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Full Length Research Paper

Molecular genetic diversity study of *Lepidium sativum* population from Ethiopia as revealed by inter simple sequence repeat (ISSR) markers

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***Lepidium sativum* L. (family Brassicaceae), is an underutilized medicinal plant with worldwide distribution. In Ethiopia, *L. sativum* occurs in all regions and agro-ecologies at different altitudinal ranges. The study was conducted to assess the genetic diversity of *L. sativum* population from Ethiopia using inter simple sequence repeat (ISSR) marker. Molecular data generated from ISSR bands recorded was used for computing gene diversity, percent polymorphism and Shannon diversity index and AMOVA. Moreover, the ISSR data was used to construct unweighted pair group method with arithmetic mean (UPGMA) and principal coordinated analysis (PCO) plot using Jaccard's coefficient. Tigray and Amhara population showed higher gene diversity (0.24) and Shannon information index (0.35). All UPGMA, neighbor-joining (NJ) and PCO analysis showed very weak grouping among individuals collected from the same regions. Generally, Tigray and Amhara regions showed moderate to high diversity in ISSR analysis. Different geographical regions of Ethiopia, showed different level of variation; thus conservation priority should be given for those regions that have high genetic diversity. This result also indicates the presence of genetic diversity that can be exploited to improve the productivity of *L. sativum* in Ethiopia.**

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

INTRODUCTION

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

L. sativum is a fast growing (30-60 cm) annual herb. Leaves are entire (upper-sessile and lower- petiolate); flowers- white, small and long racemes, fruits- small pods, obviate, two seeds per pod; seeds- brownish red and slimy when soaked in water, seed shape-elliptic (Zhan et al., 2009). This species reproduces sexually pollen; it is both self and cross pollinating plant. Insects are well known for cross pollination (Quirós and Cárdenas, 1998).

The exact origin of *L. sativum* L. is not known. However,

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it is believed to have originated in the highland of Ethiopia and central and Southwest Asia, and then spread to the rest part of the world. This species is commonly cultivated in SW Asia (perhaps Persia) from where it spread many centuries ago, to West Europe, as shown by the philosophical trace of its names in different Indo-European languages (Muhammad and Hussain, 2010).

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

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

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

MATERIALS AND METHODS

Tissue harvest and DNA extraction

The experiment was designed to characterize accessions using inter simple sequence repeat (ISSR) markers. Young leaves were collected separately from five randomly selected individual plants per accession after four weeks of planting and dried in silica gel. Approximately equal amount of the dried leaf samples were bulked for each accession and ground with pestle and mortar. Total genomic DNA was isolated from about 0.4 g of the pulverized leaf sample using modified triple cetyl trimethyl ammonium bromide (CTAB) extraction technique as described by Borsch et al. (2003) (Table 1).

Primer selection and optimization

The ISSR marker assay was conducted at Genetics Laboratory of the Microbial, Cellular and Molecular Biology Program Unit, College of Natural Sciences, Addis Ababa University, Addis Ababa. A total of 10 primers were obtained from the Genetic Research Laboratory (Primer kit UBC 900) and primers used by Kim et al. (2002) were used for the initial testing of primers variability and reproducibility. One individual was selected from each population to screen the primers with 1:5 dilutions. A total of four polymorphic and reproducible ISSR primers (812, 834, 873 and 880) were selected after testing and screening. Table 2 shows the list of primers used and tested, their annealing temperature with respective sequences and other properties.

PCR and gel electrophoresis

The polymerase chain reaction was conducted in Biometra 2003 T3 Thermo cycler. PCR amplification was carried out in a 25 µl reaction mixture containing 1 µl template DNA, 13.45 µl H_20, 5.60 µl dNTP (1.25 mM), 2.6 µl Taq buffer (10XH buffer S), 1.25 µl $MgCl_2$ (50 mM), 0.6 µl primer (20 pmol/µl) and 0.5 µl Taq Polymerase (3 u/µl). The amplification program was 4 min preheating and initial denaturation at 94°C, then 40 x 15 s at 94°C, 1 min primer annealing at 45°C/ 48°C based on primers used, 1.30 min extension at 72°C and the final extension for 7 min at 72°C. The PCR reactions were stored at 4°C until loading on gel for electrophoresis. The amplification products were differentiated by electrophoresis using an agarose gel (1.67% agarose with 100 ml 1xTBE) and 8 µl amplification product of each sample with 2 µl loading dye (six times concentrated) was loaded on gel. DNA marker 100 bp was used to estimate molecular weight and size of the fragments. The electrophoresis was done for 3 h at constant voltage of 100 V. The DNA was stained with 10 mg/ml ethidium bromide which were mixed with 250 ml distilled water for 30 min and washed with distilled water for 30 min.

Statistical analysis

The bands were recorded as discrete characters, presence '1' or absence '0' and '?' for missing data. Based on recorded bands, different software's were used for analysis. POPGENE version 1.32 software (Yeh et al., 1999) was used to calculate genetic diversity for each population as number of polymorphic loci, percent polymorphism, Gene diversity (H) and Shannon diversity index (I). Analysis of molecular variance (AMOVA) was used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006). NTSYS- pc version 2.02 (Rohlf, 2000) and Free Tree 0.9.1.50 (Pavlicek et al., 1999) software's were used to calculate Jaccard's similarity coefficient.

The unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973) was used to analyze and compare the population and generate phenogram using NTSYS- pc version 2.02 (Rohlf, 2000). To further examine the patterns of variation among individual samples on 3D, a principal coordinated analysis (PCO) was performed based on Jaccard's coefficient (Jaccard, 1908). The calculation of Jaccard's coefficient was made with PAST software version 1.18 (Hammer et al., 2001). The first three axes were used to plot the three dimensional PCO with STATISTICA version 6.0 software (Hammer et al., 2001; Statistica soft, Inc.2001).

RESULTS

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

Of the total 53 loci scored, 81.13% (43) were observed to be polymorphic. From all the population studied, Amhara

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

Number	Accession number	Altitude	Region	Region	Accession symbol
1	90018	2500	Amhara	Wello	A1
2	208030	*Uk	Amhara	Gonder	A2
3	90004	2400	Amhara	Gonder	A3
4	205162	*Uk	Amhara	Gonder	A4
5	229799	2550	Amhara	Gojam	A5
6	215713	2580	Amhara	Wello	A6
7	229205	2690	Amhara	Shewa	A7
8	241777	2110	Amhara	Wello	A8
9	214243	*Uk	Amhara	Gonder	A9
10	215714	2200	Amhara	Wello	A10
11	212628	1500	Amhara	Wello	A11
12	235892	2270	Amhara	Gonder	A12
13	229200	2150	Amhara	Shewa	A13
14	207542	*Uk	Amhara	Gonder	A14
15	229203	2620	Amhara	Shewa	A15
16	229201	2310	Amhara	Shewa	A16
17	229798	2250	Amhara	Gojam	A17
18	229204	2580	Amhara	Shewa	A18
19	229202	2600	Amhara	Shewa	A19
20	205162	2820	Amhara	Gonder	A20
21	90020	2000	Amhara	Shewa	A21
22	229199	2220	Amhara	Shewa	A22
23	208769	1900	Oromia	Wollega	O1
24	208667	1900	Oromia	Hererrege	O2
25	212852	*Uk	Oromia	Balle	O3
26	90002	*Uk	Oromia	Harerrge	O4
27	215808	1950	Oromia	Wollega	O5
28	212853	*Uk	Oromia	Balle	O6
29	215807	1720	Oromia	Wollega	O7
30	208666	*Uk	Oromia	Harerrge	O8
31	208693	*Uk	Oromia	Harerrge	O9
32	230830	2450	Oromia	Harerrge	O10
33	230831	2050	Oromia	Harerrge	O11
34	237991	2150	Oromia	Balle	O12
35	230524	*Uk	Oromia	Harerrge	O13
36	90023	*Uk	Oromia	Keffa	O14
37	90021	2620	Oromia	Shewa	O15
38	90005	*Uk	Oromia	Harrerge	O16
39	216885	1570	Oromia	Arsi	O17
40	208669	2180	Oromia	Harerrege	O18
41	90022	1480	Oromia	Wollega	O19
42	234828	*Uk	Oromia	Wollega	O20
43	216886	1570	Oromia	Arsi	O21
44	216816	*Uk	Oromia	Harerrge	O22
45	219961	*Uk	Tigray	Tigray	T1
46	219960	1940	Tigray	Tigray	T2
47	233984	1860	Tigray	Tigray	T3
48	219959	*Uk	Tigray	Tigray	T4
49	233981	2140	Tigray	Tigray	T5

Table 1. Contd

50	233986	2080	Tigray	Tigray	T6
51	207910	*Uk	Tigray	Tigray	T7
52	237512	1900	Tigray	Tigray	T8
53	242609	*Uk	Tigray	Tigray	T9
54	234355	2090	Tigray	Tigray	T10
55	207991	*Uk	Tigray	Tigray	T11
56	233983	1950	Tigray	Tigray	T12
57	233982	2210	Tigray	Tigray	T13
58	219958	2130	Tigray	Tigray	T14
59	233370	*Uk	Tigray	Tigray	T15
60	233679	*Uk	Tigray	Tigray	T16
61	240396	1950	SNNPR	Keffa	SN1
62	242916	2200	SNNPR	Keffa	SN2
63	214660	*Uk	SNNPR	Sidamo	SN3
64	202116	*Uk	SNNPR	Keffa	SN4
65	225799	2650	SNNPR	Gammo goffa	SN5
66	205141	1900	SNNPR	Shewa	SN6
67	240578	*Uk	SNNPR	Gammo goffa	SN7
68	240397	*Uk	SNNPR	Keffa	SN8
69	90016	*Uk	SNNPR	Gammo goffa	SN9
70	225725	*Uk	SNNPR	Gammo goffa	SN10
71	240808	1950	SNNPR	Gammo goffa	SN11
72	90009	*Uk	Unknown	Unknown	UN1
73	90014	*Uk	Unknown	Unknown	UN2
74	90012	*Uk	Unknown	Unknown	UN3
75	90017	*Uk	Unknown	Unknown	UN4
76	90010	*Uk	Unknown	Unknown	UN5
77	240579	*Uk	Unknown	Unknown	UN6
78	90008	*Uk	Unknown	Unknown	UN7
79	80001	*Uk	Unknown	Unknown	UN8
80	80002	*Uk	Unknown	Unknown	UN9
81	216815	*Uk	Somali	Somali	SO1
82	230829	*Uk	Somali	Somali	SO2
83	231210	1910	Somali	Somali	SO3
84	230523	*Uk	Somali	Somali	SO4
85	90007	*Uk	Somali	Somali	SO5

Source: IBC database. *Uk= Unknown.

and Tigray were 6.04%, Oromia 50.94%, SNNPR 47.17%, and Somali 45.28% polymorphic. Amhara and Tigray showed more percent polymorphism; while the least polymorphism was detected in population from Somali region. No unique bands were observed for either the accessions or the populations (Table 3).

Among the *L. sativum* accessions evaluated using ISSR marker, samples from Tigray and Amhara exhibited the highest gene diversity (H = 0.24), whereas samples from Oromia had (H = 0.17), from SNNPR (H = 0.18) and Somali (H= 0.18) gene diversity values. The average gene diversity for the total population (H_T) was 0.27 (Table 3, Figure 1).

Primer 873 showed highest gene and Shannon diversity (0.36 and 0.53, respectively) and primer 812 was the least (0.20 and 0.31, gene and Shannon diversity, respectively) (Table 4).

Analysis of molecular variance

Analysis of molecular variance was carried out on the overall ISSR data score of *L. sativum* accessions without grouping by region or geographic location. AMOVA revealed high percentage of variation (94%) that was attributed to within population variation while the remaining

Table 2. List of primers, annealing temperature, primer sequence, amplification quality and repeat motives used for optimization.

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

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

Table 3. Banding patterns generated using the four selected primers, their repeat motifs, amplification patterns and number of scored bands.

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

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

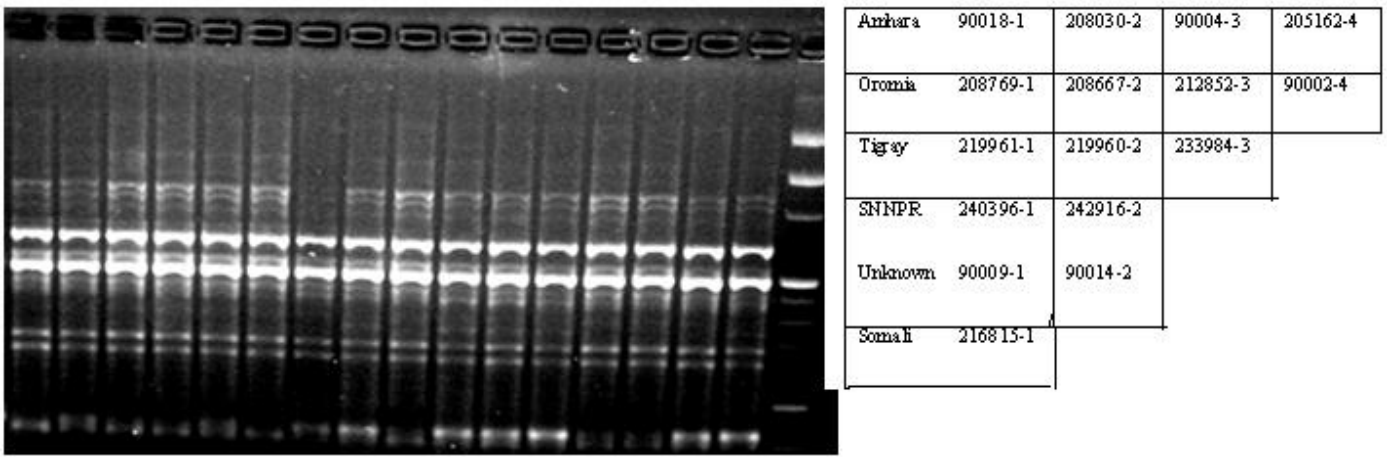

Figure 1. ISSR fingerprint generated from 16 individual accessions using primer 873.

variation was due to population variation (6%). The highest polymorphic loci (35) and percent polymorphism (66.04) were observed in Amhara and Tigray regions (Table 5). Similarly, the highest genetic diversity (0.24) and Shannon information index (0.35) were recorded in Amhara and Tigray regions (Table 5).

The variation was found to be highly significant at P = 0.00. The result shows that there was high gene flow or seed flow among population in different region; this resulted in low genetic variation and differentiation among population (Table 6).

Clustering analysis

UPGMA and neighbor joining tree construction methods

Table 4. Number of scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H), Shanon index information (I) of 85 *L. sativum* accessions based on all primers used.

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

Table 5. The number of polymorphic loci (NPL), percent polymorphism (PP), genetic diversity (H) and Shannon information index (I) among the five regions of Ethiopia.

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

Table 6. Analysis of molecular variance (AMOVA) of *L. sativum* accessions in Ethiopia without grouping.

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

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

PCO analysis

All the data obtained using the four ISSR primers were used in PCO analysis using Jaccard's coefficient of similarity. The first three coordinates of the PCO having Eigen values of 4.83, 4.55 and 1.63 with variance of 18.28, 17.26 and 6.20%, respectively were used to show the grouping of individuals using two and three coordinates. In 3D most of the individual accessions that represent different populations spread all over the plot. Using two coordinates (Figures 4 and 5) almost similar result was observed like that of three coordinates. Overall, no clear grouping was observed among individuals collected from different locality.

DISCUSSION

Molecular diversity and its implications for improvement and conservation

In the present study, ISSR was used for the first time to assess genetic variation of *L. sativum* population from Ethiopia. This method provides an alternative choice to other system for obtaining highly reproducible marker without any necessity for prior sequence information for various genetic analyses. Due to the abundant and rapidly

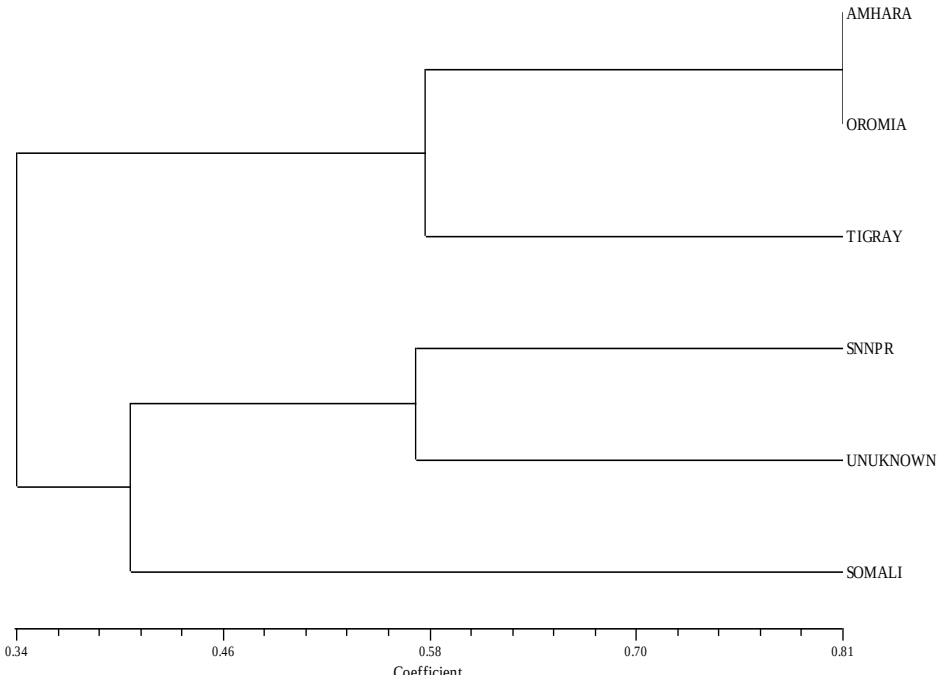

Figure 1. UPGMA based dendrogram for 6 *L. sativum* population using 4 ISSR (2 di, 1 penta and 1 tetra nucleotide) primers.

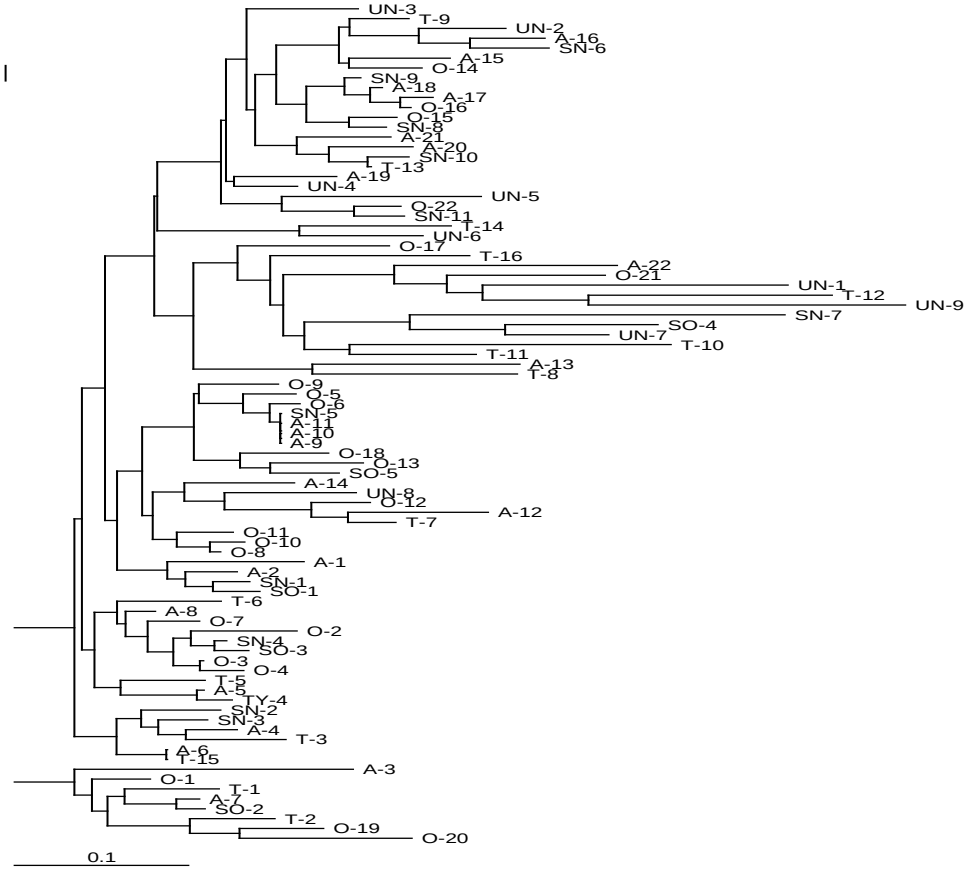

Figure 3. Neighbor-joining analysis of 85 individuals based on 53 PCR bands amplified by two dinucleotide (812 and 834); one tetranucleotide (873); and one pentanucleotide (880) primers. The neighbor joining algorithm is based on Jaccard's coefficient.

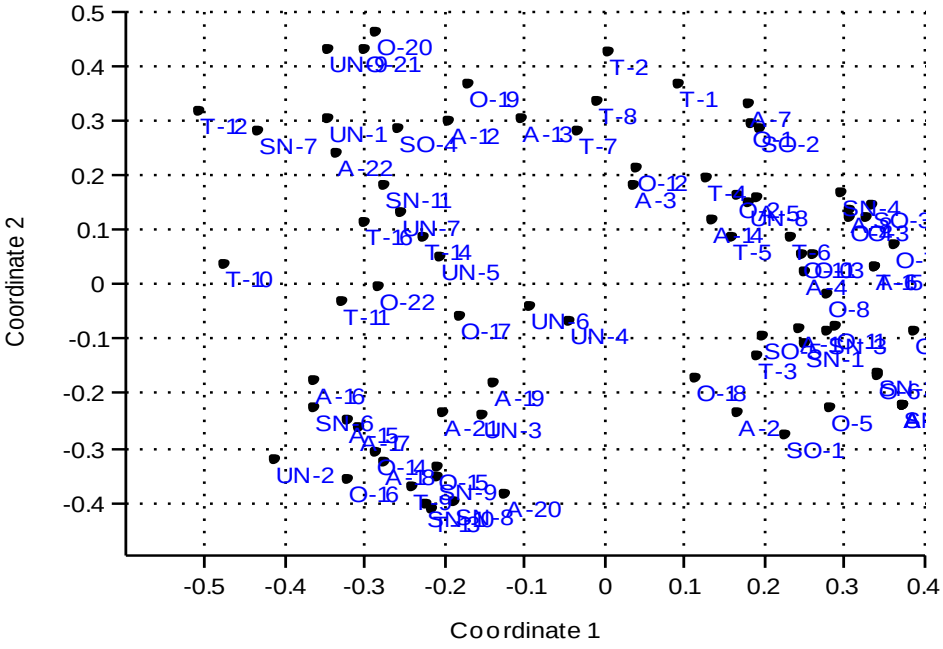

Figure 4. Two dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of L. sativum accessions using ISSR data.

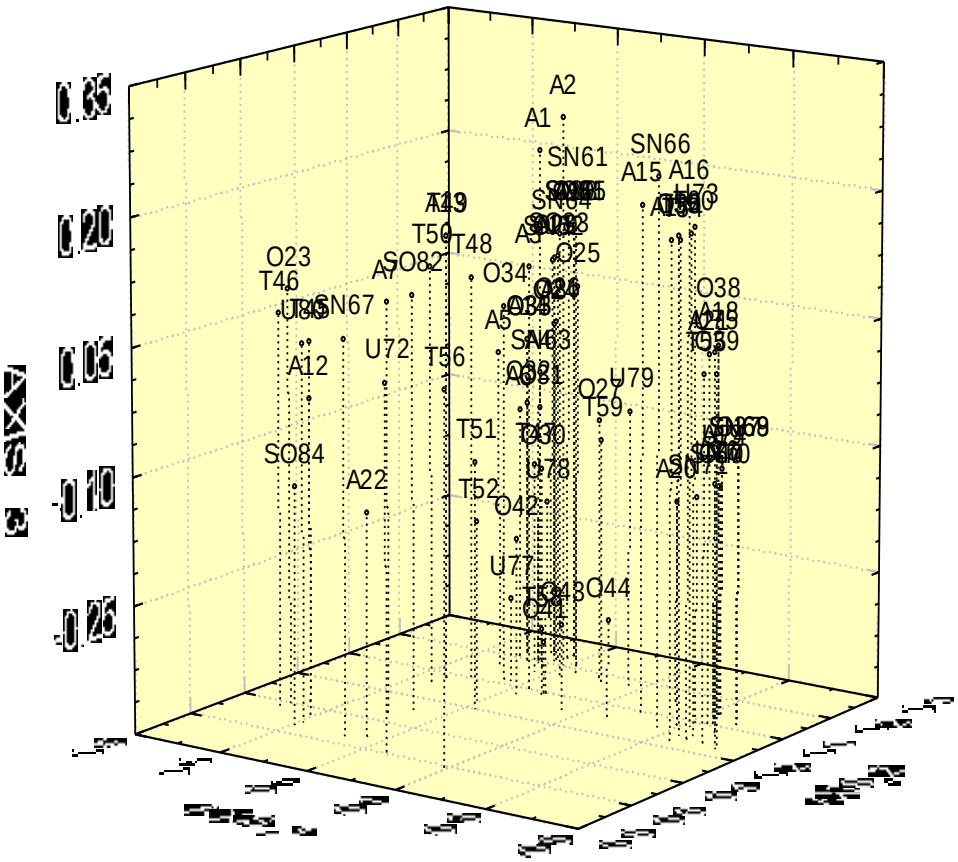

Figure 5. Three dimensional representation of principal coordinate analysis of genetic relationships among 85 accessions of *L. sativum* accessions.

evolving SSR regions, ISSR amplification has the potential of illuminating much larger number of polymorphic fragments per primer than any other marker system used such as RFLP or microsatellites. ISSRs are regions that recline within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers, since they reveal variation with- in unique regions of the genome at several loci simultaneously. Several property of microsatellite such as high variability among taxa, ubiquitous occurrence and high copy number in eukaryotic genome make ISSRs extremely useful marker for variability analysis (Morgante et al., 2002).

In this study, bulk sampling approach was chosen, since it permits representation of the vast accession by optimum number of plants. Yang and Quiros (1993) reported that bulked samples with 10, 20, 30, 40 and 50 individuals had resulted in the same RAPD profiles as that of the individual plant constituting the bulk sample. Gilbert et al. (1999) also reported that pooling of DNA from individuals within accessions is the most appropriate strategy for assessing large quantities of plant material and concluded that 2-3 pools of five genotypes is sufficient to represent the genetic variability within and between accessions in the lupin and similar collections. Edossa et al. (2010) used bulked samples for diversity assessment in lentil collected from Ethiopia. The technique revealed higher genetic diversity, and, therefore, validated the usefulness of bulk sample analyses. Dagmawi (2011) also used bulked sample in germplasm diversity study of sesame populations, and found moderate genetic diversity of both Ethiopian and exotic population.

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

Edossa et al. (2010) studied the morphological and molecular diversity of Ethiopian lentil (Lens culinaris Medikus) using four ISSR primers and found 59.57% polymorphism with higher percent variation attributed within population (56.28%). Gezahegn et al. (2009) studied wild and cultivated rice species of Ethiopia using six ISSR primers and reported 38.3 and 28.3% polymorphism of wild and cultivar rice species, respectively. Moreover, higher proportion of genetic diversity was observed within populations of rice (Gezahegn et al., 2009). Hence, the present study shows higher percent polymorphism and higher proportion of diversity within population of *L. sativum* comparable with that of Edossa et al. (2010) and Gezahegn et al. (2009). In general Amhara and Tigray had good genetic diversity than Oromia, SNNPR and Somali.

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

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

Dendrogram of the present study by using UPGMA of Jaccard's coefficient of similarity showed that Amhara and Oromia population of *L. sativum* were closely related. Based on this study, the samples with unknown origins could probably has been collected from Southern part of Ethiopia since they closely clustered with the SNNPR population. The Somali population had its own lineage far from the other population and diverted as an out lies. Genetic distance is a measure of the allelic substitutions per locus that have occurred during the separate evolution of two population or species.

Smaller genetic distances indicate a close genetic relationship, whereas large genetic distances indicate a more distant genetic relationship. Crosses between distantly related individuals are expected to give better offspring than those between closely related genotypes. Therefore, prior knowledge of the genetic distance between genotypes or accessions is important in designing breeding program.

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

Conclusion

Analysis of molecular variance for the accessions studied showed that the highest proportion of genetic variation was attributed to within population than among population. It is also highly significant. This confirms that there was a high level of gene flow and low level of genetic differentiation. Based on the UPGMA data, the Amhara, Tigray and Oromia accessions were clustered into one group, whereas the SNNPR and the unknowns to the other cluster. Samples from Somali formed a distinct cluster and showed that it is distantly related to accessions from the entire regions.

Competing interest

The authors declare that they have no competing interests.

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