

## Genetic diversity study of Ethiopian hot pepper cultivars (*Capsicum* spp.) using Inter Simple Sequence Repeat (ISSR) marker

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**ABSTRACT:** Hot pepper (*Capsicum* spp.) is an economically important spice widely cultivated and consumed in Ethiopia. In spite of its wide importance, there is no information available on the molecular genetic diversity of this crop. Cultivars characterization is an important link between the conservation and utilization of plant genetic resources in various breeding programs. Using five ISSR primers, a total of 37 scorable bands were generated of which 35 (94.6%) were polymorphic bands. The diversity of polymorphic bands within population ranged from 51.35% to 91.89 % with a mean of 66.6 %, Nei's genetic diversity of 0.19 - 0.30 with a mean of 0.28, and Shannon information index of 0.29 - 0.45 with a mean of 0.43. With all diversity parameters, the highest diversity was obtained from amhara2 populations, whilst the lowest was from Oromia2. From Jaccard's pairwise similarity coefficient, Oromia1 and oromia2 were the most related populations exhibiting 0.956 similarity and Semn omo and Amhara 2 were the most distantly related populations with similarity of 0.827. Clustering was showed that there is strong correlation between geographic distance and genetic diversity of Ethiopian hot peppers cultivars because geographically closely related species have been clustered together. Amhara 2 populations exhibited the highest genetic diversity so that the populations should be considered as the primary sites in designing conservation areas for this crop in Ethiopia. Further, it is suggested that molecular markers are valid tags for the assessment of genetic diversity in *Capsicum* spp. cultivars.

**KEYWORDS:** Capsicum, Cultivars, Cluster analysis, Diversity, Pepper

### INTRODUCTION

Hot pepper (*Capsicum* spp.) commonly known as red pepper or chili pepper is a dicotyledonous flowering plant which belongs to the family of Solanaceae [18]. It is an important commercial crop cultivated exclusively in tropical and temperate zones of the world and grown on more than 1.5 million hectares worldwide [11]. Peppers are usually classified based on fruit characteristic, including pungency, color, shape, flavor, size and use [3-5]. Pepper cultivars are grown in Ethiopia mainly in South Nation and Nationalities of Peoples (SNNP), since its introduction in the early 17th century by the Portuguese

[14]. *Capsicum annuum* is the main types of capsicum species that has grown in the country. Pepper species are grown at a medium altitude of 1,400 to 2,120 meters above sea level. In Ethiopia, it is grown under rain fed conditions in areas with 600-1,250 mm of annual rain. Hot pepper covers 67.98% of all the area under vegetables in Ethiopia. South Nations, Nationalities and Peoples Regional State (SNNPS) contribute a significant portion to the country's total pepper production [6]. It is the main part in the daily diet of most Ethiopian societies. The average daily consumption of hot pepper by

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Ethiopian adult is estimated at 15 grams, which is higher than tomatoes and most other vegetables (ARC, 2004). Hot pepper is a popular vegetable and plays an important role in the national economy of the country to reduce poverty. It also serves as raw material for the dye processing industries, important cash crop to farmers, and a source of employment to urban and rural populations. However, hot pepper production for dry pod has been low with a national average yield of 0.4 t/ha [12] and decreasing with time.

Therefore, the use of resistant or adapted varieties is recommended. Such varieties can be developed or simply searched for within the existing diversity of the cultivars [13]. Knowledge of the genetic diversity and the agronomic performances of the existing cultivars are necessary to improve the crops [1, 9, 25]. Moreover, designing the best improvement programs also needs for mastering production constraints and farmers' varietal preference criteria [7, 41]. Therefore, a study on the genetic relationship of different cultivated pepper species to find new parental cultivars is important in effective utilization of pepper cultivars and an effective way to solve the constraints of pepper breeding.

Different genetic markers are widely used by breeders and conservationists to study genetic diversity. Among the assessment of genetic diversity, the Molecular markers have the distinct advantages of being independent of environmental factors, abundant, the most convenient and popular methods to identify and study genetic polymorphism [2].

The cost of Inter simple sequence repeat (ISSR) analysis is relatively lower than that of AFLP and displays acceptable reproducibility. Therefore, it is widely used in current studies of population genetics [39]. Only the study of morphological and phenotypic characterization is conducted with limited accessions of hot peppers populations of Ethiopia. However, Morphological characterization has its own limitation like, they could be affected by changes in environmental factors and may vary at different developmental stages; moreover, their number is limited [8]. No scientific studies have been conducted on molecular diversity of Ethiopian hot pepper cultivars using ISSR marker or other molecular markers. Therefore, this study was conducted to assess the genetic diversity of *Capsicum* spp. using ISSR marker. The study provides base line information about genetic diversity within and among hot pepper cultivars collected from different pepper growing regions of Ethiopia for efficient

preservation, proper utilization of the existing genetic resources. It is also for the management of cultivars, including the classification of cultivars by known allelic constitution and detection of redundancy in collections.

The genetic diversity information will also be used in deciding which cultivar to be included in breeding programs and for the identification of promising cultivars that substantially contribute to the overall diversity of the species. Therefore, this study aimed at evaluating (assessing) the genetic diversity and relationship of hot pepper populations collected from different regions of Ethiopia using ISSR markers and generates information for its conservation and sustainable use.

## MATERIALS AND METHODS

### Plant materials

For the purpose of this study, 73 accessions of hot pepper seeds that were collected from Oromia, Amhara, Southern Nation and Nationalities Peoples (SNNPs) and Benishangul-Gumuz regional states were obtained from Ethiopian Biodiversity Institute. Those accessions were classified into eight populations based on the geographical regions they were collected from. Ten Seeds were randomly selected from each accession and planted in pot in glasshouse of Wolkite University. Watering was done once a day and, after a month, healthy and young leaves were randomly collected, dried in silica gel and used for DNA extraction and further analysis.

### DNA extraction

About 0.2-0.3g of the silica-dried bulked leaves tissues from each accessions were ground with mixer mill (model- Retsch MM400) and total genomic DNA was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide (2% Cetyltrimethyl ammonium Bromide, 1% polyvinyl pyrrolidone, 100mMTris: PH=8, 20mM EDTA, 1.4M NaCl, 0.2% beta-Mercapto-ethanol)) method described in Piccolo et al. (2012) with minor modifications.

The quality and presence of isolated DNA was checked by gel electrophoresis using 1 % agarose at 100 V constant for 30 minutes. The gel picture was taken with Bio Rad 200 and further examined and used to make selection of good quality DNA extract. Genomic DNA from the second extractions was found to be promising and was selected for ISSR-PCR analysis. The concentration of DNA was determined by Nano-drop

**Table 1.** Passport descriptors of *Capsicum* spp. samples collected from different sites in Ethiopia

No	Populations	Regions/States	Zone	Altitude (m.a.s.l)
1	Amhara 1	Amhara	West Gojam	1954 – 2050
2	Amhara 2	Amhara	East Gojam, N. Gonder	1555 – 2570
3	Mareko	SNNP	Gurage	1820 – 2060
4	Alaba	SNNP	Kembata	1820 – 1940
5	Benishangul	Benishangul Gumuz	Metekel	1640 – 1700
6	Semen Omo	SNNP	Semen Omo	1440 – 1940
7	Oromia 1	Oromia	South west Shewa	1550 – 2160
8	Oromia2	Oromia	Bale & Arssi	1953 – 2780

spectrophotometer 8000. The DNA concentration ranges from 109.5 to 11,221.1 ng/ $\mu$ l which was later diluted to optimized constant concentration of 20 ng/ $\mu$ l.

### Primer selection and optimization

The ISSR marker assay was conducted at Plant Genetics Research Laboratory of the Microbial, Cellular and Molecular Biology Department, Addis Ababa University. Ten ISSR-primers were screened on a total of sixteen (16) individuals by random selection of two individuals from each population, and five primers that produced clear, reproducible and polymorphic band pattern were selected for in-depth study using 73 individuals of the 8 populations (Table 1).

### PCR amplification and electrophoresis

The polymerase chain reaction was conducted in Biometra 2000 T3 Thermo cycler. PCR amplification was carried out in a 25  $\mu$ l reaction volume containing *Taq* DNA polymerase (3 U/reaction), *Taq* DNA polymerase buffer (1x) with 1.5 mM MgCl<sub>2</sub>, random decamer primers (10 pmol/reaction), dNTPs (25 mM) of Himedia, India and template DNA (50 ng/reaction). The amplification program was 4 min preheating and initial denaturation at 94°C, then 40X 15 s at 94°C, one min primer annealing at (45°C/ 48°C) based on primers used, 90 s extension at 72°C and the final extension for 7 min at 72°C. The PCR reactions were stored at 40°C until loading on gel for electrophoresis. The amplification products were differentiated by electrophoresis using an agarose gel (1.67%). DNA marker 100 bp was used to estimate molecular weight and size of the fragments. The gels were stained with ethidium bromide and visualized under UV.

### Data analysis

Polymorphic fragments (bands) were scored manually as binary data; '1' for the present, '0' for absent and '?' for

missing or ambiguous data. Only unambiguously amplified ISSR bands were scored while weak bands were excluded. Softwares were used for analysis of the binary data matrix filled on excel and rearranged accordingly. Percentage of polymorphic bands (PPB), Nei's (1973) gene diversity (h), Shannon-Weaver diversity index (I) were estimated using POPGENE Version 1.32 (Yeh et al., 1999) under the assumption of Hardy-Weinberg equilibrium. The two comparable estimators: Nei's gene diversity (h) and Shannon's information indices (I) were used to calculate genetic diversity for each population. Jaccard's similarity coefficient [17] was used to estimate similarity between pairs of populations from NTSYS-pc version 2.02 [31]. An analysis of molecular variation (AMOVA) was used to estimate genetic variance within and among each population using Arlequin version 3.01 [10]. Cluster analysis was performed to construct dendrograms with both Unweighted Pair Group Method with Arithmetic averages (UPGMA) tree using NTSYS-pc version 2.02 and neighbor joining (NJ) tree [24] using Free Tree 0.9.1.50 [27] using Jaccard's coefficient similarity.

## RESULTS

### ISSR band variation and level of polymorphism

Genomic DNA was amplified using ISSR markers to obtain unique fingerprints. A total of ten ISSR primers were screened, out of which five ISSR primers were selected which was giving clear and reproducible bands. A total of 37 clear and scorable bands were amplified by five primers with an average of 7.4 bands per primer having ISSR fragment size ranged from 350-1000 base pair. The number of polymorphic bands ranged from 6 to 8 attributing to 35 total polymorphic bands and 7 average polymorphic bands per primer. Percentage polymorphic bands (PPB) ranged from 85.71% for primer 818 to 100% for primers 811, 825, and 834. The dinucleotide primer

**Table 2.** List of primers, annealing temperature, primer sequence, amplification pattern and repeat motives used for optimization (Source: UBC)

Primers	Primer Sequence (5'→3')	Annealing Temp.	Band size	Scored bands			Diversity	
				Total	NPB	PPB	h±SD	I±SD
811	(GA) <sub>8</sub> C	48 <sup>o</sup> C	500-1000	7	7	100	0.3631± 0.1662	0.5327± 0.2107
818	(CA) <sub>8</sub> G	48 <sup>o</sup> C	400-1000	7	6	85.71	0.3326± 0.1862	0.4882± 0.2531
825	(AC) <sub>8</sub> T	48 <sup>o</sup> C	350-1000	7	7	100	0.3052± 0.1840	0.4571± 0.2488
834	(AG) <sub>8</sub> (C/T)T	48 <sup>o</sup> C	500-1000	8	8	100	0.1783± 0.1357	0.3037± 0.1877
857	(AC) <sub>8</sub> GGTC	56 <sup>o</sup> C	500-1000	8	7	87.50	0.2570± 0.1711	0.3968± 0.2422
Total			350-1000	37	35			
Mean				7.4	7	94.6	0.2775± 0.1735	0.4231± 0.2327

h: Nei's gene diversity, I: Shannon's information index, NPB: number of polymorphic bands, PPB: percentage of polymorphic bands

**Table 3.** Measures of genetic diversity in the 8 populations of *Capsicum* spp.

Pop	NPB	PPB	h±SD	I±SD	Gst*	Nm*
<b>Amara1</b>	23	62.16	0.2231±0.2144	0.3290±0.3008	-	-
<b>Amara2</b>	34	91.89	0.2962±0.1569	0.4523±0.2053	-	-
<b>Mareko</b>	25	67.57	0.2426±0.1946	0.3628±0.2788	-	-
<b>Alaba</b>	23	62.16	0.2189±0.2043	0.3266±0.2900	-	-
<b>Semen Omo</b>	21	56.76	0.2312±0.2166	0.3369±0.3079	-	-
<b>Benishangul</b>	25	67.57	0.2668±0.2074	0.3906±0.2925	-	-
<b>Oromia1</b>	27	72.97	0.2314±0.1809	0.3555±0.2555	-	-
<b>Oromia 2</b>	19	51.35	0.1938±0.2084	0.2866±0.2985	-	-
<b>Mean</b>	24.625	66.60	0.2830±0.1732	0.4301±0.2322	0.1751	2.3550

Nm\* estimate of gene flow from, Gst. Nm = 0.5(1-Gst)/Gst, h Nei's gene diversity, I Shannon's information index, NPB number of polymorphic bands, PPB percentage of polymorphic bands

818 generated only six polymorphic bands while the other dinucleotide primers 811, 825, and 857 generated seven polymorphic bands. Only one primer, 834, generated the highest (8) polymorphic bands (Table 2). All the five primers produced an average of 94.6 % polymorphic bands at the species level. The highest Nei's gene diversity (0.36) and Shannon information index (0.53) were exhibited by primer 811. In contrast, primer 834 showed the least Nei's gene diversity and Shannon information index with 0.18, 0.30, values, respectively. The mean Nei's gene diversity and Shannon information index for all primers were 0.28 and 0.42, respectively (Table 2).

### Population genetic diversity

Within-populations Percentage of polymorphic bands (PPBs) ranged from 51.35 % for Oromia2 to 91.89 % for Amara2 with a mean of 66.60 %. Similarly, gene diversity ranged from 0.19 for Oromia2 to 0.30 for Amara2 with a mean of 0.28, and the same patterns have been observed

for the Shannon information index which ranged from 0.29 for Oromia2 to 0.45 for Amara2 with a mean value of 0.43. Populations of Oromia1, and Semen Omo showed nearly the same gene diversity value (0.23). The least percentage of the polymorphic band (51.35 %), gene diversity (0.19) and Shannon information index (0.29) were exhibited by Oromia2 population, collected from Bale, Arsi and west wollega. Generally, populations collected from Amara2 (East Gojjam and North Gonder) showed the highest gene diversity (0.30), PPB (91.89 %), and Shannon index (0.45), respectively (Table 3). Within-populations Percentage of polymorphic bands (PPBs) ranged from 51.35 % for Oromia2 to 91.89 % for Amara2 with a mean of 66.60 %. Similarly, gene diversity ranged from 0.19 for Oromia2 to 0.30 for Amara2 with a mean of 0.28, and the same patterns have been observed for the Shannon information index which ranged from 0.29 for Oromia2 to 0.45 for Amara2 with a mean value of 0.43. Populations of Oromia1, and Semen Omo showed nearly the same gene diversity value (0.23). The least percentage

**Table 4.** Similarity matrixes for the seven Ethiopian *Capsicum* spp. populations based ISSR markers

PopID	Am1	Am2	Mk	Al	SO	Ben	OR1	OR2
Am1	****	0.9286	0.9388	0.9098	0.8269	0.8969	0.8910	0.8651
Am2		****	0.9702	0.9494	0.9134	0.9496	0.9402	0.9469
Mk			****	0.9515	0.8734	0.9402	0.9406	0.9470
Al				****	0.8799	0.9142	0.9419	0.9581
SO					****	0.9453	0.9087	0.9326
Ben						****	0.9305	0.9385
OR1							****	0.9656
OR2								****

Am1: Amara1, Am2: Amra2, Mk: Meskan, Al: Alaba, SO: Semen Omo, Ben: Benishangul, OR1: Oromia1 and OR2: Oromia2

of the polymorphic band (51.35 %), gene diversity (0.19) and Shannon information index (0.29) were exhibited by Oromia2 population, collected from Bale, Arsi and west wollega. Generally, populations collected from Amara2 (East Gojjam and North Gonder) showed the highest genediversity (0.30), PPB (91.89 %), and Shannon index (0.45), respectively (Table 3).

### Population genetic divergence

From Jaccard genetic similarity of pepper cultivars, comparatively the highest similarity was observed between Oromia1 and Oromia 2 (0.965) than other landraces combination. The lowest similarity was found in Amara 1 versus Semen Omo (0.827) landraces pair. All landraces showed an average of 0.896 genetic similarities, which could mean that the landraces share an average of 89.6% of their band fragments (Table 4).

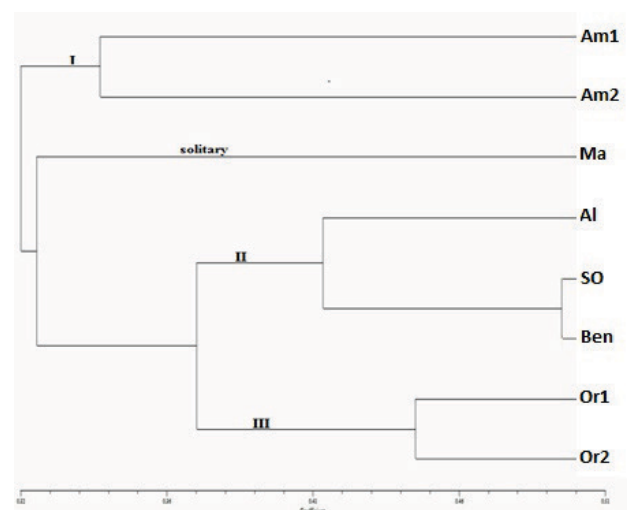
### Genetic distances and clustering analysis

The genetic distances between all combinations of any two populations were investigated and the genetic distances among the 8 populations ranged from 0.03 to 0.19. The lowest genetic distance was revealed between Oromia 1 and Oromia2 whereas the highest was between amara2 and Semen Omo. UPGMA and neighbor joining (NJ) analysis were used to construct dendrogram for the eight populations and 73 individuals based on 37 PCR bands amplified by five di-nucleotides (811, 818, 825, 834 and 857) ISSR primers. Clustering based on NJ resulted in three main clusters (I, II and III) and one solitary (Mareko). The first cluster (I) is out grouping population from Amahara (Amhara1 and Amhara2), while the second cluster (II) contained Alaba, Semen Omo and benishangul populations. The third cluster

branched into oromia 1 and oromia 2 populations. Semen Omo and Benishangul, which are highly, related populations (Figure 2).

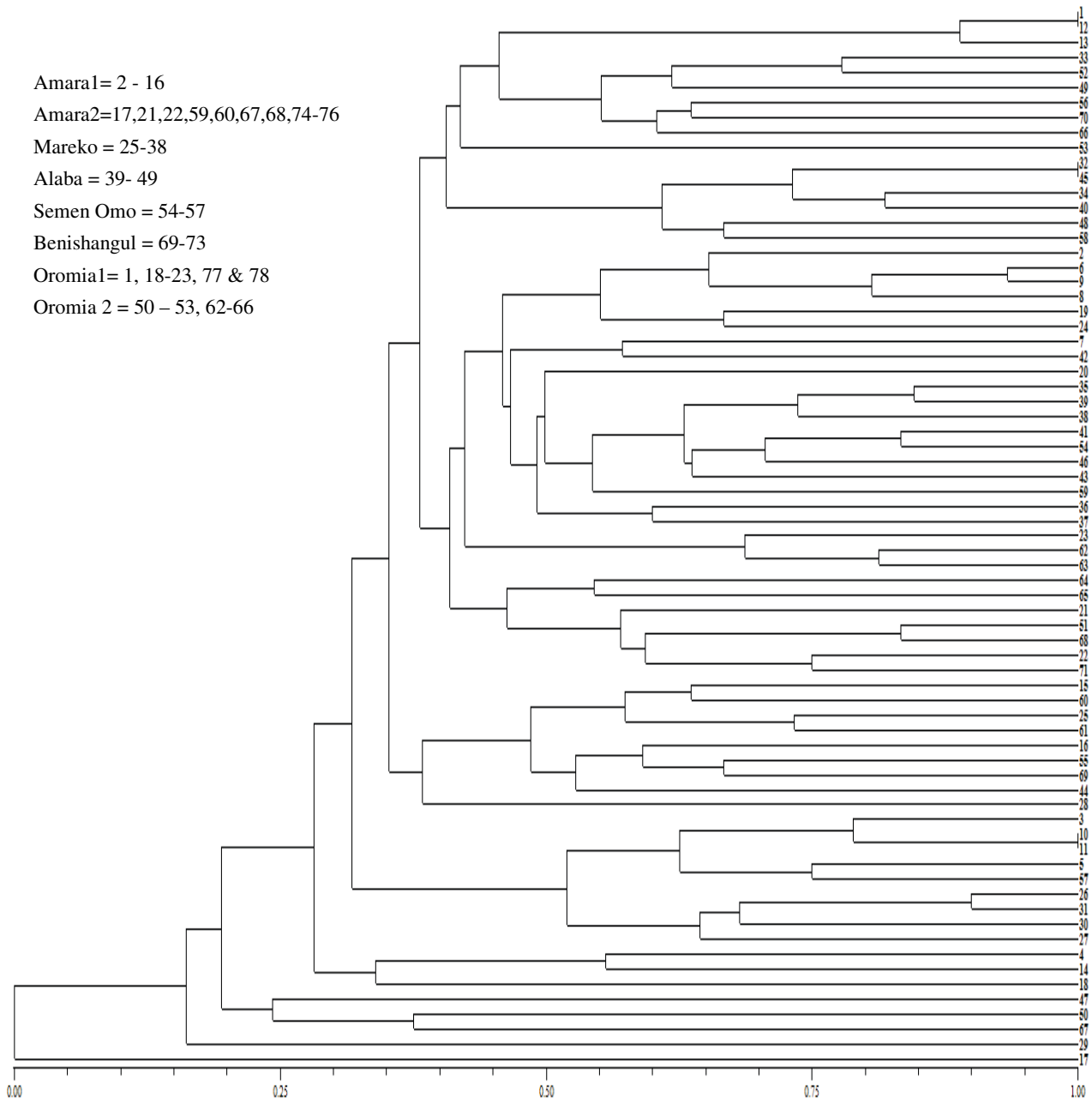
On the other hand, individual based UPGMA clustering of an overall analysis showed clustering of individuals with respect to their populations except few intermixed individuals from other population (Figure 3).

Analysis of molecular variance (AMOVA) indicated a total of 86.67 % within population and 13.13 % among population variation with 0.13 fixation index (FST) indicating about 13 % genetic differentiation among populations independent of the marker used ( $P < 0.05$ ) (Table 5).



**Figure 1.** UPGMA based dendrogram for 8 populations of *Capsicum* spp.

Am1: Amara1, Am2: Amra2, Ma: Mareko, Al: Alaba, SO: Semen Omo, Ben: Benishangul, Or1: Oromia1 and Or2: Oromia2



**Figure 3.** UPGMA based dendrogram for 73 individuals of *Capsicum* spp. using five ISSR primers. The UPGMA algorithm is based on Jaccard's coefficients obtained after pair wise comparison of the presence-absence fingerprint

**Table 5.** Analysis of molecular variance among the 8 populations of *Capsicum* spp. without grouping

Source of Variation	df	Sum of Squares	Variance components	% of variation	$F_{ST}$	P value
Among Pop.	7	68.525	0.63989 <sup>a</sup>	13.13	0.131	0.05
Within Pop.	63	266.8	4.2349 <sup>b</sup>	86.87		
Total	70	335.32	4.8748			

## DISCUSSION

### Genetic diversity

Genetic diversity study is very important to the long-term survival of species; without it, species cannot adapt to environmental changes and are more susceptible to extinction. The amount of genetic diversity available within species also determines the potential for improving species through breeding and selection programs. Knowledge of pattern of genetic variability is important for identifying heterogeneity patterns in hybrid breeding

and for relating the observed pattern with the presence of certain economically important traits. Such information can be used to design effective cultivars conservation and for setting cultivars collection mission as well as to predict the risk of genetic erosion in certain area. The pattern of genetic variability can be studied by morphological, isosyme and molecular markers. Among the molecular markers ISSR markers are important to study genetic variations in plant species, as they are effective in detecting very low levels of genetic variation [42].

Seventy-three accessions from the *Capsicum* collection maintained at the Ethiopian Biodiversity Institute, seed bank, (Addis Ababa, Ethiopia) were evaluated with a set of ISSR loci spread overall the pepper genome. High genetic diversity was observed among the genotypes of *Capsicum* spp. based on the DNA markers generated by ISSR primers. ISSR markers have been widely used to analyze plant genetic diversity and have contributed substantially for the improvement of hot peppers cultivars because of their good stability and high genetic polymorphisms [22]. In the present study, five ISSR primers succeeded to produce 35 polymorphic bands out of 37 bands. Among the five primers only one, primer 834, generated the highest number of polymorphic bands. The use of ISSR primers was also reported to be highly appreciable toward discrimination of disputed hot peppers samples [19]. These results are in accordance with other reports on diverse plants such as tomato [32], mulberry [35], and peanut [30] where higher polymorphism was unraveled with ISSR primers from closely related cultivars. The results clearly showed that ISSR-PCR analysis has been shown to be a reproducible marker that generates sufficient polymorphism with potential for large-scale DNA fingerprinting purposes [29].

The mean Nei's and Shannon information index for all primers were 0.28 and 0.42 respectively, indicating an immense genetic diversity at species level. The high percentage of overall polymorphism (94.6 %) revealed by this study suggest that ISSR is a powerful technique for genotyping *Capsicum* spp. populations as well as cultivars. Similarly, high percentage polymorphisms were found in the same crops 98.7% [37], 91.2% [9] and 89% [36] used ISSR along with AFLP and RAPD and Ballina-Gómez et al. (2013) used morphology for determining genetic variation, phylogenetic relationships among different species within the Mexicans *Capsicum* and obtained 94% of polymorphisms.

### Genetic differentiation and population structure

Genetic diversity parameters, (PPB, gene diversity (h), and Shannon's diversity (I)) were highest for amhara2 (91.89 %, 0.3, 0.543), respectively, indicating that probably these places are centers of genetic diversity for Ethiopian hot peppers cultivars. The observed heterozygosity in the entire collection was high and comparable to previous studies in *Capsicum* spp. [15, 40]. Such level of heterozygosity is expected due to high seed exchanges of hot peppers among farmers [21]. The genetic diversity, expressed as a measure of the Nei's unbiased gene diversity index, was similar to values reported in studies with larger diverse sets of *Capsicum* spp. [34] confirming that we are evaluating a sample of *Capsicum* spp. with considerable variability. The lowest percentages of polymorphic bands, gene diversity and diversity index were shown by Oromia2 indicating that individuals of oromia2 are uniform. This low genetic diversity might be explained by the loss of wild relatives mainly due to anthropogenic destruction of natural habitats; homogeneity due to single or few seed sources during its introduction to these areas [23].

In this work, a relatively high level of genetic diversity was also observed for the cultivated *Capsicum* spp., as opposed to other self-pollinated crops. This is most likely the result of its long cultivation history and different climatic and topographic factors of Ethiopia which enhanced the effects of natural and artificial selection on cultivars.

The coefficient of gene differentiation showed that the genetic variation within and among the 8 geographical regions was 82.5 and 17.5%, respectively. Therefore, low genetic differentiation between populations was observed due to migration or selection. The values obtained from gene flow (Nm) show the approximate number of individuals migrating from one population to the other. The highest gene flow (Nm=2.36) observed in this study may suggest that the seed exchanging system might be high among the farmers, resulting in low genetic differentiation observed among the populations (D=0.175). The observed gene flow in the entire collection was high and comparable to previous studies in *Triticum diccicum* species studied by (Shiferaw and Kassahun, 2017) using total seed storage protein marker. AMOVA indicated that 86.87 % of total variation was accounted for within population variation. According to compiled data of Nybom (2004), genetic diversity is strongly associated with life form, geographic range,

breeding system, seed dispersal mechanism, and successional status. The estimate of population differentiation of *Capsicum* spp. using  $F_{ST}$  was 0.13 indicating 13 % of the genetic variation was due to differences between populations

The UPGMA-based dendrogram of the 8 populations of *Capsicum* spp. generated three distinct clusters. This study showed that there is strong correlation between geographic distance and genetic diversity of Ethiopian hot peppers cultivars because geographically closely related species have been clustered together.

The similarity coefficient values and the UPGMA dendrogram revealed narrow genetic base among the tested *Capsicum* spp. This is likely due to the fact that their parental breeding lines were the same or were very close to each other, bearing in mind that pepper is a self-pollinated crop. This finding confirms the results of Kumar et al. (2001); Ilbi (2003); Yang et al. (2005) and Lijun and Xuexiao (2012) who detected that the genetic variations among five *Capsicum* spp. were mainly inter-specifically rather than intra-specifically.

## CONCLUSION

In conclusion, the results show that ISSR markers detected a considerable genetic diversity in the studied pepper cultivars, which could be used in conservation of genetic resources of Ethiopian hot pepper cultivars and crop breeding through marker assisted selection (MAS)

Our results showed that hot peppers cultivars from amhara2 (west Gojjm and North Gonder) had the highest genetic polymorphism. ISSR markers have been widely used to analyze plant genetic diversity because of their good stability, high genetic polymorphisms and it is more reliable than the morphological characteristics. Therefore, our results revealed the phylogenetic relationships of the populations and accessions cultivated species more authentically.

This study is the first attempt to address the genetic diversity of *Capsicum* spp. population Ethiopian cultivars. However, the current study has a limitation that only few populations and accessions from Ethiopia were studied. Therefore, capsicum genetic diversity and phylogeography of Ethiopian populations from different agro-ecology should be studied in detail.

Analysis of genetic diversity in *Capsicum* spp. using more than one methods helps to better understand the levels of genetic variation, the genetic structure of populations and determine migration root; when compared to the results

obtained using only one method. Analysis with co-dominant markers system, like microsatellites, needs to be conducted to better understand and estimate the gene flow, and determine the size of population and levels of inbreeding

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## مطالعه تنوع ژنتیکی ارقام فلفل قرمز اتیوپیایی با استفاده از نشانگر ISSR

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### چکیده

فلفل تند (*Capsicum spp.*) یک ادویه مهم تجاری است که بطور گسترده در اتیوپی کشت و مورد استفاده قرار می‌گیرد. علیرغم اهمیت بسیار آن، اطلاعاتی در زمینه تنوع ژنتیکی این محصول زراعی در دسترس نیست. تعیین ویژگی‌های ارقام یک پیوند مهم بین محافظت و استفاده از منابع ژنتیک گیاهی در برنامه‌های اصلاحی مختلف می‌باشد. با استفاده از ۵ آغازگر ISSR، در مجموع ۳۷ باند قابل امتیازدهی تولید شد که ۳۵ باند (۹۴/۶٪) آنها چندشکل بودند. تنوع باندهای چندشکل در جمعیت از ۵۱/۳۵٪ تا ۹۱/۸۹٪ با میانگین ۶۶/۱۶٪، تنوع ژنتیکی Ni از ۰/۱۹ تا ۰/۳۰ با میانگین ۰/۲۸، و شاخص Shanon از ۰/۲۹ تا ۰/۴۵ و میانگین ۰/۴۳ متغیر بود. با در نظر گرفتن همه پارامترهای تنوع، بالاترین تنوع از جمعیت‌های amhara2 و کمترین مقدار از Oromia2 بدست آمد. بر اساس ضریب مشابهت جفتی جاکارد، جمعیت‌های Oromia1 و Oromia2 با مشابهت ۰/۹۵۶ مرتبط‌ترین و جمعیت‌های Semn omo و Amhara2 با مشابهت ۰/۸۲۷ بیشترین فاصله از هم را داشتند. خوشه‌بندی نشان داد که همبستگی قوی بین فاصله جغرافیایی و تنوع ژنتیکی ارقام فلفل تند اتیوپیایی وجود دارد، زیرا گونه‌هایی که از نظر جغرافیایی با هم مرتبط بودند در یک گروه قرار گرفتند. جمعیت‌های Amhara2 بیشترین تنوع ژنتیکی را نشان دادند و بنابراین می‌توان آنها را بعنوان مکان‌های اصلی برای طراحی نواحی محافظت شده برای این گیاه زراعی در اتیوپی در نظر گرفت. علاوه بر این، پیشنهاد می‌شود که نشانگرهای مولکولی ابزار معتبری برای ارزیابی تنوع ژنتیکی در ارقام فلفل می‌باشند.

**کلمات کلیدی:** کیسیکوم، ارقام، تجزیه و تحلیل خوشه‌بندی، تنوع، فلفل