsi, Shewa, Bale, Gonder formed the other subcluster (I_1). Therefore, based on this result, three major groups of landraces can be identified: Group I (SEE-NWE-CE (accessions of Arsi-Gojam-Shewa-Bale) and others), Group II (accessions of NE (Tigray) and others) and Group III (accessions of NEE (Wello) and others). However, Geletu *et al.* (1996) provided geographical-region based description of lentil landrace populations of Ethiopia, where the lentil of the West Highlands was early maturing and short, that of the North Highlands was large-seeded, whereas lentils from the Central Highlands were the least distinctive group.

The cophenetic correlations of the UPGMA tree and Euclidian distance matrices of the entire plant materials (r = 0.81) and the landraces separately (r = 0.72) showed good and poor fit, respectively (interpreted according to Rohlf (2004)). However, a low cophenetic correlation coefficient does not mean that the dendrogram has no utility, but only indicates that some distortion might have occurred (Mohammadi and Prasanna, 2003).

6.2. Molecular Diversity

6.2.1. Genetic Variation

ISSR markers are important to study intraspecific variations in plant species, as they are effective in detecting very low levels of genetic variation (Zietkiewicz *et al.*, 1994). In lentil, RAPDs and microsatellite-primed PCR markers detected a low level of useful polymorphic bands, while ISSRs revealed a higher degree of variation (Sonnante and Pignone, 2001).

In this study, ISSR markers were used to assess the molecular diversity of the lentil materials based on bulked leaf sample analysis of five plants per accession/genotype. In this technique, the between accession variation is nullified due to pooling of samples, thereby minimizing the total genetic diversity. Nonetheless, the technique revealed higher genetic diversity in the present study.

ISSR primer 881 is a penta-nucleotide repeat while all the rest are di-nucleotide repeats. Among the ISSR scorable fragments, primer 881 produced high molecular weight (larger) ISSR fragment while primer 812 produced the shortest fragment. Intermediate fragment sizes were produced by 818 and 835, the latter of the two with large fragment size.

6.2.1.1. Genetic Diversity and Distance

The term gene diversity has been used in an informal sense for RAPD marker analysis since it is likely that RAPDs will often contain only noncoding DNA (Lynch and Milligan, 1994). However, the term gene diversity seems to apply more appropriately to ISSR marker (compared to RAPD) since the ISSR primers are designed from the SSR motifs, which often flank coding sequences (a gene).

In comparison to exotic genotypes, Ethiopian lentil landraces were found less polymorphic and less diverse. This is because exotic genotypes are improved varieties, which have come from a series of breeding activities. On the average, the landraces were separated from exotic genotypes by considerable genetic distance. However, the distance from exotic genotypes varied from one population (AR) to another. Samples of Shewa, Tigray and Gojam depicted a relatively higher genetic distance, where as other populations showed lower genetic distance from the exotic genotypes. Therefore, population specific improvement programs designed between exotic genotypes and distantly related populations are expected to yield good results, because more distantly related populations/plants are more heterotic.

Among the 47 bands scored in Ethiopian landrace populations, 29 (51.79 %) were polymorphic, which revealed high genetic diversity (Ht = 0.1745), which is by far greater than the within species diversity (0.049) of the cultivated lentil reported by Ferguson *et al.* (1998) from RAPD data of 100 accessions from 10 countries. In fact, a number of literatures indicate that RAPD detects low level of diversity (Abo-elwafa *et al.*, 1995; Ahmad *et al.*, 1996; Ford *et al.*, 1997; Ferguson *et al.*, 1998; Sonnante and Pignone, 2001), while ISSRs reveal a higher degree of variation (Sonnante and Pignone, 2001) in lentil. Samples from Gonder, Wello and Shewa were more polymorphic and exhibited high intra-population diversity. This result suggests that these populations have a high potential (or wider possibilities) for intra-population improvement practices based on selection. In addition, the results suggest that, in relative terms, collections at IBC covered more diversity from these ARs. On the contrary, samples from Arsi, Bale and Tigray were found less polymorphic and less diverse. This suggests that either there is a relatively narrow genetic diversity within these ARs or the available diversity within the ARs was not well represented through extensive collection activities compared to the most diverse ones. The latter case invites further germplasm collection activities from these ARs to enrich the germplasm gene pool. Furtheremore, samples of Shewa, Gojam and Tigray expressed wider genetic separation from samples of Wello Arsi and Bale. In addition, samples of Wello and Gonder showed a moderately high genetic distance between each other. The other pairwise population combinations showed moderately low to very low (relatively) genetic distance from one another. Therefore, inter-population improvement activities between the pairwise

combinations of the landrace populations (ARs) with high genetic distances are expected to produce good results.

GR-based analysis showed that samples from NEE followed by NWE and CE were more polymorphic and more diverse, whereas those from SEE were less polymorphic and less diverse. NEE-GR was found more polymorphic and diverse since it was composed of samples from Wello, with high polymorphic loci and intra-population diversity, and samples of Tigray, with low number of polymorphic loci and intra-population genetic diversity; however, the ARs are separated by high genetic distance. Therefore, the high intra-population genetic diversity of samples from Wello and its higher genetic distance from samples of Tigray contributed to the high genetic diversity observed in samples of NEE-GR. Similarly, the high within population diversity of samples from Gonder mainly contributed to the high diversity observed in samples of NWE-GR. Furthermore, all GRs showed lower genetic distances from one another with a single exception of the distance between SEE and CE. This higher genetic distance was due to the fact that each constituent ARs of the GRs were distantly related to one another in a pair-wise population comparison. In general, GR based genetic analysis of the landraces showed lower genetic distance between most pair-wise combinations of the GRs.

6.2.1.2. Genetic Differentiation and Gene Flow

Population (AR)-based analysis indicated that within population gene diversity was slightly higher than among population diversity. There was higher level of gene differentiation among populations, which could be attributed to mutation, random genetic drift and differential selection pressure (by the environment) on the loci assessed. This can be justified by the higher within population genetic diversity (H_S = 0.0952) in some populations as compared to the among population genetic diversity (D_{ST} = 0.0793). This was further confirmed by higher genetic distance between samples of Shewa, Gojam and Tigray with samples of Wello, Arsi and Bale, and between samples of Gonder and Wello. Furthermore, there was also high level of gene flow (Nm = 0.5995) among landrace populations (ARs). This was supported by the lower among population gene diversity (D_{ST} = 0.0793) as compared to the within population diversity (H_S = 0.0952) and the lower genetic distances between most of the pairwise population combinations.

GR-based analysis, too, revealed very high within GR gene diversity (H = 0.1289) than among GR diversity (Dst = 0.0479). It is expected that within GR diversity is greater than within AR diversity, while among AR gene diversity is greater than among GR diversity. Furthermore, GR-based gene differentiation (Gstg = 0.2713) was lower than the population (AR)-based gene differentiation (Gst = 0.4548). On the other hand, there was very high level of gene flow (Nmg = 1.3432) among GRs as compared to the population-based gene flow (Nm = 0.5995).

In general, results of estimates of gene flow indicate the presence of moderately high level of gene flow among the ARs and very high level of gene flow among GRs. Since lentil is a self-pollinated crop, gene flow could occur only in the form of seed exchange within or between the ARs/GRs, which could be attributed to social and economic factors. Among these factors are movement of people from one place to another along with their own seeds, purchase of lentil seeds from the neighboring administrative regions for cultivation.

6.2.2. Genetic Structure

6.2.2.1. Analysis of Molecular Variance

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation, and population isolation), genetic drift, mating system, gene flow and selection (Schaal *et al.*, 1998).

Generally, the breeding system of flowering plant species greatly affects population genetic differentiation (Hamrick and Godt, 1989). Estimates of genetic differentiation between populations of outcrossing species based on AMOVA derived by analyzing RAPD markers have usually been < 28 %. For inbred species, estimates of interpopulation genetic variation have usually been > 70 % (Nybom and Bartish, 2000). However, it might be argued that higher inter-population genetic variation estimate for self-pollinated crops suggested above might not hold in some cases (of food crops, for instance) where there is high rate of gene flow (seed exchange) through human involvement. In the present study, AMOVA analysis conducted on landrace populations (ARs) revealed lower among ARs genetic variation (43.72 %; although highly significant, $p < 0.0001$), than the within ARs genetic variation (56.28 %), which agrees with the population-based genetic diversity analysis of the present study discussed above. The result did

not agree with the general view that self-pollinated species depict higher among population genetic variation than within population. This disagreement can be attributed to the high rate of seed exchange through movement of people ("migration") from one ARs to another along their seeds, particularly in the past but it seems that the trend is still going on in Ethiopia. In addition, since lentil is a food crop it moves through complex marketing networks from one AR to another in the form of seed/grain where it might be used for cultivation. Therefore, the deviation of the result from the general view could be explained by the moderately high rate of seed exchange between the ARs due to "migration" and marketing networks.

The global *FST*, analogous to Wright's gene fixation index (Fst), is the measure of the level of genetic structuring within the total population (between subpopulations). In this study, the global *FST* (0.43721) was highly significant ($p < 0.001$, 1023 permutations) indicating that there is a moderate level of genetic structuring within the total landrace population. This also agrees with the results from genetic differentiation (Gst) analysis. Population specific *FST* indices (Weir and Hill, 2002) could be computed such that the global *FST* index would be a weighted average of population specific *FST* values. These coefficients are provided mainly to see if some populations do contribute differently than others to the average *FST*, which could be indicative of special evolutionary constraints in these populations (selection, bottleneck, etc...). Intuitively, population-specific coefficients would represent the degree of evolution of particular populations from a common ancestral population, which would have split into all the demes considered in the genetic structure (Weir and Hill, 2002). In this study, population specific *FST* values were highly significant ($p < 0.001$, 1023 permutations) for all populations and ranged from 0.41175 for samples of Gonder to 0.45081 for samples of Arsi. This indicates that all the populations contributed nearly equally to the average *FST* implying the absence of special evolutionary constaraints in Ethiopian lentil landraces. Samples from Arsi, Tigray, Bale, Wello and Gojam (in the order of magnitude of their specific *FST* values) showed *FST* values above the average *FST* suggesting that these populations show certain degree of evolution from the total landrace population.

Ethiopian landrace accessions were grouped into four based on geographical proximity of the ARs to one another in the assumption that there was gene flow (represented by seed movement in

the case of lentil) resulting from many social and economic factors. Such grouping revealed highly significant variation ($p < 0.001$, 1000 permutations) among geographical regions (GRs), among ARs within GRs and within GRs. The highest proportion of genetic variance was attributed to within GR variation (55.91%). The variation among ARs within GRs was also high (39.47%), whereas the variation among GRs was extremely low (4.63%). This result agrees with the analysis of population differentiation (Gst) above. The lower proportion of genetic variance attributed to among GRs than within GRs also indicates the high rate of gene flow (seed exchange) between the GRs. When compared with among AR variation, among GR variation is very low. This confirms the expectation that there is high rate of seed exchange (gene flow) between the neighboring ARs than those located far apart from one another.

On the other hand, geographical region (GR) specific F-st indices were highly significant ($p < 0.001$, 1023 permutations) among GRs ($FCT = 0.04625$, $p < 0.0001$), within GRs ($FST = 0.44093$; $p < 0.0001$) and ARs within GRs ($FSC = 0.41381$; $p < 0.0001$). In this case, the highest F-statistic value was obtained for within GR variation than among ARs within GRs, while that between GRs was very minimal. This indicates that there is a relatively high genetic structuring within GRs than between ARs within GRs. This also indicates the presence of a slightly higher rate of gene (seed) flow between ARs within GRs than within GRs. The minimal genetic structuring observed between GRs indicates the presence of high rate of gene (seed) flow between the GRs than among ARs within GRs.

6.2.2.2. Clustering Analysis

Two major clusters were obtained at 76 % similarity level using Dice's (1945) similarity coefficient. Most exotic genotypes (7) tended to form distinct cluster while some of them were grouped into the other major cluster along with the landrace accessions. This indicates that ISSR marker tended to distinguish exotic genotypes from indigenous landrace accessions of lentil at 76% similarity level.

Similarly, two major clusters were obtained from a separate clustering analysis conducted for Ethiopian lentil landrace accessions at 85% similarity. Most of the samples from Wello, Gonder, Bale and Arsi were grouped into the first major cluster (cluster I), while most of the samples from

Shewa, Gojam and Tigray were grouped into the second major cluster (cluster II). Cluster I further subdivided into two subclusters, which identified samples of Wello in one subcluster (subcluster I_1), and samples of Arsi and Bale in another subcluster (subcluster I_2). In general, samples Gonder were dispersed within the major clusters and did not form a distinct sort of cluster or subcluster. This confirms the result from genetic structuring that there is a moderate level of gene flow between the different ARs of Ethiopia so that all samples from the same AR did not fall into a separate cluster/subcluster. In addition, the appearance of most of the samples from the same AR in the same cluster, further confirms the AMOVA result that there is also a moderate level of gene differentiation.

The cophenetic correlations of the UPGMA tree and Dice's similarity matrices (tree matrices) for the entire plant materials ($r = 0.93$) and the landraces separately ($r = 0.82$), were very good and good, repectively (interpreted subjectively according to Rohlf, 2004).

6.3. Correlation of Morphological and Molecular Dissimilarity Matrices

The correlation between morphology based and ISSR-marker based matrices for the whole plant materials evaluated (landraces and exotic genotypes; $r = 0.42$, $p < 0.001$) and separately for landraces ($r = 0.26$, $p < 0.001$), revealed positive and significant correlation. No reports were obtained comparing the extent of agreement between morphological and any of the molecular markers in lentil. However, Yoseph *et al.* (2005) working on Ethiopian traditional maize accessions reported a correlation value of $r = 0.43$ and $r = 0.39$ between 15 morphological characters and SSR and AFLP, respectively. Furthermore, Roldan-Ruiz *et al.* (2001) working with 16 rye grass varieties reported a correlation value of $r = -0.06$ between AFLP and 15 morphological characters. In addition, Bolaric *et al.* (2005) working on 12 phenotypic characters of 22 perennial ryegrasses reported a correlation value of $r = 0.10$ between morphological and molecular (RAPD) marker. Therefore, in comparison with the ryegrasses, Ethiopian lentil landraces appear to be more stable as suggested by the higher agreement between phenotypic and ISSR based molecular distances and indicates that the observed phenotypic variation was at least partly caused by genetic factors. However, when compared to the traditional Ethiopian maize accessions, Ethiopian lentil landrace accessions seem to be less stable. Nonetheless, the positive and significant correlation obtained between the two distance matrices indicate that they likely

reflect the same pattern of genetic diversity and validate the use of the data to calculate the different diversity statistics for the lentils. Nevertheless, the genetic relationship observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais *et al.*, 2000).

7. CONCLUSION

In the present study, morphological variability and molecular diversity of the landraces separately and in comparison with exotic genotypes were assessed to provide information with respect to their genetic variation.

Morphological variability study showed the presence of significant variation within the landrace accessions and exotic genotypes altogether for all the measured agro-morphological traits. Although the variation between the accessions/genotypes was significant for days to flowering and maturity, they showed narrow level of phenotypic and genotypic coefficients of variation as compared to other characters, suggesting low opportunity for improvement of these traits through selection.

Comparison of exotic genotypes to landrace accessions revealed that both groups were important for one or more character(s) that was lacking in either of them. For instance, landraces showed higher average value and wider level of variation for number of primary branches, pods and seeds per plant, whereas exotic genotypes showed higher average values for seed yield, biomass, harvest index, days to flowering and maturity and plant height. Nevertheless, the landrace accessions showed broader variation for all the characters except for days to flowering and maturity.

Within the landraces, too, there was a broader range of variation between landrace populations from different ARs for the traits that might be lacking in either of them. Therefore, both inter- and intra-population improvement activities could be carried out to improve the character of interest. Furthermore, the Ecuilidian distance between populations could provide a landmark on which population(s) to use.

In molecular analysis of the present study, bulk samples of five individual plants per accession were used for ISSR fingerprinting. The results revealed high genetic diversity within all the plant materials considered, and therefore validate the usefulness of bulk sample analysis for diversity assessment in lentil. This technique is economical and rapid method of assessing genetic variation in a large set of germplasm by representing an accession with optimum number of

individual plants. Bulk analysis technique, originally designed for mapping experiments by Michelmore *et al.* (1992), relies on the differences between (not within) the bulked samples (accessions and genotypes in the present study).

In comparison to the landraces, exotic genotypes were found more polymorphic and diverse, which might be due to the fact that they have come from a series of improvement activities.

Separate analysis of the Ethiopian lentil landraces revealed high level of genetic diversity within the samples from Shewa, Wello and Gonder. Relatively, lower genetic diversity was observed for all other populations with those from Bale and Arsi being the least diverse. In general, populations with high intra-population genetic diversity were genetically distantly related to populations with lower intra-population genetic diversity. GR-based diversity assessment revealed the least within GR diversity for samples from SEE and the highest for samples from NEE. On the other hand, samples from CE were genetically more distantly related to samples from SEE.

There was moderately high level of genetic differentiation and gene flow among the different ARs. Furthermore, there was very high level of gene flow but lower coefficient of genetic differentiation among the different GRs of Ethiopia (relative to that observed among ARs).

Analysis of genetic structure for Ethiopian lentil landraces revealed that the highest proportion of genetic variation was attributable to within AR than among AR variation. Similarly, the highest proportion of variation was observed within GR variation, while the proportion of variation attributable to among GRs was very small, although significant. This confirmed the results of Gst that there was a moderately high level of gene flow and genetic differentiation among the different ARs; and very high level of gene flow but low level of genetic differentiation among the different GRs. This was further confirmed by *FST* values with reference to the total ARs and GRs. On the other hand, the AR-specific *FST* values indicated the absence of evolutionary constraints in all the populations where each population contributed to the average *FST* almost equally.

Clustering analysis from the standardized agro-morphological data clustered the landraces (with and without exotic genotypes) into two major clusters with three distinct groups. Similarly, the landraces (with and without exotic genotypes) were clustered into two major clusters with three distinct groups based on molecular data. There was highly significant correlation between the distance matrices derived from the Euclidian distance matrix of morphological data and Nei's (1972) genetic distance matrix derived from molecular data. The existence of highly significant correlation between the two types of data indicates the stability of the lentil landraces (and exotic genotypes), which also implies that the observed phenotypic variations were at least partly attributed to genetic factors. Furthermore, the agreement of the two methods validates the use of both approaches for characterizing the genetic diversity in lentils.

8. RECOMMENDATIONS

Plant genetic resource conservation strategies, informed by an understanding of the genetic variation within species, are likely to result in a wider representation of conserved diversity in *ex situ* gene banks and *in situ* genetic reserves.

Ethiopian lentil landraces are susceptible to various biotic and abiotic stresses, small seeded and low yielders, which discouraged breeders from designing improvement activities for the landraces. Yet, compared to exotic genotypes, they have better average performances and wider variability for some characters. Therefore, breeders should be encouraged to design improvement activities targeting improvement/amendment of the landraces for dual purposes: for utilizing the best characters from the best germplasm types (indigenous landraces vs exotic genotypes) and for *in situ* conservation of the genes and genetic diversities of indigenous landraces. This can be done by a combination of two or more breeding techniques: selection of the most appropriate individual plant from the most appropriate population and the various crossing techniques. Furthermore, the wider variability observed in the landraces should also encourage IBC for further collection activities so as to conserve more variable accessions.

Molecular analysis of the present study revealed a relatively low genetic diversity for samples of Bale, Arsi, Tigray and Gojam. Assuming that the accessions studied represent the genetic diversity at IBC collected from the specified ARs and that more diversity is available in these ARs, therefore, further collections should be made to cover more diversity from the ARs with low genetic diversity (Arsi, Bale, Gojam and Tigray). On the other hand, apart from targeting areas of low genetic diversity for further collection and other forms of conservation, an approach in which regions of high diversity contribute more accessions than those with a low diversity may be useful. In this approach, therefore, Gonder, Welllo and Shewa should be represented by more number of accessions, which invites further collection activities in these ARs.

Furtheremore, the genetic variability of Ethiopian lentil landrace accessions is not well characterized even with morphological traits. Therefore, further studies should be encouraged so as to generate information on their diversity, which is important for improvement and further conservation programs.

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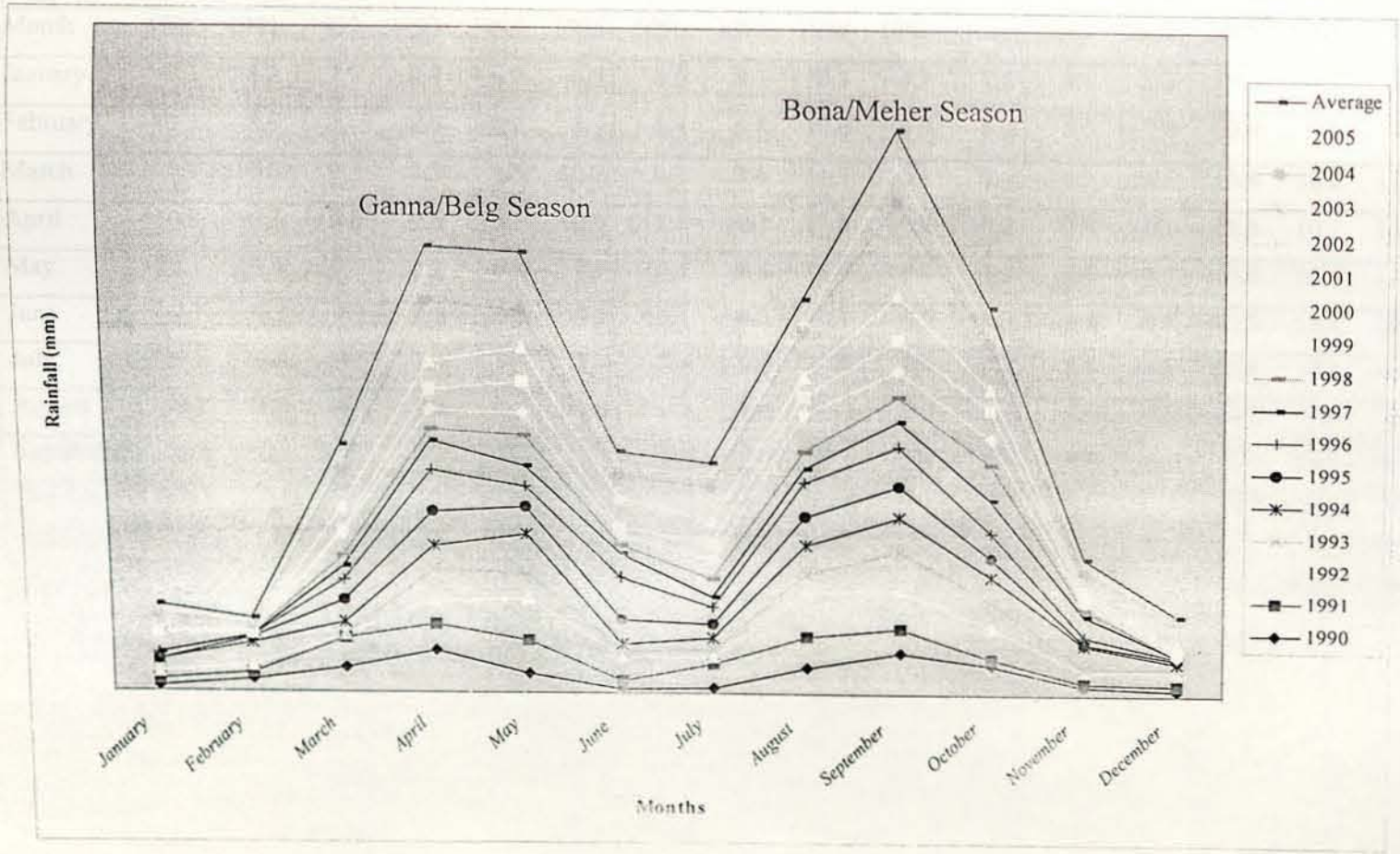
10. APPENDICES

Appendix 1. Total monthly and average annual rainfall (mm) data of 16 years at Sinana Agricultural Research Center

Month	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Average
January	23.0	22.4	29.8	47.4	0.0	0.0	24.3	1.2	75.0	0.0	1.8	0	27.6	1.7	36.5	23.3	19.6
February	46.3	27.1	29.5	85.5	0.0	16.8	7.6	0.0	5.3	0.0	0.0	12.1	0.0	0.0	23.4	11.3	16.6
March	94.7	108.2	13.4	0.0	52.6	80.6	80.8	57.4	38.3	83.7	13.3	86.6	62.4	44.3	39.1	36.4	55.7
April	162.8	95.4	95.1	117.8	96.0	133.1	158.8	111.0	48.0	75.5	76.6	103.3	66.1	70.7	90.9	105.6	100.4
May	71.2	125.8	165.2	94.5	157.5	102.4	83.6	78.1	115.9	89.0	120.4	126.2	60.5	40.6	35.2	125.6	99.5
June	16.3	31.4	93.9	18	26.2	90.6	171.4	91.4	35.2	38.4	31.4	110.9	51.2	23.1	13.3	39.1	55.1
July	21.2	90.7	30.6	25.2	46.1	53.1	65.3	41.0	74.3	71.2	68.8	81.0	42.9	45.9	46.3	40.3	52.7
August	97.8	124.3	168.7	73.5	116.8	108.5	136.6	53.6	67.1	144.4	64.2	74.9	33.8	52.2	85.5	28.0	89.4
September	157.6	93.2	156.8	148.3	137.2	116.1	154.7	96.9	94.4	101.9	121.8	162	89.5	129.3	119.7	134.2	125.9
October	114.5	25.7	118.9	98.1	103.5	73.4	100.1	124.3	138.8	97.6	116.8	76.8	81.6	41.7	51.4	43.5	87.9
November	36.5	10.1	43.5	17.0	85.0	9.2	24.0	83.3	23.5	27.8	26.6	28.3	10.6	38.0	15.2	24.0	31.4
December	22.0	16.4	58.0	8.9	22.7	9.3	10.9	5.9	0.5	0.0	10.7	12.3	36.4	47.4	28.6	0.0	18.1
Total	863.9	770.7	1003.4	734.2	843.6	793.1	1018.1	744.1	716.3	729.5	652.4	874.4	562.6	534.9	585.1	611.3	752.4

Appendix 2. Bimodal pattern of rainfall distribution at Sinana Agricultural Research Center based on 16 years' data



Appendix 3. Average monthly and annual minimum temperature (^{0}C) data of 16 years at Sinana Agricultural Research Center

Month	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Average
January	7.3	8.2	7.9	8.9	6.9	6.6	8.5	8.1	10.1	8.3	7.0	8.1	8.3	9.3	9.7	7.8	8.2
February	10.3	8.6	9.4	8.6	8.2	8.9	8.9	6.8	10.6	8.6	8.0	8.5	7.9	9.9	9.3	9.3	8.9
March	9.9	10.0	9.8	8.5	9.6	10.2	10.3	9.6	11.7	10.1	9.5	10.3	10.1	10.6	10.0	11.0	10.1
April	10.3	10.3	10.8	9.6	10.3	10.6	11.1	10.7	11.8	10.8	10.2	10.8	10.5	11.5	10.7	11.1	10.7
May	10.1	10.4	10.1	10.2	9.9	9.9	10.4	9.8	11.9	8.3	10.8	10.6	10.7	10.8	10.8	11.0	10.4
June	9.7	9.7	9.3	9.8	10.2	9.8	10.1	9.3	10.9	9.9	10.3	9.8	9.8	10.5	10.4	10.3	10.0
July	9.7	9.8	9.1	9.5	9.8	9.7	9.4	9.9	10.8	9.9	10.5	9.5	9.9	10.4	9.3	10.2	9.8
August	9.1	9.6	9.6	9.6	9.7	9.9	9.0	9.6	11.1	9.8	10.1	9.7	9.9	10.2	9.4	9.7	9.8
September	10.4	10.3	9.1	9.2	9.7	9.7	10.0	9.6	10.5	10.1	9.9	9.9	9.6	9.4	10.0	10.3	9.9
October	8.7	9.1	9.6	9.5	8.7	9.9	8.6	10.7	11.4	10.1	10.5	9.7	10.3	9.3	9.2	9.7	9.7
November	8.2	7.6	7.9	7.8	7.9	7.7	7.7	10.3	8.1	7.5	8.2	8.1	8.3	9.3	8.0	8.1	8.2
December	7.1	7.8	8.4	6.8	6.5	7.5	6.5	8.9	6.3	7.4	7.6	7.6	10.2	8.1	8.3	6.9	7.6
Average	9.2	9.3	9.2	9.0	9.0	9.2	9.2	9.4	10.4	9.2	9.4	9.4	9.6	9.9	9.6	9.6	9.4

Appendix 4. Average monthly and annual maximum temperature (^{0}C) data of 16 years at Sinana Agricultural Research Center

Month	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Average
January	21.7	22.9	21.8	19.9	22.1	23.2	21.8	22.8	20.3	22.5	22.6	22.9	22.4	24.2	21.8	22.3	22.2
February	21.9	23.3	21.2	20.4	23.7	23.6	23.5	23.4	22.2	23.1	23.1	23.5	24.2	27.6	22.3	23.4	23.2
March	20.8	22.0	22.3	22.1	22.9	20.9	21.8	22.9	23.0	20.8	23.6	22.4	23.0	23.5	22.6	22.1	22.3
April	20.5	20.9	21.3	19.4	20.8	20.8	20.3	19.9	22.6	21.5	22.0	22.0	23.2	22.0	20.0	21.1	21.1
May	21.8	20.6	20.9	20.2	20.2	21.4	19.9	20.2	21.8	20.7	21.2	22.2	23.3	22.4	22.7	20.2	21.2
June	22.7	21.7	20.5	21.3	22.0	22.3	19.4	20.4	22.2	21.0	21.2	20.3	23.0	21.4	20.9	20.8	21.3
July	22.5	20.4	20.8	20.8	21.6	21.4	19.9	20.8	21.3	20.3	21.1	20.8	22.5	20.9	21.0	20.8	21.1
August	21.4	20.5	20.4	20.7	20.4	21.2	20.2	21.2	20.7	20.2	20.2	20.8	22.3	20.4	20.6	21.4	20.8
September	17.9	20.8	19.7	20.1	20.3	19.8	20.2	20.6	20.0	19.9	19.6	20.2	22.2	20.5	19.9	20.6	20.1
October	19.8	21.7	18.9	18.4	19.5	18.7	19.7	19.0	19.3	18.7	18.7	19.7	20.4	19.9	19.4	19.2	19.4
November	20.1	20.5	18.8	19.3	20.0	19.7	21.2	19.5	19.2	19.5	19.8	19.6	22.4	21.0	21.0	19.8	20.1
December	21.5	20.6	20.0	21.4	21.4	21.1	21.1	20.2	20.8	20.8	21.3	21.8	22.0	20.1	21.7	21.3	21.1
Average	21.1	21.3	20.6	20.3	21.2	21.2	20.8	20.9	21.1	20.7	21.2	21.4	22.6	22.0	21.2	21.1	21.2

Appendix 5. Definition of the agro-morphological traits used in the study

1. DTF (Days to flowering): Number of days from planting to appearance of the first flower.
2. DTM (Days to maturity): Number of days from planting to physiological maturity.
3. PH (Plant height): The above ground height of the plant.
4. NPB (Number of primary branches): The number of branches extending from the main stem.
5. NSB (Number of secondary branches): The number of branches extending from the primary branch.
6. NP (Number of pods): Total pod count.
7. NS (Number of seeds): Total seed count.
8. HSW (Hundred seed weight): The weight of hundred randomly sampled seeds.
9. Biom (Biomass): Above ground weight.
10. SY (Seed yield): The weight of total seeds from a single plant.

Appendix 6. Average agronomic performance of 81 lentil plant materials (70 landrace accessions, 10 released varieties and 1 candidate genotype) at SARC during Bona/Meher 2005

Source	Accession/ genotype	DTF	DTM	PH	NPB	NSB	NP	NS	HSW	Biom	SY
Wello	207309	62.67	109.00	32.80	8.70	5.25	107.90	167.10	2.55	9.07	4.35
Wello	36168	66.59	108.50	28.60	8.20	14.67	124.80	192.40	2.57	10.20	4.89
Wello	36162	62.50	108.00	29.70	9.20	14.92	142.90	187.70	2.69	9.97	4.78
Wello	36151	66.75	108.50	27.40	8.20	17.25	134.90	161.70	1.97	9.00	3.83
Wello	36141	64.17	108.50	33.90	8.00	10.50	147.10	218.70	2.60	11.94	5.72
Wello	36104	63.75	109.50	32.70	5.85	6.17	119.40	180.40	2.85	10.80	5.17
Wello	36103	66.50	109.00	31.80	8.30	12.09	164.00	258.30	2.76	14.79	7.09
Wello	36101	66.17	110.50	30.70	8.50	8.50	115.40	178.30	2.39	10.32	4.23
Wello	36097	66.84	111.00	36.60	8.40	9.25	150.00	234.60	2.43	11.65	5.58
Wello	36084	69.84	110.50	30.10	8.70	10.67	150.70	228.50	2.45	11.93	5.72
Arsi	231240	67.67	111.00	27.20	7.50	8.59	97.40	127.00	2.18	5.97	2.86
Arsi	231239	66.00	110.50	28.30	7.70	10.75	134.00	183.40	2.35	9.17	4.39
Arsi	216881	64.09	110.00	28.30	5.70	2.75	69.00	106.60	2.24	6.12	2.45
Arsi	216879	65.50	111.00	27.80	7.80	6.50	99.90	153.00	2.20	7.13	3.42
Arsi	36131	69.42	110.00	28.80	8.40	11.00	135.10	209.10	2.38	11.60	5.08
Arsi	36047	63.92	110.00	29.20	7.20	7.25	94.80	143.90	2.29	6.52	3.13
Arsi	36042	65.09	110.00	29.30	7.70	11.17	98.80	151.00	2.30	8.34	3.52
Arsi	36041	65.67	111.00	27.40	8.60	6.09	107.10	150.80	2.33	7.55	3.62
Arsi	36008	70.67	111.00	27.30	8.20	7.59	108.50	180.90	2.28	8.58	4.11
Arsi	36007	64.50	110.50	28.60	9.10	13.92	134.00	204.20	2.16	9.11	4.36
ICARDA	Chalew	75.09	114.00	30.30	8.90	7.42	121.70	120.95	2.55	6.91	3.31
ICARDA	EL-142	68.34	110.50	28.80	6.80	8.84	102.00	139.10	2.41	6.74	3.23
ICARDA	Ada'a	79.25	124.50	34.00	7.85	12.92	56.80	78.20	3.80	6.09	2.92
ICARDA	Chekol	71.09	112.00	29.50	6.40	11.42	107.30	169.43	2.22	8.01	3.84
ICARDA	Gudo	62.17	126.00	35.20	6.54	4.42	77.40	86.70	4.76	8.57	4.11
ICARDA	Teshale	70.59	113.00	30.80	6.20	7.34	52.70	67.90	3.74	5.15	2.47
ICARDA	R-186	85.67	154.00	35.92	8.67	7.58	92.17	118.50	2.46	7.54	3.61

Appendix 6. Continued

Source	Accession/ genotype	DTF	DTM	PH	NPB	NSB	NP	NS	HSW	Biom	SY
ICARDA	Alemaya	63.92	112.00	29.30	8.00	8.25	105.90	121.50	3.15	9.83	4.71
ICARDA	Alemtena	70.09	114.00	28.90	6.30	11.33	67.10	82.63	3.68	8.04	3.86
ICARDA	Assano	62.42	109.00	28.40	7.20	5.75	91.10	94.00	4.61	11.11	5.33
ICARDA	FLIP-88-3L	60.50	109.00	28.90	7.20	6.58	69.60	77.60	4.38	8.61	4.36
Bale	36029	62.34	109.50	30.20	9.60	14.92	143.10	254.40	2.38	12.45	5.97
Bale	231243	64.42	109.00	31.80	7.90	13.09	159.00	290.30	2.18	13.86	6.64
Bale	237688	63.42	109.00	30.00	9.00	11.09	119.90	161.80	2.39	9.41	4.51
Bale	230020	64.67	110.00	31.40	13.40	10.59	124.00	171.80	2.61	9.37	4.49
Bale	230017	65.59	109.00	27.90	8.30	7.67	122.60	178.83	2.34	9.26	4.44
Bale	230015	66.42	110.50	26.10	8.30	11.67	107.50	133.73	2.25	5.35	2.57
Bale	212851	69.42	109.50	29.30	6.80	6.84	84.60	120.34	2.10	7.72	2.50
Bale	212848	61.50	108.00	30.80	8.20	5.92	93.20	137.70	2.52	7.24	3.47
Bale	36121	67.92	109.50	29.40	8.10	10.25	168.60	256.20	2.32	12.19	6.08
Bale	36033	64.17	108.00	31.70	9.70	7.00	153.20	194.30	2.45	10.46	5.01
Gonder	36072	64.00	108.50	25.90	8.30	12.50	119.00	166.80	2.23	9.63	3.90
Gonder	36065	64.67	109.50	30.80	9.40	13.09	196.40	289.90	2.28	13.65	6.54
Gonder	36026	65.17	111.50	30.10	9.10	7.09	123.60	175.50	2.44	8.10	4.36
Gonder	36146	65.92	110.00	29.90	8.50	8.34	125.50	201.60	2.69	11.25	5.39
Gonder	207305	69.59	111.50	28.00	6.30	5.25	62.50	80.20	2.59	4.62	2.21
Gonder	207291	66.00	110.50	29.50	7.80	5.42	105.20	157.60	2.66	9.33	4.47
Gonder	207266	62.67	109.50	30.30	7.20	8.25	101.90	135.30	2.78	7.91	3.79
Gonder	207259	68.84	112.50	30.30	7.80	5.34	70.40	96.70	2.69	5.60	2.68
Gonder	207257	63.00	110.00	27.70	7.80	7.59	113.90	173.60	2.77	11.59	4.83
Gonder	36086	68.59	109.50	28.50	8.10	7.42	97.90	124.73	2.73	7.18	3.44
Shewa	36001	65.84	110.50	28.00	9.10	9.92	110.50	136.20	2.41	7.00	3.36
Shewa	236891	65.34	111.50	27.90	8.60	4.92	111.20	156.30	2.60	8.87	4.25
Shewa	229184	63.59	111.50	29.40	7.80	7.17	114.70	168.20	2.68	10.38	4.49

Appendix 6. Continued

Source	Accession/ genotype	DTF	DTM	PH	NPB	NSB	NP	NS	HSW	Biom	SY
Shewa	36056	63.75	109.50	26.30	7.10	5.75	82.60	115.00	2.26	7.72	2.74
Shewa	36048	66.25	110.00	26.30	7.20	7.09	70.40	95.30	2.44	6.46	2.38
Shewa	36020	63.42	110.00	26.10	7.50	7.42	106.20	139.00	2.38	7.42	3.56
Shewa	36014	66.75	109.00	28.50	6.50	5.17	75.50	96.00	2.79	5.94	2.85
Shewa	36009	67.92	109.50	27.90	8.20	7.75	118.60	167.40	2.41	8.68	4.16
Shewa	36006	59.00	105.50	27.40	6.90	6.75	117.80	178.20	2.39	10.51	4.32
Shewa	36003	64.09	107.00	27.80	8.30	7.00	92.70	141.50	2.38	6.34	3.04
Gojam	36024	70.84	111.50	29.20	8.20	9.67	115.00	146.70	2.24	7.40	3.55
Gojam	36028	68.42	112.50	28.10	7.90	6.25	97.20	141.60	2.30	7.31	3.50
Gojam	36027	64.00	110.50	26.90	7.90	5.17	79.30	106.70	2.46	8.31	2.79
Gojam	36025	54.92	104.00	22.50	7.40	6.00	86.50	121.40	2.23	5.39	2.58
Gojam	36069	64.00	109.00	31.20	7.80	5.50	106.70	145.70	2.32	7.57	3.63
Gojam	241132	67.75	113.00	30.60	8.30	8.09	109.50	146.40	3.10	8.53	4.09
Gojam	238978	69.92	113.00	28.50	7.80	5.50	96.00	136.30	2.43	7.32	3.51
Gojam	219507	67.92	115.50	29.80	8.20	5.84	100.50	124.50	2.77	7.39	3.54
Gojam	212745	63.17	113.00	29.50	8.30	8.34	113.20	142.40	2.66	7.93	3.80
Gojam	36118	70.42	114.00	30.40	5.90	4.92	62.40	96.40	2.41	5.88	2.34
Tigray	223222	66.00	112.00	27.50	6.30	3.42	78.30	100.70	2.42	5.17	2.48
Tigray	213254	64.25	110.00	27.00	5.70	3.25	75.10	102.70	2.36	5.13	2.46
Tigray	223224	68.84	110.00	30.55	7.00	4.10	76.70	98.50	2.58	5.37	2.57
Tigray	219957	67.34	111.00	27.40	6.70	3.84	59.60	84.90	2.45	4.49	2.15
Tigray	223223	67.59	109.00	29.90	7.20	4.59	70.00	99.90	2.43	6.70	2.49
Tigray	223220	63.84	110.00	28.20	9.10	8.09	113.40	147.00	2.69	8.04	3.85
Tigray	207260	63.50	109.50	30.90	6.60	4.34	91.00	130.80	2.91	7.79	3.73
Tigray	219954	63.92	110.00	29.40	6.40	2.50	98.70	142.50	2.48	7.78	3.73
Tigray	221719	61.17	109.50	25.00	5.90	3.09	66.90	77.30	3.06	5.42	2.60
Tigray	219953	64.67	112.00	27.60	6.90	3.59	89.10	123.10	2.54	6.68	3.20
	Mean	66.10	111.16	29.28	7.81	7.91	105.64	149.19	2.60	8.36	3.89

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university, and that all sources of material used for the thesis has been duly acknowledged.

Candidate:

Name Edossa Fikiru

Signature

Date 02 August, 2006

Confirmation by supervisor:

Name

Signature

Date