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Full Length Research Paper

A comparative study of morphological and molecular diversity in Ethiopian lentil (*Lens culinaris* Medikus) landraces

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Genetic diversity assessment with different methods and their comparison could provide complementary information for improvement and conservation programs. However, such information is not available for Ethiopian lentil landraces. To fill this gap, nine morphological traits and four ISSR primers were used on seventy Ethiopian lentil landrace accessions to study morphological and genetic diversity among the accessions, assess the correlation between phenotypic and genetic dissimilarities, and classify the accessions into groups based on both markers. Analysis of variance of morphological data showed significant ($p < 0.01$) differences among accessions for all traits. However, days to maturity and flowering, seed weight and plant height showed low phenotypic coefficient of variation. From principal component (PC) analysis number of seeds and pods, seed yield and number of secondary branch were included in PC1, whereas, days to maturity and flowering were dominant in PC2. Seed weight and number of primary branch were also dominant in PC3 and PC4, respectively. With regard to molecular analysis, accessions from Gonder, Shewa and Wello (with I = 0.203, 0.174 and 0.162, respectively) showed moderate level of diversity. The average and overall diversity index values, respectively, were 0.145 and 0.264. The overall morphological and genetic dissimilarities ranged from 0.825 - 11.018 and 0.000 - 0.340 with average value of 3.921 and 0.125, respectively. The correlation (r) between morphological and genetic dissimilarity matrices was 0.28 ($p < 0.001$) indicating that either of the markers could be used for genetic diversity study. Cluster analysis based on morphological and molecular markers, respectively, revealed two and three major groups of lentil landrace accessions. The result of the present study would be useful for further collection and conservation, and improvement programs.

Key words: Clustering, correlation, genetic diversity, ISSR, lentil landraces, morphological variation, principal component.

INTRODUCTION

Ethiopia is among the centers of diversity for lentil (Vavilov, 1956; McGuire, 2000; Tanto and Demissie, 2000; Edossa et al., 2007) and much of the acreage allocated to the crop is covered by landrace cultivars that are small seeded (microsperma type) and generally early maturing (Geletu and Yadeta, 1994; Edossa et al., 2006). Ethiopia is also one of the major lentil producing countries in the world (Erskine, 1985; Edossa et al., 2006) and the first in Africa (FAO Statistical Yearbook, 2009). On average basis, the crop was grown annually on 1.345 million hectares of land with average seed yield of 1.416 million tons per annum during the years 2005 - 2007 (FAO Statistical Yearboook, 2009).

Landraces are the most diverse populations of cultivated plants (Frankel et al., 1995). Besides being adapted to their environments, natural and man-made, landrace genotypes are co-adapted. Hence, genetic variation within a landrace may be considerable, but is far from random (Qualset et al., 1997). The genetic diversity among and within landraces makes them a valuable

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resource as potential donors of genes for the development and maintenance of modern crop varieties, and for direct use by farmers (Soleri and Smith, 1995).

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

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; Muthusamy et al., 2008), 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 since it requires no complicated laboratory facilities and procedures.

Characterization of plant 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 (Weising 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).

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), random amplified DNA (RAPD), and amplified fragment length polymorphism (AFLP) techniques have all been used to produce molecular markers for studies of genetic variation at the population level (Wolf and Liston, 1998; Muthusamy et al., 2008).

Another molecular marker in use is the inter-simple sequence repeat (ISSR) (Zietkiewicz et al., 1994). This polymerase chain reaction (PCR)-based technique uses single anonymous primers to produce markers from genomic DNA. 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. Most studies have utilized ISSRs to study genetic diversity of germplasm collections and to differentiate cultivars (Wolf and Liston, 1998). Popularity of ISSR is growing; ISSRs are alternative methods to isozymes and RAPDs in terms of population genetic studies (Meekins et al., 2001).

The value of molecular markers 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 method must be combined with an appropriate strategy for handling large sample sizes. 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 technique compared to the bulk approach. However, the technique is advantageous in that it allows the assessment of intra-accession diversity (Edossa et al., 2007). 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). 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. However, this technique is more costly compared to leaf sample bulking strategy since it requires extraction of DNA from all representative plants of an accession (Edossa et al., 2007).

Molecular analyses in conjunction with morphological and agronomic evaluation of germplasm are recommended because they increase the resolving power of genetic diversity analyses and provide complementary information (Singh et al., 1991), which would be useful for germplasm collection, management and improvement. Lentil germplasm collected from different parts of Ethiopia by the Institute of Biodiversity Conservation (IBC) are held at the gene bank of the Institute. However, diversity of the germplasm has not yet been fully studied by using different marker systems. The present study was, therefore, conducted with the following objectives: (1) To study the morphological and genetic diversity of Ethiopian lentil landrace accessions, (2) To classify lentil landrace accessions into groups based on both morphological traits and molecular profiles, and (3) To assess the correlation between phenotypic and genotypic distances.

MATERIALS AND METHODS

Morphological analyses

Plant materials

Seeds of seventy lentil landrace accessions collected from ten districts of seven different administrative regions (ARs) of Ethiopia (Table 1) were obtained from Institute of Biodiversity Conservation

Table 1. Passport data of Ethiopian lentil landrace accessions used in the study.

Accession name	Regional state	District	Administrative region	Latitude	Longitude	Altitude
Acc 36007	Oromiya	Dodota Sire	Arsi	8-09-N	39-21-E	2050
Acc 36008	Oromiya	Gedeb	Arsi	7-10-N	39-12-E	2440
Acc 36041	Oromiya	Merti	Arsi	8-24-N	39-52-E	2040
Acc 36042	Oromiya	Chole	Arsi	08-22-N	39-53-E	2520
Acc 36047	Oromiya	Sude	Arsi	07-53-N	39-44-E	2520
Acc 36131	Oromiya	Robe	Arsi	7-49-N	39-47-E	2480
Acc 216879	Oromiya	Bekoji	Arsi	---------	---------	------
Acc 216881	Oromiya	Dodota Sire	Arsi	08-07-N	39-27-E	2370
Acc 231239	Oromiya	Sherka	Arsi	Aug-35	39-52-	2330
Acc 231240	Oromiya	Jeju	Arsi	Aug-37	39-41-	1920
Acc 36029	Oromiya	Agarfa	Bale	7-18-N	39-58-E	2580
Acc 36033	Oromiya	Sinana Dinsho	Bale	---------	---------	2620
Acc 36121	Oromiya	Dodola	Bale	---------	---------	------
Acc 212848	Oromiya	Goro	Bale	7-00-N	40-28-E	1800
Acc 212851	Oromiya	Nansebo	Bale	---------	---------	------
Acc 230015	Oromiya	Kokosa	Bale	---------	---------	2620
Acc 230017	Oromiya	Agarfa	Bale	7-10-N	34-11-E	------
Acc 230020	Oromiya	Ginir	Bale	07-08-N	40-36-E	2020
Acc 231243	Oromiya	Adaba	Bale	---------	---------	------
Acc 237988	Oromiya	Adaba	Bale	---------	---------	2460
Acc 36026	Amhara	Este	Gonder	11-34-N	38-45-E	2590
Acc 36065	Amhara	Dembia	Gonder	---------	---------	------
Acc 36072	Amhara	Farta	Gonder	11-48-N	35-28-E	3114
Acc 36086	Amhara	Lay Gayint	Gonder	11-41-N	38-29-E	3120
Acc 36146	Amhara	Debark	Gonder	13-13-N	38-01-E	3220
Acc 207257	Amhara	Wegera	Gonder	---------	---------	------
Acc 207259	Amhara	Fogera	Gonder	---------	---------	------
Acc 207266	Amhara	Kemekem	Gonder	---------	---------	------
Acc 207291	Amhara	Janamora	Gonder	---------	---------	------
Acc 207305	Amhara	Simada	Gonder	---------	---------	------
Acc 36024	Amhara	Dega Damot	Gojam	---------	---------	------
Acc 36025	Gulomahada	Wenbera	Gojam	---------	---------	1580
Acc 36027	Amhara	Enarj Enawga	Gojam	10-38-N	38-10-E	2510
Acc 36028	Amhara	Hulet Ej Enese	Gojam	11-4-N	37-051-E	2270
Acc 36069	Amhara	Guzamn	Gojam	10-20-N	37-44-E	2460
Acc 36118	Amhara	Shebel Berenta	Gojam	10-27-N	38-21-E	2420
Acc 212745	Amhara	Enemay	Gojam	38-11-N	10-34-E	2580
Acc 219507	Amhara	Banja	Gojam	---------	---------	------
Acc 238978	Amhara	Bahir Dar Zuria	Gojam	11-38-N	37-13-E	1930
Acc 241132	Amhara	Achefer	Gojam	---------	---------	2030
Acc 36001	Oromiya	Berehna Aleltu	Shewa	9-50-N	39-13-E	2820

Table 1. Cont'd.

Accession name	Regional state	District	Administrative region	Latitude	Longitude	Altitude
Acc 36003	Amhara	Moretna Jiru	Shewa	9-57-N	39-13-E	2820
Acc 36006	Oromiya	Gimgichu	Shewa	8-57-N	39-5-E	2370
Acc 36009	Oromiya	Ejere	Shewa	9-2-N	38-10-E	2270
Acc 36014	Oromiya	Wuchalena Jido	Shewa	09-39-N	38-49-E	2695
Acc 36020	Oromiya	Walisona Goro	Shewa	8-39-N	37-54-E	2260
Acc 36048	Oromiya	Kembibit	Shewa	09-19-N	39-16-E	2890
Acc 36056	Oromiya	Ambo	Shewa	---------	---------	------
Acc 229184	Amhara	Lay Betna Tach Bet	Shewa	---------	---------	2720
Acc 236891	Oromiya	Girar Jarso	Shewa	39-42-N	38-48-E	2650
Acc 207260	Tigray	Gulomahda	Tigray	---------	---------	-----
Acc 213254	Tigray	Wukro	Tigray	---------	---------	-----
Acc 219953	Tigray	Laelay Maychew	Tigray	---------	---------	-----
Acc 219954	Tigray	Asegede Tsimbela	Tigray	---------	---------	-----
Acc 219957	Tigray	Adwa	Tigray	14-09-N	38-56-E	2330
Acc 221719	Tigray	Enderta	Tigray	---------	---------	2500
Acc 223220	Tigray	Saesi Tsaedaemba	Tigray	14-15-N	39-28-E	2580
Acc 223222	Tigray	Alaje	Tigray	22-43-N	39-32-E	2600
Acc 223223	Tigray	Endamehoni	Tigray	12-42-N	39-32-E	2450
Acc 223224	Tigray	Ganta Afeshum	Tigray	14-14-N	39-28-E	2360
Acc 36084	Amhara	Guba Lafto	Wello	---------	---------	------
Acc 36097	Amhara	Dawuntna Delant	Wello	11-32-N	39-15-E	2200
Acc 36101	Amhara	Wadla	Wello	---------	--------	2830
Acc 36103	Amhara	Kutaber	Wello	11-20-E	39-18-E	2620
Acc 36104	Amhara	Tenta	Wello	11-16-N	39-15-E	2900
Acc 36141	Amhara	Were Ilu	Wello	10-44-N	39-28-E	2660
Acc 36151	Amhara	Dessie Zuria	Wello	11-6-N	39-38-E	2260
Acc 36162	Amhara	Legambo	Wello	---------	---------	3230
Acc 36168	Amhara	Tehuledere	Wello	---------	---------	------
Acc 207309	Amhara	Ambasel	Wello	---------	---------	------

Source: IBC.

(IBC) (Addis Ababa, Ethiopia) and grown at Sinana Agricultural Research Center (Bale Zone, Ethiopia) on station field plot. Planting was done in two replications of 0.62 m^2 plot size (2 rows of 1.55 m length spaced at 0.2 m) during 'Bona' or 'Meher' season that extends from August to December. All plants used in this study were generated from seeds under natural conditions. Fifteen individual plants were randomly selected from each of the two replications and marked just before flowering. Morphological data were collected from all marked plants.

Phenotypic data

Phenotypic data were recorded on number of days to flowering and maturity, plant height (cm), number of primary and secondary branches, number of pods and seeds, 100-seed weight (g) and seed yield (g). All data were recorded on individual plant basis and the mean of fifteen plants was used to represent an accession for each replication.

Morphological data analyses

Homogeneity of variance across accessions on phenotypic data was tested using Bartlett's homogeneity test (Snedecor and Cochran, 1989). One-way analysis of variance (ANOVA) was conducted for all characters according to Gomez and Gomez (1984). Phenotypic coefficient of variation (PCV) was computed according to Burton and de Vane (1953) for each morphological trait. PC analysis was computed from the correlation matrix with SPSS v10.01 (SPSS Inc., 1999) computer software to provide an empirical basis for judging the structure of the morphological traits.

Euclidian distance between the landrace accessions was calculated from the standardized trait mean values (mean of each trait subtracted from the data values and the result divided by the standard deviation of the trait) over each accession using NTSYSpc Version 2.11 (Rohlf, 2004). Similarly, the standardized trait mean values over each accession were used to perform cluster analysis with the same software. To group the accessions based on morphological dissimilarity, cluster analysis was conducted on 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) using NTSYSpc2.11.

Molecular analyses

DNA extraction

Since the ISSR marker study did not aim to estimate the degree of genetic diversity within accessions, a bulk of 15 plant samples were analyzed in order to represent the genetic variability within each accession. Young leaves were collected separately from randomly selected individual plants of each accession before flowering and dried in silica gel. Approximately equal amounts of the dried leaf samples were bulked for each accession and ground with pestle and mortar. Total genomic DNA was isolated from about 0.2 g of the fine ground leaf sample using a modified cetyltrimethyl-ammoniumbromide (CTAB) extraction technique (Borsch et al., 2003). The isolated DNA samples were visualized using 1% ethidium bromide stained agarose gel under UV light. Determination of the quantity and quality of DNA was done by comparing gel photographs of the first, second and third extracts with one another. Extracts with more intense band are considered to possess high DNA concentration while bands with no or low amount of smear represent high quality DNA extracts. Based on this method of quantification and quality analysis, the second or third extractions were selected for PCR amplification. The selected genomic DNA samples were diluted with sterile distilled water in 1:5 ratio.

ISSR analyses

A total of 12 ISSR primers (UBC primer set # 9, Vancouver, BC, Canada) were selected based on published results in lentil (Ford and Taylor, 2003; Kahraman et al., 2004) and related legume species such as common bean (de la Cruz et al., 2004; González et al., 2005), *Ammopiptanthus* (*Ammopiptanthus* is known by various common names such as milk-vetch, locoweed and goat's-thorn, etc.) (Ge et al., 2005) and chickpea (Flandez-Galvanez et al., 2003). These primers were screened for the amplification of unambiguously visible and polymorphic ISSR bands on seven representative landrace accessions, which were expected to represent a high level of genetic diversity due to difference in collection sites and morphological traits. Finally, ISSR primers UBC 812 [$(GA)_8A$], UBC 818 [$(CA)_8G$], UBC 835 [$(AG)_8YC$] and UBC 881 [$(G_3TG)_3$], which produced unambiguously visible and polymorphic bands across the seven accessions were chosen for further analyses.

Optimization of PCR reaction components for ISSR genotyping was done using DNA extracted from seven representative accessions used for screening primers. The optimum reaction components were 16.7 µl dH_2O, 250 µM of each dATP, dGTP, dCTP and dTTP, 2.6 µl of 10X *Taq* buffer, 1 U *Taq* polymerase, 0.23 µM primer and 1 µl template DNA. The final reaction volume per sample was 26 µl. PCR amplification conditions were set as: Initial denaturing at 94°C for 4 min followed by 40 cycles of 94°C for15 s, 45/48°C for 1 min, and 72°C for 1½ min and ended with extension phase of 72°C for 7 min. The lid temperature was held at 105°C. The amplified PCR products were stored at 4°C until the time of electrophoresis. PCR amplification was performed with Biometra® T3 thermorecycler.

The ISSR-PCR product was resolved on 1.7% agarose gel in 1X TBE buffer. Genomic DNA of 9 µl was loaded with 2 µl of 6X loading dye and 100 bp DNA ladder (PEQLAB Biotechnologie GmbH) was used on each side of the gel as a marker. Electrophoresis was conducted at 100 V for about 2:00 hours in 1X TBE buffer. The resultant gel was visualized by ethidium bromide staining under UV light and photographed with Biodoc Analyzer (035 - 300).

ISSR bands were scored manually for each individual accession from the gel photograph. Unambiguously scored bands were recorded as discrete characters, presence '1' or absence '0'. Matrix of binary data was constructed with rows accessions and columns, respectively, representing marker data.

ISSR data analyses

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), Shannon information index (I) (Lewontin, 1972) and Nei's standard genetic distance (D) (Nei, 1972) were computed with POPGENE ver 1.32 (Yeh et al., 2000). The haploid option of the software was used for analysis in accordance with the assumption of Ferguson et al. (1998) that each individual of a highly inbreeding species, such as *Lens* species, is homozygous. Since ISSRs are dominant markers, only the presence or absence of an allele can be determined and, therefore, each band position corresponds to a locus with two alleles represented by the presence or absence of a band (Powell et al., 1996).

Cluster analysis of the ISSR data was conducted based on the standard genetic distance matrix using UPGMA procedure of the SAHN clustering methods with NTSYSpc2.1. The goodness of fit of clustering to the data set was tested by comparing the co-phenetic matrix generated from the UPGMA tree with the dissimilarity matrix using the two-way test method (Mantel, 1967) of the software.

RESULTS

Morphological variability

Bartlett's chi-square test revealed non-significant ($p < 0.05$) difference among accessions for their variance in all traits, indicating homogeneity of variance between accessions for the measured traits. Analyses of variance showed highly significant ($p < 0.01$) difference among accessions for all traits justifying the appropriateness of further analysis. Number of primary and secondary branches, pods and seeds, and seed yield showed high phenotypic coefficient of variation (PCV). On the other hand, plant height, hundred seed weight, days to flowering and maturity revealed low PCV with days to flowering showing the least PCV (Table 2).

Compared to the overall average phenotypic performance, accessions from Gojam and Tigray were late in maturity, short in stature, and low in number of primary and secondary branches, pods and seeds, but with high seed weight and low yield. Similarly, accessions from Gonder and Wello represented tall and late flowering

Table 2. Analyses of variance and PCV for the agro-morphological traits measured in 70 Ethiopian lentil landrace accessions.

Morphological traits	Source of variation	Degrees of freedom	Sum of squares	Mean square	CV %	PCV
Days to flowering	Between accessions	69	1091.94	15.83**	3.81	6.09
	Within accessions	70	433.10	6.19		
	Total	139	1525.04			
Days to maturity	Between accessions	69	486.77	7.06**	1.75	2.41
	Within accessions	70	260.10	3.73		
	Total	139	747.77			
Plant height (cm)	Between accessions	69	1003.65	14.55**	9.61	12.98
	Within accessions	70	558.17	7.97		
	Total	139	1561.81			
Number of primary branch	Between accessions	69	302.66	4.39**	17.31	26.93
	Within accessions	70	125.70	1.80		
	Total	139	428.35			
Number of secondary branch	Between accessions	69	2210.94	32.04**	27.65	61.67
	Within accessions	70	472.88	6.76		
	Total	139	2683.81			
Number of pods	Between accessions	69	260329.28	3772.89**	10.26	50.40
	Within accessions	70	10953.07	156.47		
	Total	139	271282.35			
Number of seeds	Between accessions	69	756072.00	10957.57**	12.09	58.86
	Within accessions	70	32388.35	462.69		
	Total	139	788460.36			
100 seed weight (g)	Between accessions	69	6.69	0.10**	9.44	12.82
	Within accessions	70	3.79	0.05		
	Total	139	10.48			
Seed yield (g)	Between accessions	69	283.91	4.12**	26.49	50.38
	Within accessions	70	85.034	1.22		
	Total	139	368.945			

** Significant at $p < 0.01$.

plants with high seed weight and yield. However, accessions from Arsi and Bale were early in maturity, and with high number of primary and secondary branches, pods and seeds, low seed weight and high yield. On the other hand, accessions from Shewa were late in flowering and maturity, short plants with low number of primary and secondary branches, pods and seeds, but with high seed weight and low yield (Table 3).

In principal component analysis, as stated by Chatfield and Collins (1980), components with an Eigen value of less than one (1) should be eliminated so that fewer computations are dealt with. In the present study, the first three PCs which accounted for 79.7% of the variation in the original phenotypic data satisfied this requirement. However, the associated communalities showed that higher proportion (52.7%) of the variance due to number of primary branch was not properly described by the three principal components. For this reason, the fourth principal component in which 91.8% of the total variance due to number of primary branch was described and taken into consideration (Table 4). When the fourth PC is considered, all the communalities were larger than 0.7 and, therefore, it can be assumed that all the variables were described to an acceptable level. The first four PCs accounted for 86.3% of the total phenotypic variation. In the first PC, which explained 41.7% of the total variation, the most important traits were number of seeds, number of pods, seed yield and number of secondary branch per plant. In the second PC which explained 18.1% of the total variation, predominant traits were days to flowering

Table 3. Administrative Region (AR) -based average performance and overall summary statistics of the agromorphological traits measured in 70 Ethiopian lentil landrace accessions.

ARs	Average morphological performance								
	DTF	DTM	PH	NPB	NSB	NP	NS	HSW	SY
Arsi	66.01	110.83	28.52	8.64	10.93	135.65	205.79	2.27	4.21
Bale	64.78	109.20	30.02	8.87	11.77	154.35	232.24	2.33	4.99
Gojam	66.03	111.60	28.67	7.26	6.99	96.63	131.01	2.49	3.36
Gonder	65.35	110.30	29.55	7.61	9.34	128.08	189.95	2.59	4.64
Shewa	64.45	109.40	28.07	7.12	7.43	100.02	143.64	2.48	3.70
Tgray	64.98	110.30	28.35	6.53	6.53	81.88	110.74	2.59	2.93
Wello	65.49	109.64	32.53	8.18	12.90	156.76	231.65	2.52	5.28
Summary statistics over all accessions									
Minimum	54.90	104.00	22.50	4.70	2.80	59.60	77.30	2.00	2.10
Maximum	70.80	115.50	37.60	15.20	27.40	299.40	446.90	3.10	9.50
Range	15.90	11.50	15.10	10.50	24.60	239.80	369.60	1.10	7.40
Average	65.30	110.18	29.39	7.78	9.19	121.87	177.83	2.47	4.03
Standard deviation	2.81	1.88	2.69	1.63	4.55	43.39	74.04	0.23	1.43

In this table, DTF, DTM, PH, NPB, NSB, NP, NS, HSW and SY, respectively represent number of days to flowering, number of days to maturity, plant height (cm) number of primary branch, number of secondary branch, number of pods per plant, number of seeds per plant, hundred seed weight (g) and seed yield per plant (g).

Table 4. Initial Eigen vectors, Eigen values, communalities, individual and cumulative percentage of variation explained by the first four principal components (PC) derived from morphological traits in Ethiopian lentil landrace accessions.

Morphological traits	Initial Eigen vectors				Communalities
	PC1	PC2	PC3	PC4	
NS	0.973	0.026	0.030	-0.090	0.832
NP	0.960	0.013	0.002	-0.077	0.804
SY	0.913	0.100	0.250	-0.114	0.748
NSB	0.849	-0.064	-0.168	-0.071	0.981
DTM	-0.236	0.850	-0.146	0.074	0.758
DTF	-0.049	0.792	-0.431	-0.127	0.927
HSW	-0.282	0.221	0.796	0.278	0.957
PH	0.473	0.464	0.555	-0.047	0.838
NPB	0.649	0.013	-0.325	0.674	0.918
	Eigen values				
Total	4.203	1.629	1.345	0.587	
Individual % of Variance	46.705	18.097	14.942	6.517	
Cumulative % of variance	46.705	64.802	79.744	86.262	

*Traits corresponding to underlined numbers are the most significant ($p < 0.05$) ones that contributed much of the variation in each PC. In this table, DTF, DTM, PH, NPB, NSB, NP, NS, HSW and SY, respectively represent number of days to flowering, number of days to maturity, plant height (cm) number of primary branch, number of secondary branch, number of pods per plant, number of seeds per plant, hundred seed weight (g) and seed yield per plant (g).

and maturity. The third PC which accounted for 14.9% of the total variation was dominated by hundred seed weight, while number of primary branch was the dominant trait in the fourth PC which accounted for 6.5% of the total variation. These traits were significant ($p < 0.05$) according to Hair et al. (1998).

Clustering analysis based on morphological data revealed two major and four minor clusters at an average dissimilarity value of 3.80. Clusters I, II, III, and IV were minor clusters and together consisted twelve accessions

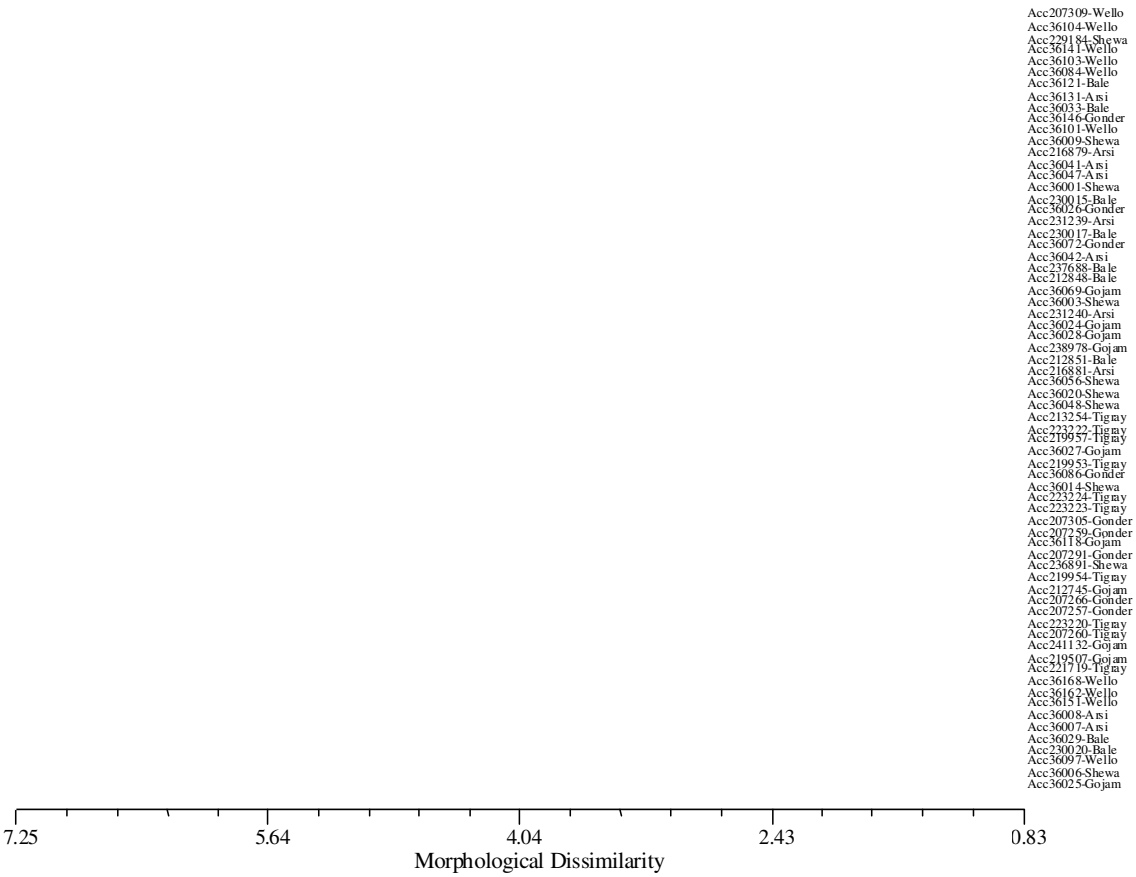
Acc207309-Wello
Acc36104-Wello
Acc229184-Shewa
Acc36141-Wello
Acc36103-Wello
Acc36084-Wello
Acc36121-Bale
Acc36131-Arsi
Acc36033-Bale
Acc36146-Gonder
Acc36101-Wello
Acc36009-Shewa
Acc216879-Arsi
Acc36041-Arsi
Acc36047-Arsi
Acc36001-Shewa
Acc230015-Bale
Acc36026-Gonder
Acc231239-Arsi
Acc230017-Bale
Acc36072-Gonder
Acc36042-Arsi
Acc237688-Bale
Acc212848-Bale
Acc36069-Gojam
Acc36003-Shewa
Acc231240-Arsi
Acc36024-Gojam
Acc36028-Gojam
Acc238978-Gojam
Acc212851-Bale
Acc216881-Arsi
Acc36056-Shewa
Acc36020-Shewa
Acc36048-Shewa
Acc213254-Tigray
Acc232222-Tigray
Acc219957-Tigray
Acc36027-Gojam
Acc219953-Tigray
Acc36086-Gonder
Acc36014-Shewa
Acc232224-Tigray
Acc223223-Tigray
Acc207305-Gonder
Acc207259-Gonder
Acc36118-Gojam
Acc207291-Gonder
Acc236891-Shewa
Acc219954-Tigray
Acc212745-Gojam
Acc207266-Gonder
Acc207257-Gonder
Acc223220-Tigray
Acc207260-Tigray
Acc241132-Gojam
Acc219507-Gojam
Acc221719-Tigray
Acc36168-Wello
Acc36162-Wello
Acc36151-Wello
Acc36008-Arsi
Acc36007-Arsi
Acc36029-Bale
Acc230020-Bale
Acc36097-Wello
Acc36006-Shewa
Acc36025-Gojam
7.25
5.64
4.04
2.43
0.83
Morphological Dissimilarity

Table 5. Number and percentage of polymorphic loci (P), and Shannon's information index (I) values in 7 AR (administrative region) -based Ethiopian lentil landrace populations.

Accessions	Number of polymorphic loci	P	I ± St. dev.
Arsi	10	21.28	0.107 ± 0.218
Bale	11	23.40	0.115 ± 0.218
Gojam	13	27.66	0.136 ± 0.234
Gonder	16	34.04	0.203 ± 0.291
Shewa	15	31.91	0.174 ± 0.262
Tigray	10	21.28	0.114 ± 0.232
Wello	16	34.04	0.162 ± 0.241
Average	13	27.66	0.145 ± 0.242
Over all ARs	28	59.57	0.264 ± 0.280

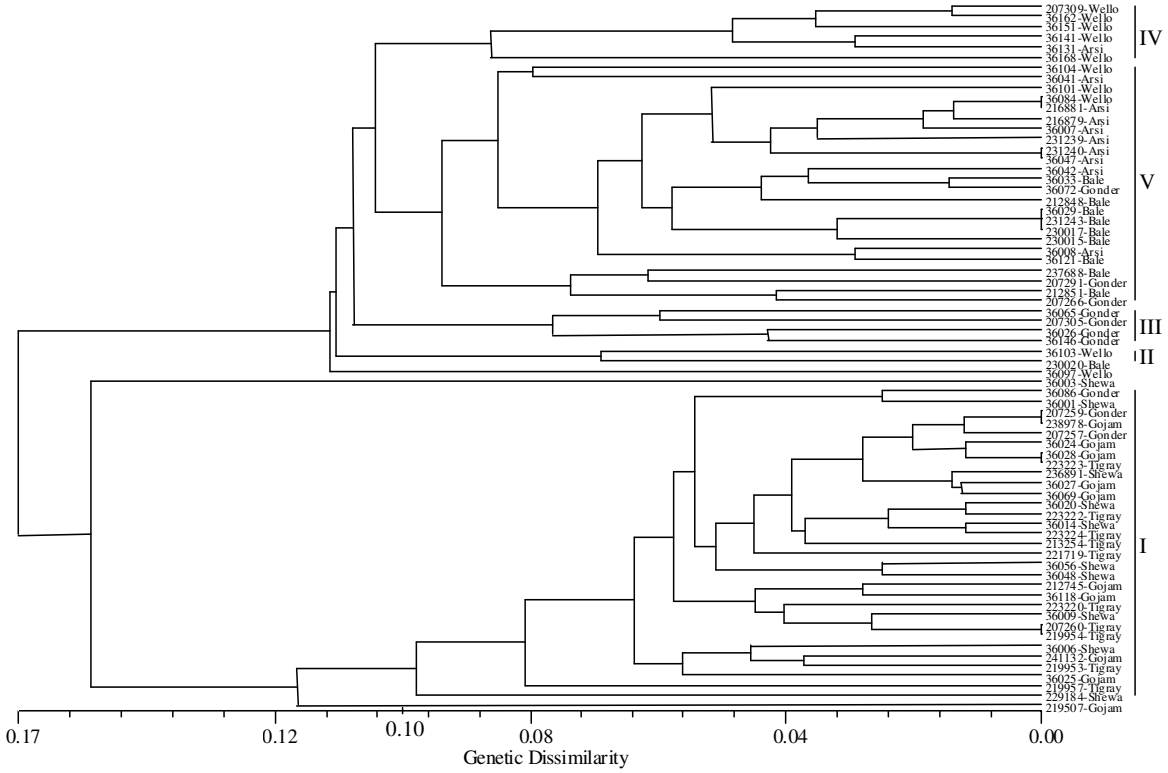

Figure 2. A dendrogram of 70 Ethiopian lentil landrace accessions derived by UPGMA from Nei's (1972) standard genetic distance based on data generated using four ISSR primers.

good fit to the data set, and hence validated the observed clusters as interpreted according to Rohlf (2004).

Correlation between morphological and molecular dissimilarity matrices

The Euclidian distance generated from the standardized morphological data revealed that accessions from Wello (3.882) followed by those from Gonder (3.436) showed the highest average interaccession morphological dissimilarity. Similarly, accessions from Gonder showed the largest range of Euclidian distance (1.477 - 10.369) while those from Tigray showed the least (0.937 - 4.340). The overall range of interaccession Euclidian distance was 0.825 - 11.018 (Table 6).

On the other hand, genetic dissimilarity matrix generated from the ISSR data using Nei's standard genetic distance revealed that accessions from Gonder (0.104) showed the highest interaccession genetic dissimilarity

Table 6. Mean, range and standard deviation of Nei's genetic dissimilarity (calculated using ISSR marker) and Euclidian distance (calculated using morphological traits) in Ethiopian lentil landrace accessions.

Accessions	Morphological dissimilarity			ISSRs dissimilarity		
	Mean	Range	St. dev.	Mean	Range	St. dev.
Arsi	3.246	0.825 - 6.306	1.214	0.055	0.000 - 0.110	0.026
Bale	3.271	1.827 - 7.082	1.859	0.063	0.000 - 0.111	0.029
Gojam	3.240	0.837 - 8.507	2.246	0.064	0.012 - 0.146	0.035
Gonder	3.436	1.477 - 10.369	2.636	0.104	0.012 - 0.194	0.036
Shewa	2.271	1.196 - 4.469	1.247	0.083	0.026 - 0.180	0.042
Tigray	2.187	0.937 - 4.340	1.256	0.051	0.000 - 0.098	0.025
Wello	3.882	1.462 - 7.089	1.279	0.087	0.015 - 0.189	0.034
Mean	3.076	1.223 - 6.880	1.677	0.072	0.009 - 0.147	0.032
Overall	3.921	0.825 - 11.018	1.622	0.125	0.000 - 0.340	0.058

(D), while those from Tigray (0.051) showed the least. Similarly, accessions from Gonder showed the largest range of interaccession genetic distance (0.012 - 0.194), while those from Tigray showed the least (0.000 - 0.098). The overall range of interaccession genetic distance was 0.000 - 0.340 (Table 6).

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. Accordingly, the correlation (r) between morphological and ISSR dissimilarity matrices of all accessions was positive (0.28) and highly significant ($p < 0.001$, 1000 random permutations). The overall average Euclidian distance from the standardized morphological data (3.921) was greater than the average ISSR-based genetic dissimilarity (0.125) (Table 6).

DISCUSSION

Morphological variation and implications for improvement

In the present study, seventy lentil landrace accessions collected from seven different administrative regions of Ethiopia were used to assess morphological variation. The presence of highly significant morphological variation between the landrace accessions indicates the existence of high degree of phenotypic diversity implying great potential of the landraces in improvement programs. This was evidenced by high phenotypic coefficient of variation (PCV) for traits such as number of primary and secondary branches, pods and seeds, and seed yield, which indicates a wide phenotypic variation. Similar results were reported by Jain et al. (1995) and Chakrabotry and Haque (2000) in other lentils. However, plant height, hundred seed weight, days to flowering and maturity revealed low PCV, indicating narrow phenotypic variation and hence very low opportunity to improve these traits through inter-accession improvement activities. This result agrees with the report of Thakur and Banjpai (1993) for days to maturity, Singh and Singh (1991) for days to flowering and plant height in other lentil materials.

PC analysis, on the other hand, showed that the first four PCs could be considered significant. In the present study, the most significant variables in the components represented by high loadings were taken into consideration in evaluating each PC. In addition to this, there exists a difference between the components, where those with larger variances are more desirable since they give more information about the data. The most important traits in PC1 were the most interrelated variables of all, and hence could preferably be represented by seed yield. The first PC, therefore, could be identified as yield related variables. The most significant variables of the second PC, showed higher interrelationship to one another and could be identified as maturity variables. Interestingly enough, seed weight and number of primary branch did not show a significant ($p < 0.05$) interrelationship with the dominant variables of PC1 other than forming their own PCs. Similar findings were reported by Toklu et al. (2009) for the first two PCs. The overall PC analysis of the present study suggests that seed yield, days to maturity, seed weight and number of primary branch could suffice as parameters for data collection in further studies since they represent much of the variability in each PC.

From the analyses of variance, all traits showed significant variation among accessions. Nevertheless, unlike other traits, plant height did not show significant contribution in PC analysis. Thus, ANOVA does not provide insight onto the underlying dimensions of variability, nor does it provide guidance for the selection of variables suitable for emphasis in further studies. PCV values and PC analysis, on the other hand, produced comparable results for the most important traits in PC1, but with differences in their order. However, traits such as days to flowering and maturity, and hundred seed weight showed low PCV, but were the most important in PC2.

This could be explained by their interdependence with other traits.

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. Furthermore, they reported lentil of the west highlands as early maturing with short stature, that of the north highlands as large-seeded, and lentils from the central highlands as the least distinctive group. Results of the present study also confirmed their finding that lentils of the north highlands (Tigray, Gojam, Gonder and Wello) were large seeded.

Clustering analysis from the standardized morphological data revealed two major and three minor groups of the lentil landraces under consideration. The largest major cluster (V) accessions from all the administrative regions, except those from Wello. One of the subclusters (V_1) of this major cluster constituted accessions from north, northwest and central part of Ethiopia, while the other subcluster (V_2) constituted accessions from southeastern parts Ethiopia. The other major cluster was constituted by accessions from Wello, indicating that these accessions tended to form a distinct cluster. However, few accessions from Wello and Gojam were dispersed all over the tree indicating that some accessions of these ARs have a close resemblance with those of other ARs for the variables under consideration. Generally, however, the dendrogram showed a tendency of grouping the accessions in accordance with their geographical regions of origin.

Molecular diversity and its implications for improvement and genetic conservation

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 the present study, ISSR markers were used to assess the molecular diversity of Ethiopian lentil landrace accessions based on bulked leaf sample analysis of fifteen plants per accession. Bulk sample analysis is useful to handle large sample sizes cost effectively (Gilbert et al., 1999; Edossa et al., 2007).

The present study revealed moderate diversity index (I = 0.264) value for the landraces under examination as compared to gene diversity index (I = 0.376) reported by Sonnante and Pignone (2007) using ISSR markers in Italian landraces of lentil. Accessions from Gonder followed by those from Shewa and Wello were found to be of high diversity as opposed to those from Gojam, Tigray, Bale and Arsi. The existence of moderate level of diversity within the total landrace population and in some ARs has a good implication for improvement programs. The result also suggests that high priority should be given to areas with high genetic diversity for further collection activities in an approach in which areas of high genetic diversity contribute more accessions than those with a low diversity. On the other hand, the maximum inter-accession genetic distance in the present study (0.34) is comparable to that reported by Ford et al. (1997) (0.36) in 16 accessions and cultivars of lentil using RAPD marker. Three major and two minor clusters were obtained at 0.10 average genetic dissimilarity. Few accessions from Gonder and Wello were distributed all over the tree, while some of the accessions tended to form their own cluster.

This could be explained by gene flow from one administrative region to the other due to various factors. For instance, from the year 1632 - 1855 when Gonder was a capital city of Ethiopia (Bahru, 2002), seeds of some lentil germplasm might have been transported to and from Gonder for social, economic and political purposes. On the other hand, all accessions of Shewa, Tigray and Gojam appeared together in the same cluster, which may suggest the presence of network(s) of genetic exchange which probably converged this genetic base. The major reason for such genetic mix-up could probably be the fact that seeds of various crops, including lentil, are shipped to and from Addis Ababa (the capital of Ethiopia since 1886 (Getahun, 2000)). This result confirms the report of Edossa et al. (2007) that stated the existence of an intermediate level of gene flow among administrative region-based populations of Ethiopian lentil landraces. Accessions from Arsi and Bale constituted cluster V and this may be attributed to the ease of seed movement between the two ARs because of geographical and cultural factors.

Correlation of phenotypic and ISSR-based molecular distances

The correlation between morphological and molecular dissimilarity matrices of the present study was positive and significant ($r = 0.279$, $p < 0.001$). Piergiovanni and Taranto (2004) reported that the result from SDS-PAGE analysis agrees with that of the previous agronomic studies in lentil. Although no additional reports comparing the extent of agreement between morphological and any of the molecular markers in lentil were obtained, few similar works were conducted on some other crops.

Yoseph et al. (2005) working on sixty-two Ethiopian traditional maize accessions reported a correlation value (r) of 0.43 and 0.39 between fifteen morphological characters and SSR and AFLP, respectively. Furthermore, Roldan-Ruiz et al. (2001) working with sixteen rye grass varieties reported a correlation value of r = -0.06 between AFLP and fifteen morphological characters. In addition, Bolaric et al. (2005) working on twelve phenotypic characters of twenty-two perennial

rye grasses 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. In any case, 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).

Conclusion

In conclusion, the present study showed that Ethiopian lentil landrace accessions possess a wide range of phenotypic and genetic diversity. This is an important landmark both for improvement and conservation programs. However, due to some drawbacks of the landraces, such as small seededness and low yielding potential as compared to the exotic ones, breeders did not focus on improving the landraces. Yet, they have good average performance and wider variability for some characters. Furthermore, the wider phenotypic and molecular variability observed represents a good indication for the importance of lentil landraces in breeding programs. This is an encouraging result for further collection activities so as to capture more variability from other agroecologies of the country. In addition, the congruence between morphological and molecular methods of diversity study suggests that both marker systems are applicable for genetic diversity study in lentil landraces and, hence, provide complementary information.

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