International Scholars Journals

African Journal of Ecology and Ecosystems ISSN: 2756-3367 Vol. 9 (9), pp. 001-009, September, 2022.
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Full Length Research Paper

Genetic diversity and population structure of Ethiopian lentil (*Lens culinaris* Medikus) landraces as revealed by ISSR marker

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Accepted 4 August, 2022

Characterization of genetic resources maintained at genebanks has important implication for future utilization and collection activities. The objective of this study was to assess the level of genetic diversity, genetic structure and genetic distance, and to indirectly estimate the level of gene flow among populations of Ethiopian lentil landraces using intersimple sequence repeat (ISSR) marker. For this purpose, seeds of seventy landrace accessions collected from seven different administrative regions of Ethiopia were obtained from Inistitute of Biodiversity Conservation and grown at Sinana Agricultural Research Center (Bale-Robe, Ethiopia) on-station field plot. DNA was extracted from a bulk leaf sample of fifteen randomly selected seed-raised plants using a triple CTAB extraction technique. ISSR data were generated using four primers. The total genetic diversity (H_T), the intrapopulation genetic diversity (H_S) and the interpopulation genetic diversity (D_{ST}) were 0.175, 0.095 and 0.079, respectively. High intrapopulation genetic diversity was observed for Gonder, Shewa and Wello populations, while Arsi, Bale, Tigray and Gojam populations showed low intrapopulation diversity. There was high genetic differentiation (G_{ST} = 0.455) but intermediate gene flow level (*Nm* = 0.60) among populations. The genetic distance (D) between populations ranged from 0.012 – 0.228. Cluster analysis revealed two groups of Ethiopian lentil landrace populations. The results provide important baseline for future germplasm conservation and improvement programs.

Key words: Bulk sampling, gene flow, genetic differentiation, genetic distance, ISSR, genetic diversity and lentil landrace accessions.

INTRODUCTION

Lentil belongs to the genus *Lens* of the *Viceae* tribe in the *Legumnosae* (*Fabaceae*) family, commonly known as the legume family. The cultivated lentil, *Lens 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). All members of *Lens* are self-pollinating diploids (2n = 2x = 14) (Sharma et al., 1995) and annual herbaceous plants.

The center of origin of *L. culinaris* is the Near East and was first domesticated in the Fertile Crescent around 700 BC (Zohary, 1972) . According to Cubero (1981, 1984), 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. Ethiopia is amongst the centers of diversity for lentil.

Lentil is currently an important pulse crop grown widely throughout the Indian Subcontinent, Middle East, Northern Africa, Southern Europe, North and South America, Australia and West Asia (Ford and Taylor, 2003; 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 et al., 1998).

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In Ethiopia, lentil is grown for human consumption as a rich source of protein (23 – 24%) (Addise and Asfaw, 1993) and, therefore, may correct 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 for Ethiopia. The crop is generally grown in rotation with cereals to break cereal disease cycles and to 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 2001/2002, lentil was grown on 60,120 ha with a total production of 3.84×10^4 tonnes. However, its average seed yield has remained very low, 0.64 tonnes/ha (CACC, 2002). Among the major reasons are: susceptibility of the landraces to various diseases and their inherently low yield potential (Geletu and Yadeta, 1994). These problems necessitated introduction of lentil germplasms to improve production and productivity. Accordingly, ten improved varieties were released for production in Ethiopia. However, in the long run, these varieties may replace indigenous landraces ultimately resulting in genetic erosion. In addition, farmers are eager to replace lentil with remunerative crops, which could exacerbate the threat of genetic erosion. These facts suggest the importance of exhaustive germplasm collections before possible loss of genetic diversity. Prior knowledge of genetic variability within the germplasm available at genebank is important to identify areas of major priority for conservation and for improvement programs.

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). There are numerous DNA- based molecular marker systems suitable for genetic diversity assessment. Inter-simple sequence repeat (ISSR) marker, in addition to its suitability to genetic diversity study, is highly polymorphic, reproducible, cost effective, 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 genetic diversity of Ethiopian lentils using any of the molecular markers with the exception of some studies in which very small number of Ethiopian landrace accessions were included. Therefore, it is important to generate baseline information on this aspect.

In order to obtain a good estimate of the genetic diversity of accessions cost effectively, the choice of appropriate sampling technique is important. Individual plant and bulk sampling (Michelmore et al., 1991) are the two methods of sampling plants for DNA extraction. Analysis based on individual plant sampling requires 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 advantageeous in that it allows 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). 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 each individual plant of the accessions to be represented.

The aim of this study was to analyze the levels of genetic diversity, genetic structure and genetic distance of administrative region (AR)-based populations of Ethiopian lentil landrace accessions and to indirectly detect possible gene flow among the populations using ISSR marker and bulk leaf sample analyses.

MATERIALS AND METHODS

Plant materials and DNA extraction

Seeds of seventy lentil landrace accessions collected from seven different administrative regions (ARs) of Ethiopia were obtained from Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia (Table 1). Most seeds and plants of each accession showed distinct morphological variation from one another in terms of seedcoat colour, stem and leaf pigment and pigmentation pattern, stem thickness, vigorousity in vegetative growth, canopy coverage, etc. All accessions from each AR were considered as a single population and, therefore, samples (accessions) collected from ten randomly selected districts of an AR were used to represent a population. All plants of each accession used in this study were generated from seeds at Sinana Agricultural Research Center on station field plot under natural conditions.

Since this study did not aim to estimate the degree of genetic diversity within the accessions, 15 plant bulks were analyzed in order to represent the genotypic variability present within each lentil accession. Young leaves were collected separately from fifteen randomly selected individual plants of each accession just before flowering and dried in silica gel. Approximately equal amounts of the dried leaf samples were bulked for each accession and ground with pistle and mortar. Total genomic DNA was isolated from about 0.2 g of the pulverized leaf sample using tripple cetyltrimethyl-ammoniumbromide (CTAB) extraction technique. The isolation procedure was modified to yield optimal quantities of high-quality DNA from tissues with considerable amounts of secondary compounds (Borsch et al., 2003). The isolated DNA samples were

Table 1. Passport data of Ethiopian lentil landrace accessions used in the study (Source: IBC).

S/N	Accession	District	AR	Latitude	Longitude	Altitude
1	Acc 36007	DodotaSire	Arsi	8-09-N	39-21-E	2050
2	Acc 36008	Gedeb	Arsi	7-10-N	39-12-E	2440
3	Acc 36041	Merti	Arsi	8-24-N	39-52-E	2040
4	Acc 36042	Chole	Arsi	08-22-N	39-53-E	2520
5	Acc 36047	Sude	Arsi	07-53-N	39-44-E	2520
6	Acc 36131	Robe	Arsi	7-49-N	39-47-E	2480
7	Acc 216879	Bekoji	Arsi	---	---	---
8	Acc 216881	Dodota Sire	Arsi	08-07-N	39-27-E	2370
9	Acc 231239	Sherka	Arsi	Aug-35	39-52-	2330
10	Acc 231240	Jeju	Arsi	Aug-37	39-41-	1920
11	Acc 36029	Agarfa	Bale	7-18-N	39-58-E	2580
12	Acc 36033	Sinana Dinsho	Bale	---	---	2620
13	Acc 36121	Dodola	Bale	---	---	---
14	Acc 212848	Goro	Bale	7-00-N	40-28-E	1800
15	Acc 212851	Nansebo	Bale	---	---	---
16	Acc 230015	Kokosa	Bale	---	---	2620
17	Acc 230017	Agarfa	Bale	7-10-N	34-11-E	---
18	Acc 230020	Ginir	Bale	07-08-N	40-36-E	2020
19	Acc 231243	Adaba	Bale	---	---	---
20	Acc 237988	Adaba	Bale	---	---	2460
21	Acc 36026	Este	Gonder	11-34-N	38-45-E	2590
22	Acc 36065	Dembia	Gonder	---	---	---
23	Acc 36072	Farta	Gonder	11-48-N	35-28-E	3114
24	Acc 36086	Lay Gayint	Gonder	11-41-N	38-29-E	3120
25	Acc 36146	Debark	Gonder	13-13-N	38-01-E	3220
26	Acc 207257	Wegera	Gonder	---	---	---
27	Acc 207259	Fogera	Gonder	---	---	---
28	Acc 207266	Kemekem	Gonder	---	---	---
29	Acc 207291	Janamora	Gonder	---	---	---
30	Acc 207305	Simada	Gonder	---	---	---
31	Acc 36024	Dega Damot	Gojam	---	---	---
32	Acc 36025	Wenbera	Gojam	---	---	1580
33	Acc 36027	Enarj Enawga	Gojam	10-38-N	38-10-E	2510
34	Acc 36028	Hulet Ej Enese	Gojam	11-4-N	37-051-E	2270
35	Acc 36069	Guzamn	Gojam	10-20-N	37-44-E	2460
36	Acc 36118	Shebel Berenta	Gojam	10-27-N	38-21-E	2420
37	Acc 212745	Enemay	Gojam	38-11-N	10-34-E	2580
38	Acc 219507	Banja	Gojam	---	---	---
39	Acc 238978	Bahir Dar Zuria	Gojam	11-38-N	37-13-E	1930
40	Acc 241132	Achefer	Gojam	---	---	2030
41	Acc 36001	Berehna Aleltu	Shewa	9-50-N	39-13-E	2820
42	Acc 36003	Moretna Jiru	Shewa	9-57-N	39-13-E	2820
43	Acc 36006	Gimbichu	Shewa	8-57-N	39-5-E	2370
44	Acc 36009	Ejere	Shewa	9-2-N	38-10-E	2270
45	Acc 36014	Wuchalena Jido	Shewa	09-39-N	38-49-E	2695
46	Acc 36020	Walisona Goro	Shewa	8-39-N	37-54-E	2260
47	Acc 36048	Kembibit	Shewa	09-19-N	39-16-E	2890
48	Acc 36056	Ambo	Shewa	---	---	---
49	Acc 229184	Lay Betna Tach Bet	Shewa	---	---	2720
50	Acc 236891	Girar Jarso	Shewa	39-42-N	38-48-E	2650

Table 1. Contd

51	Acc 207260	Gulomahda	Tigray	---------	---------	-----
52	Acc 213254	Wukro	Tigray	---------	---------	-----
53	Acc 219953	Laelay Maychew	Tigray	---------	---------	-----
54	Acc 219954	Asegede Tsimbela	Tigray	---------	---------	-----
55	Acc 219957	Adwa	Tigray	14-09-N	38-56-E	2330
56	Acc 221719	Enderta	Tigray	---------	---------	2500
57	Acc 223220	Saesi Tsaedaemba	Tigray	14-15-N	39-28-E	2580
58	Acc 223222	Alaje	Tigray	22-43-N	39-32-E	2600
59	Acc 223223	Endamehoni	Tigray	12-42-N	39-32-E	2450
60	Acc 223224	Ganta Afeshum	Tigray	14-14-N	39-28-E	2360
61	Acc 36084	Guba Lafto	Wello	---------	---------	------
62	Acc 36097	Dawuntna Delant	Wello	11-32-N	39-15-E	2200
63	Acc 36101	Wadla	Wello	---------	--------	2830
64	Acc 36103	Kutaber	Wello	11-20-E	39-18-E	2620
65	Acc 36104	Tenta	Wello	11-16-N	39-15-E	2900
66	Acc 36141	Were Ilu	Wello	10-44-N	39-28-E	2660
67	Acc 36151	Dessie Zuria	Wello	11-6-N	39-38-E	2260
68	Acc 36162	Legambo	Wello	---------	---------	3230
69	Acc 36168	Tehuledere	Wello	---------	---------	------
70	Acc 207309	Ambasel	Wello	---------	---------	------

visualized using 1% ethidium bromide stained agarose gel under UV light. The second or third extractions were selected for PCR amplification based on DNA quantity and quality. The selected genomic DNA samples were diluted with sterile distilled water in 1:5 ratio.

ISSR analysis

A total of 12 ISSR primers (UBC primer set #9, Vancouver, BC, Canada) were selected based on published experimental 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* (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. A final set of 4 ISSR primers (Table 2), which produced unambiguously visible and polymorphic bands across the seven accessions were chosen for further analyses.

Optimization of PCR reaction components for ISSR genotyping in lentils 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 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 for 15 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. PCR amplification was performed with Biometra® T3 thermorecycler.

The ISSR-PCR product was resolved on 1.7% agarose gel in 1X TBE buffer. 9 µl genomic DNA per sample was loaded with 2 µl of 6X 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 conducted at 100 V for about 1:40 - 2:00 h in 1X TBE buffer. The resultant gel was visualized by ethidium bromide staining under UV light and photographed with Biodoc Analyzer (035 - 300). Laboratory analysis was conducted at Genetics Research Laboratorty of the Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

Statistical analysis

ISSR profiles/bands were scored manually for each individual landrace accession from the gel photograph. The bands were recorded as discrete characters, presence '1' or absence '0'. Matrix of binary data was constracted with rows equal to accessions and columns equal to distinct molecular marker band. Only bands that could be unambiguously scored across all the sampled populations were used in this report.

Since ISSRs are dominant markers 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 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*), within population diversity (H_S), between population diversity (D_{ST}), coefficient of gene differentiation (G_{ST}) (Nei, 1973), Nei's standard genetic distance (D) (Nei, 1972) and gene flow estimates (McDermott and McDonald, 1993) were computed with POPGENE ver 1.32 (Yeh et al., 2000) computer software. The haploid option of the software was used for analysis in accordance with the assumption of Ferguson et al. (1998) above.

The analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was used to estimate variance components for the ISSR

Table 2. Primer-based marker size and polymorphysm of Ethiopian lentil landrace populations.

Primer		Marker size amplified (bp)	No. of loci		P
Name	Sequence		Total	Polymorphic	
UBC 812	$(GA)_8A$	280-1400	13	8	61.54
UBC 818	$(CA)_8G$	350-1800	9	4	44.44
UBC 835	$(AG)_8YC$	300-2200	12	9	75.00
UBC 881	$(G_3TG)_3$	790-3500	13	7	53.85
Average			11.75	7	59.57
Total			47	28	59.57

Table 3. Number and percentage of polymorphic loci (P), and Nei's mean gene diversity (H) in Ethiopian lentil landrace populations.

Population	P	H±SD	H_S	D_{ST}	G_{ST}	N_m
Arsi	21.28	0.070 ± 0.146				
Bale	23.40	0.075 ± 0.144				
Gojam	27.66	0.088 ± 0.158				
Gonder	34.04	0.140 ± 0.203				
Shewa	31.91	0.116 ± 0.177				
Tigray	21.28	0.076 ± 0.159				
Wello	34.04	0.104 ± 0.163				
Average	27.66	0.096 ± 0.013	0.095 ± 0.013	0.079		
Total population	59.57	0.175 ± 0.040			0.455	0.600

SD stands for standard deviation.

phenotypes using Arlequin ver 3.01 (Excoffier et al., 2006) computer software to describe the genetic structure at population level. Significance of the result was tested by 1000 random permutations with the same software.

Cluster analysis was conducted based on the standard genetic distance matrix (Nei, 1972) with NTSYSpc ver 2.1 (Rohlf, 2004) computer software. A dendrogram was constructed from the dissimilarity matrix using Unweighted Pair Group of Arithmetic Mean (UPGMA) procedure of the Sequencial, Agglomerative, Hierarchical and Nested (SAHN) clustering 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 cophenetic matrix generated from the UPGMA tree was compared with the dissimilarity matrix (from which the tree has been produced) using two-way Mantel (1967) test method.

RESULTS

Genetic diversity

The DNA amplification pattern using ISSR primers in this study was very reproducible across gels based on the results from 70 DNA samples. A total of 47 bands were amplified in Ethiopian lentil landrace accessions using the four ISSR primers. Of the total bands, 28 (*P* = 59.57%) were polymorphic. No population specific bands were observed. The size of ISSR fragments ranged from 280 to 3500 bp. On the average, each primer amplified 11.75 bands of which 7 were polymorphic. The highest percentage polymorphic loci was obtained for primer 835 (*P* = 75.00 %), followed by primer 812 (*P* = 61.54 %), while the least P was observed for primer 818 (44.44%) (Table 2).

The highest percentage polymorphic loci was obtained for samples from Gonder (P = 34.04%), followed by samples from Wello (P = 34.04%) and Shewa (P = 31.91%), while samples from Arsi and Tigray showed the least percentage polymorphic loci (*P* = 21.28 %). Samples from Gonder were the most diverse (H = 0.140) followed by samples from Shewa (H = 0.116) and Wello (H = 0.104). Among the landrace populations evaluated, samples from Gojam (H = 0.088), Tigray (H = 0.076), Bale (H = 0.075) and Arsi (H = 0.070) were the least diverse. The average gene diversity relative to the total landrace population (H_T) was 0.175 (Table 3).

Genetic differentiation and gene flow

The average gene diversity among populations (D_{ST} = 0.079) was slightly lower than the average gene diversity within populations (H_S = 0.095). The extent of gene differentiation relative to the total population (G_{ST}) was

Table 4. Analysis of molecular variance (AMOVA) for Ethiopian lentil landrace populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value
Among populations	6	127.19	1.88	43.72	0.000
Within populations	63	152.3	2.42	56.28	
Total	69	279.49	4.3		

Table 5. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) in Ethiopian lentil landrace populations.

Population	Arsi	Bale	Gojam	Gonder	Shewa	Tigray	Wello
Arsi	****	0.964	0.836	0.936	0.828	0.828	0.956
Bale	0.037	****	0.841	0.953	0.835	0.831	0.922
Gojam	0.179	0.174	****	0.937	0.976	0.988	0.809
Gonder	0.066	0.048	0.065	****	0.929	0.937	0.898
Shewa	0.189	0.181	0.025	0.074	****	0.971	0.796
Tigray	0.189	0.185	0.012	0.065	0.030	****	0.811
Wello	0.045	0.081	0.212	0.108	0.228	0.209	****

0.455. The extent of gene flow (*Nm*) among populations of Ethiopian lentil landraces was 0.600 (Table 3). In agreement with the analysis from Nei's diversity statistics, AMOVA analysis showed highly significant ($p < 0.001$) variation among populations (43.72%), which was lower than the within population (56.28 %) genetic variation (Table 4).

Genetic distance

Inter-population genetic distance (D) ranged from 0.012 to 0.228. Samples from Wello were distantly related to samples of Shewa (0.228), Gojam (0.212) and Tigray (0.209) . In general, among the pairwise population comp-arsons made, samples of Shewa, Tigray and Gojam were distantly related to samples of Wello, Arsi and Bale. Genetic distance between the other pairwise combinat-ions of populations was very low with the least genetic distance between samples of Tigray and Gojam (0.012) (Table 5).

Cluster analysis

Cluster analysis revealed two major groups of Ethiopian lentil landrace populations. The first cluster contained Tigray, Gojam and Shewa populations, while the second cluster contained Gonder, Bale, Arsi and Wello popula-tions (Figure 1). The correlation between Nei's standard genetic distance matrix and cophenetic (ultrametric) dissi-milarity values generated from the UPGMA tree (=0.88) was good (interpreted subjectively according to Rohlf, 2004), validating the observed clusters.

DISCUSSION

The value of molecular biology for monitoring the genetic status of germplasm collections is subject to practical limitations. The large number and variability of acces-sions held usually dictates the approach that can be employed. A quick, simple but reliable molecular protocol must be combined with an appropriate strategy for hand-ling large sample sizes (Gilbert et al., 1999). In this study, bulk sampling approach was chosen because it permits representation of an accession by optimum number of plants. 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 (Hou et al., 2005). Yang and Quiros (1993) reported that bulked samples with 10, 20, 30, 40 and 50 individuals had resulted in the same RAPD profiles as that of the individual plants constituting the bulk sample. Bustos et al. (1998) also reported that bulks of 10 to 20 individuals resulted in the same RAPD pro-files. 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 genetic diversity in landraces and wild forms of barley. In this study, bulked leaf sample analysis of fifteen individual plants per accession was used to assess the genetic diversity of 70 Ethiopian lentil landrace accessions. The technique revealed higher genetic diversity, and, therefore, valid-ated the usefulness of bulk sample analyses for diversity assessment in lentil.

ISSR markers are important to study intraspecific var-iations in plant species, as they are effective in detecting very low levels of genetic variation (Zietkiewicz et al.,

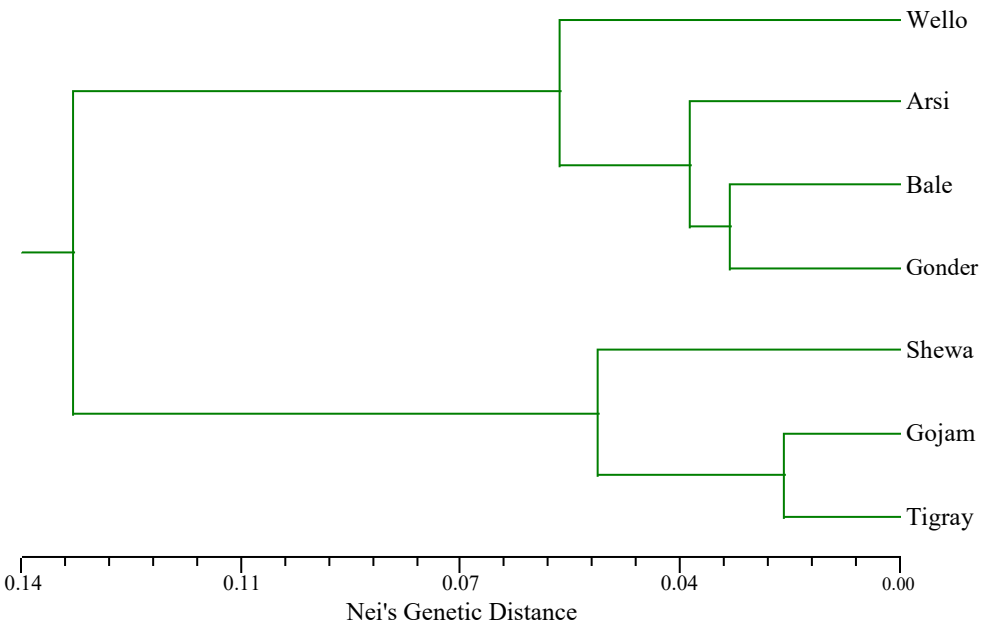

Figure 1. A dendrogram of 7 Ethiopian lentil landrace populations derived by UPGMA from Nei's (1972) standard genetic distance based on data generated using four ISSR primers.

1994). In the present study, ISSRs revealed higher genetic diversity (H_T = 0.175) in Ethiopian lentil landraces. This was greater than the within species diversity (H_T = 0.049) of the cultivated lentil reported by Ferguson et al. (1998) from RAPD data of 100 lentil landrace 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; Sonnante and Pignone, 2001), while ISSRs reveal a higher degree of variation (Sonnante and Pignone, 2001) in lentil. These differences could be explained mainly by the difference in sensitivity of different molecular markers.

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation, and population isolation), genetic drift, mating system, gene flow and selection (Schaal et al., 1998). In the present study, there was high level of genetic differentiation (G_{ST} = 0.455) among populations. This could be attributed to mutation of SSR loci (annealing site for ISSR primers), random genetic drift and differential selection pressure (by the environment) on the loci assessed. For most markers, the contribution of mutation to differentiation has generally been ignored because mutation rates of neutral (traditional isozyme) markers are negligible when compared to migration rates. This may not be the case when dealing with ISSR, which uses SSRs (with high mutation rate) as primer annealing sites.

Estimates of genetic differentiation between populations of inbred species based on AMOVA derived by analyzing RAPD markers have usually been > 70% (Nybom and Bartish, 2000). However, this might not hold in some cases (of food crops, for instance) where there is high gene flow represented by seed movement through human involvement. In accordance with this argument, AMOVA analysis in the present study revealed lower among population genetic variation (43.72%), than within population genetic variation (56.28%). Nei's diversity statistics revealed similar result.

The values obtained from Nm show the approximate number of individuals migrating from one population to the other, in a typical island model. Generally, if Nm < 1, then local differentiation of populations will result, and if Nm > 1, then there will be little differentiation among populations (Wright, 1951). The fact that estimates of Nm was < 1 suggests that gene flow between popula-tions is insufficient to counter the effects of random drift (Real, 1994). Genetic differentiation results from genetic drift only if Nm < 1, but not if Nm > 1 (Slatkin, 1987). According to Slatkin (1981, 1985), Caccone (1985) and Waples (1987) Nm values can be grouped into three categories: high (Nm ≥ 1.000), intermediate, (0.250 – 0.990), and low (0.000 – 0.249). In this study, the rela-tively high genetic differentiation and intermediate level of gene flow (Nm = 0.600) detected strongly indicate that genetic drift has greatly affected the genetic composition of individual populations. The lower gene flow among populations could be attributed to geographical, social or cultural isolation/barrier and limited use of lentil seeds purchased from one AR for cultivation in another AR.

The wide range of interpopulation genetic distance shows the potential for landrace improvement program.

In general, populations with high intra-population genetic diversity were genetically distantly related to populations with low intra- population diversity. Similar finding was reported by de la Cruz et al. (2004) in 5 wild populations of *Phaseeolus vulgaris* L. using ISSR marker.

Implications for improvement

The study of genetic diversity would be important for designing appropriate improvement program. The present study showed high genetic diversity in Gonder, Shewa and Wello populations. Intrapopulation improvement programs should, therefore, target selection of individual plants with desirable traits from these populations, since the populations are more diverse. However, this does not mean that less diverse populations do not consist plants with important trats. But it only means that the probability of getting individual plants with good traits from such populations is very low as compared to those that are more diverse. Therefore, a more detailed sort of selection should also be practiced in such populations.

The genetic distance between populations, on the other hand, is a vauable parameter for germplasm improvement programs. Hybridization/crossing between any distantly related populations is expected to yield more heterotic and vigorous plants constituting much of the different traits contained in the two parental lines. Therefore, hybridization or crossing between distantly related populations of the present study could be an appropriate strategy for interpopulation landrace improvement programs.

Using both (genetic diversity and distance) parameters simultaneously for improvement programs would be the best approach. In this case, the best individuals selcted from the highly diverse population(s) and crossed with individuals selected from population(s) that is/are comparatively distant from the former population could yield good results.

Implications for conservation

The ultimate goal of conservation is to ensure continuous survival of populations and to maintain their evolutionary potential. Information on current levels of genetic diversity of germplasm at gene bank is essential for designing appropriate strategies for future conservation. According to the results of the present study, the high genetic differentiation among the ARs or populations suggests that all the ARs/populations should be well represented by more samples/accessions *ex situ*. Furtheremore, the high genetic diversity within some populations (Gonder, Shewa and Wello) indicate that the most effective strategy for preserving genetic variation would be to conserve a large number of individuals for more diverse population(s).

Conclusion

In conclusion, the present study shows that bulk sampling strategy and ISSR marker are important for genetic diversity study in lentil. The results also provide important milestone for future conservation and improvement programs of Ethiopian lentil landraces. However, it is recommended that the present study be complemented with a parallel investigation on adaptive traits (drought, pest and insect resistance) and other economic traits (growth and form) before a definite strategy is adopted. Furtheremore, results from gene flow estimates suggest that there could be a possibility of sampling plants with the same genetic constitution from different administrative regions. Therefore, representing a population by as many collections as possible would be the best approach.

ACKNOWLEDGEMENTS

Edossa Fikiru is thankful to: the Agricultural Research and Training Project of Ethiopian Inistitute of Agricultural Research and Addis Ababa University for funding the research; Inistitute of Biodiversity Conservation (Addis Ababa, Ethiopia) for kindly providing seeds and passport data of the landrace accessions; Sinana Agricultural Research Center (Bale-Robe, Ethiopia) for provision of physical resources and other facilities; and, Mr. Tamiru Oljira and the stuff of Pulse and Oil Crops Breeding and Genetics Section of Sinana Agricultural Research Center for their respective support. All the authors especially thank Dr. Thomas Borsch and Dr. Tadesse Weldemariam for their valuable assistance.

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