OPEN ACCESS International Journal of Agricultural Research

ISSN 1816-4897
DOI: 10.3923/ijar.2018.

Research Article
Genetic Diversity Study of Ethiopian Hot Pepper Cultivars (*Capsicum species*) Using Inter Simple Sequence Repeat (ISSR) Marker

Shiferaw Aemu Alayachew, Destaw Mulualem Atnafu and Samuel Augna Gedefa

Department of Applied Biology, College of Natural and Computational Sciences, Wolkite University, Ethiopia

Abstract

Background and Objective: Hot pepper is an economically important spice that is widely cultivated and consumed in Ethiopia. In spite of its wide importance, there is no/few information available on molecular genetic diversity of this crop and less attention has been given for its improvement. Cultivars characterization is an important link between the conservation and utilization of plant genetic resources in various breeding programmes. The present experiment was conducted to determine the amount of genetic diversity of Ethiopian hot peppers cultivars using ISSR markers. **Materials and Methods:** The experimental site was located at Wolkite, Southern Nation and Nationalities Peoples (SNNPs), Ethiopia. Ten Seeds were randomly selected from each accession and planted 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. **Results:** Using five ISSR primers, a total of 37 scorable bands were generated of which 35 (94.6%) were polymorphic. Within population diversity based on polymorphic bands ranged from 51.35-91.89% 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 most related populations exhibiting 0.956 similarity and Semen omo and Amhara2 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. **Conclusion:** Amhara2 populations (from west Gojjam and north Gonder) exhibited the highest genetic diversity so that the populations should be considered as the primary sites in designing conservation areas for this crop. Further, it was suggested that molecular markers are valid tags for the assessment of genetic diversity in *Capsicum species* cultivars.

Key words: Hot pepper, amhara2, *Capsicum species* cultivars, cluster analysis, ISSR primers

Citation: Shiferaw Aemu Alayachew, Destaw Mulualem Atnafu and Samuel Augna Gedefa, 2018. Genetic diversity study of Ethiopian hot pepper cultivars (*Capsicum species*) using inter simple sequence repeat (ISSR) marker. Int. J. Agric. Res., CC: CC-CC.

Corresponding Author: Shiferaw Aemu Alayachew, Department of Applied Biology, College of Natural and Computational Sciences, Wolkite University, Ethiopia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hot peppers *(Capsicum species)* are also known as red pepper or chili pepper. It is a dicotyledonous flowering plant which belongs to the family of Solanaceae[1] and an important commercial crop cultivated exclusively in tropical and temperate zones of the world. It has been grown on more than 1.5 million ha worldwide annually[2]. They are usually classified based on their 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 state (SNNPs), since its introduction in the early 17th century by the Portuguese[6]. In Ethiopia, *Capsicum annum* is the main types of capsicum species that has grown in the country. Pepper species are grown at a medium altitude of 1,400-2,120 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 ($SNNP_S$) contribute a significant portion to the country's total pepper production[7]. The average daily consumption of most adult Ethiopians has been about 15 g, which is higher than tomatoes. Now a day's, hot pepper plays an important role in the national economy of the country by generating high revenue and contribute in reducing poverty. It is also a raw material for the dye processing industries and a source of employment to urban and rural populations. However, the production of hot peppers for dry pod has been low with a national average yield of 0.4 t ha^{-1} [8] and decreasing with time due to diseases and monoculture. Therefore, the use of resistant varieties is recommended to overcome uniformity and diseases[9]. Such varieties can be developed for within the existing diversity of the cultivars[10].

Knowledge of the genetic diversity of the existing cultivars is necessary to improve the crops through marker assisted breeding[11-13]. Moreover, applying the best improvement programs also needs for knowing production constraints and farmers' varietal preference criteria[14,15]. Therefore, a study on the genetic relationship and gene diversity of different cultivated pepper species to find new parental cultivars is important in proper utilization of pepper cultivars and an effective way to solve the constraints of pepper breeding.

Different genetic markers are widely used by breeder to study genetic diversity. Among those markers, the molecular markers are advantageous of being independent of environmental factors, abundant, the most convenient and popular methods to identify and study genetic polymorphism[16]. When compare the cost of Inter simple sequence repeat (ISSR) analysis, it is relatively lower than that of AFLP and produces acceptable reproducibility. Therefore, it is widely used in current studies of population genetics among geneticists[17]. Only morphological and phenotypic characterization of pepper cultivars was conducted with limited accessions from Alaba, Ethiopia. However, morphological study has its own limitation like, they could be influenced by changes in environmental factors and may vary at different growth stages of plants; moreover, their number is limited[18]. No scientific studies have been conducted on molecular diversity of Ethiopian hot pepper cultivars using ISSR marker or other molecular markers. Therefore, this study also 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 (EBI). Those accessions were classified into 8 populations based on the geographical regions they were collected from Table 1. Ten Seeds were randomly selected from each accession and planted using pot in glasshouse of Wolkite University, Wolkite, Ethiopia, from

Table 1: Passport descriptors of *Capsicum species* populations collected from different sites in Ethiopia

Populations	Regions/States	Zone	Altitude (masl)
Amhara1	Amhara	West Gojam	1954-2050
Amhara2	Amhara	East Gojam, N. Gonder	1555-2570
Mareko	SNNPs	Gurage	1820-2060
Alaba	SNNPs	Kembata	1820-1940
Benishangul	Benishangul Gumuz	Metekel	1640-1700
Semen Omo	SNNPs	Semen Omo	1440-1940
Oromia1	Oromia	South west Shewa	1550-2160
Oromia2	Oromia	Bale and Arssi	1953-2780

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. (°C)	Band size	Scored bands		Diversity		
				Total	NPB	PPB	h±SD	I±SD
811	(GA)8C	48	500-1000	7.0	7	100.00	0.3631±0.1662	0.5327±0.2107
818	$(CA)_8G$	48	400-1000	7.0	6	85.71	0.3326±0.1862	0.4882±0.2531
825	(AC)8 T	48	350-1000	7.0	7	100.00	0.3052±0.1840	0.4571±0.2488
834	(AG)8 YT	48	500-1000	8.0	8	100.00	0.1783±0.1357	0.3037±0.1877
857	$(AC)_8GGTC$	56	500-1000	8.0	7	87.50	0.2570±0.1711	0.3968±0.2422
Total			350-1000	37.0	35			
Mean				7.4	7	94.60	0.2775±0.1735	0.4231±0.2327

*Single-letter abbreviations for mixed base positions: R = (A, G) Y = (C, T), NBP: Number of polymorphic band

November, 2017 to June, 2018. 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 0.25 g of the silica-dried bulked leaves tissues from each accessions were ground with mixer mill (model- Retsch MM400) and the total genomic DNA was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide (2% Cetyltrimethyl ammonium Bromide, 1% polyvinyl pyrrolidone, 100 mMTris: PH: 8, 20 mM EDTA, 1.4 M NaCl, 0.2% beta-Mercapto-ethanol)) method described in Lo Piccolo *et al*.[19] with minor modifications.

The presence and quality of isolated DNA bands for the first 10 accessions was checked by gel electrophoresis using 1% agarose at 100 volt constant for 30 min. The gel picture was taken with Bio Rad 200 and used to check the presence of genomic DNA and 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 spectrophotometer 8000. The DNA concentration ranges from 109.5-11,221.1 ng μL^{-1} which was later diluted to optimized constant concentration of 20 ng μL^{-1}.

Primer selection and optimization: The ISSR marker assessment was carried out at Plant Genetics Research Laboratory of the Microbial, Cellular and Molecular Biology Department, Addis Ababa University. Ten ISSR-primers were screened by using a total of 16 randomly selected accessions i.e., two accessions from each population and 5 primers that produced clear, reproducible and polymorphic band pattern were selected for in-depth study using 73 individuals of the 8 populations (Table 2).

PCR amplification and electrophoresis: The Polymerase Chain Reaction (PCR) was conducted in Biometra 2000 T3 Thermo cycler. The amplification was carried out in a 25 µL reaction volume containing *Taq* DNA polymerase (3 U/reaction), *Taq* DNA polymerase buffer (1x) with 1.5 mM $MgCl_2$, random decamer primers (10 pmol/reaction), deoxynucleotide triphosphate (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 sec at 94°C, 1 min primer annealing at (45/48°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 1×TE) and 8 µL amplification product of each sample with 2 µL loading dye (6 times concentrated) was loaded on gel.

DNA marker 100 bp was used to estimate molecular weight and size of the fragments. Electrophoresis were run for 3 h at constant voltage of 100 V. The DNA was stained with 30 µL (10 mg mL^{-1}) ethidium bromide which was mixed with 450 mL distilled water for 30 min and washed with distilled water for 30 min.

Data analysis: Polymorphic fragments (bands) were scored manually as binary data; (1) for the present, (0) for absent and (?) for missing data. Only unambiguously amplified ISSR bands were scored while weak bands were excluded. Different softwares were used for analysis of the binary data matrix filled on excel and rearranged accordingly. Percentage of polymorphic bands (PPB), Nei[20] reported that gene diversity (h), Shannon-Weaver diversity index (I) were calculated using POPGENE Version[21] 1.32 under the assumption of Hardy-Weinberg equilibrium. Nei's gene diversity (h) and Shannon's information indices (I) estimators were used to calculate genetic diversity for each population. Jaccard's similarity coefficient[22] was used to estimate similarity between pairs of populations from NTSYS- pc version[23] 2.02. An analysis of molecular variation (AMOVA) was used to estimate genetic variance within and among each population using Areliquin version 3.01 software[24]. Cluster analysis was done 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[20] using Free Tree[25] 0.9.1.50 using Jaccard's coefficient similarity.

RESULTS

ISSR band variation and level of polymorphism: Genomic DNA was amplified using ISSR markers to obtain unique fingerprints (Fig. 1). A total of 10 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 varied from 6-8 attributing to 35 total polymorphic bands and 7 average polymorphic bands per primer. Percentage polymorphic bands (PPB) ranged from 85.71% for primer 818-100% for primers 811, 825 and 834. Primer 818 was generated only 6 polymorphic bands while the other di-nucleotide primers 811, 825 and 857 were revealed 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 0.17 and 0.30 values, respectively. The mean Nei's gene diversity and Shannon information index for all primers were 0.27 and 0.42, respectively (Table 2).

Population genetic diversity: Within-populations, percentage of polymorphic bands (PPBs) ranged from 51.35% for Oromia 2-91.89% for Amhara2 with a mean of 66.60%. Similarly, gene diversity ranged from 0.19 for Oromia 2-0.296 for Amhara2 with a mean of 0.28 and the same patterns had been observed for the Shannon information index which ranged from 0.29 for Oromia 2-0.45 for Amhara2 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 Amhara2 (East Gojjam and North Gonder) revealed the highest gene diversity (0.30), PPB (91.89%) and Shannon information index (0.45), respectively (Table 3).

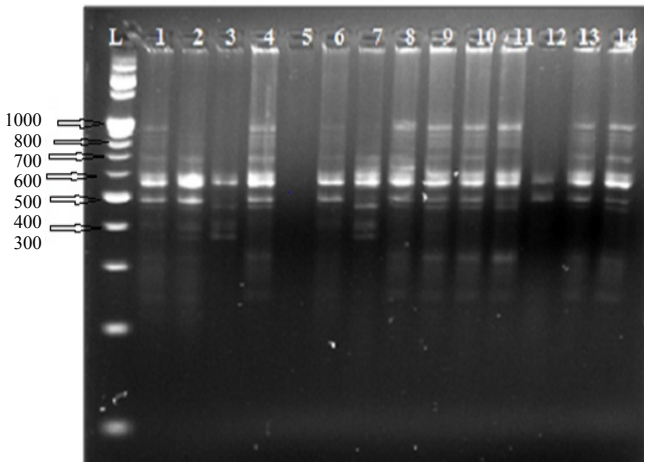

Fig. 1: Sample of ISSR fingerprint generated using primer 811
ISSR fingerprints generated by primer 811 using agarose gels. L: Ladder, the lanes represents samples of Oromia2, Semen Omo , amhara2 and Benishangul, individuals

Population genetic divergence: From Jaccard genetic similarity of hot peppers cultivars, comparatively the highest similarity was observed between Oromia1 and Oromia2 (0.965) than other cultivars combination. The lowest similarity was found in Amhara1 vs. Semen Omo (0.827) cultivars pair. All cultivars showed an average of 0.896 genetic similarities, which could mean that they shared 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 0.03-0.19. The lowest genetic distance was revealed between Oromia1 and Oromia2 whereas the highest was between amhara2 and Semen Omo. UPGMA and neighbor joining (NJ) analysis were used to construct dendrogram for the 8 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) was 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 Ormia1 and Oromia2 populations. Semen Omo and Benishangul, which were highly, related populations (Fig. 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 (Fig. 3).

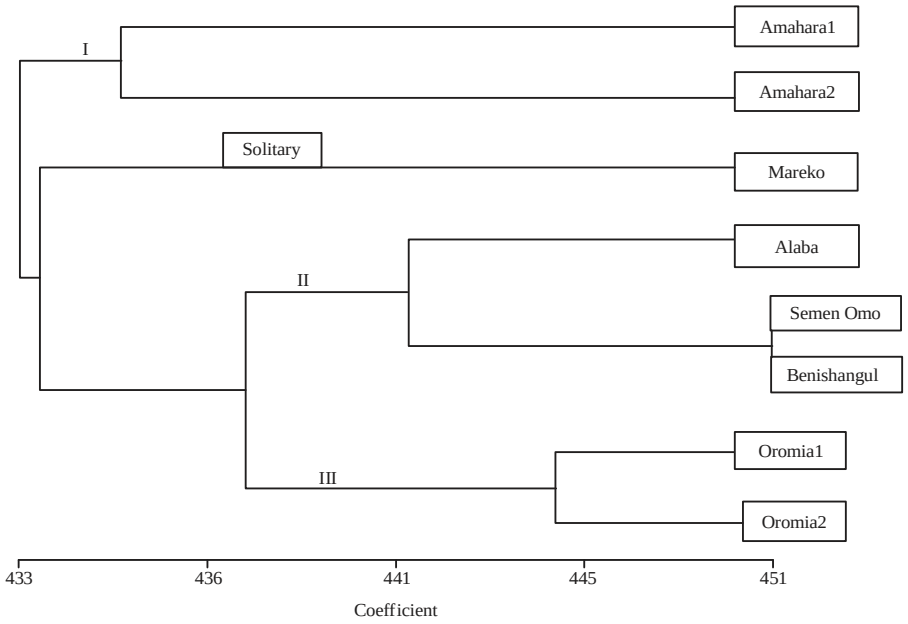

Fig. 2: UPGMA based dendrogram for 8 populations of *Capsicum species*

Table 3: Number of polymorphic band (NPB), percentage polymorphism (PP), genetic diversity (h) and shannon index (I) for each population

Pop	NPB	PPB	h±SD	I±SD	Gst*	Nm*
Amhara1	23	62.16	0.2231±0.2144	0.3290±0.3008	-	-
Amhara2	34	91.89	0.2962±0.1569	0.4523±0.2053	-	-
Mareko	25	67.57	0.2426±0.1946	0.3628±0.2788	-	-
Alaba	23	62.16	0.2189±0.2043	0.3266±0.2900	-	-
Semen Omo	21	56.76	0.2312±0.2166	0.3369±0.3079	-	-
Benishangul	25	67.57	0.2668±0.2074	0.3906±0.2925	-	-
Oromia1	27	72.97	0.2314±0.1809	0.3555±0.2555	-	-
Oromia 2	19	51.35	0.1938±0.2084	0.2866±0.2985	-	-
Mean	24.625	66.60	0.2830±0.1732	0.4301±0.2322	0.1751	2.3550

Table 4: Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Pop ID	Am1	Am2	Mrk	Al	SO	Ben	OR1	OR2
Am1	****	0.9286	0.9388	0.9098	0.8269	0.8969	0.8910	0.8651
Am2	0.0740	****	0.9702	0.9494	0.9134	0.9496	0.9402	0.9469
Mrk	0.0632	0.0302	****	0.9515	0.8734	0.9402	0.9406	0.9470
Al	0.0945	0.0519	0.0497	****	0.8799	0.9142	0.9419	0.9581
SO	0.1900	0.0905	0.1353	0.1279	****	0.9453	0.9087	0.9326
Ben	0.1088	0.0517	0.0617	0.0897	0.0563	****	0.9305	0.9385
OR1	0.1154	0.0617	0.0612	0.0599	0.0957	0.0720	****	0.9656
OR2	0.1449	0.0545	0.0544	0.0428	0.0697	0.0634	0.0350	****

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

Table 5: Analysis of molecular variance among the 8 populations of *Capsicum species* without grouping

Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}	p-value
Among populations	7	68.525	0.63989[a]	13.13	0.131	0.05
Within populations	63	266.799	4.23491[b]	86.87		
Total	70	335.324	4.87480			

df: Degree of freedom, F_{st}: Fixation index

Analysis of molecular variance (AMOVA) indicated a total of 86.87% within population and 13.13% among population variation with 0.13 fixation index (F_{ST}) indicating about 13% genetic differentiation among populations independent of the marker used ($p<0.05$) (Table 5).

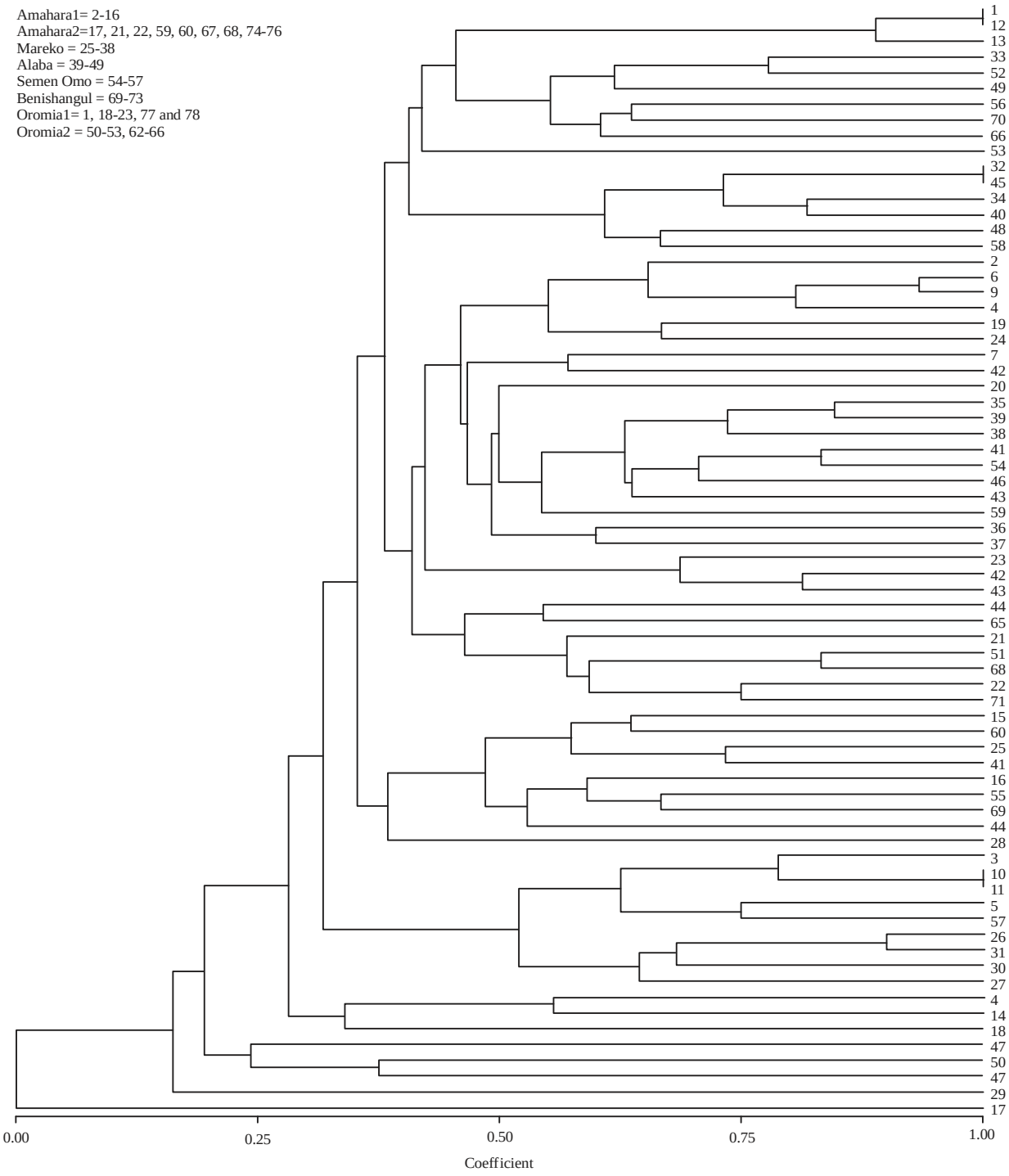

Fig. 3: UPGMA based dendrogram for 73 individuals of *Capsicum* sp. using five ISSR primers. The UPGMA algorithm is based on Jaccard's coefficients obtained after pair wise comparison of the presence-absence fingerprint

DISCUSSION

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, isozyme 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[26].

About 73 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* sp. based on the DNA markers generated by ISSR primers. In the present study, five ISSR primers succeeded to produce 35 polymorphic bands out of 37 bands. The use of ISSR primers was also reported to be highly appreciable toward discrimination of disputed hot peppers samples[27]. These results are in accordance with other reports on diverse plants such as tomato[23], mulberry[28] and peanut[29] by using ISSR markers. 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[30].

The mean Nei's (0.28) and Shannon information index (0.42) for all primers were indicating an immense genetic diversity at species level. The high percentage of overall polymorphism (94.6%) revealed by this study suggested that ISSR is a powerful technique for genotyping peppers populations as well as cultivars. Similarly, high percentage polymorphisms were found in the same crops 98.7% by Thul *et al.*[31], 91.2% by Dias *et al.*[13] and 89% by Tanksley[32] used ISSR along with AFLP and RAPD and Ballina-Gomez *et al.*[16] used morphology for determining genetic variation, phylogenetic relationships among different species within the Mexicans *Capsicum* and obtained 94% of polymorphism*s.*

The highest genetic diversity parameters for Amhara2 (91.89%, 0.3, 0.543) were indicating that probably these places are centers of genetic diversity for Ethiopian peppers cultivars. The observed heterozygosity in the entire collection was high and comparable to previous studies in peppers[33,15]. Such level of heterozygosity is expected due to high seed exchanges of hot peppers among farmers[34]. The Nei's genetic diversity was similar to values reported in studies with larger diverse sets of *Capsicum species*[35]. The lowest genetic diversity parameters were shown by Oromia2 indicating that individuals of Oromia2 are uniform. This low genetic diversity might be explained by the loss of wild relatives[36] mainly due to homogeneity i.e., single or few seed sources were introduced during its introduction to these areas.

In this work, a relatively high level of overall genetic diversity was observed for the cultivated *Capsicum* sp., 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 populations was 82.5 and 17.5%, respectively. Therefore, low genetic differentiation between populations was observed due to migration or selection. The highest gene flow (Nm = 2.36) observed in this study may suggested 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 diccocum* species studied by Alayachew and Geletu[37] using total seed storage protein marker.

AMOVA indicated that 86.67% of total variation was accounted for within population variation. According to compiled data of Nybom[12], 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* sp. using F_{ST} was 0.13 indicating 13% of the genetic variation was due to differences between populations

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 peppers species. 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.*[27], Ilbi[38], Yang *et al.*[39] and Lijun and Xuexiao[40] who detected that the genetic variations among five peppers species were mainly inter-specifically rather than intra-specifically.

CONCLUSION AND RECOMMENDATIONS

The results revealed that hot peppers cultivars from Amhara2 (west Gojjm and North Gonder) had the highest genetic diversity so that the populations should be considered

as the primary sites in designing conservation of genetic resources of Ethiopian hot pepper cultivars and crop breeding through marker assisted selection (MAS).

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 identified the highest genetic diversity center of this crop that can be beneficial for conservation and marker assisted breeding. This study will help the researcher to uncover the critical areas of genetic diversity using other markers like Simple Sequence Repeats (SSR) that more reliable than the markers used by this study, needs to be conducted to better understand and estimate the gene flow and determine the size of population and levels of inbreeding.

SIGNIFICANCE STATEMENT

The study provides ground information about genetic diversity within and among hot pepper cultivars collected from different peppers 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.

ACKNOWLEDGMENT

The work was supported through funding from wolkite University and we also acknowledge Ethiopian Biological Societies, wolkite university research directorate and medicinal chemistry conference organizers to presented this original research article for the research communities.

REFERENCES

1. Knapp, S., 2002. Tobacco to tomatoes: A phylogenetic perspective on fruit diversity in the Solanaceae. J. Exp. Bot., 53: 2001-2022.
2. FAO., 2013. FAOSTAT database. Food and Agriculture Organization of the United Nations. http://faostat.fao.org/default.aspx.
3. Bosland, P.W. and E.J. Votava, 2000. Peppers, Vegetables and Spices *Capsicum*. CABI Publishing, New York, Pages: 198.
4. Bosland, P.W., 1992. Chiles: A diverse crop. HortTechnology, 2: 6-10.
5. Bosland, P.W., 1994. Chillies History, Cultivation and Uses. In: Species, Herbs and Edible Fungi, Charalambous, G. (Ed.)., Elsevier Publication, New York, USA., pp: 347-366.
6. Huffnagel, H.P., 1961. Agriculture in Ethiopia. Food and Agriculture Organization of the United Nation Organization, Rome, Pages: 465.
7. CSA., 2011. Agricultural sample survey 2010-2011. Vol. I. Report on Area and Production of Major Crops. Central Statistics Agency, Federal Democratic Republic Ethiopia, Statistical Bulletin. Addis Ababa, pp: 12-96.
8. Mariame, F. and D. Gelmesa, 2006. Review of the status of vegetable crops production and marketing in Ethiopia. Uganda J. Agric. Sci., 12: 26-30.
9. Houimli, S.I.M., M. Denden and S.B. El Hadj, 2008. Induction of salt tolerance in pepper (*Capsicum annuum*) by 24-epibrassinolide. Eur. Asia J. BioSci., 2: 83-90.
10. Truong, L., S.C. Tilton, T. Zaikova, E. Richman, K.M. Waters, J.E. Hutchison and R.L. Tanguay, 2013. Surface functionalities of gold nanoparticles impact embryonic gene expression responses. Nanotoxicology, 7: 192-201.
11. Ajjapplavara, P.S., 2009. Genetic diversity in chilli (*Capsicum annuum* L.). Asian J. Hortic., 4: 29-31.
12. Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol., 13: 1143-1155.
13. Dias, G.B., V.M. Gomes, T.M.S. Moraes, U.P. Zottich and G.R. Rabelo *et al*., 2013. Characterization of *Capsicum* species using anatomical and molecular data. Genet. Mol. Res., 12: 6488-6501.
14. Dansi, A., H. Adoukonou-Sagbadja and R. Vodouhe, 2010. Diversity, conservation and related wild species of Fonio millet (*Digitaria* spp.) in the Northwest of Benin. Genet. Resourc. Crop Evol., 57: 827-839.
15. Yumnam, J.S., W. Tyagi, A. Pandey, N.T. Meetei and M. Rai, 2012. Evaluation of genetic diversity of chilli landraces from North Eastern India based on morphology, SSR markers and the Pun1 locus. Plant Mol. Biol. Rep., 30: 1470-1479.
16. Ballina-Gomez, H., L. Latournerie-Moreno, E. Ruiz-Sanchez, A. Perez-Gutierrez and G. Rosado-Lugo, 2013. Morphological characterization of *Capsicum annuum* L. accessions from southern Mexico and their response to the *Bemisia tabaci-Begomovirus* complex. Chilean J. Agric. Res., 73: 329-338.
17. Yeteh, F.C., R.C. Yang, T.J.B. Boyle, Z. Ye and J.X. Mao, 1999. POPGENE, the user friendly shareware for population genetics analysis. Version 1.3.1. Molecular Biotechnology Center, University of Aleberta, Canada.

18. De Vicente, M.C. and T. Fulton, 2003. Using molecular marker technology in studies on plant genetic diversity. Illus. Nelly Giraldo. IPGRI, Rome, Italy.
19. Lo Piccolo, S., A. Alfonzo, G. Conigliaro, G. Moschetti, S. Burruano and A. Barone, 2012. A simple and rapid DNA extraction method from leaves of grapevine suitable for polymerase chain reaction analysis. Afr. J. Biotechnol., 11: 10305-10309.
20. Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.
21. Yuan, C.Y., C. Zhang, P. Wang, S. Hu and H. Chang *et al*., 2014. Genetic diversity analysis of okra (*Abelmoschus esculentus* L.) by Inter-Simple Sequence Repeat (ISSR) markers. Genet. Mol. Res., 13: 3165-3175.
22. Jaccard, P., 1908. Nouvelles researches sur la distribution florale. Bull. Soc. Vaudoise Sci. Natl., 44: 223-270.
23. Rus-Kortekaas, W., M.J.M. Smulders, P. Arens and B. Vosman, 1994. Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome, 37: 375-382.
24. Excoffier, L., G. Laval and S. Schneider, 2006. Arlequin Ver3.01: An integrated software package for population genetics data analysis. Computational and Molecular Population Genetics Lab (CMPG), Institute of Zoology, University of Berne, Switzerland, http://cmpg.unibe.ch/software/arlequin3
25. Pavlicek, A., S. Hrda and J. Flegr, 1999. Free-Tree-freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biol. (Praha), 45: 97-99.
26. Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by Simple Sequence Repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.
27. Kumar, L.D., M. Kathirvel, G.V. Rao and J. Nagaraju, 2001. DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. Forensic Sci. Int., 116: 63-68.
28. Srivastava, P.P., K. Vijayan, A.K. Awasthi and B. Saratchandra, 2004. Genetic analysis of *Morus alba* through RAPD and ISSR markers. Indian J. Biotechnol., 3: 527-532.
29. Raina, S.N., V. Rani, T. Kojima, Y. Ogihara, K.P. Singh and R.M. Devarumath, 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome, 44: 763-772.
30. Prevost, A. and M.J. Wilkinson, 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appied Genet., 98: 107-112.
31. Thul, S.T., M.P. Darokar, A.K. Shasany and S.P.S. Khanuja, 2012. Molecular profiling for genetic variability in *Capsicum* species based on ISSR and RAPD markers. Mol. Biotechnol., 51: 137-147.
32. Tanksley, S.D., 1984. High rates of cross-pollination in chile pepper. HortScience, 19: 580-582.
33. Ibiza, V.P., J. Blanca, J. Canizares and F. Nuez, 2012. Taxonomy and genetic diversity of domesticated *Capsicum* species in the Andean region. Genet. Resour. Crop Evol., 59: 1077-1088.
34. McLeod, M.J., W.H. Eshbaugh and S.I. Guttman, 1979. A Pre-Liminary Biochemical Systematic Study of the Genus *Capsicum*-Solanaceae. In: The Biology and Taxonomy of the Solanaceae, Hawkes, J.G., R.N. Lester and A.D. Skelding (Eds.)., Academic Press, New York, pp: 701-714.
35. Shirasawa, K., K. Ishii, C. Kim, T. Ban and M. Suzuki *et al*., 2013. Development of *Capsicum* EST-SSR markers for species identification and *in silico* mapping onto the tomato genome sequence. Mol. Breed., 31: 101-110.
36. National Research Council, 1991. Managing Global Genetic Resources: The U.S. National Plant Germplasm System. Committee on Managing Global Genetic Resources: Agricultural Imperatives, Board on Agriculture. National Research Council. National Academy Press, Washington DC., USA.
37. Alayachew, S.A. and K.T. Geletu, 2017. Genetic diversity of Ethiopian emmer wheat Triticum dicoccum Schrank landraces using seed storage proteins markers. Afr. J. Biotechnol., 16: 889-894.
38. Ilbi, H., 2003. RAPD markers assisted varietal identification and genetic purity test in pepper, *Capsicum annuum*. Scient. Hortic., 97: 211-218.
39. Yang, R.L., J. Kong, X. Wu, Z.R. Deng, Q. Chen and W.X. Liu, 2005. Application of ISSR markers in genetic polymorphism of *Capsicum frutescens* L. J. Shanghai Univ. University (Natl. Sci. Edn.), 11: 423-426.
40. Lijun, O. and Z. Xuexiao, 2012. Inter simple sequence repeat analysis of genetic diversity of five cultivated pepper species. Afr. J. Biotechnol., 11: 752-757.