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Evaluation of Genetic Diversity of Iranians Populations of Hazlnut (*Corylus avellana* L.) Using SSR Markers

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A B S T R A C T

Hazelnut (*Corylus avellana* L.) is mainly cultivated in the north and northwest of Iran. In this study, the genetic structure and diversity of 52 hazelnut accessions were investigated using 9 simple sequence repeats (SSR) loci. The number of alleles per locus ranged from 2 to 3, with a total number of 19 alleles. The highest and lowest values of observed alleles were found in the Fandogloo-Ardabil (*Na*=2.22) and 6th population (*Na*=1.77), respectively. The highest and lowest effective alleles were observed in Fandogloo-Gilan (*Ne*=1.94) and Hatammeshasi (*Ne*=1.56) populations, respectively. The highest value of the Shanon index (*I*=50%) was found in Hatammeshasi. A high level of genetic diversity, including expected heterozygosity (*He*), was found in Hatammeshasi population (*He*=0.50), while the highest value of observed heterozygosity (*Ho*) was found in Talesh population (*Ho* =0.25). The polymorphic information content (PIC) varied from 0.87 to 0.98 per locus. The mean values of *PIC*, F_{is}, and F_{st} for all loci were 0.88, 0.53, and 0.15, respectively. Overall, gene flow between populations (*Nm*=1.57) was observed in the studied populations. The highest correlations (r=0.94, p<0.05) were observed between the Eshkavar and Fandogloo-Gilan, and Hatammeshasi and Fandogloo-Ardabil populations. Regarding the X^2 test at p<0.05, the studied populations did not follow the Hardy-Weinberg equilibrium. Cluster analysis based on UPGMA method divided hazelnut genotypes into four groups. The results confirm that SSR is a reliable DNA marker that can be used to accurately study genetic diversity in hazelnut populations.

Introduction

Hazelnut (*Corylus avellana* L.) (2n =2x =22) of the Betulaceae family is one of the most widespread and popular nut crops in the world. Hazelnut not only adds a pleasant taste and texture to various foods, but also can play an important role in human nutrition and health due to their high content of oil, proteins, vitamins, and minerals (Ozdemir and Akinci, 2004). In Asia, hazelnut habitants extend from Turkey through Caucasus to Iran in the east and from the Anti-Taurus Mountains in Anatolia to Syria and Lebanon in the south (Kasapligil, 1964). It seems that hazelnut was domesticated independently in three regions, namely

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the Mediterranean, Turkey, and Iran (Boccacci and Botta, 2009). Iran is one of the most important hazelnuts producing countries in the world with more than 15000 tons (FAOSTAT, 2019). Hazelnut is an ancient nut crop in Iran for which there is an extremely abundant germplasm resource. The north and northwest of Iran are the main areas of the natural distribution of C. *avellana* (Thompson *et al.,* 1996). This species is present in Fandogloo, Hatammeshasi, Talesh, Eshkavar, Tarom, Dinochal, and Goli-daq, extending from west to east along the Caspian Sea coast. Different hazelnut populations grown in Iran show tremendous diversity in terms of plant size, growth habit, nut size, nut shape, involucre length, and many other morphological characteristics (Hosseinpour *et al.,* 2013; Aghapoor *et al.,* 2018), but these resources of *C. avellana* have experienced severe genetic erosion in recent years. Therefore, native Iranian germplasm needs to be well characterized in order to manage it most efficiently and utilize it effectively. In most cases, the identification of genotypes, cultivars, lines, and hybrids is based on morphological traits (Hajnajari *et al.*, 2012; Sadat-Hosseini *et al.*, 2019; Sarikhani and Vahdati, 2019). However, the number of these traits is limited and unstable and they do not always allow discrimination between closely related accessions or cultivars (Konarev *et al.,* 2000). DNA-based molecular markers are excellent tools for exploring the genetic diversity in plants (Karimi *et al.*, 2010; Mohsenipoor *et al.*, 2010; Vahdati *et al.*, 2015; Fiore *et al.,* 2022). The use of molecular markers in hazelnut has been limited mainly to RAPD markers and a few topics, including self-incompatibility (Bassil and Azarenko, 2001), genetic relationships (Erdogan et al., 2010), resistance to eastern filbert blight (EFB) (Muehlbaueret et al., 2014), and marker-assisted selection for EFB gene pyramids (Sathuvalli and Mehlenbacher, 2012). SSR markers are second-generation DNA markers that are commonly used in breeding programs and for research (Sharifani *et al*., 2017; Hassani *et al*., 2022). Microsatellites or simple sequence repeat (SSR) markers are short, tandem repeated DNA sequences that are highly polymorphic in eukaryotic genomes (Sharma *et al.,* 2007). Due to their high variability, codominant inheritance, suitability for inter-laboratory exchange, automated allele sizing, and cross-species transferability, they are convenient markers for many applications such as hazelnut cultivar identification and parentage analysis (Gökirmak *et al.,* 2009), genetic mapping (Dirlewanger *et al.,* 2004; Bhattarai *et al.,* 2017), diversity assessment (Bassil *et al.,* 2005; Gökirmak *et al.,* 2009; Gürcan *et al.,* 2009; Sathuvalli and Mehlenbacher, 2012, Zong *et al.,* 2015; Fiore *et al.,* 2022), and discrimination of plant material before long maturation process (Öztürk *et al.,* 2018). Several genetic diversity studies have been previously conducted using SSR on native Iranian hazelnuts (Ghanbari *et al.,* 2005; Erfatpour *et al.,* 2011; Ahmadi *et al.,* 2012). This study aimed to evaluate the genetic diversity among the native genotypes from different populations in the north and northwest of Iran and some commercial cultivars using SSR markers.

Materials and Methods

Plant material

Samples were collected from six populations of *C. avellana* from the north and northwest of Iran. The studied plants included eleven accessions from Gilan parts of Fandogloo (FG) as population 1, eleven accessions from Ardabil parts of Fandogloo (FA) as population 2, eight accessions from Talesh (T) as population 3, ten accessions from Hatammeshasi (H) as population 4, eight accessions from Eshkavar (E) as population 5 and four commercial cultivars including: 'Ronde du Piemonte', 'Long despine', 'Daviana' and 'Cosford' as population 6. Leaf samples were collected in spring and stored at -80°C until DNA extraction. Populations 1-5 consisted of old hazelnut trees from open pollinated seedlings. Elevation of the sampled areas of the studied populations ranged from 1067 to 1380 m above sea level. The geographic

characteristics of the populations are shown in Table 1. Hazelnut genotypes that were less than 10 km apart were considered to belong to the same deme.

Table 1. Geographical coordinates and some climatic conditions of studied hazelnut populations.

Pop-No.	Sampling regions	Individual No.	Longitude	latitude	Altitude (m)	Annual average temperature (C°)	Annual rainfall (mm)	Province
Pop1	Fandogloo-Gilan (FG)	11	48°35'	38°20'	1090-1250	11.5	372	Gilan
Pop2	Fandogloo-Ardabil(FA)	11	48°32'	38°18'	1340-1380	8.9	295.5	Ardabil
Pop3	Talesh (T)	8	48°37'	37°50'	1080-1220	14.2	1199	Gilan
Pop4	Hatammeshasi(H)	10	48°23'	38°18'	1109-1203	5.9	361.2	Ardabil
Pop5	Eshkavar (E)	8	50°25'	36°57'	1067-1213	15.8	2453	Gilan
Pop 6[1]	Commercial cultivars	4	48°86'	38°31'	27	-	-	-

1-From hazelnut collection of Astara agriculture research station located in Gilan province

DNA extraction

Genomic DNA was extracted from young leaves (approximately 0.1 g) according to Doyle and Doyle method (Doyle and Doyle, 1987) with some modifications. CTAB buffer (CTAB 2%, 1.4 M NaCl, 20 mM EDTA, pH: 8.0, 100 mM Tris-HCl, pH: 8.0, 1% PVP 40, 2% mercaptoethanol) containing 2 µL proteinase K (20 mg/mL) was used for DNA extraction. Additional extraction with chloroform: isoamyl alcohol (24:1) was also performed, and RNA was removed by incubation with 2 µL RNase (20.3 mg Ml^{-1}) for 30 min at 37°C. Finally, after purification, total DNA was suspended in 100 µl TE buffer. The quality of DNA was checked by running 1 µl DNA in 0.8% (w/v) gels in 0.5X TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). DNA samples that showed a smear in the gel were discarded.

Polymerase chain reaction (PCR) and fragment analysis

Fourteen primer pairs related to genomic SSRs were extracted from the hazelnut genomic library (Bassil *et al.,* 2005; Boccacci *et al.,* 2006), from which only nine were amplified in the genotypes studied and were selected and used in this study. Each PCR amplification was performed in 25 µl containing 1X reaction buffer [200 mM Tris-HCl, pH: 8.55, 160 mM $(NH4)_2SO_4$ 0.1% (v/v)], 2.0 mM $MgCl_2$, 0.4 mM dNTPs (CinnaGen Inc, Iran), 0.16 µM of each SSR primer, 1.0 units of Taq DNA polymerase (CinnaGen Inc., Iran), and 25 ng genomic DNA template. DNA amplifications were performed in a Gene Amp PCR System 9700 Thermocycler (PerkinElmer-Applied Biosystems) programmed for a preliminary step at 95ºC for 2 min, followed by 35 cycles at 93ºC for 45 s, 50-55ºC for 60 s, and 72ºC for 2 min; a final extension was performed at 72ºC for 10 min. Samples were then kept at 4ºC until SSR fragments were separated by electrophoresis using 3% agarose gel in 0.5X TBE buffer and visualized with ethidium bromide (1.0µg ml^{-1}) under UV light.

Data analysis

Banding profiles generated by SSR assays were compiled separately into a data matrix based on the presence (1) or absence (0) of bands. The binary matrices were used to estimate DNA polymorphisms and genetic relatedness of hazelnut genotypes. The efficiency of the clustering algorithms and their goodness of fit was determined using the cophenetic correlation coefficient. Accordingly, the Jaccard similarity coefficient was used to generate the similarity coefficient and the dendrogram (data not mentioned). Mean number of alleles per locus (*Na*), effective allele number (*Ne*), allele frequency, F-statistics (F_{is} and F_{st}) (Wright, 1978). Shannon index

(*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and Hardy-Weinberg equilibrium test (*HWE*) were estimated using the program PopGene32 (Yeh *et al*., 1997). Allelic polymorphism information content (*PIC*) was calculated as described by Anderson *et al*. (1992). Polymorphism information content (PIC) was calculated as $PIC = \sum_{i=1}^{n} P_i^2$ where Pi is the proportion of the population carrying the ith allele, calculated for each microsatellite locus. Data analyses were performed using NTSYS-pc version 2.11 software (Rohlf, 1998).

Results

Five populations of *C.avellana*, including 48 native Iranian hazelnut genotypes from Ardabil and Gilan provinces and four commercial cultivars as the sixth population, including 'Cosford', 'Long despine', 'Ronde du Piemonte' and 'Daviana', were sampled (Tables 1 and 2) and analyzed using nine polymorphic SSR markers (Table 3). Polymorphism information content value varied from (*PIC*=0.87) at loci CaCB028, CaCB109, and CaCB113 to (*PIC*=0.98) at locus CaCC001a (Table 4). F_{is} values ranged from -0.11 at locus CaTC502 to 0.86, 0.80, and 0.78 at loci CaCB011, CaTB509, and CaCB028, respectively (Table 4). F_{st} varied from 0.00 in CaTC502 to 0.26 in CaCB011 with an average of 0.15 (Table 4). In our study, the gene flow of *C. avellana* was *Nm*=1.57, which gives an estimate of the average number of migrants between all studied populations per generation (Table 4). In this study, the mean values for *I*, *Na*, *Ne*, *He,* and *Ho* were 0.62, 2.07, 1.81, 0.49, and 0.19, respectively (Table 5). Estimated null allele frequencies ranged from r=-0.005 to r=0.36 (Table 5). The highest and lowest values of fixation index were obtained in Fandogloo-Ardabil (F_{is}=0.59), and 6th populations (F_{is}=0.41), respectively (Table 5). Studied populations did not follow the Hardy-Weinberg equilibrium (*HWE*) (Table 6). Identification of genetic distances between the studied populations showed the highest correlations (r= 0.94, $p < 0.05$) between Eshkavar and Fandogloo-Gilan and Hatammeshasi and Fandogloo-Ardabil populations (Table 7, Fig. 1). According to Jaccard's correlation, the average pairwise genetic similarity was 0.80 and the highest correlation value (r= 0.87) was observed between genotypes 'Eshkavar 6' and 'Talesh 3', and 'Eshkavar 5' and 'cosford' (Table 8). According to a dendrogram generated by Ward's method based on the Euclidean squared distance, the 52 studied hazelnut accessions were divided into four separate groups. In the created dendrogram, cluster II was the largest with 42 genotypes, followed by cluster III, Cluster I and IV (Fig. 2).

Discussion

Two provinces of Gilan and Ardabil in the north and northwest of Iran account for 74% of the total area under hazelnut cultivation in Iran (Hosseinova and Shamskia, 2013; Aghapoor *et al.,* 2018). The mean Shannon-Weaver diversity index was *I*= 0.62, which represents a low level of genetic diversity compared to values previously reported for *C.heterophylla* using SRAP markers (Yao Di *et al.,* 2014) and *C. mandshurica* using SSR markers (Zong *et al,* 2015). The high genetic diversity observed in these studies might be related to the biological characteristics and living conditions of these species. However, a perennial species can maintain its genetic diversity over a long period (Yao Di *et al.,* 2014). Yang et al. (2012) also found that SRAP markers generate more polymorphic alleles than SSR markers. In this study, a total of 19 alleles were observed at the 9 loci. Our results displayed 2 to 3 alleles per locus with an average of 2.11 (Table 3). The highest number of alleles (*Na*=3) was observed at locus CaCB113. *PIC* value varied from 0.87 at loci CaCB028, CaCB109, and CaCB113 to 0.98 at locus CaCC001a (Table 4). Markers with a high *PIC* can be effectively used in genetic diversity studies of hazelnut. In our study, some hazelnut markers such as CaCB011, CaCB113, CaTB509 and CaCC001 showed higher values of *PIC* compared to previous reports on Iranian hazelnut genotypes by Erfatpour et al. (2011) and Ahmadi *et al.*

(2012). The discriminatory power of each marker was estimated using the *PIC* value. In addition, the discriminative power of each locus was also assessed by *Na*, *He*, and *Ho*. In our study, the mean values for *Na*, *Ne*, *He,* and *Ho* were 2.07, 1.81, 0.49, and 0.19, respectively, which were lower than those reported in other hazelnut studies (Table 5). For example, in a study of 78 hazelnut cultivars from various germplasm repositories using 16 SSR loci, the mean values of *Na* and *He* were 9.4 and 0.78, respectively (Boccacci *et al.,* 2006). In a study of genetic diversity in 270 clonal *C. avellana* accessions using 21 SSRs, the values of *Na*, *He*, *Ho,* and *PIC* were 9.81, 0.72, 0.67, and 0.68, respectively (Gökirmak *et al.,* 2009). A high level of genetic diversity was found in 33 Spanish hazelnut genotypes using 16 SSR markers (*Na*= 7.1, *He* = 0.7, *Ho* = 0.80) (Boccacci and Botta, 2009). The study of genetic diversity in 88 accessions from Turkey, Azerbaijan, Georgia, and Spain using 12 SSR markers revealed *Na*= 9.67, *He*=0.71, *Ho*=0.70, and *PIC*=0.65 (Gürcan *et al*., 2009). In a study on 16 Iranian genotypes and 7 internationally known cultivars, the average of *Na*, *H*e, and Ho were reported to be 9.2, 0.78, and 0.84, respectively (Ghanbari *et al.,* 2005). A study on an Iranian population of 90 hazelnut accessions from the Talesh Mountains in Gilan province revealed the values for *Na*, *Ne*, *PIC*, *He*, and *Ho* were 6.53, 3.75, 0.72, 0.70, and 0.62, respectively (Erfatpour *et al.,* 2011). Genetic diversity in 28 hazelnut cultivars, using 10 SSR markers, was reported as *Na*= 6.3, *He*=0.70, *PIC* =0.97 (Ahmadi *et al.,* 2012). The lower values obtained in the present study could be due to the use of a small number of markers. Another reason could be the use of agarose gel electrophoresis for microsatellite screening. Agarose gel is non-toxic and safe to handle, but an automated detection system and polyacrylamide would be able to resolve allelic variations at a finer scale than agarose gel electrophoresis analysis. In some studies on SSRs, the reason for a larger number of alleles was duplication of some loci resulting in more alleles, as in the case of apricot (Hormaza, 2002).

In our study, the estimated null allele frequencies ranged from r=-0.005 for CaCB113 to r=0.36 for the CaTB509 locus (Table 5). Bruford *et al*. (1998) confirmed that high frequencies of null alleles lead to low allele frequencies and heterozygosity. F_{is} values ranged from -0.11 in locus CaTC502 to 0.86, 0.80, and 0.78 in loci CaCB011, CaTB509, and CaCB028, respectively (Table 4). Erfatpour *et al*. (2011) reported F_{is} values ranging from -0.15 for CaCB113 to 0.48 for the CacB028 locus. Gökirmak *et al*. (2009) reported positive F_{is} values for CaCB028 and CaTB508, which is consistent with the present study. The F statistics or inbreeding coefficients, including F_{is}, Fa_{st}, and F_{it}, refer to the extent of heterozygosity at different levels of population structure. F_{it} is the inbreeding coefficient of an individual relative to the total population, F_{is} is the inbreeding coefficient of an individual relative to the subpopulation, and F_{st} is the effect of subpopulations relative to the total population. The fixation index (F_{is}) can vary from -1 (all individuals are heterozygous) to +1 (all individuals are homozygous) (Wright, 1978). In this study, the mean value of F_{is} in Fandogloo-Gilan, Fandogloo-Ardabil, Talesh, Hatammeshasi, Eshkavar, and 6th populations was 0.51, 0.59, 0.42, 0.56, 0.58, and 0.41, respectively. The positive values of F_{is} in genetic diversity indicate excessive homozygosity in hazelnut populations, which could be attributed to the hazelnut mating system. It could also be caused by selection or disassortative mating (Karimi *et al.,* 2014). F_{st} varied from 0.00 in CaTC502 to 0.26 in CaCB011 with an average of 0.15 (Table 4). In the genetic variation of *C. heterophylla* populations at different altitudes in China, the mean value of F_{st} was reported to be 0.22 (Yao Di *et al.,* 2014). F_{is} and F_{st} are the critical points in evaluating gene flow. When gene flow between populations is higher than 1, it plays an important role in discriminating between populations. Gene flow is an important factor affecting the genetic structure of *C.avellana*. In our study, the

gene flow of *C.avellana* was Nm=1.57, which gives an estimate of the average number of migrants between all studied populations per generation (Table 4). The relatively high gene flow may be caused by wind-pollinated flowers, highly nutritious seeds, and self-incompatible mating system. In a study of 348 individuals of *C. mandshurica* from 12 populations in China using SSR markers, the coefficient of genetic differentiation and average gene flow were reported to be F_{st} = 0.12 and Nm = 1.80, respectively (Zong *et al.,* 2015). In a study of 300 specimens of *C.avellana* from 20 different populations in Finland using SSR markers, most of the genetic diversity was within populations (F_{st} = 0.83) (Tanhuanpää *et al*., 2019).

The Chi-square test (X^2) revealed that the studied populations did not follow the Hardy-Weinberg equilibrium (*HWE*) (Table 6). Hardy-Weinberg disequilibrium and positive F_{is} in populations can generally be observed in open-pollinated species where there is a possibility that non-random mating may result in low heterozygosity (Jothi, 2008). The observed values suggest that gene exchange between populations is low. The current genetic structure of hazelnut in Iran suggests that the impact of gene flow from human activities and hybridization is lower than would be expected due to domestication since ancient times and the abundant trade in hazelnut germplasm. Our results indicate a general trend toward isolation by distance and genetic differentiation among hazelnut populations in the different geographic regions of Iran. In the different geographical regions, differentiation between individual populations at different altitudes was partly caused by genetic drift. Geographic isolation due to different environmental conditions could be the main reason for genetic differentiation among *C.avellana* populations. Variations in elevation above sea level, climate, temperature, and soil would result in differences in genetic composition among populations. Genetic variation among populations distributed over limited geographic areas is due to habitat selection pressure (Jin and Li, 2005; Nematollahi *et al.*, 2009). Genetic mutations are often not considered a major factor in differentiating between populations due to their very low frequency (Yao Di et al., 2014). Identification of genetic distances between the studied populations showed the highest correlations (r= 0.94, p < 0.05) between Eshkavar and Fandogloo-Gilan and Hatammeshasi and Fandogloo-Ardabil populations (Table 7, Fig. 1). The Eshkavar and Fandogloo-Gilan populations reside in Gilan province in northern Iran, while the Hatammeshasi and Fandogloo-Ardabil populations reside in Ardabil province in northwestern Iran. Gilan province is under the influence of dense fog and high humidity from the Caspian Sea and has different climatic conditions than Ardabil. Significant climatic differences were evident along an altitudinal gradient, resulting in shortened plant life span and delayed pollination phenology, which affected gene flow and led to genetic differentiation among populations of *C.avellana*. The genetic structure along altitudinal gradients may also be affected by interspecific hybridization (Huang *et al.,* 2008). Overall, alleles of some genotypes may introgress into other genotypes, causing a genetic cline at specific loci along elevational gradients. Such phenomena may also affect the distribution of genetic diversity within populations, as the inflow of new alleles may increase allelic richness.

The construction of similarity matrices and dendrograms is a useful tool for studying genetic diversity. Among the different methods, the cophenetic correlation, a measure of the correlation between the similarity represented in the dendrograms and the actual degree of similarity, was calculated for each dendrogram (Ahmadi *et al.,* 2012). The highest cophenetic correlation (r= 0.76) was observed for UPGMA based on Jaccard's coefficient. According to Jaccard's correlation, the average pairwise genetic similarity was 0.80 and the highest correlation value (r= 0.87) was observed between 'Eshkavar 6' and 'Talesh 3', and 'Eshkavar 5' and 'cosford' genotypes (Table 8). A high correlation coefficient (r=0.85) was observed between 'Hatammeshasi 5' and

'Hatammeshasi 2', 'Fandogloo-Gilan 8' and 'Fandogloo-Gilan7', 'Eshkavar 6' and 'Talesh 6', 'Eshkavar 6' and 'Talesh 4', 'Hatammeshasi 5' and 'Hatammeshasi 2', 'Talesh 5' and 'Fandogloo-Ardabil 8', and 'Fandogloo-Gilan 4' and 'Eshkavar 3'. Therefore, the dendrogram created by this method was used to show the genetic diversity of the genotypes (Fig. 2). According to a dendrogram generated by Ward's method based on the Euclidean squared distance, the 52 studied hazelnut accessions were divided into four separate groups. In the created dendrogram, cluster II was the largest with 42 genotypes, followed by cluster III with 6 genotypes. Cluster I contained genotypes 'Daviana' and 'Fandogloo-Gilan 11'. Finally, 'Ronde du Piemonte' and 'Long despine' were classified in cluster IV (Fig. 2). In a study of genetic diversity of 16 Iranian hazelnut genotypes and 7 internationally known cultivars, the dendrogram produced was divided into three main clusters. The third cluster included 'Daviana', 'Cosford', and 4 foreign cultivars along with 3 Iranian accessions (Ghanbari *et al.,* 2005). In the current study, the foreign cultivars, except ‘Cosford’, were separately classified into clusters I and IV, while 'Cosford' along with 41 Iranian hazelnut accessions were classified into group II. Six genotypes of Hatammeshasi population were classified in group III. The highest genetic diversity is generally found in genotypes belonging to populations that are large and have been outbred over many generations, and the lowest in individuals belonging to populations that have experienced severe demographic decline and/or inbreeding over time (Soulé, 1976). Correlation analysis revealed a spatial genetic structure in four distant classes, with Hatammeshasi far from the rest of the Caspian Sea population studied. Across the altitudinal gradient, it appears that some factors such as low annual mean temperature and mountainous boundary isolate Hatammeshasi genotypes, while the genotypes in group II are influenced by Caspian Sea moisture. Neutral genetic variation within these populations often changes over spatial distance because individuals within populations generally mate with other individuals in the same population. Genetic variation among populations of a species may exhibit a "geographic structure" that is generally correlated with spatial distance (Fine *et al.,* 2013). Results suggest that local accessions are closely related but relatively distant from foreign cultivars. Selected hazelnuts from northern and northwestern Iran could be directly valuable as new cultivars or as parents in breeding programs. Therefore, it would be important to collect and preserve these accessions with high genetic diversity.

Table 2. Name and origin of 52 studied hazelnut accessions used for SSR fingerprinting

Name	Origon	Name	Origin	Name	Origin	Name	Origin	Cultivar name
FG1	Fandogloo-Gilan	**FA2**	Fandogloo-Ardabil	**T3**	Talesh	**H7**	Hatammeshasi	D Daviana
FG2	Fandogloo-Gilan	**FA3**	Fandogloo-Ardabil	**T4**	Talesh	**H8**	Hatammeshasi	LD Longdespine
FG3	Fandogloo-Gilan	**FA4**	Fandogloo-Ardabil	**T5**	Talesh	**H9**	Hatammeshasi	R Ronde du Piemonte
FG4	Fandogloo-Gilan	**FA5**	Fandogloo-Ardabil	**T6**	Talesh	**H10**	Hatammeshasi	C Cosford
FG5	Fandogloo-Gilan	**FA6**	Fandogloo-Ardabil	**T7**	Talesh	**E1**	Eshkavar	
FG6	Fandogloo-Gilan	**FA7**	FandoglooArdabil	**T8**	Talesh	**E2**	Eshkavar	
FG7	Fandogloo-Gilan	**FA8**	Fandogloo-Ardabil	**H1**	Hatammeshasi	**E3**	Eshkavar	
FG8	Fandogloo-Gilan	**FA9**	Fandogloo-Ardabil	**H2**	Hatammeshasi	**E4**	Eshkavar	
FG9	Fandogloo-Gilan	**FA10**	Fandogloo-Ardabil	**H3**	Hatammeshasi	**E5**	Eshkavar	
FG10	Fandogloo-Gilan	**FA11**	FandoglooArdabil	**H4**	hatammeshasi	**E6**	Eshkavar	
FG11	Fandogloo-Gilan	**T1**	Talesh	**H5**	Hatammeshasi	**E7**	Eshkavar	
FA1	Fandogloo-Ardabil	**T2**	Talesh	**H6**	Hatammeshasi	**E8**	Eshkavar	

Table 3. Primer sequences, SSR repeat, annealing temperature, number of alleles of the 9 simple sequence repeat (SSR) loci applied to hazelnut accessions

Primer	Reverse Seq	Forward Seq	Allele No.	Annealing temperature	SSR repeat
CaCB028	CCTGTTTCTCTTTGTTTTCGAG	ATGGACGAGGAATATTTCAGC	2	55	$(AG)_{16}$
CaCB011	CACTGGTGATCTCACAGGTTTA	GTCCTCAAAAGCTAAGCACAAG	2	62	$(GA)_{16}$
CaCB109	AATCCAAGCCTTTTCACTACC	ACCCATCAAGTTCACCAATC	2	58	(GA)21
CaCB113	TTGAGGAAGTCCAGGAAAAT	GCCAGAGAGAGCAAGAGTTAG	3	60	$(GA)_{16}$
CaTB509	GTCTGGCATGGTTTTGAGAAGA	CTTTCCCGCCCAAACCAC	2	50	(GA) 14
CaTB106	CCAATCGCCAATGAATCATC	CCCTTTCCAAACTGGGCAT	2	55	(CT) 14
CaTB507	CTAAGCTCACCAAGAGGAAGTTGAT	GCTTCTGGGTCTCCTGCTCA	2	55	$-(GC)(GA)GC_2(GA)_{14}$ $(GA)_1$
CaTC502	GCATGSAAGGTGGTCGGT	TTTGGCACCCAACAACTCTAGA	2	55	$_1T_1$ (CTT) $_{11}C$ $_2T_4CTT$ (CTT)
CaCC001a	CCCGTAACTAACCAATCACAAT	TGGAGAAGAGGAGAGCTTAGTG	2	53	(CACAGAG)3

Table 4. Polymorphism information content, F-statistics and the estimation of gene flow in 9 studied loci among populations

Primer	PIC	F_{is}	F_{st}	Nm
CaCB028	0.87	0.78	0.10	2.25
CaCB011	0.90	0.86	0.26	0.71
CaCB109	0.87	0.43	0.21	0.94
CaCB113	0.87	0.20	0.05	4.75
CaTB509	0.88	0.80	0.12	1.83
CaTB106	0.88	0.64	0.08	2.87
CaTB507	0.89	0.60	0.25	0.75
CaTC502	0.88	-0.11	0.00	-
CaCC001a	0.98	0.63	0.23	0.83
Mean	0.88	0.53	0.15	1.57

The estimation of gene flow $Nm= 0.25(1-F_{st})/F_{st}$

Table 5. Characterization of 9 simple sequence repeat (SSR) loci in hazelnut accessions from different populations.

	Pop1							Pop2							Pop3						
Locus	**Na**	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**	**Na**	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**	**Na**	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**
CacB028	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.86	0.65	0.48	0.36	0.21	0.08	2.00	1.80	0.63	0.47	0.00	1.00	0.31
CacB011	2.00	1.34	0.42	0.26	0.10	0.60	0.12	2.00	1.93	0.67	0.50	0.09	0.81	0.27	2.00	1.24	0.34	0.20	0.00	1.00	0.16
CacB109	2.00	1.98	0.68	0.52	0.30	0.39	0.14	3.00	1.96	0.79	0.51	0.27	0.44	0.15	2.00	1.90	0.66	0.50	0.55	-0.16	-0.03
CacB113	3.00	2.98	1.09	0.70	0.40	0.39	0.17	3.00	2.87	1.05	0.67	0.54	0.14	0.07	3.00	2.70	1.06	0.67	0.68	-0.03	-0.005
CaTB509	2.00	1.92	0.67	0.50	0.20	0.58	0.20	2.00	1.65	0.58	0.41	0.00	1.00	0.29	2.00	1.52	0.52	0.36	0.00	1.00	0.26
CaTB106	2.00	1.98	0.68	0.52	0.30	0.39	0.14	2.00	1.76	0.58	0.41	0.00	1.00	0.29	2.00	1.90	0.66	0.50	0.33	0.29	0.11
CaTB507	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.54	0.62	0.45	0.27	0.37	0.12	2.00	1.52	0.52	0.36	0.22	0.35	0.10
CaTC502	2.00	1.60	0.56	0.39	0.50	-0.33	-0.07	2.00	1.86	0.53	0.36	0.09	0.74	0.19	2.00	1.38	0.45	0.29	0.33	-0.20	-0.03
CacC001a	2.00	1.72	0.61	0.44	0.20	0.52	0.16	2.00	1.89	0.65	0.48	0.18	0.60	0.20	2.00	1.97	0.68	0.52	0.22	0.55	0.19
Mean	2.11	1.94	0.67	0.48	0.24	0.51	0.15	2.22	1.89	0.68	0.47	0.20	0.59	0.20	2.11	1.78	0.62	0.43	0.25	0.42	0.11
	Pop4							**Pop5**							**Pop6**						
	Na	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**	**Na**	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**	**Na**	**Ne**	**I**	**He**	**Ho**	**F_{is}**	**r**
CacB028	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.60	0.56	0.40	0.00	1.00	0.28	2.00	1.60	0.56	0.42	0.00	1.00	0.29
CacB011	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.60	0.56	0.45	0.00	1.00	0.31	2.00	1.60	0.56	0.42	0.00	0.37	0.29
CacB109	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.75	0.62	0.45	0.12	0.70	0.22	2.00	1.00	0.00	0.00	0.00	-	0.00
CacB113	3.00	2.17	0.89	0.66	0.60	-0.11	-0.10	3.00	2.66	1.03	0.66	0.25	0.60	0.24	3.00	2.60	1.03	0.71	0.50	0.25	0.12
CaTB509	2.00	1.92	0.67	0.50	0.20	0.58	0.20	2.00	1.75	0.62	0.45	0.12	0.70	0.22	2.00	2.00	0.69	0.57	0.00	0.78	0.36
CaTB106	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.89	0.69	0.53	0.25	0.50	0.18	2.00	1.60	0.56	0.42	0.00	0.73	0.29
CaTB507	2.00	1.92	0.67	0.50	0.00	1.00	0.33	2.00	1.60	0.56	0.40	0.25	0.33	0.10	2.00	1.00	0.00	0.00	0.00	-	0.00
CaTC502	2.00	1.60	0.56	0.39	0.50	-0.33	-0.07	2.00	1.43	0.48	0.32	0.37	-0.23	-0.03	2.00	1.60	0.56	0.42	0.50	-0.33	-0.05
CacC001a	2.00	1.98	0.68	0.52	0.10	0.79	0.27	2.00	1.75	0.62	0.45	0.12	0.70	0.22	2.00	1.00	0.00	0.00	0.00	0.32	0.00
Mean	2.11	1.94	0.69	0.50	0.20	0.56	0.19	2.11	1.79	0.64	0.45	0.16	0.58	0.19	1.77	1.56	0.44	0.29	0.11	0.41	0.14

Mean of alleles (Na), mean of effective alleles (Ne), Polymorphism Information Content (PIC), Shannon index (I), observed heterozygosity (H_O), expected heterozygosity (He), Nei index, F_{is}, Null allele (r), See population code in Table 1.

Table 6. Chi-square (X^2) test and Hardy-Weinberg Equilibrium test.

	Individual No.	df	X^2	Probability level	[1](HWE)	In equilibrium
Pop1	10	9	10.25	0.33	0.45	It is not
Pop2	11	9	15.28	0.08	0.62	It is not
Pop3	9	9	11.45	0.24	0.48	It is not
Pop4	10	9	15.97	0.06	0.55	It is not
Pop5	8	9	11.20	0.26	0.58	It is not
Pop6	4	9	5.20	0.81	0.40	It is not

[1]Hardy-Weinberg Equilibrium (HWE). See population name in table 1

Table 7. Estimation of genetic distances among studied populations using Nei (1973)

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6
Pop1	0.00					
Pop2	0.92	0.00				
Pop3	0.81	0.91	0.00			
Pop4	0.93	0.94	0.91	0.00		
Pop5	0.94	0.89	0.85	0.88	0.00	
Pop6	0.80	0.66	0.65	0.79	0.75	0.00

See population name in Table 1.

Table 8. Coefficient matrix for hazelnut genotypes based on SSR data using Jaccard coefficient (Jaccard, 1908)

	FG11	T2	FA6	FA10	T3	H1	FG7	T8	T1	E7	T6	FA4	FA1	E3	T7	T4	FA5	H8	FG3	FA2	FA9	E2	Daviana	FA8	Long Despine	E5	Ronde	H9	FG2	H2	FG6	H4	E1	FG4	E8	FG5	H5	FG10	H6	FA11	FG8	FA3	E6	T5	FA7	FG1	FG9	H7	H3	H10	Cosford	E4
FG11	0.00																																																			
T2	0.20	0.00																																																		
FA6	0.00	0.57	0.00																																																	
FA10	0.12	0.87	0.62	0.00																																																
T3	0.20	0.33	0.42	0.28	0.00																																															
H1	0.16	0.44	0.57	0.25	0.55	0.00																																														
FG7	0.12	0.66	0.44	0.50	0.77	0.62	0.00																																													
T8	0.50	0.00	0.16	0.00	0.11	0.40	0.33	0.00																																												
T1	0.14	0.55	0.33	0.37	0.66	0.33	0.62	0.66	0.00																																											
E7	0.16	0.62	0.57	0.66	0.75	0.57	0.50	0.75	0.14	0.00																																										
T6	0.00	0.71	0.25	0.50	0.62	0.25	0.37	0.62	0.00	0.50	0.00																																									
FA4	0.00	0.37	0.12	0.33	0.33	0.12	0.42	0.50	0.20	0.57	0.25	0.00																																								
FA1	0.14	0.55	0.500	0.22	0.666	0.500	0.62	0.66	0.28	0.55	0.44	0.33	0.00																																							
E3	014	0.75	0.71	0.57	087	0.50	0.62	0.66	0.12	0.55	0.85	0.50	0.22	0.00																																						
T7	0.00	0.85	0.37	0.66	0.75	0.37	0.33	0.75	0.00	0.44	0.71	0.83	0.42	0.44	0.00																																					
T4	0.16	0.62	0.83	0.42	0.75	0.37	0.71	0.55	0.14	0.44	0.71	0.37	0.25	0.62	0.85	0.00																																				
FA5	0.00	0.50	0.42	0.50	0.44	0.25	0.57	0.44	0.16	0.50	0.37	0.25	0.50	0.50	0.50	0.37	0.00																																			
H8	0.00	0.57	0.50	0.33	0.50	0.28	0.25	0.50	0.00	0.22	0.42	050	0.33	0.37	0.37	0.66	0.42	0.00																																		
FG3	0.00	0.37	0.28	0.33	0.33	0.28	0.66	0.50	0.20	0.37	0.42	0.50	0.60	0.37	0.37	0.42	0.42	0.50	0.00																																	
FA2	0.00	0.62	0.22	0.66	0.57	0.37	0.33	0.70	0.14	0.62	0.50	0.57	0.66	0.44	0.44	0.71	0.33	0.57	0.42	0.00																																
FA9	0.00	062	0.37	0.42	0.55	0.22	0.52	0.55	0.14	0.62	0.33	0.37	0.66	0.62	0.44	0.50	0.50	0.83	0.42	0.42	0.00																															
E2	0.00	0.00	0.20	0.00	0.12	0.20	0.16	0.12	0.50	0.14	0.16	0.00	0.00	0.14	0.14	0.00	0.16	0.00	0.00	0.00	0.00	0.00																														
Daviana	0.52	0.85	0.35	0.66	0.75	0.32	0.33	0.55	0.00	0.62	0.50	0.57	0.42	0.44	0.62	0.71	0.50	0.57	0.42	0.25	0.71	0.71	0.00																													
FA8	0.00	0.28	0.40	0.20	0.25	0.16	0.14	0.11	0.00	0.12	0.14	0.00	0.00	0.28	0.28	0.14	0.33	0.40	0.20	0.00	0.14	0.33	0.00	0.00																												
LongDespine	0.32	0.25	0.33	0.00	0.37	0.14	0.28	0.22	0.25	0.42	0.12	0.14	0.16	0.42	0.25	0.12	0.28	0.14	0.16	0.00	0.12	0.28	0.33	0.28	0.00																											
E5	0.00	0.14	0.20	0.00	0.12	0.00	0.16	0.00	0.00	0.14	0.00	0.00	0.00	0.14	0.14	0.00	0.16	0.20	0.00	0.00	0.00	0.16	0.00	0.16	0.50	0.00																										
Ronde	0.20	0.33	0.25	0.12	0.44	0.42	0.37	0.44	0.40	0.71	0.22	0.25	0.28	0.50	0.33	0.22	0.22	0.25	0.12	0.12	0.37	0.37	0.20	0.37	0.16	0.60	0.00																									
H9	0.00	0.37	0.28	0.14	0.33	0.50	0.25	0.50	0.20	0.22	0.25	0.28	0.33	0.57	0.22	0.42	0.25	0.28	0.60	0.33	0.42	0.42	0.00	0.25	0.20	0.16	0.00	0.00																								
FG2	0.25	0.37	0.50	0.14	0.50	0.50	0.42	0.33	0.20	0.37	0.42	0.28	0.00	0.37	0.57	0.25	0.42	0.12	0.14	0.14	0.11	0.11	0.25	0.25	0.20	0.40	0.25	0.50	0.00																							
H2	0.16	0.44	0.37	0.25	0.57	0.35	0.71	0.75	0.33	0.66	0.62	0.44	0.50	0.50	0.35	0.42	0.66	0.50	0.50	0.16	0.33	0.00	0.28	0.00	0.37	0.42	0.25	0.37	0.47	0.00																						
FG6	0.25	0.22	0.28	0.33	0.33	0.50	0.42	0.50	0.50	0.57	0.42	0.28	0.33	0.22	0.37	0.25	0.22	0.28	0.14	0.33	0.42	0.25	025	0.25	0.00	0.16	0.00	0.50	0.14	0.33	0.00																					
H4	0.00	0.50	0.25	0.28	0.44	0.25	0.37	0.44	0.16	0.50	0.22	0.25	0.50	0.71	0.33	0.37	0.37	0.66	0.28	0.28	0.57	0.83	0.00	0.57	0.40	0.33	0.20	0.42	0.50	0.12	0.37	0.00																				
E1	0.20	0.33	0.42	0.50	0.44	0.42	0.57	0.62	0.40	0.50	0.57	0.25	0.50	0.50	0.50	0.37	0.57	0.66	0.28	0.50	0.57	0.57	0.20	0.37	0.16	0.14	0.00	0.25	0.28	0.12	0.57	0.50	0.00																			
FG4	0.16	0.44	0.57	0.11	0.55	0.57	0.50	0.55	0.33	0.44	0.33	0.22	0.25	0.85	0.44	0.33	0.50	0.37	0.42	0.25	0.33	0.50	0.16	0.33	0.33	0.50	0.16	0.57	0.66	0.42	0.50	0.25	0.57	0.00																		
E8	0.20	0.33	0.25	0.28	0.44	0.25	0.57	0.62	0.40	0.71	0.37	0.42	0.80	0.50	0.33	0.37	0.37	0.42	0.28	0.50	0.57	0.57	0.20	0.37	0.00	0.33	0.00	0.42	0.28	0.12	0.83	0.50	0.42	0.66	0.00																	
FG5	0.37	0.50	0.42	0.50	0.44	0.42	0.37	0.44	0.16	0.50	0.37	0.26	0.28	0.33	0.50	0.37	0.37	0.66	0.28	0.28	0.57	0.57	0.00	0.57	0.40	0.14	0.20	0.42	0.28	0.28	0.22	0.50	0.42	0.42	0.37	0.00																
H5	0.14	0.55	0.50	0.37	0.66	0.50	0.62	0.87	0.28	0.55	0.62	0.50	0.57	0.75	0.55	0.62	0.62	0.50	0.57	0.57	0.62	0.14	0.44	0.12	0.24	0.00	0.33	0.57	0.22	0.85	0.37	0.50	0.71	0.62	0.71	0.33	0.00															
FG10	0.00	0.42	0.33	0.40	0.37	0.00	0.50	0.22	0.00	0.42	0.28	0.33	0.40	0.25	0.42	0.28	0.50	0.60	0.16	0.40	0.28	0.50	0.00	0.50	0.25	0.20	0.33	0.14	0.00	0.16	0.28	0.16	0.33	0.33	0.12	0.33	0.33	0.00														
H6	0.14	0.55	0.50	0.57	0.66	0.71	0.62	0.82	0.28	0.55	0.85	0.50	0.37	0.50	0.75	0.62	0.62	0.50	0.37	0.57	0.62	0.44	0.14	0.44	0.12	0.11	0.00	033	037	0.37	0.62	0.57	0.33	0.71	0.44	0.50	0.50	0.75	0.00													
FA11	0.00	0.42	0.14	0.40	0.37	0.33	0.28	0.37	0.00	0.25	0.50	0.60	0.16	0.25	0.42	0.50	0.28	0.14	0.16	0.40	0.28	0.12	0.00	0.28	0.00	0.00	0.00	0.14	0.16	0.40	0.28	0.16	0.14	0.14	0.12	0.14	0.14	0.25	0.20	0.00												
FG8	0.00	0.50	0.42	0.28	0.44	0.25	0.83	0.44	0.16	0.50	0.37	0.42	050	0.50	0.50	0.37	0.57	0.66	0.28	0.80	0.37	0.57	0.00	0.37	0.16	0.14	0.20	0.25	0.28	0.28	057	0.28	0.42	0.42	0.37	0.42	0.42	0.50	0.60	0.50	0.00											
FA3	0.16	0.62	0.57	0.42	0.75	0.37	0.33	0.57	0.14	0.62	0.50	0.37	0.25	0.44	0.62	0.50	0.50	0.37	0.42	0.11	0.50	0.50	0.16	0.71	0.33	0.50	0.16	0.57	0.25	0.42	0.33	0.42	0.37	0.37	0.50	0.37	0.57	0.44	0.28	0.44	0.12	0.00										
E6	0.14	0.75	0.50	0.57	0.87	0.50	0.44	0.87	0.12	0.55	0.85	0.71	0.35	0.55	0.75	0.85	0.62	0.33	0.57	0.37	0.62	0.44	0.14	0.62	.012	0.25	0.00	0.33	0.37	0.37	0.62	0.37	0.33	0.50	0.44	0.50	0.33	0.75	0.25	0.75	0.72	0.33	0.00									
T5	0.00	0.85	0.37	0.66	0.75	0.22	0.33	0.57	0.00	0.62	0.50	0.57	0.42	0.44	0.62	0.71	0.50	0.57	0.42	0.25	0.71	0.71	0.00	0.85	0.33	0.25	016	037	028	0.25	0.33	0.25	0.57	0.37	0.37	0.37	0.57	0.44	0.50	0.44	0.28	0.37	0.71	0.00								
FA7	0.00	0.57	0.28	0.33	0.50	0.28	0.50	0.50	0.00	0.22	0.42	050	0.33	0.57	0.37	0.66	0.42	0.28	0.60	0.33	0.42	0.42	0.00	0.42	0.20	0.16	0.00	0.12	0.60	0.14	0.42	0.00	0.50	0.28	0.42	0.28	0.12	0.57	0.16	0.37	0.40	0.28	0.25	0.57	0.00							
FG1	0.00	0.50	0.11	0.28	0.44	0.42	0.37	0.62	0.16	0.50	0.37	0.66	0.50	0.50	0.33	0.57	0.22	0.25	0.28	0.50	0.57	0.37	0.00	0.37	0.00	0.14	0.00	0.42	0.50	0.28	0.57	0.28	0.42	0.25	0.37	0.42	0.25	0.50	0.14	0.50	0.60	0.42	0.22	0.50	0.37	0.00						
FG9	0.00	0.37	0.28	0.14	0.33	0.28	0.25	0.33	02.0	0.37	0.11	0.12	0.33	0.57	0.22	0.25	0.25	0.50	0.33	0.14	0.42	0.66	0.00	0.42	0.50	0.40	0.25	0.50	0.60	0.14	0.25	0.14	0.28	0.80	0.66	0.28	0.50	0.37	0.16	0.22	0.00	0.28	0.42	0.22	0.43	0.32	0.00					
H7	0.00	0.38	0.25	0.14	0.33	0.50	0.42	0.33	0.20	0.37	0.25	0.28	0.14	0.37	0.37	0.25	0.25	0.28	0.14	0.33	0.25	0.25	0.00	0.25	0.20	0.16	0.25	0.50	0.33	0.60	0.25	0.33	0.28	0.12	0.42	0.12	0.50	0.22	0.16	0.37	0.40	0.50	0.25	0.22	0.25	0.14	0.50	0.00				
H3	0.20	0.50	0.42	0.28	0.62	0.25	0.37	0.44	0.16	0.50	0.37	0.25	0.28	0.71	0.50	0.37	0.58	0.42	0.28	0.12	0.37	0.57	0.20	0.57	0.40	0.60	0.20	0.42	0.28	0.28	0.37	0.12	0.66	0.42	0.57	0.42	0.25	0.50	0.33	0.33	0.14	0.25	0.57	0.50	0.57	0.50	0.25	0.50	0.00			
H10	0.20	0.50	0.42	0.28	0.62	0.42	0.22	0.44	0.16	0.50	0.37	0.25	0.12	0.50	0.50	0.37	0.37	0.50	0.28	0.00	0.37	0.37	0.20	0.57	0.40	0.60	0.20	0.66	0.28	0.50	0.22	0.28	0.42	0.25	0.57	0.25	0.42	0.33	0.14	0.33	0.14	0.11	0.83	0.50	0.57	0.28	0.25	0.50	0.28	0.00		
Cosford	0.33	0.25	0.33	0.00	0.37	0.14	0.28	0.22	0.25	0.42	0.12	0.14	0.16	0.42	0.25	0.12	0.28	0.14	0.16	0.00	0.12	0.28	0.33	0.28	0.25	0.87	0.23	0.60	0.16	0.40	0.28	0.16	0.33	0.14	0.50	0.33	0.14	0.25	0.20	0.11	0.00	0.14	0.50	0.25	0.28	0.16	0.14	0.40	0.16	0.60	0.00	
E4	0.16	0.33	0.33	0.50	0.42	0.50	0.23	0.33	0.14	0.00	0.22	0.16	0.60	0.44	0.25	0.41	0.50	0.71	0.00	0.37	0.42	0.25	0.42	0.42	0.50	0.66	0.14	0.22	0.60	0.45	0.25	0.37	0.28	0.37	0.25	0.37	0.14	0.12	0.50	0.44	0.12	0.28	0.52	0.40	0.25	0.00	0.60	0.57	0.28	0.11	0.29	0.00

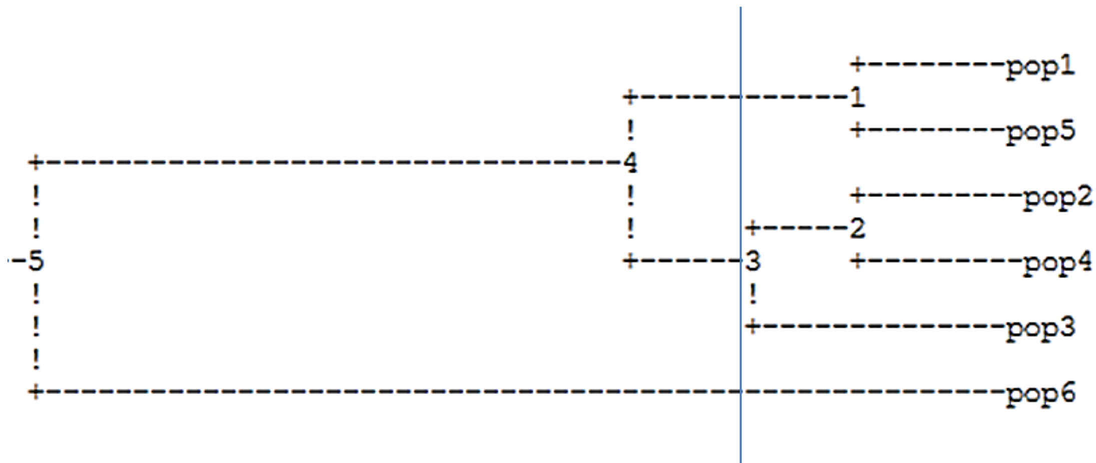

Fig. 1. Dendrogram for the 6 studied hazelnut populations produced by Nei's clusters analysis; See population names in Table 1.

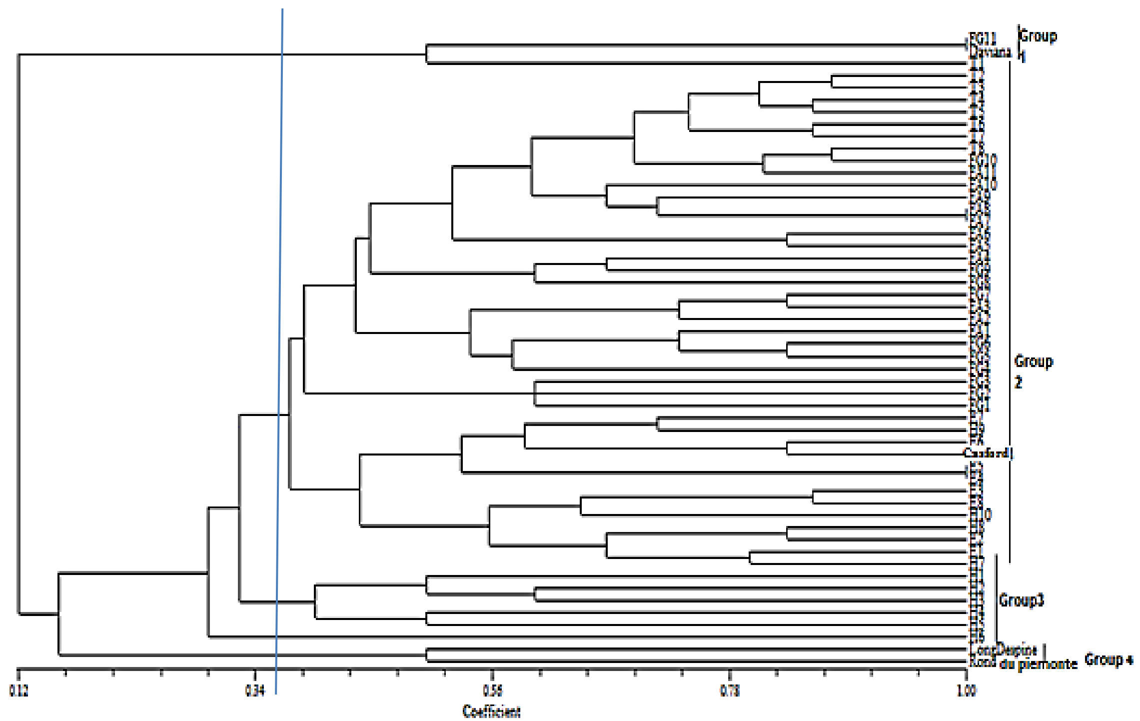

Fig. 2. Dendrogram of 52 hazelnut genotypes generated by the UPGMA clustering method based on a Jaccard's coefficient of similarity matrix. For genotype names corresponding to each code, see Table 2.

Conclusions

Fandogloo jungle is located in the north and northwest of Iran and hosts one of the most important gene pools of hazelnut (*Corylus avellana*) in Iran. Therefore, this study aimed to evaluate the genetic diversity of hazelnut germplasm from Fandogloo by DNA fingerprinting using SSR markers. Of the fourteen SSR primer pairs, nine were amplified and were polymorphic in the accessions studied. These SSRs were used to identify 52 individuals from five Iranian hazelnut populations of the Fandogloo jungle and four foreign cultivars. Our results showed high genetic diversity among accessions, mainly within populations. The chi-square test (X^2) showed that the populations studied did not follow Hardy-Weinberg

equilibrium. The UPGMA dendrogram showed that the classification of accessions based on SSR markers was independent of their geographic distance for some genotypes. The results indicate that SSR is a useful marker for characterizing and determining genetic relationships among Iranian hazelnut accessions. The current study highlights the need to conserve this valuable resource. The results may also help breeders to select the appropriate individual genotypes and use them as parents in a breeding program to improve plants well adapted to harsh climatic conditions.

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