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Genetic diversity in pepper (*Capsicum annum L.*) germplasms using SSR markers

Motbaynor Terefe[1*], Sisay Kidane Alemu[1], Gamachu Olani[2], Abel Debebe[2], Shimelis Aklilu[2] and Biruktait Berhanu[1]

[1]Ethiopian Institute of Agricultural Research (EIAR), National Agricultural Biotechnology Research Center, Holeta, Ethiopia.
[2]Ethiopian Institute of Agricultural Research (EIAR), Melkasa Agricultural Research Center, Adama, Ethiopia.

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Analysis of pepper genetic diversity and genetic relationship is important in selecting genetically diverse parental lines drawn from several genetic populations, and also helps to implement effective conservation strategies. For this purpose, 25 pepper genotypes comprising both accessions and improved varieties were examined using 16 SSR markers. The markers were polymorphic and showed a mean PIC value of 52% with a range of 8 to 80%, and generated a total of 67 alleles, with an average of 4.19 alleles per marker. The gene diversity ranged from 0.09 to 0.82, with an average of 0.57. Interestingly, pairwise genetic dissimilarity was the highest (1.00) between PBC-731 and Acc-22, and the lowest (0.25) between Acc-13 and Acc-11 genotypes. This is expected because improved varieties are genetically far from accessions than accessions are from each other. Neighbor-joining (NJ) tree produced three major clusters consisting of C1=100% accessions, C2= 67% improved varieties, and 33% accessions, whereas C3= 50% accessions and 50% improved varieties. The principal coordinate analysis (PCoA), showed a scatter plot with a wide dispersion of the genotypes in all the quadrants without forming a clear cluster, and some genotypes like PBC-731, Acc-45, Acc-9, and Acc-22 are plotted far from the central axis. The population structure generated an optimal groups of ΔK=4 with a high level of admixtures. The analysis of molecular variance (AMOVA) both based on STRUCTURE results and grouping into the accessions and improved varieties partitioned the total variance into 9% among groups, and 91% among individuals in the groups. The high level of genetic diversity found in Ethiopian pepper genotypes in the present study will help breeders to utilize the genotypes for further improvements in pepper germplasm.

Key words: *Capsicum annum*, genetic diversity, hybridization, pepper, SSR markers.

INTRODUCTION

Pepper (*Capsicum annum* L.) is one of the species from the Solanaceae family and genus Capsicum. *Capsicum* comprises around 38 recognized species believed to have originated in the tropical South American Regions,

*Corresponding author. E-mail: motbaynor2008@gmail.com.

of which only five are domesticated and cultivated, namely *C. annum*, *C. baccatum*, *C. chinense*, C. *frutescens* and *C. pubescent* (Bosland et al., 2012; Moscone et al., 2007). *C. annum* is a self-pollinating diploid crop having a varied chromosome number of pungent type (2n=2x=24 and non-pungent (2n=2x=26) with comparatively large genome size (Moscone et al., 2007; Kim et al., 2014). The Pungent (chilli or hot pepper) and non-pungent (sweet pepper) variants of *Capsicum annuum* L. are the most popular vegetable and spices, with worldwide commercial distribution. Pepper is one of the ancient crops which has been domesticated for thousands of years contributing great importance to human welfare (Bosland et al., 2012).

In Ethiopia, it was first introduced by the Portuguese in the 17th century and subsequently from all over the world and it has since been cultivated for centuries and adapted to varied agro-ecological regions in the country (Geleta et al., 2005). The complex geographic environment and climatic conditions helped abundant germplasms of pepper to be evolved with different features, such as fruit type, pungency, and pests and disease resistance (Marame et al., 2009). For instance, there are reports on which Ethiopian origin small-fruited and pungent *C. annum* to be the most important and persistent source of powdery mildew (*Leveillula taurica*) (Jo et al., 2017) and wilt disease resistance (Woubit et al., 2021). Pepper in Ethiopia is grown in different agro-ecologies at altitudinal range from 1400 to 2120 m under rained and irrigated conditions mainly in South Nation Nationalities and Peoples, central (Eastern and Southern Shoa), western, north-western (Wellega, Gojjam) (Nigussie and Zewdia, 2021). According to the FAOSTAT report, Ethiopia produces 4511 metric tons regarded as one of the top five pepper (hot Pepper) producing country in Africa (FAOSTAT, 2016). The total estimated area covered under green pepper and red pepper is 11,409 and 174,463.62 ha, respectively, which is about 73% of the total vegetable production of the country (CSA, 2019), contributing an important role in the national economy. Pepper is a popular vegetable and spice crop in Ethiopia, and it is consumed in different forms. It is widely used in the Ethiopian diet, mainly used in traditional foods known locally as “Karia’’ the green fruit, eaten raw as a salad and dried red fruit grounded into powder, named ‘‘berbere” added as a sauce to “wot”. Pepper consumption is strongly ingrained in Ethiopian dietary habits, with an average daily consumption of 15 g by Ethiopian adults, which is higher than eating of other vegetable crops (Woubit et al., 2021). It is an excellent source for bioactive compounds, vitamins, dietary fibers and some essential minerals (Bosland et al., 2012). In addition, pepper has a wide variety of uses in pharmaceuticals, cosmetics, natural coloring agents, and as an ornamental plant. Despite its wide range of possible applications, Ethiopia's average pepper yield is low as compared to the global scenario (CSA, 2019). The scarcity of appropriate high-yielding varieties, the use of unknown seed sources and low-quality seeds, a poor irrigation system, insufficient knowledge about soil fertility, and the prevalence of fungal, bacterial, and viral diseases are some of the yield-limiting factors (Belay et al., 2019). As a result, pepper breeding goals are focused on overcoming those constraints in order to enhance national production and productivity. In Ethiopia, promising efforts are made to develop improved varieties, and some enhanced cultivars are under production (MANR, 2016).

Crop improvement heavily relies on a comprehensive understanding of the genetic variability and their genetic relationships, which could then be used in breeding programs. The information on genetic relationships has been used to estimate the genetic distance between the genotypes/species evaluated, allowing the species to be classified into distinct groups based on their genetic similarity (Ve and Palloix, 2013). This in turn necessitates the selection of genetically diverse parental lines drawn from several genetic populations and the varietal identification and purity test (Shapturenko et al., 2014). Whenever divergent parents are used in crossing programs, the progenies are expected to have a lot of heterosis (Jagosz, 2011) and increase the chance of obtaining superior segregants in advanced generations, and is important to enhance the genetic base. In addition, knowing the genetic resources of crops is crucial for implementing effective conservation strategies (Gollin, 2020).

The use of molecular markers for plant genetic diversity analysis is thought to be an appropriate tools (Collard and Mackill, 2008), because they are independent of environmental factors and can detect differences in alleles or changes in DNA sequence. Various molecular marker systems such as RAPD (Bhadragoudar and Patil, 2011; Devi et al., 2018), AFLP (Geleta et al., 2005; Aktas et al., 2009), ISSR (Patel et al., 2011; Alayachew et al., 2017; López Castilla et al., 2019) and SSR (Nagy et al., 2007; Dhaliwal et al., 2014; Christov et al., 2021; Woubit et al., 2021), have been utilized to examine the genetic diversity and phylogenetic relationships of pepper germplasms. The use of polymorphic, multi-allelic, reproducible, and widely distributed microsatellite markers in pepper accessions could assist in the selection of traits of interest and potential breeding materials for introgression through the use of molecular marker-assisted breeding and germplasm conservation (Mimura et al., 2012). Therefore, a more accurate analysis employing co-dominant microsatellite (SSR) markers is required to determine the genetic diversity and to infer the genetic relationship of Ethiopian peppers. To date, SSR markers have only been used in a few studies in Ethiopian pepper germplasms to assess the genetic diversity (Rabuma et al., 2020; Woubit et al., 2021) and the improved pepper cultivars have not been studied using SSR markers. Thus, the study aims are to identify and characterize *Capsicum* spp., as well as to capture

Table 1. Lists of pepper genotypes used for the study.

S/N	Accession number and/ genotype names	Local name	Area collected	Source
1	Acc-3	Mi-Alaba2	Kuleto (Halaba)	Halaba market
2	Acc-9	Mi-Alaba1	Halaba- (Adama)	Adama market
3	Acc-10	Ha-Bedessa	Bedessa (W/Hararghe)	W/Hararghe market
4	Acc-11	Tad-Halaba	Ansia (Halaba)	Halaba market
5	Acc-12	Tad-Ybale (Agarfa)	Agarfa (Bale)	Bale market
6	Acc-13	Wfo-Gojam	Finote-Selam (Gojam)	Gojam market
7	Acc-17	Har-Milkay	Mechara(W/Hararghe)	W/Hararghe market
8	Acc-21	Marko-Kumo Almin8	Marko (Gurage)	Hawasa Research
9	Acc-22	Marko-Didamidore9	Marko (Gurage)	Hawasa Research
10	Acc-24	Awa-Dalle1	Awassa-Dalle2	Hawasa Research
11	Acc-26	Awa-Gello2-	Gello-Argessa	Hawasa Research
12	Acc-40	Turu-11	Bati-Futo	Farmers seed lot
13	Melka Awaze	Improved variety	-	MARC
14	Melka shola	Improved variety	-	MARC
15	Melka Oli	Improved variety	-	MARC
16	PBC 602	Improved variety	-	MARC
17	Mareko Fana	Improved variety	-	MARC
18	PBC 731	Improved variety	-	MARC
19	Melka Zala	Improved variety	-	MARC
20	Melk Dhera	Improved variety	-	MARC
21	Melka Eshete	Improved variety	-	MARC
22	Melka Shote	Improved variety	-	MARC
23	Acc-41	Walga-2	Abishege	Farmers seed lot
24	Acc-8	Na-Ybale	Bale2 (Adama)	Adama market
25	Acc-45	Assossa-2	Benishangule	Assossa Research

Source: Aklilu et al. (2016) and Melkassa Agricultural Research Center (MARC).

the potential genetic divergence and genetic relationships among pepper accessions along with the improved varieties.

MATERIALS AND METHODS

Plant materials

A total of twenty-five (25) pepper genotypes (*Capsicum annum L.*) were used for this study, which comprises of fifteen (15) accessions and ten (10) improved varieties (Table 1). Seed samples were obtained from Melkassa Agricultural Research Center (MARC).

Genotyping

Seeds from each genotype were sown in seedling raising tray in the greenhouse at National Agricultural Biotechnology Research Center (NABRC), Holeta. Young healthy leaves from a single seedling at the 2 to 3 leaf stage were collected in an Eppendorf tube and immediately dried using liquid nitrogen. The dried leaves were then pulverized using a Geno grinder (MM-200, Retsch) at 25 rpm for 3 min. Genomic DNA was extracted following plant DNA extraction protocol (DARTs, 2000) with minor modifications. The quality and quantity of the isolated DNA were checked by gel-electrophoresis using 0.8% agarose at 100 constant voltages for 45 min. The gels were visualized under UV light and photographed with a camera mounted on the UV Transilluminator. The quality and concentration of the DNA were further confirmed by a spectrophotometer (8 pedestal, Nano drop) at 260/280 nm wavelength absorbance. Good-quality DNA from each sample was used for PCR analysis after normalization to approximately 50 ng/µl (the normalization was carried out based on the concentration of each sample from Nano drop result).

A set of 16 SSR markers previously reported by Nagy et al. (2007), Dhaliwal et al. (2014) and Sharmin et al. (2018) were obtained and used for the final genotyping of pepper collections (Table 2). Prior to whole sample amplification, gradient PCR was applied to each primer pair on BIO-RAD T100^{TM} thermal cycler to get an optimum annealing temperature and other PCR setup. The polymerase chain reaction (PCR) was then carried out in a 12.5 µl final reaction volume containing 6.25 µl of Taq DNA polymerase, 0.5 µl of each forward and reverse primers, 3.25 µl of nuclease-free water, and finally 2 µl of gDNA. The PCR condition was adjusted at initial denaturation of 95°C for 5 min, 36 cycles of denaturation 94°C for 45 s, annealing varied with the primers (Table 2) for 45 s, extension 72°C for 90 s, and final extension 72°C for 10 min.

The amplified products stained with 6X loading dye-containing gel red, were separated by 3% agarose gel electrophoresis with 1×TAE buffer at 100v constant voltage run for 2:30 hrs. A 100 and 50 bp DNA ladder (SMOBIO, DM2100 and DM1100) was used to estimate the molecular weight of the fragments. The gels

Table 2. Lists of SSR markers used for the study and their detail information.

S/N	Markers	Forward sequence (5'-3')	Reverse sequence (5'-3')	Expected size (in bp)	Ta (^{o}C)	Reference
1	*AVRDC PP-18*	GCTAGGCTTGATCCTTCACC	CGCTTGAAATCATGCTCACT	83-113*	47.9	Dhaliwal et al. (2014)
2	*AVRDC PP-32*	ATGGAGGATTACCTCGCAAC	CATGATGACCATCCATCCAT	102-177*	46	>>
3	*AVRDC PP-65*	GTGAGGCCGAGAATGAAGAT	AACGACCATGTGTGGTTGA	425-562*	48.2	>>
4	*AVRDC PP-167*	TCATCTTACACGGCTTGCTC	AGCTCCTCAACTGCCTTTTA	254-341*	55.7	>>
5	*AVRDC PP-67*	TATTCCTTCTTCACCCCTCC	GAAAGAGGCGCTAACTGGAC	153-310*	55.5	>>
6	*CAMS-806*	GCTAGGCTTGATCCTTCACC	CGCTTGAAATCATGCTCACT	170-210	54.3	Sharmin et al. (2018)
7	*GPMS8*	TGATGATAAGGCCATGATAAAATG	CCAGATTCTTTAGCAAGGTTTACC	159-229	54.3	Nagy et al. (2007)
8	*GPMS6*	CAGAGCACTTGACATGCCTT	GATCTTTATAGTAGCTCATCAATA	122-172	52.5	>>
9	*GPMS112*	TCCCTCAGCAGCAACAATTT	GTCGGGCTCTTTGATTGTGT	203-280	54.3	>>
10	*GPMS117*	GATGTTAGGTCCGTGCTTCG	AAGCCCCATGGAAGTTATCC	111-177	53.2	>>
11	*GPMS178*	GATTTTTGACATGTCACATTCATG	AACGTTGAAAAATAAAGTAAGCAAG	230-261	58.2	>>
12	*GPMS197*	GCAGAGAAAATAAAATTCTCGG	CAATGGAAATTTCATCGACG	272-344	54.2	>>
13	*EPMS303*	AAAACTCCAAACTACCCCTGG	TTAAGCGTAGCGCTTGTGTG	292-330	53.2	>>
14	*EPMS331*	AACCCAATCCCCTTATCCAC	GCATTAGCAGAAGCCATTTG	97-107	53.2	>>
15	*EPMS376*	ACCCACCTTCATCAACAACC	ATTTGTGGCTTTTCGAAACG	235-259	53.2	>>
16	*EPMS418*	ATCTTCTTCTCATTTCTCCCTTC	TGCTCAGCATTAACGACGTC	178-210	54.8	>>

*= observed fragment size in the present study; the others were reported in previous studies.
Source: Author

were visualized under UV light and image capture was done by a gel documentation system (UV Transilluminator).

Data scoring and analysis

The fragment sizes detected by each SSR region were scored using PyElph 1.4 software package (Pavel and Vasile, 2012) with respect to the size marker. For a single locus, fragments with the same mobility were treated as the same fragment size and treated as the same allele, while bands of differing molecular weight were treated as distinct alleles. To determine gene diversity (GD), observed heterozygosity (Ho), the number of alleles (Na), and polymorphic information content (PIC) in each marker, Power marker v3.25 software (Liu and Muse, 2005) was used. The PIC value for each primer was estimated using the formula:

$$PIC = \frac{1 - \sum Pij^2}{j=1}$$

Where Pij is the frequency of j^{th} allele in the i^{th} primer and summation extends over 'n' patterns. The genetic relationships within and among pepper genotypes, simple matching pairwise dissimilarity across each genotype, and a biplot display of principal coordinate analysis (PCoA) were estimated using DARwin ver 6.0.21 software (Perrier and Jacquemoud-Collet, 2006). The pairwise dissimilarity was calculated based on the following formula:

$$dij=1-1/L\sum_{i=1}^{L} ml/\pi$$

Where, dij=dissimilarity between units i and j, L=number of loci, π= ploidy level, ml= number of matching alleles for locus L.

Analysis of molecular variance (AMOVA) to estimate population genetic differentiation of among and within pepper accessions and improved varieties were computed by GenAlex 6.5 software (Peakall and Smouse, 2012).

A model-based population structure analysis was carried out with STRUCTURE ver.2.3.1 Software (Pritchard et al., 2000) using the admixture model with correlated allele frequencies. The number of possible *K* was set from 1 to 10 with 20 runs for each K, and each run had a burn-in period of 250,000 and 500,000 MCMC iterations. The optimum value of *K* was determined using ΔK simulation (Evanno et al., 2005) implemented in the web-based Analysis tool STRUCTURE HARVESTER v6.93 (Earl and vonHoldt, 2012).

RESULTS AND DISCUSSION

Gene diversity and markers polymorphism

16 SSR markers were successfully implemented

Table 3. Summary of gene diversity indices at 16 polymorphic loci in 25 Pepper genotypes.

Marker	MAF	Na	Ho	GD	PIC (%)
AVRDC PP-18	0.48	6	0.00	0.69	65
AVRDC PP-32	0.52	5	0.74	0.60	53
AVRDC PP-65	0.52	5	0.59	0.66	62
AVRDC PP-167	0.50	2	1.00	0.50	38
AVRDC PP-67	0.32	9	0.00	0.82	80
CAMS-806	0.40	4	0.54	0.71	66
GPMS8	0.61	4	0.00	0.57	53
GPMS6	0.95	2	0.00	0.09	8
GPMS112	0.93	2	0.14	0.13	12
GPMS117	0.56	4	0.00	0.55	47
GPMS178	0.46	4	0.00	0.66	60
GPMS197	0.52	3	0.00	0.54	44
EPMS303	0.30	4	0.00	0.74	70
EPMS331	0.50	4	0.12	0.64	58
EPMS376	0.32	6	0.00	0.79	75
EPMS418	0.77	3	0.00	0.38	34
Mean	**0.54**	**4.19**	**0.20**	**0.57**	**52**

MAF=major allele frequency, Na=number of alleles, GD=gene diversity, Ho= observed heterozygosity, PIC=polymorphic information content.
Source: Author

in 25 pepper genotypes to evaluate the gene diversity and levels of polymorphism within and among the accessions and improved varieties. The gene diversity indices varied across the entire markers in tested genotypes. The 16 SSR markers generated a total of 67 alleles, ranging from 9 for marker *AVRDC PP-67* to 2 for markers *AVRDC PP-167*, *GPMS6,* and *GPMS112* with a mean of 4.19 alleles per marker (Table 3).

Various results have been reported from similar studies on varying number of genotypes and markers. For instance, Rabuma et al. (2020) reported a lower mean number of alleles (2.2) in 32 Ethiopian and Indian accessions using 14 SSR markers. In contrast, a higher number of alleles were reported by Woubit et al. (2021) that identified a mean of 8.54 alleles in 75 Ethiopian pepper germplasms using 13 SSR markers. Similarly, a mean number of alleles of 6.9 are reported from a large *Capsicum annum* collection in 179 individuals from six countries, other than Ethiopian origins using 21 SSR markers (Christov et al., 2021). A relatively lower (2.7) mean number of alleles were reported in 64 Indian pepper accessions using 27 polymorphic SSR markers. Obviously, the number of alleles detected in the germplasm or population is influenced by the species' genetic backgrounds and the molecular markers difference deployed.

The major allele frequency (MAF) ranged from 30 to 95% with a mean of 54%. A mean value of 0.20 was obtained for observed heterozygosity (Ho) with the highest record (1.00) was attained by marker *AVRDC PP-167* and no (0.00) observed heterozygosity by the ten markers (Table 3). The lowest Ho observed in most of the markers in our study is directly correlated with the fact that the majority of improved varieties and accessions are being homozygous. This can also be attributable to the high level of inbreeding that improved varieties are expected to exhibit. A variation in the levels of markers polymorphism has been observed (Figure 1), with a PIC value ranging from 8 to 80% and a mean of 52% (Table 3).

Molecular markers with PIC values of >0.50 (50%) are considered to be highly informative for genotyping studies (Botstein et al., 1980). Except for the two markers (*GPMS6* and *GPMS112),* the others showed high PIC values, implying the presence of immense genetic diversity among studied genotypes and the very suitability of the markers for molecular characterization of pepper genotypes. Similar results were obtained with a mean PIC value of 0.57 using different SSR markers (Rabuma et al., 2020).

A gene diversity index is one of the most important measures of genetic divergence and is useful for determining the amount of diversity in the genotypes. The gene diversity (GD) in this study ranged from 0.09 to 0.82 with an average of 0.57 (Table 3), indicating the presence of high genetic diversity among Ethiopian pepper genotypes. The highest GD value (GD=0.82) was recorded for marker *AVRDC PP-67* while the lowest

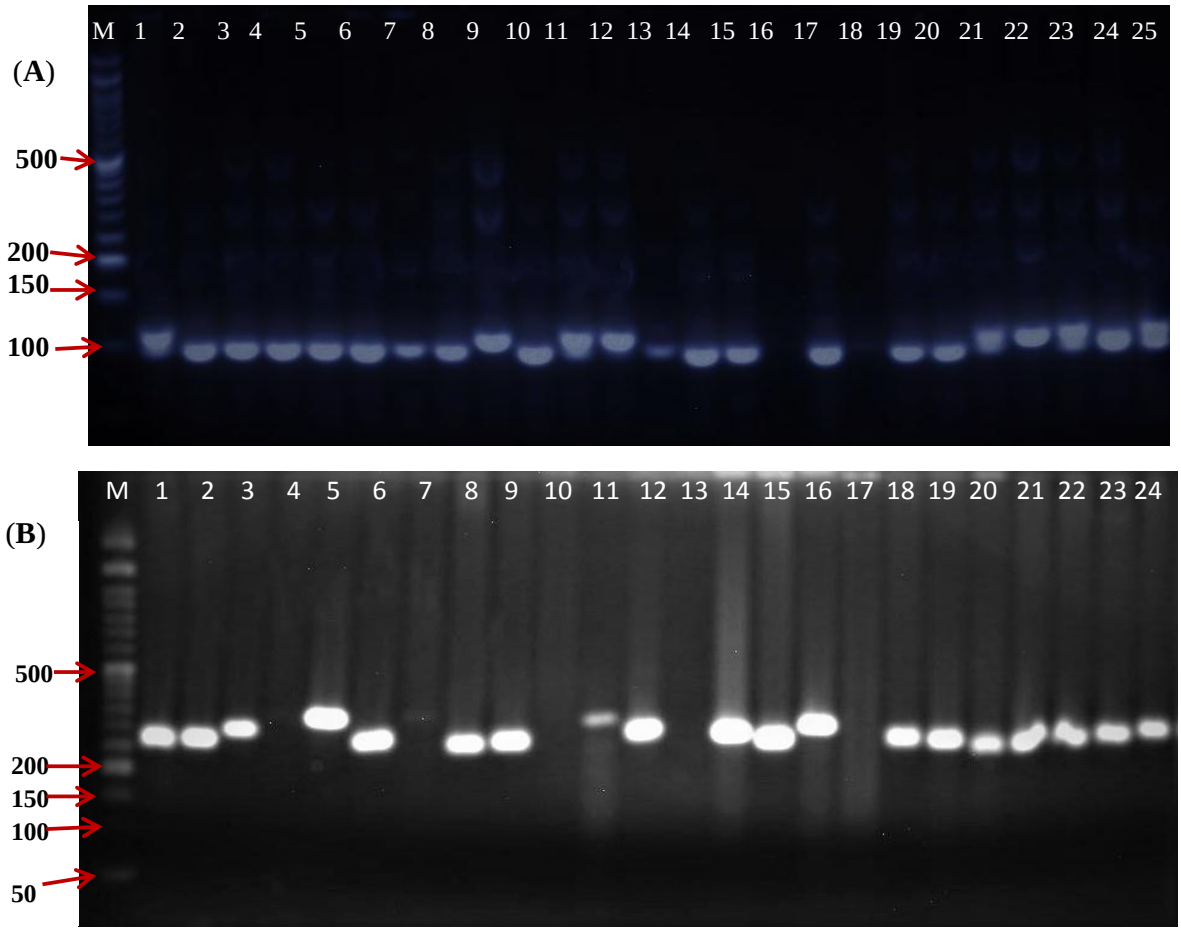

Figure 1. PCR amplification profile of pepper genotypes with a marker *AVRDC PP-18* (A) and *EPMS376* (B), M; represents DNA ladder (50 bp, SMOBIO, DM1100) and lane 1-25/24 are pepper genotypes.
Source: Author

(GD=0.09) was for marker *GPMS6.*

Genetic dissimilarity and phylogenetic relationshionship

The pairwise genetic dissimilarity coefficient determines the genetic relatedness among the genotypes. The highest genetic distance value of 1.00 was observed between PBC-731 and Acc-22 and the lowest (0.25) was between Acc-13 and Acc-11 genotypes (Table 4). The dissimilarity coefficient value of 1.00 indicates that the two genotypes are genetically different; while, the the value 0.25 indicates that the two genotypes have a higher genetic similarity. In general, most of the pairwise dissimilarities obsereved were higher across the 25 genotypes implying a broad range of genetic variability basis among the tested pepper genotypes.

Several evolutionary factors influence genetic diversity among and within species, including seed dispersal, gene flow, natural selection, geographic range, and the diversity center (Sork, 2016). Cluster analysis was used to find the best possible grouping based on genetic distance. In the present study, a neighbor-joining (NJ) tree was built to determine the genetic relationship of the 25 pepper genotypes using 16 SSR markers, and the analysis deployed all of the genotypes into three major clusters and formed different sub-clusters (Figure 2). The first cluster (C1) comprised of 9 (100%) genotypes all of them are accessions, while the second cluster (C2) contained 12 genotypes of which 8 (67%) are improved varieties and 4 (33%) are accessions. The third cluster (C3) is composed of 4 genotypes, 2 (50%) are improved varieties while the other 2 (50%) are accessions. Although the first cluster comprises the majority of the accessions, some of the accessions were found dispersed in all of the other clusters, depicting the presence of a high genetic distance between accessions. Likewise, most of the improved varieties fell in the second cluster; while, some were in the third cluster, showing presence of considerable genetic distance between varieties though most seems relatively close. The current

Table 4. A pairwise genetic dissimilarity across the 25 pepper genotypes.

	Acc-3	Acc-9	Acc-10	Acc-11	Acc-12	Acc-13	Acc-17	Acc-21	Acc-22	Acc-24	Acc-26	Acc-40	Melka awaze	Melka shola	Melka oli	PBC 602	Mareko fana	PBC 731	Melka zala	Melka dera	Melka eshete	Melka shote	Acc-41	Acc-8	Acc-45
Acc-3	***																								
Acc-9	0.56	***																							
Acc-10	0.56	0.41	***																						
Acc-11	0.47	0.50	0.34	***																					
Acc-12	0.44	0.44	0.44	0.31	***																				
Acc-13	0.31	0.41	0.34	**0.25**	0.28	***																			
Acc-17	0.69	0.59	0.69	0.72	0.63	0.72	***																		
Acc-21	0.50	0.44	0.47	0.50	0.38	0.31	0.66	***																	
Acc-22	0.44	0.59	0.41	0.47	0.53	0.38	0.75	0.47	***																
Acc-24	0.47	0.59	0.47	0.50	0.47	0.41	0.59	0.34	0.53	***															
Acc-26	0.59	0.81	0.69	0.72	0.53	0.53	0.81	0.50	0.59	0.44	***														
Acc-40	0.47	0.72	0.53	0.56	0.50	0.47	0.72	0.47	0.50	0.47	0.34	***													
Melka awaze	0.50	0.72	0.56	0.53	0.50	0.47	0.53	0.53	0.63	0.34	0.50	0.38	***												
Melka shola	0.69	0.75	0.56	0.72	0.56	0.56	0.81	0.56	0.63	0.59	0.47	0.44	0.63	***											
Melka oli	0.53	0.72	0.69	0.63	0.47	0.56	0.59	0.56	0.75	0.53	0.56	0.38	0.41	0.56	***										
PBC 602	0.81	0.75	0.84	0.78	0.72	0.75	0.69	0.78	0.75	0.81	0.72	0.78	0.72	0.69	0.72	***									
Mareko fana	0.66	0.84	0.72	0.69	0.59	0.66	0.75	0.53	0.69	0.53	0.47	0.47	0.56	0.44	0.47	0.59	***								
PBC 731	0.88	0.66	0.88	0.81	0.84	0.88	0.75	0.78	**1.00**	0.81	0.88	0.75	0.72	0.81	0.81	0.84	0.75	***							
Melka zala	0.63	0.66	0.53	0.59	0.59	0.50	0.75	0.63	0.69	0.47	0.59	0.69	0.59	0.47	0.66	0.69	0.69	0.78	***						
Melka dera	0.66	0.81	0.56	0.59	0.56	0.53	0.78	0.47	0.63	0.38	0.44	0.53	0.50	0.50	0.56	0.72	0.47	0.78	0.28	***					
Melka eshete	0.53	0.78	0.69	0.75	0.59	0.69	0.66	0.59	0.56	0.56	0.47	0.34	0.50	0.47	0.41	0.78	0.53	0.75	0.63	0.41	***				
Melka shote	0.34	0.72	0.69	0.63	0.59	0.56	0.53	0.63	0.53	0.47	0.50	0.44	0.47	0.69	0.44	0.72	0.53	0.75	0.66	0.56	0.28	***			
Acc-41	0.69	0.72	0.59	0.66	0.63	0.63	0.69	0.53	0.44	0.63	0.63	0.47	0.72	0.53	0.59	0.78	0.63	0.94	0.78	0.63	0.50	0.59	***		
Acc-8	0.50	0.69	0.66	0.63	0.63	0.59	0.66	0.50	0.66	0.41	0.63	0.56	0.47	0.69	0.56	0.78	0.59	0.75	0.56	0.47	0.34	0.31	0.66	***	
Acc-45	0.81	0.78	0.81	0.75	0.66	0.75	0.88	0.66	0.84	0.72	0.72	0.81	0.81	0.78	0.88	0.97	0.81	0.56	0.78	0.78	0.84	0.81	0.81	0.63	***

Source: Author

study did not split the genotypes in to only accessions and improved varieties. Besides, the cluster of the accessions was not based on their geographic proximity. Most likely, this could be due to seed mixture as some genotypes were collected from local markets (Aklilu et al., 2016). Additionally, lack of a formal seed system in the country (Abebe and Lijalem, 2011) may contribute to seed exchange across different geographic locations.

Phylogenetic analysis is useful not only for estimating the genetic distance of genotype collections but also for selecting crossing parental lines. Varieties with a greater genetic distance are generally recommended as parents to produce

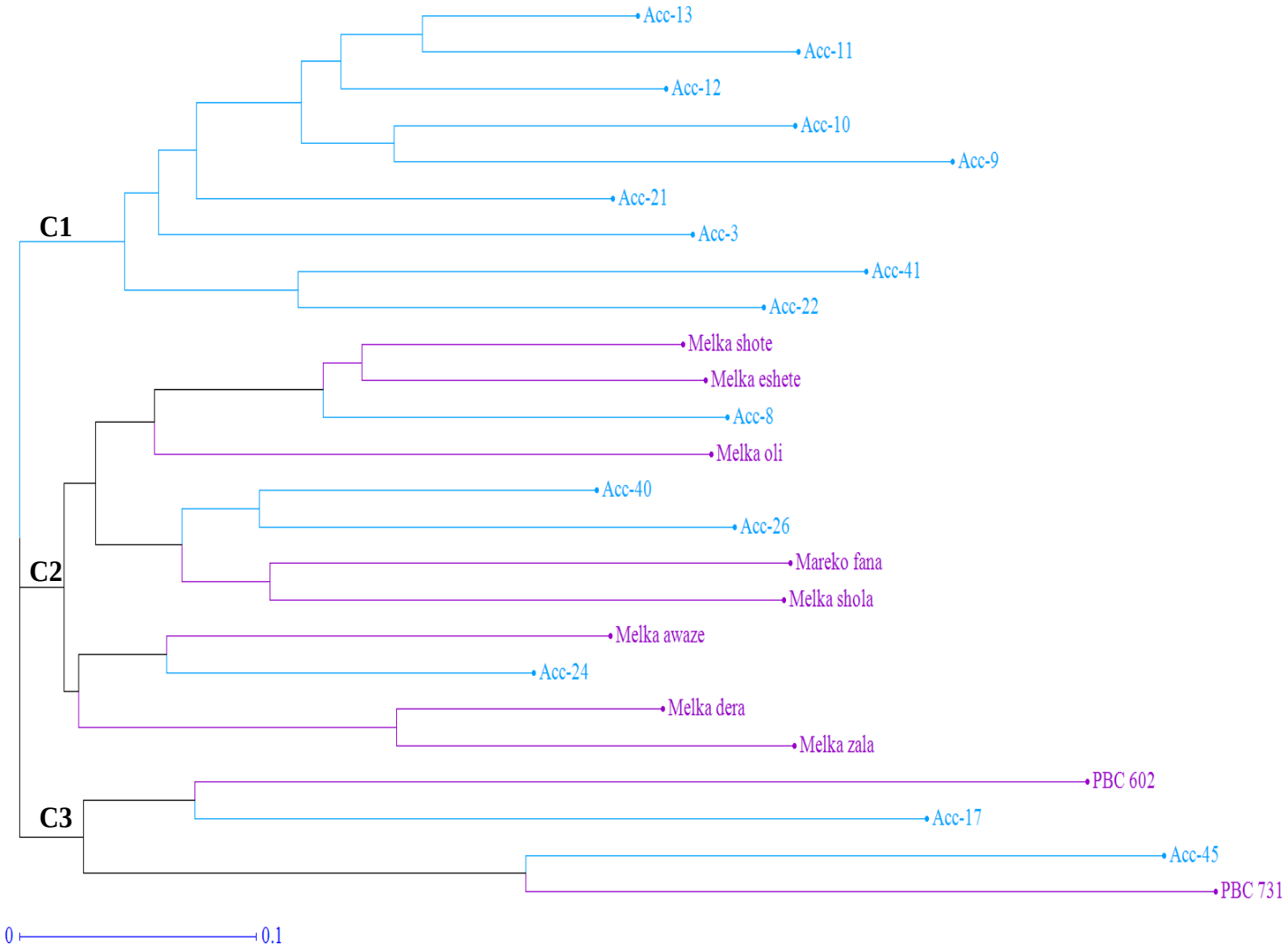

Figure 2. Phylogenetic relationship of 25 Pepper genotypes using 16 SSR markers. The colors, light blues are accessions and purple are improved varieties.
Source: Author

progeny with a heterosis effect. Indeed, the wide range of diversity in our tested genotypes could be important for broadening the genetic base because it enhances the chances of discovering more unique genes. Moreover, it provides the opportunity of hybridization between distant genotypes which helps in the production of heterozygous individuals with desirable traits.

A two-dimensional display of principal coordinate analysis (PCoA) was also performed to further investigate the genetic relationship of pepper genotypes and the result showed the first three principal coordinate axes explained 44.33% of the total genotypic variation in the studied genotypes. The first and the second explained 17.01 and 16.06% of the total variation, respectively. The PCoA displayed a scatter plot with a wider dispersion of the genotypes in all the quadrants without forming a clear cluster, and some genotypes like PBC-731, Acc-45, Acc-9, and Acc-22 are plotted far from the central axis, indicating the individuals' genetic distance among pepper genotypes and such kind of genotypes are highly recommended for future pepper breeding (Figure 3). In most cases, even though the genotypes are displayed scattered across all quadrants in the PCoA, the majority of the accessions and improved varieties are somewhat separated and formed three clusters (C1, C2, and C3) based on their genetic background, as similar as the dendrogram.

Population structure and analysis of molecular variance

The population structure analysis was inferred on the 25 genotypes (15 accessions and 10 improved varieties).

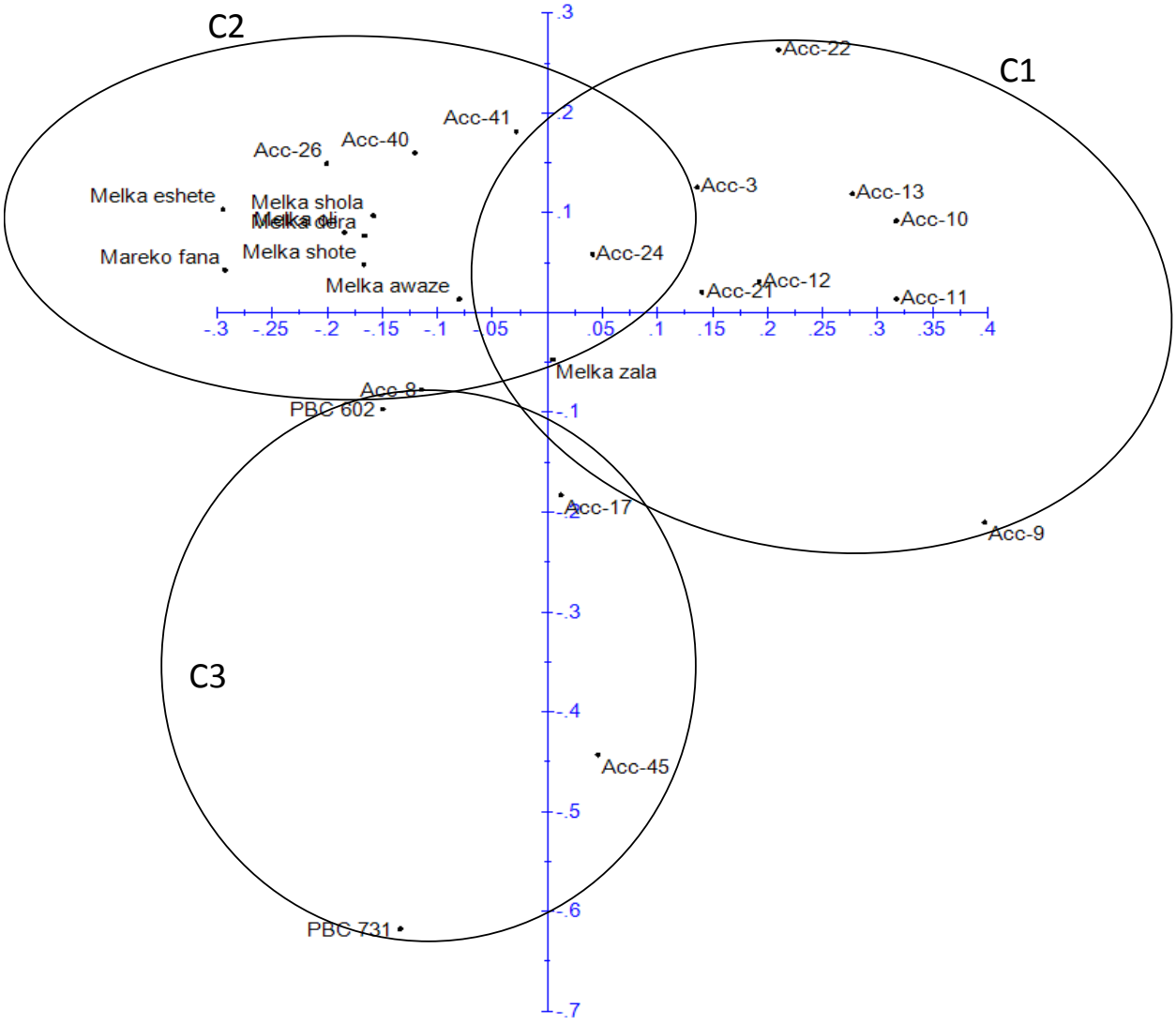

Figure 3. A biplot display of the axis 1 and 2 of the principal coordinate analysis based on the dissimilarity matrix of 16 SSR markers for the 25 Pepper genotypes.
Source: Author

The highest value of delta K (ΔK) was obtained for K=4, revealing the existence of four genetic groups (Group I, Group II, Group III and Group IV) of pepper genotypes (Figure 4A and B). Group I comprised 9 genotypes, of which 5 were improved varieties and 4 were accessions. Group II and Group III consists of 8 and 1 accessions, respectively. Whereas Group IV constituted 7 genotypes, 5 of which are improved varieties while 2 are accessions. However, this structure analysis displayed that the group I and IV, and genotypes from the predicted genetic groups had a high level of admixture (Figure 4C). We speculate that the reason for this is maybe pepper genotypes were acquired from the different gene pools with a high-level of mixture, as reflected by a high level of gene flow (Nm=2.4) and low genetic differentiation between groups (Table 5). The grouping at K=4 showed less concordance with that of the dendrogram and PCoA, this is because the very few distantly related genotypes could have contributed for the less concordance. Reports from population structure analysis in other pepper diversity panel indicated the existence of well-differentiated population groups (Solomon et al., 2019; Rabuma et al., 2020). The same is true in the present study except we used relatively small number of genotypes.

Analysis of molecular variance (AMOVA) was used to quantify the genetic divergence within and among groups. We partitioned the total molecular variance based on the K=4 from STRUCTURE result and the priory grouping information into accessions and improved varieties (Table 5A and B). As a result, the total variation was partitioned in to 9% among the four groups, and 91% within groups.

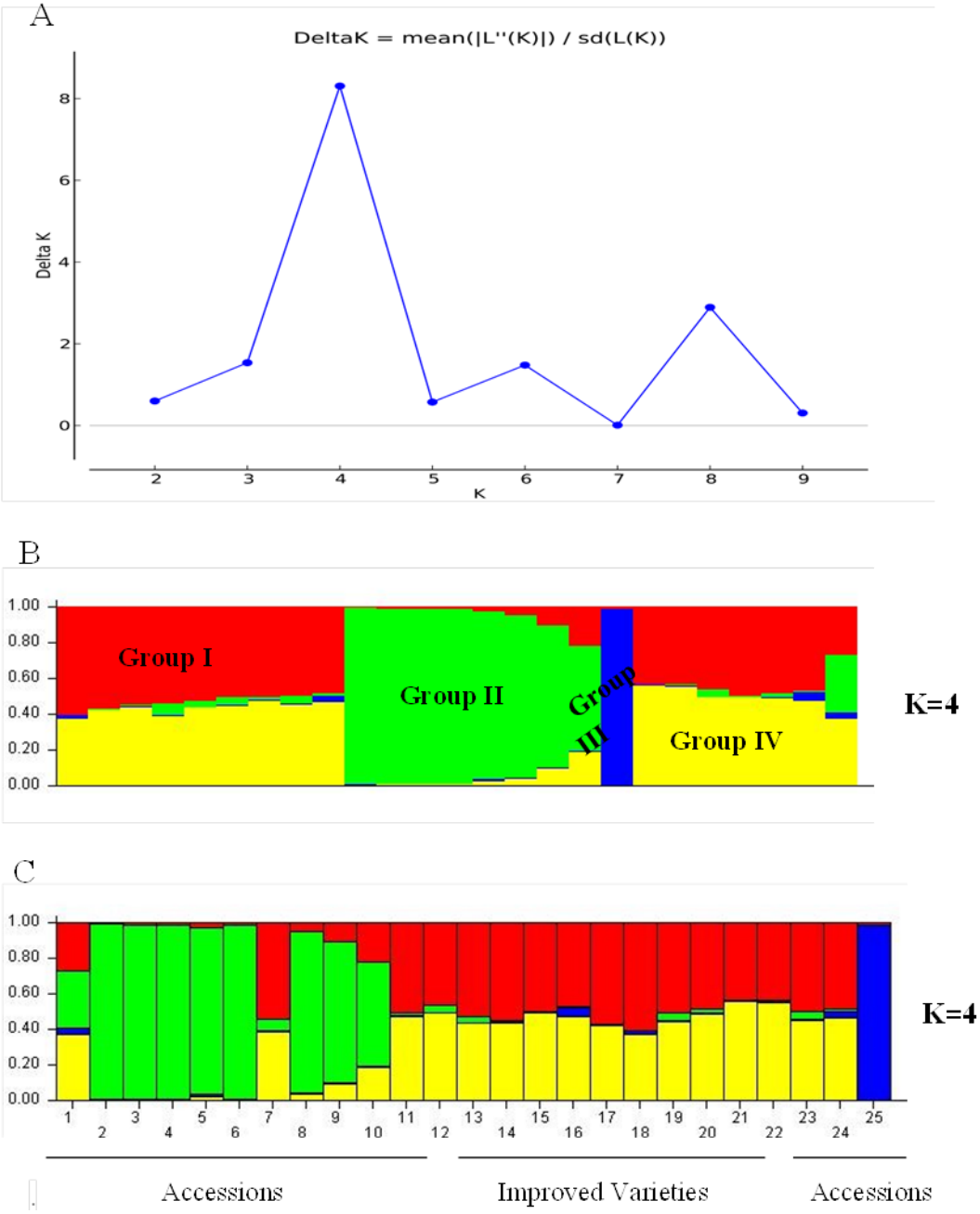

Figure 4. Population structure analysis of 25 pepper genotypes (A) inferred optimal Δ*K* based on the Evanno method (Evanno et al., 2005), (B) bar plot showing four of the groups (*k=4*) and their estimated membership built by *Q matrix,* (C) bar plot of the individual genotypes and their admixture. Each accession is represented by a vertical bar. Each color represents one ancestral group, and the length of each colored segment of each vertical bar represents the group contributed by ancestral groups.
Source: Author

Apparently, the high genetic differentiation has been demonstrated among individuals (Fis=1.00) as it is evident from Table 5. Almost the same result is obtained from the AMOVA based on the grouping with priory information in to improved varieties and accessions (Table 5B). Except for the negligible difference in estimated variance and F-statistics, exactly identical % of variations were found for among population (9%) and among individuals (91%) sources. This result may suggest that the ΔK based grouping of the genotypes is somehow related to grouping in to the accessions and improved varieties which are genetical grouping as well.

Table 5. Analysis of molecular variance (AMOVA) based on grouping from STRUCTURE results and a priory information into accessions and improved varieties.

Source	df	SS	MS	Est. Var.	% of variation	F-statistics	P-value	Gene flow (Nm)
A. Grouping based on structure analysis results; K=4								
Among Pops	3	43.742	14.581	0.470	9%	Fst=0.094		2.4
Among individuals	21	189.778	9.037	4.519	91%	Fis=1.00	0.001	
Total	24	233.520	23.618	4.989	100%			
B. Grouping based on a priory information in to accessions and improved varieties								
Among Pops	1	20.653	20.653	0.475	9%	Fst=0.093	0.001	2.4
Among individuals	23	212.867	9.255	4.628	91%	Fis=1.00		
Total	24	252.280	29.908	5.436	100%			

Df, degree of freedom; SS, sum square; MS, mean square; Est. Var., estimated variance.
Source: Author

Several variable results have been reported from previous studies conducted by various authors. Woubit et al. (2021) reported a partitioning of the total molecular variance in to 7% among eight-geographic groups, 63% among individuals, and 26% within individuals of Ethiopian pepper accessions. Similarly, in 32 Ethiopian and Indian accessions grouped in 9 populations by SSR markers, Rabuma et al. (2020) reported 32% of the total variation among populations and 68% within populations of the total variation. In another study conducted by SSR markers on Mexican pepper populations, of the total molecular variance among the population, 10% was among wild, landrace, and hybrids, 15% was among individuals within populations, and 74% was within individuals in the populations (Pacheco-Olvera et al., 2012). In a nut shell, levels of molecular variations explained by sources of variations in diversity studies are a function of the grouping compositions (varieties, landrace accessions, hybrids, advanced breeding lines etc.) the number of individuals, polymorphic power of the markers used etc.

Conclusion

Conclusively, the genetic diversity of Ethiopian pepper accessions and improved varieties are effectively investigated using SSR markers. Our result revealed that the SSR markers used were polymorphic suggesting their potential use for genetic studies of pepper collections. The markers detected a high genetic diversity in the studied pepper genotypes, which could be used as a source for breeding and genetic improvements. The results can aid breeders in effectively selecting genetically distant parents and applying hybridization. It is recommended that a large number of collections from all over the country have to be studied using efficient marker tools to generate more comprehensive information.

CONFLICT OF INTERESTS

The authors declare that they have no any conflict of interest.

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