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Molecular diversity study of black cumin (*Nigella sativa* L.) from Ethiopia as revealed by inter simple sequence repeat (ISSR) markers

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***Nigella sativa* L. (commonly known as black cumin) belonging to family Rannunculaceae is an important medicinal plant with worldwide distribution. In Ethiopia, *N. sativa* occurs in all regions and agro-ecologies at different altitudinal ranges. This plant has a lot of importance in Ethiopia. However, there is no information available on molecular genetic diversity of this crop in respect to Ethiopia. Therefore, the aim of this study was to investigate the genetic diversity of black cumin populations collected from Amhara, Oromia, Tigray, Benshangul and South Nation's Nationality People regions of Ethiopia using ISSR marker. A total of 84 black cumin accessions were obtained from Institute of Biodiversity Conservation (IBC). The ISSR marker was used for computing gene diversity, percent polymorphism, Shannon diversity index and AMOVA. Overall, accessions from Oromia showed the highest gene diversity (H = 0.35) and Shannon information index (I= 0.52), followed by Amhara with gene diversity and Shannon index values of (0.35) and (0.51), respectively. NJ and UPGMA results showed strong grouping among accessions collected from the Oromia and Amhara region. The five geographical regions of Ethiopia showed different levels of genetic variation. Thus, conservation priority should be given for those regions that have low genetic diversity.**

Key words: Conservation, indigenous, molecular markers, primers.

INTRODUCTION

The genus Nigella contains about 14 species of annual herbs. Among the medicinal plants in use from prehistoric times, black cumin (*Nigella sativa*) is being used for healthcare. It is commonly known as black cumin, fennel

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flower, Roman coriander and Kalonji (Weiss, 2002). Different bioactive compounds have been reported in this spice and these are well known for their beneficial effects on health (Hussain et al., 2006). These contain essential macro and micronutrients which play vital roles as structural and functional components of metalloproteins and enzymes in the living cells (Ansari et al., 2004).

N. sativa has been used traditionally for centuries in the Middle East, Northern and east Africa and India for the treatment of various diseases (Worthen, 1998; Burits and Bucar, 2000; Al-Ghamdi, 2001; Gilani et al., 2004). Research from around the globe is giving increasing support for black cumins widespread healing powers. Extracts of the black cumin have many therapeutic effects such as anti-diabetes, antibacterial, and antitumor (Khan et al., 2003; Kanter et al., 2003; Hussein et al., 2005).

Assessment of the genetic diversity in black cumin is therefore, of crucial importance for developing conservation strategy for this economically important spice species. With the development of the polymerase chain reaction (PCR), many molecular techniques have been, and still are being developed for plant genome analysis (Esayas and Bryngelsson, 2006). They have been replacing the traditional morphological and agronomic characterization, since they virtually cover the whole genome, are not influenced by the environment, and less time consuming (Esayas et al., 2005).

Molecular techniques have been applied in the analysis of specific genes, as well as to increase understanding of gene action, generate genetic maps and assist in the development of gene transfer technologies. Molecular techniques have also had critical roles in studies of phylogeny and species evolution, and have been applied to increase our understanding of the distribution and extent of genetic variation within and between species. Markers such as inter-simple sequence repeat (ISSR) (Zietkiewicz et al., 1994) are widely used in genetic diversity studies because they need no prior DNA sequence information, development costs are low and laboratory procedures can easily be transferred to any plant species.

Black cumin is found in different parts of Ethiopia at various altitude ranges. This shows that there is the existence of genetic diversity of black cumin species that deserve conservation. However, no information on molecular genetic diversity of this herb has been generated for the Ethiopian population. One of the important factors restricting their large-scale production and development of better varieties is that very little information is available about their genetic diversity, inter and intra-specific variability and genetic relationships among these species. Therefore, attempts to analyze possible untapped genetic diversity become extremely essential for breeding and crop improvement. This study investigated the genetic diversity and population structure of black cumin germplasms collected from Ethiopia using ISSR markers.

MATERIALS AND METHODS

Sampling locations and plant materials

The present study was conducted at Genetics, laboratory Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. A total of 84 *N. sativa* accessions were provided by Institute of Biodiversity Conservation (IBC). The samples were originally collected from different parts of Ethiopia. Soil was prepared with a PH range from 5-8 and all accessions were planted in pots at College of Natural Sciences greenhouse and watering every day to maintain the normal growth of the plant in the green house for fifteen days. A total of 34, 24, 10, 9 and 7 accessions were included from Oromia, Amhara, Benshangul gumuz, Tigray and South Nation's Nationality Peoples Regions, respectively (Table 1).

DNA extraction

The study was designed to characterize the 84 black cumin accessions using inter simple sequence repeat (ISSR) markers. Young fresh leaves were collected separately from seven randomly selected individual plants per accession after three weeks of planting in the greenhouse. 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).

ISSR Primer selection and optimization

A total of ten primers, obtained from the Genetics Laboratory (Primer kit UBC 900) were initially tested for primers variability and reproducibility through optimization process. The optimization process was repeated three times to found the optimum conditions of the PCR and the same band were observed. To represent wider geographic locations, two individuals from Amhara, two from Oromia, two from Benshangul Gumuz, two from SNNP and two from Tigray were selected to screen the ten primers. Finally, a total of five polymorphic and reproducible ISSR primers (UBC-809, UBC-810, UBC-811, UBC-835 and UBC- 880) were selected after testing its reproducibility and polymorphism.

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, 17.8 µl H_20, 0.2 µl dNTP (20 mM), 2.6 µl Taq buffer (10X buffer B), 2.5 µl $MgCl_2$ (25 mM), 0.6 µl primer (20 pmol/ l) and 0.3 µl Taq Polymerase (5 u/µl) for all the five primer namely 809, 810, 811, 835 and 880. The amplification program was 4 min preheating, 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 then stored at 4°C until loading on gel for electrophoresis.

The amplification products were separated by electrophoresis using an agarose gel (1.67% agarose with 100 ml 1xTBE) with 8 µl amplification product of each sample plus 2 µl loading dye (6X concentrated) was loaded on gel. DNA marker with 3000 bp was used to estimate molecular weight and size of the fragments. Electrophoreses was done for 3 h at constant voltage of 100 V. The ISSR gel was then stained with 10 mg/ml ethidum bromide which

Table 1. *N. sativa* accessions, latitude, longitude and regions of collection used in the study.

Number	Accession	Region	Zone	Latitude	Longitude	Symbol	
1	8502	Oromia	Bale	37-00-00-N	39-48-00-E	T1	oro-1
2	9067	Amhara	Mirab Gojam	11-41-08-N	37-01-12-E	T2	amh-1
3	9068	Amhara	Mirab Gojam	11-45-40-N	37-05-4 –E	T3	amh-2
4	9069	Amhara	Mirab Gojam	10-38-48-N	37-05-09-E	T4	amh-3
5	9071	Amhara	Mirab Gojam	10-38-21-N	37-05-13-E	T5	amh-4
6	90501	Amhara	Mirab Gojam	10-38-21-N	37-05-13-E	T6	amh-5
7	90502	Amhara	Debub Gondar	11-57-00-N	37-42-00-E	T7	amh-6
8	90503	Amhara	Debub Gondar	11-59-00-N	37-46-00-E	T8	amh-7
9	90504	Oromia	Arssi	08-03-N	38-47-00	T9	oro-2
10	90505	Amhara	Misrak Gojam	10-20-00-N	38-00-00-E	T10	amh-8
11	90506	Amhara	Misrak Gojam	10-20-00-N	38-00-00-E	T11	amh-9
12	90507	Amhara	Semen Gondar	12-16-00-N	37-05-00-E	T12	amh-10
13	90508	Oromia	Mirab Wellega	09-00-00-N	35-15-00-E	T13	oro-3
14	90509	Oromia	Bale	07-19-00-N	39-48-00-E	T14	oro-4
15	90510	Oromia	Mirab Shewa	09-10-00-N	37-50-00-E	T15	oro-5
16	90511	Oromiya	Arssi	08-03-N	38-47-00	T16	oro-6
17	90512	Benshangul Gumuz	Metekel	08-22-00-N	39-53-00-E	T17	bgu-1
18	90513	Oromia	Arsi	08-01-00-N	39-50-00-E	T18	oro-7
19	90514	Benishangul Gumuz	Metekel	11-00-00-N	35-45-45-E	T19	bgu-2
20	90515	Tigray	Misrakawi	13-03-00-N	39-13-00-E	T20	tig-1
21	90516	Oromia	Bale	07-01-00-N	39-59-00-E	T21	Oro-8
22	90517	SNNP	Semen Omo	60-60-54-N	36-87-94-E	T22	snn-1
23	205167	SNNP	Semen Omo	60-60-54-N	36-87-94-E	T23	snn-2
24	207538	Amhara	Semen Gondar	12-20-00-N	37-14-00-E	T24	amh-11
25	207539	Amhara	Semen Gondar	12-20-00-N	37-14-00-E	T25	amh-12
26	207540	Amhara	Debub Gondar	12-20-00-N	37-14-00-E	T26	amh-13
27	208032	Amhara	Semen Gondar	12-20-00-N	37-14-00-E	T27	amh-14
28	208688	Oromia	Mirab Harerge	08-49-00-N	40-25-00-E	T28	oro-9
29	208771	Oromia	Mirab Wellega	37-56-25-N	38-67-11-E	T29	oro-10
30	212520	Tigray	Egnaw	14-06-00-N	38-42-00-E	T30	tig-2
31	212859	Oromia	Bale	07-01-00-N	39-59-00-E	T31	oro-11
32	212437	Benshangul Gumuz	Asosa	10-03-44-N	34-32-50-E	T32	bgu-3
33	215319	Amhara	Misrak Gojam	11-00-08-N	37-00-11-E	T33	amh-15
34	219970	Tigray	Mirabawi	14-08-12-N	38-18-34-E	T34	tig-3
35	223069	Amhara	Misrak Gojam	11-00-08-N	37-00-11-E	T35	amh-16
36	223070	Benishangul Gumuz	Metekel	11-00-00-N	35-45-45-E	T36	bgu-4
37	223071	Benishangul Gumuz	Metekel	11-00-00-N	35-45-45-E	T37	bgu-5
38	223072	Benishangul Gumuz	Metekel	11-00-00-N	35-45-45-E	T38	bgu-6
39	229806	Benishangul Gumuz	Asosa	10-03-44-N	34-32-50-E	T38	bgu-7
40	229807	Benishangul Gumuz	Asosa	10-04-48-N	36-31-40-E	T40	bgu-8
41	230037	Tigray	Egnaw	14-06-00-N	38-42-00-E	T41	tig-4
42	230038	Tigray	Egnaw	14-10-00-N	38-45-00-E	T42	tig-5
43	230039	Tigray	Egnaw	14-04-00-N	38-05-00-E	T43	tig-6
44	230040	Tigray	Egnaw	14-05-00-N	39-06-00-E	T44	tig-7
45	230777	Oromia	Borena	05-07-00-N	39-29-00-E	T45	oro-12
46	236832	Oromia	Mirab Shewa	38-01-00-N	38-05-00-E	T46	oro-13
47	237989	Oromia	Bale	08-03-N	38-47-00	T47	oro-14
48	239730	Oromia	Bale	07-00-03-N	40-27-68-E	T48	oro-15
49	240403	SNNP	Keficho Shekicho	07-13-63-N	35-41-93-E	T49	snn-3
50	240404	SNNP	Keficho Shekicho	07-15-00-N	36-00-00-E	T50	snn-4
51	242220	Amhara	Debub Wello	10-50-28-N	39-48-60-E	T51	amh-17

Table 1. Contd

52	242221	Amhara	Debub Wello	10-50-28-N	39-48-60-E	T52	amh-18
53	242222	Amhara	Semen Wello	11-57-65-N	39-03-80-E	T53	amh-19
54	242223	Tigray	Mirabawi	14-06-75-N	38-27-89-E	T54	tig-8
55	242224	SNNP	Arbaminch	06-06-67-N	37-66-67-E	T55	snn-5
56	242225	SNNP	Arbaminch	06-06-67-N	37-66-67-E	T56	snn-6
57	242226	SNNP	Soddo	06-51-10-N	37-45-40-E	T57	snn-7
58	242227	SNNP	Soddo	10-54-32-N	39-47-29-E	T58	snn-8
59	242528	Benishangul Gumuz	Asosa	09-59-09-N	34-40-03-E	T59	bgu-9
60	242824	Tigray	Debubawi	12-13-00-N	38-43-00-E	T60	tig-9
61	242825	Oromia	Arssi	07-35-00-N	39-32-00-E	T61	Oro-16
62	242826	Oromia	Arssi	07-40-00-N	40-12-00-E	T62	Oro-17
63	242827	Oromia	Bale	07-17-00-N	39-50-00-E	T63	Oro-18
64	242828	Oromia	Bale	06-58-00-N	40-33-00-E	T64	Oro-19
65	242829	Oromia	Bale	07-07-00-N	40-36-00-E	T65	Oro-20
66	242830	Oromia	Bale	06-53-00-N	40-42-00-E	T66	Oro-21
67	242831	Oromia	Bale	06-56-00-N	40-39-00-E	T67	Oro-22
68	242832	Oromia	Borena	04-58-00-N	38-13-00-E	T68	Oro-23
69	242833	Oromia	Arssi	07-39-03-N	39-29-46-E	T69	Oro-24
70	242834	Oromia	Arssi	07-35-73-N	39-33-43-E	T70	Oro-25
71	242835	Oromia	Arssi	07-35-73-N	39-33-49-E	T71	Oro-26
72	242836	Oromia	Arssi	07-36-22-N	39-33-04-E	T72	Oro-27
73	242837	Oromia	Arssi	07-36-22-N	39-33-04-E	T73	Oro-28
74	242838	Oromia	Arssi	07-35-71-N	39-32-29-E	T74	Oro-29
75	242839	Oromia	Arssi	07-33-79-N	39-31-42-E	T75	Oro-30
76	242840	Oromia	Arssi	07-33-16-N	39-31-60-E	T76	Oro-31
77	242841	Oromia	Arssi	07-32-64-N	39-31-87-E	T77	Oro-32
78	242842	Oromia	Arssi	07-32-08-N	39-32-11-E	T78	Oro-33
79	242843	Oromia	Arssi	07-32-08-N	39-32-11-E	T79	Oro-34
80	242844	Benishangul Gumuz	Asosa	07-36-22-N	39-31-24-E	T80	bgu-10
81	242845	Amhara	Semen Gonder	07-35-53-N	39-30-73-E	T81	Amh-21
82	242846	Amhara	Semen Gonder	07-35-87-N	39-29-33-E	T82	Amh-22
83	244653	Amhara	Semen Gondar	12-50-00-N	37-35-00-E	T83	Amh-23
84	244654	Amhara	Semen Gondar	12-50-00-N	37-05-00-E	T84	Amh-24

was mixed with 250 ml distilled water for 30 min and distained with distilled water for 30 min.

Data analysis

ISSR fragments were scored visually for each individual accession from the gel photograph. The fragments were scored as discrete characters; presence 1 or absence 0 data. Based on recorded bands, different software's are used for statistical analysis. POPGENE version1.32 software was used to calculate genetic diversity for each population (accessions were grouped based on their geographic origin), number of polymorphic loci, percent polymorphism, gene diversity (H) and Shannon diversity index (I) (Yeh et al., 1999).

Analysis of molecular variance (AMOVA) was also used to calculate variation among and within population using Areliquin version 3.01 (Excoffier et al., 2006). 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). The Neighbor Joining (NJ) method (Saitou and Nei, 1987; Studier and Keppler, 1988) was used to compare individual genotypes and evaluate patterns of genotype clustering using Free Tree 0.9.1.50 Software (Pavlicek et al., 1999).

RESULTS AND DISCUSSION

ISSR Primers and their banding patterns

Out of the ten ISSR primers initially tested, only five primers (UBC-809, UBC-810, UBC-811 and UBC-835 and UBC-880) that produced clear, reproducible and polymorphic bands were selected as informative primers (Table 3). The other five ISSR primers that resulted in either smeared, failed to give amplification products, poor or non-reproducible bands were excluded from the

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

Primer	Annealing temperature (°C)	Primer sequence	Amplification quality	Repeat motives	Status of Primers
809	48	(AG)8G	Polymorphic reproducible	Dinucleotide	selected
810	45	(GA)8T	Polymorphic reproducible	Dinucleotide	selected
811	48	(GA)8C	Polymorphic reproducible	Dinucleotide	selected
812	45	(GA)8A	Monomorphic, reproducible	Dinucleotide	Not selected
818	48	(CA)8G	Monomorphic, reproducible	Dinucleotide	Not selected
826	48	(AC)8C	Polymorphic, reproducible	Dinucleotide	Selected
834	45	(AG)8YT	Polymorphic, reproducible	Dinucleotide	Selected
835	48	(AG)8YC	Polymorphic, reproducible	Dinucleotide	Selected
873	45	(GACA)4	monomorphic, reproducible	Tetra- nucleotide	Not Selected
880	45	(GGAGA)3	Polymorphic, reproducible	Penta- nucleotide	Selected

Table 3. Banding patterns generated using the five selected primers, amplification quality and number of scored bands.

Primers	Primer Sequence	Amplification Quality	Number of scored bands
UBC-809	(AG)8G	Good	13
UBC-810	(GA)8T	Very good	18
UBC-811	(GA)8C	Good	12
UBC-835	(AG)8YC	Very good	17
UBC-880	(GGAGA)3	Good	12
Total			72

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

analysis. The amplicon (band) size of the five primers used in this study varied from 200 to 1400 bp (Table 2).

A total of 72 clear and scorable bands were recorded for the 84 black cumin accessions of which 72 (100%) were found to be polymorphic. The least number of scorable bands were scored for primer UBC-811 and UBC-880 (12); the highest number of bands were scored from primer UBC-810 (18), whereas 17 and 13 numbers of bands were scored for primer UBC-835 and UBC-809, respectively. The average number of bands and polymorphic bands per primer was 14.4 (Table 3).

The Nei's gene diversity (H) and the Shannon Information Index (I) calculated for each of the ISSR Primers showed Primer UBC-835 had the lowest gene diversity (0.31); primer UBC-809 had the highest gene diversity (0.43). UBC-880, UBC-811 and UBC- 810 had (0.42), (0.37) and (0.33) Nei's gene diversity. The Shannon's Information index ranged from 0.46 to 0.62. In the case of genetic diversity estimation which was due to gene diversity (h) and Shannon's information index (I), the highest values were from ISSR-880 primer and the least were ISSR-835 (Table 4).

The black cumin populations with the highest values of Nei's and the Shannon index were Amhara populations with 0.35 and 0.51, respectively and SNNP populations were the lowest (0.25 and 0.37, respectively for gene diversity and Shannon's Information index). The highest level of percent polymorphism was obtained from samples collected from Oromia (100) followed by Amhara, Tigray, Benshangul Gumuz and SNNPR; 88.89, 75, 70.83 and 63.89, respectively (Table 4).

Table 4. Number of scorable bands (NSB), number of polymorphic loci (NPL), percent polymorphism (PPL), gene diversity (H), Shanon information Index (I) of black cumin accessions in the present study.

Primers	NSB	NPL	PPL	H±SD	I±SD
UBC-809	13	13	100	0.43±0.10	0.62±0.12
UBC-810	18	18	100	0.32±0.16	0.48±0.20
UBC-811	12	12	100	0.37±0.16	0.55±0.21
UBC-835	17	17	100	0.31±0.14	0.47±0.17
UBC-880	12	12	100	0.42±0.11	0.61±0.13
Over all	72	72	100	0.42±0.11	0.54±0.17

In regions based analysis, accessions collected from Oromia showed the highest number of polymorphism with 100% polymorphism followed by Amhara with 88.89% polymorphism followed by Tigray with 75% polymorphism

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

Regions	Number of samples (N)	NPL	PP	H±SD	I±SD
Oromia	34	72	100.00	0.36±0.13	0.52±0.16
Amhara	24	64	88.89	0.35±0.18	0.51±0.24
Benshangul	10	51	70.83	0.26±0.21	0.38±0.28
Tigray	9	54	75.00	0.26±0.19	0.39±0.27
SNNP	7	46	63.89	0.25±0.21	0.36±0.29
Overall	84	57.4	79.70	0.29±0.18	0.44±0.25

Table 6. Analysis of Molecular Variance (AMOVA) of black cumin accessions in Ethiopia without grouping into regions of origin.

Variation source	Sum of squares	Variation Components	Percentage of variation	P
Among Population	97.34	0.72	4.96	0.001
Within Population	1059.24	13.43	95.04	
Total	1156.58	14.15	100	

followed by Benshangul Gumuz with 70.83%. The least percent polymorphism was detected in the accessions collected from SNNP region with 63.89% polymorphism (Table 5).

Similarly, black cumin samples from Amhara exhibited the highest gene diversity (H= 0.35) followed by accession from Oromia, Benshangul Gumuz, Tigray and SNNP with 0.35, 0.26, 0.26 and 0.25 values, respectively. In general, Oromia and Amhara had good genetic diversity than Benshangul Gumuz,Tigray and SNNPR. But this has to be further studied using proper sampling strategy and multi-location comparison (Table 5).

Analysis of molecular variance

Analysis of molecular variance was carried out on the overall ISSR data score of black cumin accessions without grouping by region or geographic location (Table 6). Accordingly, AMOVA revealed that 95.04269% of the variation was within population while 4.95731% of variation remained among population. The variation is highly significant at P = 0.001. Unlike other landraces of cultivated plants, *N. sativa* in Ethiopia is not restricted to a given area rather it is wildly exchanged among local community and markets. This could be caused by relatively higher seed exchange rate among different regions at its regional and central markets which could lead to the intermix of genotypes (Polhil and Raven, 1981; Davoud et al., 2010) and also defined insect pollinators could be another agent to facilitate gene flow among populations.

Genetic diversity of plant populations 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).

The present study also confirmed the presence of higher diversity as revealed by higher percent of polymorphism which is higher than Tunisian fenugreek germplasms (Nidhal et al., 2011) (94.12%). The present study also revealed higher polymorphism than said by Rakhee et al. (2004) in which 72% of polymorphism for *T. foenum graecum* was revealed by ISSR markers. Mohammed and Tesfaye (2015) studied the spice plant on *Lepidium sativum* populations by using ISSR marker and showed that there was 94% within population and very low genetic diversity among population (6%). The present study showed higher percent polymorphism and higher proportion of diversity within population of *N. sativa* comparable with that ascertained by Mohammed and Tesfaye (2015). Tewodros (2013) studied other spice plant fenugreek (*T. foenum-graecum*) populations by using ISSR marker and showed 64.1% polymorphism within populations and 35.9% polymorphism among populations. As compared to the present study, there was difference between the two spices according to AMOVA analysis. Thus, in the present study there was high gene flow among regions by different mechanisms.

Clustering analysis

UPGMA analysis based on regions of collection of *N. Sativa* revealed two major groups. The first cluster

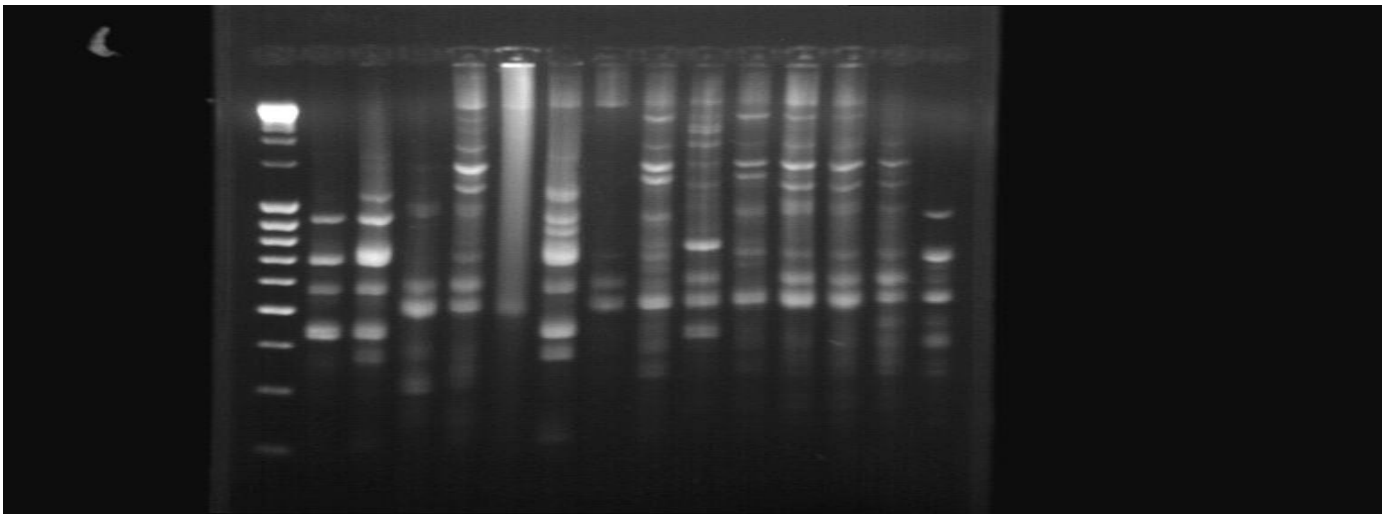

Figure 1. ISSR fingerprint generated from 14 individual accessions using primer UBC- 809.

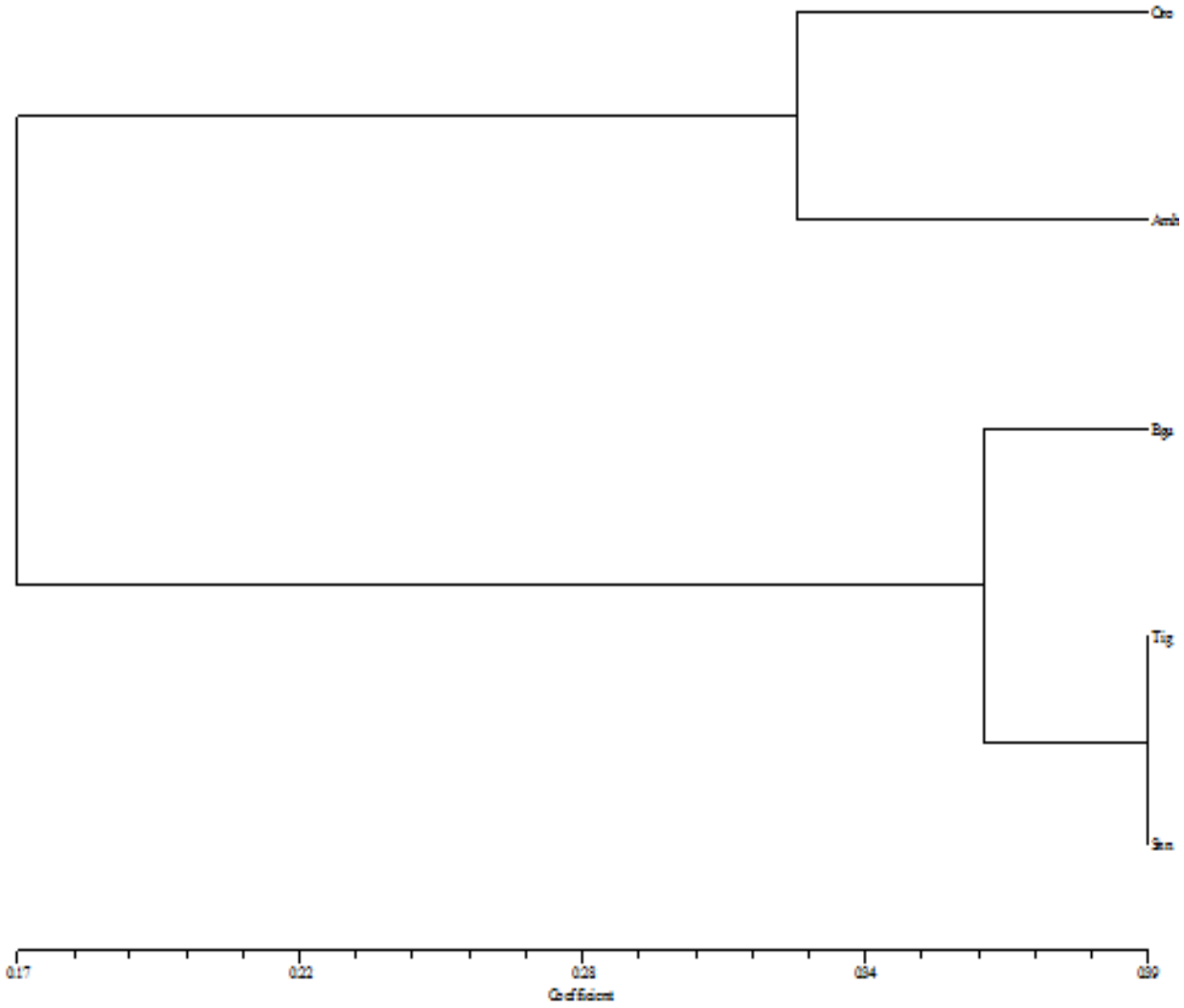

Figure 2. UPGMA based dendrogram for 5 *N. sativa* populations using 5 ISSR (4 di, 1 penta nucleotide) primers.

Figure 3. Neighbor-joining result of 84 individuals based on 72 PCR bands amplified by 4 di nucleotide (809, 810,811, and 835), and 1 penta nucleotide (880) primers using Jaccard's coefficient. Oro = Oromia; Amh = Amhara; Bgu=benshangul Gumuz; Tig = Tigray; snn=south nation's nationality peoples.

contains Oromia and Amhara while the second cluster contains Benshangul Gumuz, Tigray and SNNP. UPGMA with individual accessions showed group formation or clustering based on regions (Figure 2).

All individual accessions collected from Oromia and accessions collected from Amhara region tend to form their own groups. On the other hand, those accessions collected from Benshangul gumuz, Tigray and SNNP region mixed with each other's (Figure 3).

Conclusion

N. sativa is cultivated in different world parts including

Ethiopia. *N. sativa* is have medicinal value among local people. Because of insufficient amount of modern medicine, most of the Ethiopia population relies on traditional medicine. In Ethiopia, it is not commonly cultivated using separate farm plot rather planted along with different boarder crop. The present study was conducted with the main objective of assessing the extent of genetic diversity among Ethiopian accessions of *N. sativa* provided by the Institute of Biodiversity Conservation (IBC) using Inter Simple Sequence Repeat marker. This study shows that, ISSR markers are important markers for genetic diversity study in *N. sativa* accessions. Accessions collected from Oromia and Amhara regions were highly diversified than that of the other regions based on different softwares'. ISSR marker is also highly important for detecting the genetic diversities of black cumin plant according to this study.

Conflict of interests

The Authors declare that they have no competing interests.

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