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RESEARCH ARTICLE

Molecular Diversity and Population Structure of the Ethiopian Lentil (*Lens Culinaris* Medikus) Genotype Assessment Using SSR Markers

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Abstract

Knowledge of genetic diversity in germplasm is essential for formulating effective germplasm collection, conservation, utilization strategies in and crop improvement programs. It also provides an opportunity to take corrective steps infusing new genes to avoid risks associated with a narrow genetic bases. Genetic diversity analysis of 119 lentil genotypes of including 83 germplasm and 36 exotic genotypes from International Center for Agricultural Research in the Dry Areas was studied using 27 primers of simple sequence repeat (SSR) marker. Molecular analysis of variance showed variations of 82% within and 18% of the among population variance was explained. Degree of polymorphism observed among the populations was 100%. A total 122 alleles were detected, with 2 to 7 alleles per locus, with a mean of 4.52 alleles per locus. The estimated gene diversity value for 27 loci was 0.64. The average Shannon's information index value of 1.19 was obtained showed the existence of high genetic variation within the genotypes. The genetic similarity indices ranged from 0.21 to 1.00. The SSR markers showed an average polymorphic information content (PIC) value of 0.58. Cluster analysis grouped the genotypes into five major clusters as distinct genetic populations. Diversity analyses revealed the existence of a high level of genetic variation among genotypes. This molecular diversity information provides a basis for future germplasm collection, utilization, and conservation strategies in gene banks and introducing exotic germplasm to widen the genetic base of the current lentil breeding population.

Key words : crop growth model, maize, simulation performance, supra-optimal temperature

Introduction

Lentil (*Lens culinaris*Medik subsp. *culinaris*) is a diploid (2n=2x=14 chromosomes), self-pollinating annual species, with a haploid genome size of 4,063 Mbp (Arumuganathan and Earle 1991). The leading lentil producing countries in Africaare Ethiopia, Morocco, and Tunisia, but 61% of the production areas and 68% of the production is in Ethiopia (FAOSTAT 2009). Global lentil productivity is 0.9 ton ha^{-1} (Erskine 2009), African productivity is 0.6 ton ha^{-1} (Erskine 2009), and Ethiopian productivity about is 1.2 ton ha^{-1}(CSA 2012). Lentil is an important dietary source of macro- and micro-nutrients for both rural and urban dwellers. It provides sufficient amounts of the most essential amino acids to meet nutrient requirements. Being a cash crop, it fetches the highest price compared to other food legumes and major cereal crops in the domestic markets (Geletu et al. 1996). Despite its importance, numerous factors limit the yield of lentil. These include the inherent poor yield potential of landraces and susceptibility to biotic and abiotic stresses (Muehlbauer et al. 2006) and genetic erosion (Solh and Erskine 1984).

Success in plant breeding is directly dependent on the

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exploitable genetic variability available in the germplasm accessions available to breeders. The study of genetic variation is a primary step in the improvement of any crop (Allard 1960). Several markers have been used to study the genetic diversity of lentil, e.g. restriction fragment length polymorphisms (RFLPs) (Havey and Muehlbauer 1989), random amplified polymorphic DNAs (RAPDs) (Duran and Perez 2004; Sharma et al. 1995), amplified fragment length polymorphism (AFLP) (Hamwieh et al. 2005; Sharma et al. 1996; Torricelli et al. 2011), inter simple sequence repeat (ISSR) (Sonnante and Pignone 2007), SSR (Babayeva et al. 2009, Hamwieh et al. 2009; Reddy et al. 2009), and sequence-tagged microsatellite sites (STMS) (Datta et al. 2011). Erskine and Witcombe (1984) reported that Ethiopian germplasm was diverse in earliness, seed yield, harvest index, number of seeds per pod, and cold tolerance. Other researchers found that Ethiopian landraces had genetic variability for some important agronomic traits and disease resistance (Bayaa et al. 1997; Edossa et al. 2010; Geletu et al. 1996; Kumar et al. 2010;).

Comprehensive information on the genetic diversity of Ethiopian lentils using molecular markers is not available, except for some preliminary studies on a small number of selected Ethiopian landraces using ISSR markers (Edossa et al. 2007). Microsatellites or SSR markers are extremely useful in estimating genetic diversity because of their inherent capability to resolve a high degree of variability (Chris et al. 2009; Gupta et al. 1999; Powell et al. 1996; Varshney et al. 2005). Microsatellites have been used successfully in lentil population genetic studies, genetic diversity analyses, genetic mapping, and genome analysis (Chen 2007; Furman et al. 2009; Hamwieh et al. 2005; Hamwieh et al.2009; Mamta et al. 2012; Saha et al. 2010).

A lack of genetic information regarding the Ethiopian lentil population has limited their use as a source of useful traits in lentil breeding programs. Prior knowledge of genetic variability and characterization of genetic resources within the germplasm available at the Ethiopian Biodiversity Institute (EBI) have important implications for future use and collection activities; this knowledge will help to identify areas of major priority for conservation and breeding programs (Allard 1960). In addition, the assessment of genetic diversity among cultivars helps explain the patterns of germplasm use in breeding programs and provides an opportunity to take corrective steps such as introgression of new genes to avoid the risks associated with a narrow genetic base (Avise 1994). Thus, there is an urgent need to focus on diversity and genetic relationships among the Ethiopian gene pool to increase the overall production and productivity of this crop through varietal improvement and suitable agronomic practices under Ethiopian conditions. The objectives of this study were to analyze the genetic diversity and population structure of the Ethiopian lentil genotypes and exotic lines using SSR markers.

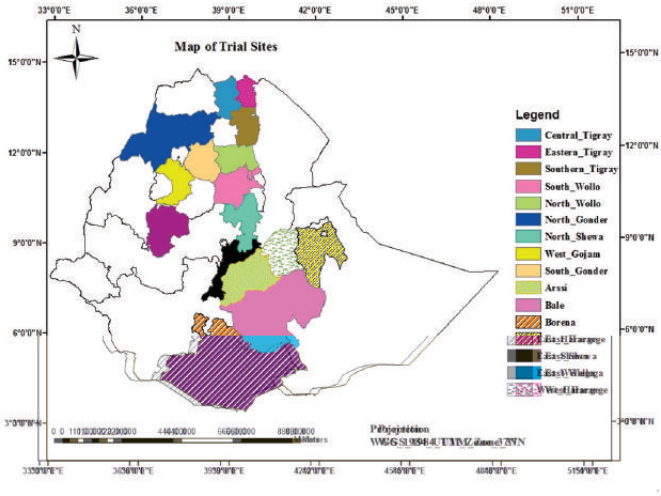

Fig. 1. Map of Ethiopia showing zonal area of collection sites (shaded region) of the 83 genotypes.

Materials and Methods

Plant materials

In this study, a total of 119 lentil genotypes of which 83 genotypes represented the Ethiopian lentil landraces collected from six major lentil-producing regions (Fig. 1) and maintainedby the Ethiopia Biodiversity Institute (EBI) were used. The local genotypes represent over 14% of the total 618 lentil germplasm holdings of EBI (Tanto and Tefera 2006). The remaining 36 genotypes were commercial varieties and exotic elite lines (Table 1).

Genomic DNA extraction

The genotypes were planted in a greenhouse at the Department of Agricultural Biotechnology, CSK Himachal Pradesh Agricultural University, Palampur, India. The DNA was extracted two weeks after planting with an equal amount of bulk leaf sample collected from five to ten plants of each genotype according to the protocol described by Edwards et al. (1991). The samples were crushedin liquid nitrogen with a pestle and mortar and genomic DNA was extracted using the cetyltriethylammoniumbromide (CTAB) method (Edwards et al. 1991) with some minor modifications. Then the DNA pellet was air dried and dissolved in 100 µL of 1 × TE buffer. The DNA samples were stored at -20°C until needed for the PCR reactions.

Screening SSR markers

The SSR primers developed by Hamwieh et al. (2005, 2009) and Saha et al. (2010) were used for the amplification of the lentil genome (Table 2). The length of the primers ranged from 18 to 23 bases. Sixty-two SSRs primers were used for selective genotyping for polymorphism using five representative genotypes of different genetic backgrounds based on the previous phenotypic variability study (Fikru et

Table 1. Passport description and list of genotypes used in the study.

Name of genotypes	Number of genotypes	Sources	Type of population
Accession number 219957, 223221, 235383, 237503, 237504, 241785, 242604, 243447	8	Ethiopia /Tigray/	Landrace
P160/ILL 2704/ / Chekol/, /ILL 1 x ILL 1169//ILL 6027/ /Adaa /, /ILL4225 x ILL4605/ /ILL 6821/ /*Alemaya*/, /ILL 7978/ /*Teshale*/, /Alemaya x FLIP88-41L /*Derash* /, /ILL 7981//*AlemeTena*/,	6	DZARC Ethiopia	Improved commercial varieties
Accession number 36003, 36025, 36028, 36041, 36061, 36071, 36088, 36097,36103,36104, 36105, 36137, 36139, 36150, 36162, 36165, 36168, 207258,207309, 211078, 215248, 215249, 228242, 229179, 229182, 229183, 231247, 235013, 235015, 235016, 235017, 236484, 236487, 237502, 238978, 238979, 241784, 241786, 243433, 243436, 243440, 243443, 244606, 244615, 244619, 244623,	45	Ethiopia /Amahara /	Landrace
Accession number 36001, 36007, 36015, 36029, 36033, 36042, 36048, 36104, 36110, 36120, 36131, 203141, 211131, 215806, 216877, 230521, 230833, 230834, 230837, 231248, 233349, 233973, 235698, 236438, 236892, 237027 and 238971	27	Ethiopia /Oromaya /	Landrace
L-9-12, X2002S 219 /shehor-74/ /ILL 7554/, X2002S 221/7979/, X2002S 221 /7980/, X2003S 222/ILL 213/, X2003S 233 /8009/, X2003S 238 /ILL 4605/, X2005S 215 /6002/, X2006S 122 /9932/, X2006S 122 /ILL 2573 x ILL 7537/ /FLIP 2003-62 L/ /ILL 9951/, X2006S 127, X2006S 127 /ILL 7620 x ILL9151/ /FLIP 2003-56L/ /ILL 9945/, X2006S 128/5480/, X2006S 129 /F2, X2006S 130/FLIP 93-46L/ /ILL 7547/, X2006S 130/FLIP 96-46 L//ILL 7978/, X2006S 133/FLIP87-21L/ /ILL 4349 x ILL4605//ILL 6211/, FLIP-2004-7L, X2006S 134/ILL 8174/	19	ICARDA	Parent[2]
Accession number 36147 and Acc. no. 228243	2	Ehiopia /PSNNP	Landrace
Accession number 230832	1	Ethiopia /Somali /	Landrace
Pedigree number EL-142 , ILL-NE-2704 / *Chekol* / x R-186-8-1, ILL-358 X ILL-2573-2-2000, 87S-93549XEL-1O3-4, ILL-NE-2704 /*Chekol*/ x R-186-8-1, EXOTIC #DZ/2008 X AK	6	EBL from DZARC[4]	Elite breeding lines
ILL-590, ILL-10045, FLIP-97-61L, L-830, Precoze	5	ICARDA	Elite breeding lines

[1]Genotypes: represent different population group of lentil germplasm such as landrace, elite breeding lines, putative parents and commercial national varieties
[2]Parent: represent genotypes with agronomically putative traits used in the crossing block
[3]PSNN: People of southern nation and nationality
[4]DZARC: DebereZeit Agricultural Research Center

al. 2014). A total of 27 polymorphic SSR primers covering nearly all the linkage groups were used for DNA amplification and genetic diversity analysis.

Polymerase chain reaction

The PCR was carried out in a final volume of 12.5 µl containing 7.13 µl of sterilized distilled water, 2.0 µl template DNA (25 ngµl^{-1}), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 0.75 µl $MgCl_2$ (25 mM), 1.25 µl 10× PCR buffer (10 mMTris-HCl, 50 mMKCl, pH 8.3), 0.2 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP, and dTTP), and 0.17 µl Taq polymerase (5 U µl^{-1}) (Invitrogen, Carlsbad, CA). The amplifications were carried out in a Gene Amp PCR System 9700® (Applied Biosystems) and a 2720 Thermal Cycler (Applied Biosystems) by using protocols suggested by Hamwieh et al. (2005, 2009).

The Touch Down PCR profile was used for the amplification of SSRs following the initial denaturing cycle of 95°C for 5 min, followed by 35 cycles, each cycle consisting of a denaturation step at 94°C for 1 min, an annealing temperature of 49 to 55°C for each primer for 1 min, and an extension step at 72°C for 2 min, followed by final extension at 72°C for 7 min, before cooling to 4°C. The amplicon separation was performed using 6% polyacrylamide gel electrophoresis.

Marker data scoring and analysis

The genetic diversity parameters were estimated using POPGENE 1.32 software (Yeh et al. 2000). For deducing the

Table 4. Diversity statistics for 27 SSR loci in 119 lentil genotypes.

No.	Primer	Previous linkage group	Sample size	Band size /bp/	Na†	Ae††	I†††	PIC‡
1	SSR59-2	5	230	350-450	7	4.13	1.63	0.72
2	SSR66	1/8/	232	300-340	6	3.89	1.54	0.71
3	SSR90	2	228	350-500	4	2.70	1.09	0.56
4	SSR96	9	222	300-340	6	3.70	1.51	0.69
5	SSR130	7/5/	238	210-325	6	2.98	1.31	0.61
6	SSR132RN	4	236	350-390	6	4.67	1.65	0.76
7	SSR154	2	236	300-500	4	2.25	0.99	0.50
8	SSR156	5	234	70-500	5	3.24	1.30	0.64
9	SSR183	1	224	70-450	6	2.44	1.21	0.54
10	SSR184	2	234	60-400	5	2.77	1.21	0.58
11	SSR191	6	232	50-290	7	3.84	1.49	0.70
12	SSR199	8	224	180-550	5	2.92	1.18	0.59
13	SSR207	1	224	225-275	3	1.83	0.80	0.41
14	SSR212	1	220	210-800	4	3.36	1.29	0.65
15	SSR253	5	234	150-850	5	3.13	1.33	0.63
16	SSR 317-1	1/8/	226	225-300	5	3.90	1.47	0.70
17	SSR 317-2	2	226	125-150	3	2.19	0.93	0.49
18	GLLC 106	2	224	100-130	3	2.03	0.87	0.45
19	GLLC 527	8	230	150-800	5	2.92	1.16	0.59
20	GLLC 538	11	230	360-750	4	2.40	1.02	0.52
21	GLLC 541	11	234	130-250	4	2.40	1.07	0.53
22	GLLC 562	2	226	150-250	3	2.67	1.03	0.55
23	GLLC 563	11	232	270-600	3	2.56	1.00	0.53
24	GLLC 595	14	228	75-400	3	1.99	0.86	0.45
25	GLLC 598	11	226	75-200	4	3.78	1.36	0.69
26	GLLC 609	2	238	75-250	2	1.99	0.69	0.38
27	GLLC 614	12	238	275-650	4	2.57	1.07	0.53
	Total				122			
	Mean ± SD		230		4.56 ± 1.37	2.94 ± 0.76	1.19 ± 0.26	0.58

† Na: observed number of alleles per locus
††Ae: effective number of alleles per locus
††† I : Shannon's information index
‡ PIC: polymorphic information content

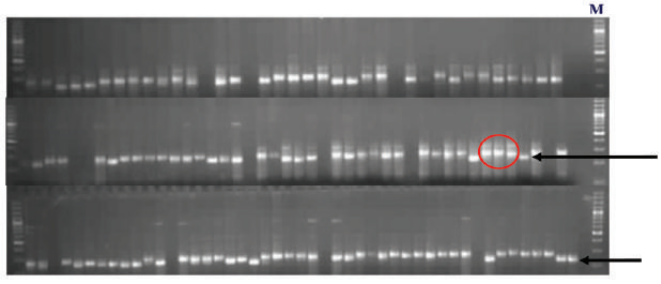

Fig. 2. Autoradiograph of Lentil DNA of 119 genotypes as revealed by a polymorphic SSR marker, GLLC-527.Mstands for DNA ladder GeneRuler™100 bp and arrows indicate polymorphic bands. Some accessions (e.g. circled) revealed more than two bands.

the population and higher among populations (43.7%) using ISSR for Ethiopian landraces.

The 27 polymorphic SSRs produced 122 alleles (Table 4). The size of the amplified fragments varied from 50 bp to 850 bp.The number of detected alleles over all loci (or markers) across genotypes ranged from two (GLLC 609) to seven (SSR59-2 and SSR191), with an average of 4.5 alleles per locus. The effective number of alleles per locus ranged from 1.8 to 4.7, with an average of 2.94 (Table 4). Babayeva et al. (2009) also reported an equivalent amount of polymorphism and alleles per locus in 39 genotypes of lentil.The lowest number of alleles (32) was observed in the breeding lines obtained from the cross 'ILL-358 × ILL-2573-2-2000' originating from DebereZeit Agricultural Research Center (DZARC), Ethiopia, whereas the largest number of alleles (50) was observedin a landrace, accession number-244623 (Supplementary Data 2). The total number of amplified alleles, average number of alleles per locus, and the polymorphic loci in this study were higher than those reported by Datta et al. (2007).

The lowest and the highest He values were 0.46 and 0.76 for SSR207 and SSR59-2, respectively, with an average of gene diversity 0.64. Earlier studies by Babayeva et al. (2009) reported a comparable gene diversity value of 0.66 for 33 loci, with genetic similarity indices among 39 lentil genotypesranging from 0.24 to 1.0. However, results contrary to ours were reported by Edossa et al. (2007), showing a very low average gene diversity (0.18) for the Ethiopian lentil germplasm with ISSR markers. The lowest observed heterozygosity values of 0.25 was recorded for SSR 183, and the highest value of 1.0 was recorded for SSR130 and GLLC 614; the average was 0.68 (Table 5). Besides previous reports indicated that the level of polymorphism depends on the type of germplasm included in the study (He et al. 2011), type of primers selected (Sharma et al. 2011) and the sampling strat-

Table 5. Summary of the parameters for genetic diversity in lentil populations from different sources.

Name of primer	Sample size	Ho⁺	He	Fst
SSR59-2	230	0.37	0.76	0.77
SSR66	232	0.34	0.75	0.78
SSR90	228	0.91	0.63	0.34
SSR96	222	0.72	0.74	0.56
SSR130	238	1.00	0.67	0.25
SSR132RN	236	0.89	0.79	0.44
SSR154	236	0.67	0.56	0.41
SSR156	234	0.63	0.69	0.56
SSR183	224	0.25	0.59	0.82
SSR184	234	0.42	0.64	0.68
SSR191	232	0.70	0.74	0.55
SSR199	224	0.90	0.66	0.39
SSR207	224	0.49	0.46	0.55
SSR212	220	0.69	0.71	0.57
SSR253	234	0.60	0.68	0.57
SSR317-1	226	0.99	0.75	0.39
SSR317-2	226	0.51	0.55	0.59
GLLC 106	224	0.49	0.51	0.59
GLLC 527	230	0.56	0.66	0.6
GLLC 538	230	0.78	0.59	0.38
GLLC 541	234	0.82	0.59	0.32
GLLC 562	226	0.61	0.63	0.56
GLLC 563	232	0.90	0.61	0.31
GLLC 595	228	0.42	0.50	0.63
GLLC 598	226	0.74	0.74	0.54
GLLC 609	238	0.87	0.50	0.12
GLLC 614	238	1.00	0.61	0.18
Mean	230	0.68	0.64	0.50
SD		0.22	0.09	

H_o-observed heterozygosity, H_e-expected heterozygosity, AvHe-average heterozygosity, Fst-Wright fixation index

egy (Kong et al. 2011) that we made. Genetic similarity indices among 119 genotypes ranged from 0.21 to 1.0 (Supplementary Data File 1).

The highest Shannon's index (*I*) value of 1.65 was recorded for SSR 132-RN and the lowest 0.69 was recorded for GLLC 609. A high average *I* value of 1.19 was recorded across the SSR loci (Table 4). The PIC values ranged from 0.38 (GLLC 609) to 0.76 (SSR 132RN), with an average value of 0.58 (Table 4). GLLC 609 showed the lowest number of alleles locus^{-1}, observed number of alleles (Na), effective number of alleles (Ae), and the lowest *I* value, while SSR 132-RN showed the highest value. The PIC values observed in the present study were comparable with the study by Datta et al. (2011), where PIC values ranging from 0.02 to 0.99 were reported for lentil using the 39 STMS markers.

The SSR183 showed the highest (0.82) Wright fixation index (Fst) whereas, SSR marker GLLC 609 showed the lowest Fst (0.12) (Table 5). However, in contrast to these findings, Alabboud et al. (2009), reported 62.5% lower percentage of polymorphic loci. Whereas Ford et al. (1997), reported a low level of mean genetic variation between any two cultivars/accessions with a value of 23%. In another study, Edossa et al. (2007) and Salehe et al. (2012), reported

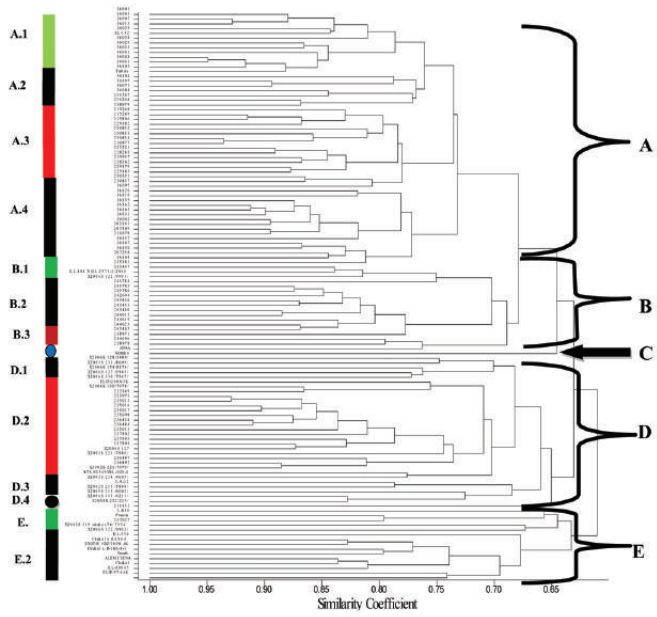

Fig. 3. A dendrogram showing the genetic similarity of lentil genotypes based on UPGMA method using Genstat software package. Numbers in the dendrogram shows the lentil genotype.

lower values of Wright's fixation index 0.18 and 0.45, respectively. These findings implied that the SSR markers are an efficient molecular tool for lentil diversity studies.

Visualizing the the genotypes relationship using cluster analysis

Dice similarity matrices of lentil genotypes were calculated from SSRs data. The relationship among the 119 individuals of lentil genotype based on genetic similarity (genetic distance) values was further determined by UPGMA cluster analysis. The UPGMA dendrogram was generated from genetic similarity coefficients. Both analyses revealed a varying degree of genetic relationship for genotypes belonging to different origin and the breeding status. The Dice similarity coefficient among cultivars ranged from 0.21 ("Acc. no. 36150" and Acc.No.243443) to 0.999 ("Acc. No. 237502" and "Acc. No. 237502") (Suplementary File 1). Dice similarity matrices showed both the divergent and complete similarity coefficients of the genetic relationship among the genotypes (supplementary File 1). The wide range of genetic distances recorded among genotypes indicated the Ethiopian lentil germplasm had a broad genetic base and the genetic similarity observed between some genotypes could be attributed to their common origin. These relationships were supported by cluster analysis. Cluster analysis clearly separated the genotypes into five major clusters as distinct genetic populations.

The UPGMA dendrogram, based on Nei's genetic distances as defined by the SSR markers, showed that most of the genotypes were grouped together because of their phylo-

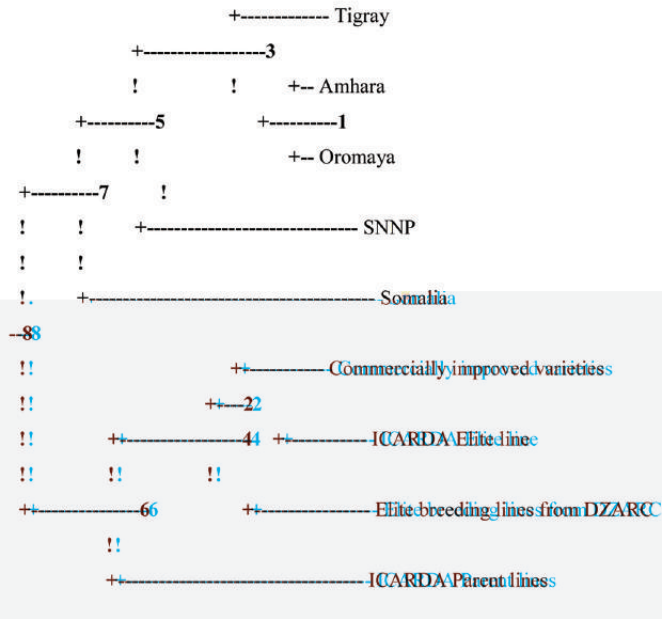

Fig. 4. A dendrogram among lentil population from different source of origin using 27 SSR primers.

genetic relation and agronomic performance such as earliness, biomass production, pod setting character and other morphological identity, wilt (*Fusarium oxysporum* Shlecht), and rust (*Uromycesviciafabae* (Pers.) Schroet) disease reaction response based on similarity coefficient levels. The dendrogram showed the existence of a definite pattern of relationships within the genotypes with respect to the breeding status and, to a lesser extent, with their geographical origins-specifically the Ethiopian landrace collection (Fig. 3). Landrace from Amhara and Oromiya regionswhere in one group, and the rust and wilt-resistant parents, elite breeding line from ICARDA, and high-yielding commercially improved varieties were clustered together. Being different from the other genotypes, *'Adaa',* an old commercial variety, was distinctly groupedinto cluster C because of its unique gene combination such as late maturity, high yield, and rustresistance. The commercial varieties '*Derash*, *AlemeTena,* and *Chekol'*and the wilt-resistant breeding lines (EXOTIC #DZ/2008 AK and Chekol × R-186-8-1)were also clustered into Cluster D, indicating their close relationship and common genetic background (Fig. 3).

High levels of intra-regional similarities were noted within each origin or, in other cases, between adjoining geographical origins. Populations from the same geographical origin were observed to characteristically fall exclusively in a single cluster or two clusters, typically cluster A, cluster B, and subcluster D-3,whereas sub-cluster D-2 and cluster E had populations from more than one source. The number of entries varied from cluster to cluster. The cross-border similarities among the Ethiopian genotypes between a few adjoining regions may be attributed, at least in part, to seed movements among neighboring regions. Tyagi and Khan (2011) also

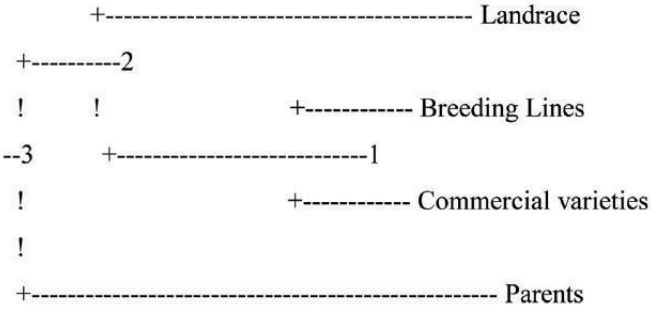

Fig. 5. A dendrogram of the different population based on the breeding status ofthe genotypes groups using Nei's (1978) genetic distance.

observed the same kind of clustering pattern for lentil genotypes belonging to the same origin distributed over different clusters, indicating the non-parallelism existing between geographic distribution and genetic diversity. Babayeva et al. (2009) also observed the same kind of clustering pattern for lentil genotypes belonging to the same origin distributed over different clusters, indicating the non-parallelism existing between geographic distribution and genetic diversity. Babayeva et al. (2009) also grouped 39 genotypes from Central Asian and Caucasian origin into six major clusters at 0.5 similarity coefficients using the same UPGMA technique (Nei 1973, Nei 1978).

The first cluster (A) was the largest group comprising 52 genotypes and had four sub-clusters. The second cluster (B) comprised 19 genotypes and had three sub-clusters. The third cluster C was only represented by one improved commercial variety *'Adaa'*, released by DZARC. The fourth cluster (D) constituted the second largest group comprised of 32 genotypes and had four sub-clusters. The fifth cluster (E) comprised 15 very divergent genotypes from the rest of the clusters and was sub-divided into two sub-clusters. This cluster had comprised very divergent genotypes separated by high inter-cluster distance as compared with the other four clusters. Genotypes in this cluster possessed important morphological and gene based attributes such as accessions with high yield per plant, high number of pods per plant, and a putative gene source for susceptibility and resistance response for wilt and rust that can be used as parents for lentil improvement (Fikru et al. 2014). In addition, three improved commercial varieties (*Derash, AlemeTena,* and *Chekol*) developed at DZARC were included in this cluster (Fig. 3). Clusters D and E comprised genotypes from two or more geographical origins. We can deduce that genotypes from different origins can have similar genetic background as revealed by SSR markers because they are exposed to similar events of domestication, both natural and artificial selection.

As noticed in Fig. 3 and the Dice similarity matrix (Supplementary File 1) as one goes from cluster A to cluster Ethe within divergence increases as indicated by length of the node. From the intra-population cluster analysis, we

Table 6. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) based on source of origin of the genotypes for 27 SSR primers

	Tigray	Elite breeding lines from DZARC	Amhara	Oromaya	ICARDA	SNNP	Somalia	EBL/DZARC/	ICARDA Core
Tigray		0.87	0.96	0.94	0.82	0.83	0.83	0.9	0.87
NCLIP	0.14		0.9	0.89	0.86	0.83	0.75	0.94	0.95
Amhara	0.04	0.11		0.99	0.82	0.91	0.85	0.87	0.87
Oromaya	0.06	0.11	0.01		0.83	0.93	0.86	0.87	0.87
ICARDA	0.19	0.15	0.2	0.19		0.74	0.73	0.85	0.91
SNNP	0.18	0.19	0.1	0.08	0.3		0.88	0.77	0.75
Somalia	0.19	0.28	0.17	0.15	0.31	0.12		0.77	0.72
EBL/DZARC/	0.11	0.06	0.14	0.14	0.16	0.26	0.26		0.93
ICARDA Core	0.14	0.05	0.14	0.14	0.09	0.29	0.33	0.07	

Table 7. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) based on breeding status of the population

Name of primer	Landrace	Parent	Breeding lines	Commercially improved varieties
Landrace		0.83	0.87	0.9
Parent	0.19		0.89	0.86
Breeding lines	0.13	0.11		0.96
Commercially improved varieties	0.11	0.15	0.04	

could infer that genotypes from the Amahara region shared a significant portion of their common ancestral gene pool with genotypes from the adjoining Oromya region. This could be ascribed to massive seed movement through the main market channels of the major lentil production belt of the central highlands of Ethiopia to other regions. The other probable reason for these notable genetic similarities between genotypes from neighboring administrative zones could be the massive seed movement associated with similar agro-ecological systems. In the other regions, there was no clear pattern of geographical similarity with adjoining administrative zones. The probable reason for these less noticeable genetic similarities within some regions (SNNP and Somalia) may point out the crop having been introduced and adapted as a new geographical niche through extension and due to low sample representation. Edossa et al. (2007 and 2010) also noticed similar patterns of gene diversity in those regions.

Population structure analyses based on source of origin

The genetic similarity coefficients and UPGMA dendrogram revealed varying degrees of genetic relationships based on geographic origins. Pair-wise genetic similarity coefficients among the sources of origin estimated by Dice's coefficient (Nei and Li 1979) ranged from 0.72 to 0.99 (Table 6). The lowest level of genetic similarity (72%) was obtained between a landrace originating from the Somalia region and a genotype introduced from the ICARDA core sets gene pool. The maximum level of genetic similarity (99%) was recorded between the landrace collected from the Amharaand landrace obtained from Oromiyaregions.The Ethiopian germplasm accessions had closer affinity with each other than with genotypes introduced from ICARDA.

Cluster analysis clearly separated the genotypes from the two sources into two major groups based on geographic origin (Fig. 4). One group included the landrace collection from Ethiopia originating from Amhara, Oromiya, Tigray, SNNP, and Somali regions. The second group comprised improved varieties, breeding lines from DZARC, elite lines, and parent materials introduced from ICARDA. The materials introduced from ICARDA were clearly distinct from the Ethiopian genotypes. Those differences observed between the Ethiopian landraces and exotic genotypes could be the result at least partly from combined effects of natural and artificial selection, geographical isolation, mutation, and migration in the respective regions.

Pattern of genetic differentiation based on breeding status of the genotypes

The pair-wise genetic similarity coefficients of different population groups based on breeding status varied from 0.83 to 0.96 (Table 7). The elite breeding lines and improved commercial varieties had similar genetic backgrounds which indicated that population types from different origins might have similar genetic background as revealed by SSR markers. This may be because they were exposed to similar events of domestication and artificial selection. The different population groups based on breeding statuswere distinctly separated into three major groups (Fig. 5). Group I included landraces of the Ethiopian collection; group II consisted of improved commercial varieties and elite breeding lines, and group III was comprised of parental lines from ICARDA.

The Ethiopian lentil landraces were distantly separated from the ICARDA parental lines based on the UPGMA clustering analysis. However, there were some exceptions to the Ethiopian germplasm which distinctly shared similar genetic backgrounds with the exotic lines from ICARDA. The presence of large genetic variations can provide the opportunity to select and breed new varieties with high yield and better resistance to biotic and abiotic stresses. A high level of genetic variations in the genotypes make them good candi-

dates as parents in lentil breeding programs in Ethiopia. Erskine and Whitcombe (1984) reported that the Ethiopian germplasm had interesting characteristics such as diversity in earliness, seed yield, harvest index, number of seeds per pod, and cold tolerance.

The present study highlighted the following key findings. First, the landrace from Ethiopia and breeding lines from ICARDA showed a highgenetic diversity. The high gene diversity coefficient and the percentage of polymorphic bands (100%) with the use of SSR markers indicatedthe presence of potentially useful sources for breeding. In line with these findings Edossa et al. (2007), indicated that the present lentil genotypes from the Ethiopian region have a broader genetic base. Secondly, the genotypes were clustered into five major clusters. Elite breeding lines with superior agronomic traits showed closer genetic relationships with the improved varieties. Genotypes with specific agronomic attributes such as disease resistance and susceptibility to rust and wilt, were clearly separated from one another. Clustering resulted in better grouping of the genotypes according to their population structure and gene attributes rather than geographic origin. The genotypes grouped into the same cluster presumably diverged very little from one another. Crossing of genotypes belonging to the same cluster is not expected to yield hetrotic recombinants. Therefore, crosses between parents with a maximum divergence could result in better yield and agronomic performance since they are likely to produce heterotic and desirable genetic recombinants.

The third finding was that it was explicit that no definite relationship was observed between geographic origins and genetic diversity of lentil in this study. Clusters A and B comprised large number of genotypes from same origin from the Amahara, Oromya, and Tigray regions were found to be closely associated with one another, as they were collected from similar geographical and environmental conditions. Hiyaly et al. (1993) and Wu et al. (2010) have noted similar report thatthere is a clear association between population characteristics and the environments in which they occur. The rest did not follow the grouping based on source of origin because some of the Ethiopian genotypes characteristically showed a similar genetic background with that of the exotic lines from ICARDA. We can deduce from the cluster analysis, the grouping of genotypes was largely contributed by their genetic background rather than their geographic origin. In earlier studies on crops like field pea (Gemechu et al. 2005), and safflower (Khan et al. 2009),no definite correspondence between geographic origin and genetic diversity was observed. This finding suggests that parental selection should be made based on a systematic assessment of genetic distance in a specific population rather than on geographic considerations.

Lastly, clusters D and E were comprised of genotypes with different genetic backgrounds from diverse origin such as exotic lines, improved varieties, and with a few Ethiopian landraces as revealed by SSR markers. According to (Fikru et al. 2014), these two clusters hadcharacteristically superior agro-morphological traits and better performing genotypes, which are indicative of the likelihood of their exploitability for lentil improvement through recombination breeding.

Conclusion

This study is the first step towards the characterization of lentil germplasm in Ethiopia using microsatellite markers. A successful attempt has been made to examine genetic diversity in lentils using SSR loci, and has been found to be advantageous in showing sufficient polymorphism and a powerful tool for analyzing genetic diversity. A high degree of genetic diversity in Ethiopian lentil germplasmwas identified and could be further exploited in crop improvement. The outcomes also indicate the need for developing a mini core set of Ethiopian germplasm. The current molecular diversity information also provides the basis for enhancing conservation strategies in gene banks and a need for introducing exotic germplasm to widening the genetic base of lentil breeding programs by focusing on biotic and abiotic resistance.

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