Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

An Appraisal of Phenotypic Diversity Among Hazelnut Wild Germplasm from Northwest Iran

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A R T I C L E I N F O

Keywords:

Cluster analysis;
Correlation analysis;
Genetic diversity;
Germplasm;
Morphological Description

A B S T R A C T

The Fandogloo jungle is located in the Ardabil province of northwestern Iran, and contains the largest in situ germplasm collection of hazelnuts (*Corylus avellana* L.) in Iran. In this study, 12 quantitative and 21 qualitative traits of 70 wild hazelnut genotypes native to Fandogloo were studied, including traits of nuts, kernel and tree morphology. Results showed that, hazelnut genotypes from Fandogloo were phenotypically diverse with significant variation found across most traits studied. Interestingly, they had a smaller nut and kernel size and weight, compared to some hazelnut germplasm pools previously studied around the world, overall they exhibited a higher kernel to shell ratio. Results also showed, there were strong correlations between kernel length and kernel thickness (r=0.878), nut length and nut thickness (r=0.875), and kernel percentage and chlorophyll index (r=0.617). Positive correlations were also found between branching density and suckering (r=0.487) and between the curvature of the nut basal scar and size of the pistil scar (r=0.352). Principal component analysis of quantitative variables revealed that the first four principal components (PCs) accounted for 74.2% of the total variation. Regarding qualitative variables, the first nine PCs accounted for 64.3% of the total variation. Cluster analysis based on quantitative and qualitative traits resulted in a dendrogram with seven and eight main clusters, respectively. Some of the genotypes were not grouped according to their geographical distribution. Overall, this study revealed the presence of high phenotypic diversity in the hazelnut genotypes from Fandogloo region, supporting their breeding value for possible use in future.

Introduction

The European hazelnut (*Corylus avellana* L.) is one of the major tree nut crops in the world with a total worldwide production of 1,006,178 MT (FAOSTAT, 2018). Hazelnuts can play an important role in human nutrition and health due to their high unsaturated fat, protein, vitamin, and mineral content (Ozdemir and Akinci, 2004). In Asia, the native area of hazelnut distribution extends from Turkey through Caucasia to Iran in the east and from the Anti-Taurus Mountains of Anatolia to Syria and Lebanon in the south. Towards the West, it spans a wide area across most of Europe, bounded by the Atlantic Ocean and reaching coastal Norway and Finland, east to the Ural Mountains in Russia at its most northern limits (Kasapligil, 1964).

Researches showed that, hazelnuts have been domesticated independently in three regions, including the

[*]**Corresponding author**: Email address: ershadi@basu.ac.ir
Received: 11 June 2020; Received in revised form: 5 August 2020; Accepted: 20 October 2020
DOI: 10.22034/jon.2020.1901939.1089

Mediterranean Basin, Turkey, and Iran (Boccacci and Botta, 2009). Northern and northwestern Iran are among the natural distribution areas of *C. avellana* (Thompson *et al*., 1996) and due to their climatic conditions they are suitable for commercial hazelnut production. In 2018, the area under hazelnut cultivation in Iran was 24,718 hectares and its production was 23,293 tons, ranking fourth for harvested area and seventh for production among the main world producer Countries (FAOSTAT, 2018). The Fandogloo jungle, a forested region with an area of 208km^2 is located in Ardabil province of northwestern Iran (longitude 48°11' to 48°42' E and latitude 38°05' to 38°39' N, altitude of 1450-1600 m asl), holds significant *Corylus avellana* genetic resources (Emkani NaneKaran *et al*., 2013). Hazelnut genotypes found in this area express significant diversity in plant size, growth habit, nut size, nut shape, involucre length, and many other morphological traits (Hosseinpour *et al*., 2013). Unfortunately, this genetic resource base has undergone a widespread genetic erosion in recent years (Emkani NaneKaran *et al*., 2013). Hence, for germplasm management and better utilization of its genetic potential for breeding programs, it is important to identify, describe, and classify the existing genetic resources in the region.

Morphological evaluation is a useful tool primary step to help achieving the goals of characterizing genetic resources (Thompson *et al*., 1996). Estimating diversity and determining relationships among variables in hazelnut germplasm can enhance efficiency of its management and support effective genetic improvement efforts (Yao and Mehlenbacher, 2000). Multivariate analysis, such as principal component analysis (PCA) and cluster analysis (CA) is a useful approach within this context (Mohammadi and Prasanna, 2003) and has been used frequently for genetic diversity analysis in many horticultural crops such as olive (*Olea europaea*) (Cantini *et al*., 1999), date palm (Hassanzadeh Khankahdani and Bagheri, 2019), iris (Azimi et al., 2018), tulips (Pourkhaloee et al., 2017), pomegranate (*Punica granatum*) (Mars and Marrakchi, 1999), apricot (*Prunus domestica*) (Gurrieri *et al*., 2001), peach (*Prunus persica*) (Nikolic *et al*., 2010), apple (*Malus* sp.) (Mratinić and Fotirić-Akšić, 2012), thyme (Ashrafi et al., 2018) and walnut (*Juglans regia*) (Mosivand *et al*., 2012; Ebrahimi *et al*., 2015). In hazelnut, PCA was previously used in morphological studies of *C. avellana* in Europe (Boccacci *et al*., 2013; Baccheta *et al*., 2015) and India (Srivastava *et al*., 2010). PCA was also employed to differentiate Italian cultivars (Menesatti *et al*., 2008) and to examine tocopherol and tocotrienol content in hazelnuts from Portugal (Amaral *et al*., 2006). The aim of this study was to investigate and document the phenotypic diversity of native hazelnut genetic resources in the Fandogloo jungle using quantitative and qualitative morphological and phenological traits.

Materials and Methods

Plant material

Hazelnut genotypes in Fandogloo are mainly distributed in two separate regions with different climatic conditions (Fig. 1). The eastern region is located in Gilan province that is characterized by dense fog and moisture from the Caspian Sea. It has an annual average temperature and rainfall of 14.5˚C and 372 mm, respectively. The western region is located in Ardabil province, 90 km away from the Caspian Sea. It has a colder and drier climate with an annual temperature and rainfall 8.9°C and 295.5 mm, respectively. In this study, 70 hazelnut genotypes naturally growing in Fandogloo region were studied, including 56 genotypes from the eastern region and 14 genotypes from the western region. Trees were randomly selected and morphological and phenological variables recorded in 2014-2015. Geographical coordinates and altitudes above sea level of hazelnut genotypes evaluated in this research has been shown in Table 1.

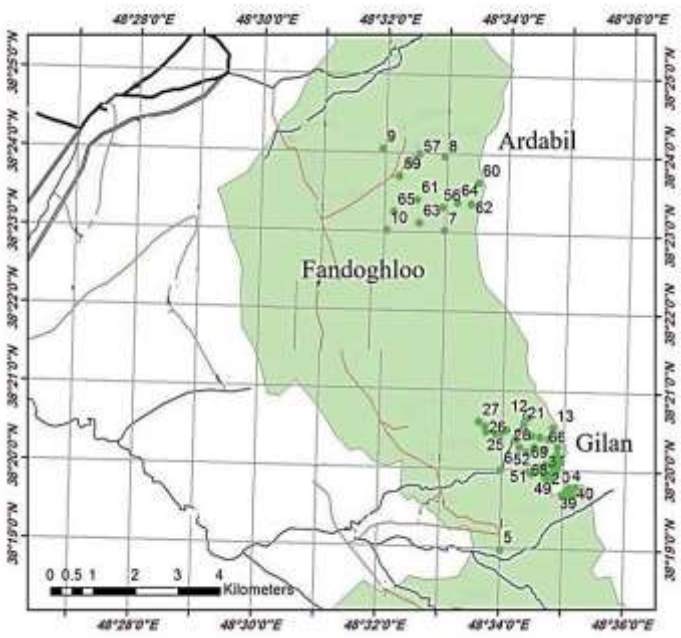

Fig.1. Samples were collected from Gilan and Ardabil parts of the Fandoghloo jungle in northwest Iran

Table 1. Geographical coordinates and altitude above sea level of studied hazelnut genotypes of Fandoghloo, Iran

Genotype	Altitude (meter)	Latitude (N)	Longitude (E)	Province	Genotype	Altitude (meter)	Latitude (N)	Longitude (E)	Province
1	1363	38˚ 20' 4.27"	48˚ 34' 57.22"	Gilan	36	1335	38˚ 20' 4.17"	48˚ 34' 55.53"	Gilan
2	1383	38˚ 20' 0.64"	48˚ 34' 54.71"	Gilan	37	1403	38˚ 20' 6.23"	48˚ 34' 53.74"	Gilan
3	1388	38˚ 19' 53.27"	48˚ 34' 51.19"	Gilan	38	1399	38˚ 19' 44.92"	48˚ 35' 6.21"	Gilan
4	1361	38˚ 19' 58.11"	48˚ 34' 48.62"	Gilan	39	1399	38˚ 19' 46.07"	48˚ 35' 7.71"	Gilan
5	1407	38˚ 18' 58.25"	48˚ 33' 59.73"	Gilan	40	1399	38˚ 19' 46.22"	48˚ 35' 11.46"	Gilan
6	1318	38˚ 19' 57.65"	48˚ 34' 48.21"	Gilan	41	1384	38˚ 19' 42.52"	48˚ 35' 13.51"	Gilan
7	1323	38˚ 19' 59.82"	48˚ 33' 58.27"	Ardabil	42	1386	38˚ 19' 58.04"	48˚ 34' 51.19"	Gilan
8	1413	38˚ 23' 02.41"	48˚ 32' 59.02"	Ardabil	43	1383	38˚ 19' 57.52"	48˚ 34' 50.09"	Gilan
9	1480	38˚ 23' 57.12"	48˚ 32' 56.27"	Ardabil	44	1390	38˚ 19' 56.72"	48˚ 34' 51.28"	Gilan
10	1440	38˚ 24' 08.19"	48˚ 31' 57.72"	Ardabil	45	1390	38˚ 19' 58.12"	48˚ 34' 51.02"	Gilan
11	1363	38˚ 23' 01.52"	48˚ 32' 07.91"	Gilan	46	1382	38˚ 19' 58.73"	48˚ 34' 49.92"	Gilan
12	1398	38˚ 20' 27.06"	48˚ 34' 50.56"	Gilan	47	1378	38˚ 19' 52.28"	48˚ 34' 48.16"	Gilan
13	1365	38˚ 20' 41.94"	48˚ 34' 27.62"	Gilan	48	1388	38˚ 19' 55.73"	48˚ 34' 47.23"	Gilan
14	1386	38˚ 20' 36.21"	48˚ 34' 53.19"	Gilan	49	1369	38˚ 19' 53.04"	48˚ 34' 52.19"	Gilan
15	1370	38˚ 20' 27.82"	48˚ 34' 38.07"	Gilan	50	1369	38˚ 19' 57.19"	48˚ 34' 42.19"	Gilan
16	1355	38˚ 20' 23.27"	48˚ 34' 27.52"	Gilan	51	1346	38˚ 19' 55.26"	48˚ 34' 41.34"	Gilan
17	1340	38˚ 20' 27.62"	48˚ 34' 21.82"	Gilan	52	1346	38˚ 19' 57.67"	48˚ 34' 32.26"	Gilan
18	1376	38˚ 20' 28.02"	48˚ 34' 56.12"	Gilan	53	1362	38˚ 19' 59.02"	48˚ 34' 30.88"	Gilan
19	1328	38˚ 20' 29.57"	48˚ 34' 18.65"	Gilan	54	1351	38˚ 20' 07.34"	48˚ 34' 42.17"	Gilan
20	1347	38˚ 20' 18.37"	48˚ 34' 55.02"	Gilan	55	1346	38˚ 20' 05.17"	48˚ 34' 39.63"	Gilan
21	1341	38˚ 20' 30.47"	48˚ 34' 29.34"	Gilan	56	1359	38˚ 20' 06.72"	48˚ 34' 37.06"	Ardabil
22	1338	38˚ 20' 38.91"	48˚ 34' 23.17"	Gilan	57	1351	38˚ 23' 18.09"	48˚ 32' 58.62"	Ardabil
23	1357	38˚ 20' 33.16"	48˚ 34' 07.19"	Gilan	58	1474	38˚ 23' 59.17"	48˚ 32' 34.17"	Ardabil
24	1356	38˚ 20' 30.65"	48˚ 33' 58.62"	Gilan	59	1439	38˚ 23' 51.47"	48˚ 32' 26.13"	Ardabil
25	1370	38˚ 20' 26.35"	48˚ 33' 48.17"	Gilan	60	1404	38˚ 23' 42.08"	48˚ 32' 17.13"	Ardabil

26	1389	38˚ 20' 31.66"	48˚ 33' 42.59"	Gilan	61	1405	38˚ 23' 44.41"	48˚ 33' 31.64"	Ardabil
27	1397	38˚ 20' 37.26"	48˚ 33' 38.18"	Gilan	62	1436	38˚ 23' 28.31"	48˚ 32' 34.15"	Ardabil
28	1389	38˚ 20' 42.03"	48˚ 33' 33.08"	Gilan	63	1462	38˚ 23' 22.51"	48˚ 33' 28.91"	Ardabil
29	1360	38˚ 20' 24.17"	48˚ 34' 32.11"	Gilan	64	1356	38˚ 23' 10.37"	48˚ 32' 38.56"	Ardabil
30	1342	38˚ 20' 18.31"	48˚ 34' 56.11"	Gilan	65	1389	38˚ 23' 27.18"	48˚ 32' 10.72"	Ardabil
31	1364	38˚ 19' 59.71"	48˚ 34' 44.25"	Gilan	66	1438	38˚ 23' 17.43"	48˚ 32' 09.37"	Gilan
32	1376	38˚ 19' 41.97"	48˚ 35' 08.19"	Gilan	67	1355	38˚ 20' 23.85"	48˚ 34' 40.28"	Gilan
33	1392	38˚ 19' 47.18"	48˚ 35' 15.23"	Gilan	68	1341	38˚ 20' 12.27"	48˚ 34' 55.76"	Gilan
34	1398	38˚ 19' 42.81"	48˚ 34' 59.12"	Gilan	69	1352	38˚ 20' 10.73"	48˚ 34' 51.92"	Gilan
35	1335	38˚ 20' 27.63"	48˚ 34' 56.27"	Gilan	70	1351	38˚ 20' 10.92"	48˚ 34' 40.74"	Gilan

Variable measurement

Based on standard phenotypic characteristics described for hazelnut in Biodiversity international and FAO (2008), 12 quantitative variables: leaf length, leaf width, involucres weight, nut length, nut thickness, nut weight, kernel weight, kernel length, kernel thickness, kernel ratio, chlorophyll index, trunk cross sectional area, along with 21 qualitative traits: tree growth vigor, tree growth habit, branching density, suckering, leaf bud break date, first male bud bloom date, first female bud bloom date, leaf blade shape, under leaf hairiness, petiole hairiness, involucre length/nut length ratio, involucre indentation, serration of indentations on the involucre, involucre thickness at base of involucre, predominant nut number per cluster, nut shape, shape of nut apex, size of pistil scar, curvature of nut basal scar, nut maturity date, kernel shape were measured for all genotypes. Ten fruits (including nut and involucre) per genotype were hand harvested randomly around the canopy of each plant at ripening stage and were transferred to the lab for further analysis.

Statistical analysis

Descriptive statistics including mean, minimum, maximum, standard deviation, and coefficient of variation (CV) were calculated for each character per genotype. To avoid scaling error, the mean of each character was normalized prior to cluster analysis using Z-scores. Correlations between quantitative and qualitative characters were determined using Pearson's and Spearman's correlation coefficients, respectively. In order to identify patterns of morphological variations, principal component, analysis (PCA) was conducted through a correlation matrix of both quantitative and qualitative characters. PCA was then used to construct a two-dimensional scatter plot for a graphical overview of the relationships among genotypes. The grouping of genotypes was performed using the Ward method based on squared Euclidean distances for quantitative and qualitative variables, separately. All of the calculations were processed using SPSS® software version 20 (SPSS Inc., Chicago, IL, USA, Norusis, 1998).

Results

Descriptive statistics

Results showed that, the highest CV among characters examined belonged to kernel length (30.50%), kernel weight (29.68%), involucre indentation (29.52%), involucre thickness at base of involucres (28.80%), kernel ratio (28.60%), while the lowest CVs were observed in nut thickness (14.96%) and nut maturity date (13.01%) (Table2). The mean leaf length, kernel ratio, chlorophyll index, trunk cross sectional area, suckering, and involucre thickness at base of involucres were 11.86 cm, 42.3%, 24.7, 14.1cm^2, 6.4, and 6.25, respectively (Table 2). Some traits showed a high variation, for instance, leaf length ranged from 7.5 cm, in genotype 2, to 20.2 cm in genotype 57. The TCSA varied from 10 cm^2 in genotypes 29, 53 and 63 to 29 cm^2 in genotype 26. The lowest tree vigor was registered in nine genotypes, namely 60, 62, 50, 58, 9, 64, 41, 43, and 62. The suckering potential of genotypes ranged from weak level in genotypes 18, 20, 21, and 45 to very strong in genotypes 6, 7, 10, 13, 28, 41, 43, 44, 46, 51, 62, 63, 68, 69, 70. Date of leaf bud break

ranged from5th March to 15th April across the genotypes and only genotypes 2 and 27 showed late season leafing date, whereas the rest of genotypes were whether early or mid-season leafing. Nut weight ranged from 0.71 g (genotype 13) to 2.70 g (genotype 2), and kernel weight ranged from 0.25 g (genotype 13) to 1.2 g (genotype 43). Kernel to shell ratio ranged from 22.70% in genotype 9 to 51.67% in genotype 25 with a mean of 42.3%.

Table 2. The descriptive analysis of 33 studied variables in 70 hazelnut genotypes of Fandoghloo, Iran

Quantitative traits	Min	Max	Mean	Standard deviation	CV%
Leaf length (cm)	7.50	20.2	11.86	2.25	18.97
Leaf width (cm)	5.70	13.2	8.55	1.70	19.88
Involucres weight (g)	0.15	0.38	0.24	0.05	20.83
Nut length (cm)	1.01	2.71	1.38	0.26	18.84
Nut thickness (cm)	1.00	2.46	1.47	0.22	14.96
Nut weight (g)	0.71	2.70	1.56	0.41	26.28
Kernel weight (g)	0.25	1.20	0.64	0.19	29.68
Kernel length (cm)	0.11	1.11	0.59	0.18	30.50
Kernel thickness	0.19	0.99	0.62	0.17	27.41
Kernel ratio (%)	22.7	51.67	42.30	12.1	28.60
Chlorophyll index (SPAD)	15.23	35.94	24.70	5.61	22.63
Trunk cross sectional area (cm^2)	10.00	29.00	14.10	3.77	26.58
Tree growth vigor	3.00	7.00	5.28	1.37	25.94
Tree growth habit	3.00	7.00	5.00	1.27	25.43
Branching density	3.00	7.00	5.94	1.35	22.72
Suckering	3.00	9.00	6.40	1.78	27.81
Leaf bud break date	3.00	7.00	3.62	1.05	29.00
First male bud bloom date	1.00	5.00	3.48	1.04	27.88
First female bud bloom date	3.00	9.00	5.91	1.34	22.67
Leaf blade shape	1.00	3.00	2.61	0.59	22.60
Under leaf hairiness	3.00	5.00	3.51	0.88	25.07
Petiole hairiness	3.00	7.00	5.54	1.48	26.71
ıvolucre length compared to nut length	3.00	7.00	5.85	1.42	24.23
Involucre indentation	3.00	7.00	5.08	1.50	29.52
Serration of indentations on the involucre	3.00	7.00	5.88	1.16	19.72
ıvolucre thickness at base of involucre	3.00	7.00	6.25	1.08	28.80
ɛdominant nut number per cluster	1.00	5.00	3.42	0.91	26.60
Nut shape	2.00	5.00	4.60	1.26	27.31
Shape of nut apex	2.00	4.00	3.44	0.73	21.22
Size of pistil scar	3.00	7.00	5.20	1.13	21.73
Curvature of nut basal scar	1.00	3.00	2.31	0.52	22.51
Nut maturity date	1.00	5.00	2.95	0.39	13.01
Kernel shape	2.00	6.00	3.77	0.78	20.68

Relationships between variables

Simple correlations among 12 quantitative and 21 qualitative variables were calculated and presented in Tables 3 and 4. Significant correlations were found between some of quantitative variables. Nut length showed strong correlations with nut thickness (r=0.875, P<0.01), nut weight (r =0.485, P<0.01), kernel thickness (r=0.468, P<0.01) and kernel length (r=0.615, P<0.01). Nut weight showed relatively high correlations with nut thickness (r=0.547, P<0.01), kernel thickness (r=0.400, P<0.01), and kernel length (r=0.494, P<0.01) but low

correlation with kernel weight (r=0.252, P<0.05). Kernel length had positive correlation with kernel thickness (r=0.878, P<0.01) (Table 3). Most of the qualitative variables showed weak or non-significant correlations. However, curvature of nut basal scar was positively correlated with size of pistil scar (r= 0.352, P<0.01). Moreover, branching density showed positive correlations with tree growth vigor (r= 0.289, P<0.05) and suckering (r= 0.487, P<0.01), (Table 4).

Table 3. Correlation matrix of 12 quantitative variables using Pearson method in 70 hazelnut genotypes of Fandoghloo, Iran

Variable	LL	LW	IW	NL	NT	NW	KW	KL	KT	K%	CI	TCSA
LL	1.000											
LW	0.774**	1.000										
IW	0.064	-0.028	1.000									
NL	-0.189	-0.240*	0.065	1.000								
NT	-0.160	-0.225	0.192	0.875**	1.000							
NW	-0.102	-0.191	0.298*	0.485**	0.547**	1.000						
KW	0.111	0.061	0.113	0.149	0.095	0.252*	1.000					
KL	-0.114	-0.190	0.160	0.615*	0.616**	0.494**	0.231	1.000				
KT	-0.065	-0.234	0.148	0.468**	0.487**	0.400**	0.250*	0.878**	1.000			
K%	-0.074	-0.086	-0.079	0.093	0.004	-0.075	-0.068	0.162	0.122	1.000		
CI	0.920	-0.026	-0.028	0.051	0.035	-0.201	-0.100	0.053	0.061	0.617**	1.000	
TCSA	-0.237*	-0.309*	-0.047	0.205	0.180	0.079	0.032	0.104	0.047	0.339**	0.187	1.000

*, ** Correlations significant at P<0.05 and P<0.01, respectively.

Table 4. Correlation matrix of 21 qualitative variables using Spearman method in 70 hazelnut genotypes of Fandoghloo, Iran

Variable	TGV	TGH	BD	S	LBD	FMBD	FFBD	LBS	ULH	PH	IL/NL	II	SII	ITBI	PN/C	NS	SNA	SPS	CNBS	NMD	KS
TGV	1.000																				
TGH	-.101	1.000																			
BD	.289*	-.179	1.000																		
S	.129	-.123	.487**	1.000																	
LBD	-.041	-.097	-.148	-.045	1.000																
FMBD	.060	-.057	.050	-.096	-.103	1.000															
FFBD	-.092	.121	-.197	-.101	-.026	-.034	1.000														
LBS	.013	-.074	-.164	-.073	-.110	-.024	.072	1.000													
ULH	.064	-.052	.006	.123	-.090	.177	-.095	.190	1.000												
PH	-.040	.036	-.049	.176	-.046	-.224	-.060	-.090	.039	1.000											
IL/NL	.092	.096	.033	-.016	-.079	-.006	001	.034	.007	.019	1.000										
II	.100	.183	-.134	-.032	-.050	-.129	.004	-.027	.009	-.117	-.029	1.000									
SII	.135	.000	.133	-.115	-.084	.103	-.121	.097	-.025	.044	.252*	-.340**	1.000								
ITBI	.169	-.076	-.042	-.059	-.024	.119	.066	.063	-.090	.050	.056	-.049	.038	1.000							
PN/C	.058	.012	.107	-.018	-.082	.080	-.085	.108	.069	.164	.197	.087	.085	-.062	1.000						
NS	-.038	.045	-.182	-.136	.103	103	-.134	.215	.049	.050	-.171	.094	.060	-.013	.034	1.000					
SNA	.125	-.114	-.074	-.032	-.039	-.072	-.172	.208	.124	-.087	.264*	-.129	.028	.051	.089	.120	1.000				
SPS	.058	-.116	-.100	.158	-.177	.221	-.057	.084	.126	-.032	.003	-.053	-.063	.138	.044	-.079	.157	1.000			
CNBS	-.159	-.105	.092	.123	-.101	.216	-.077	.030	.077	.050	-.049	-.277*	-.066	.000	.027	-.031	.033	.352**	1.000		
NMD	-.215	.090	-.137	-.048	-.133	.085	-.049	.001	-.025	-.001	-.143	.225	-.266*	-.119	-.116	.082	-.133	.026	000	1.000	

KS	.158	.045	.230	-.029	.041	.061	.040	-.013	-.184	-.047	.190	-.108	.090	-.150	-.028	.010	.177	.146	.052	-.054	1.000

growth vigor; TGH: Tree growth habit; BD: Branching density; S: Suckering; LBD: Leaf bud break date; FMBD: First male bud bloom date; FFBD: First female bud bloom date; LBS: Leaf blade shape; ULH: Under leaf hairiness; PH: Petiole hairiness; IL/NL: Involucre length compared to nut length; II: Involucre indentation; SII: Serration of indentations on the involucre; ITBI: Involucre thickness at base of involucre; PN/C: Predominant nut number per cluster; NS: Nut shape; SNA: Shape of nut apex; SPS: Size of pistil scar; CNBS: Curvature of nut basal scar; NMD: Nut maturity date; KS: Kernel shape.

Principal Component Analysis (PCA)

The PCA showed that, the first four components, based on quantitative variables, (λ1= 4.09, λ2=2.01, λ3 =1.71, λ4= 1.07) and the first nine components, based on qualitative variables, (λ1= 2.43, λ2=1.80, λ3= 1.65, λ4= 1.53, λ5= 1.39, λ6= 1.32, λ7=1.27, λ8=1.08, λ9=1.01) explained 74.2% and 64.3% of the total variation, respectively (Tables 5 & 6). Regarding quantitative characters, nut length, nut thickness, nut weight, kernel length and kernel thickness were found influential in the first component (PC1), explaining 34.1% of the total variation, this component was involved in nut and kernel traits. Characters that positively loaded on PC2, kernel percentage, kernel weight, chlorophyll index and TCSA, explained 16.8% of the total variation (Table 5). Based on quantitative traits, genotypes: 34, 16, 41, 70, 20, 8, 65, 62, 2, 3, 67, 27, 54, 68, 37, and 41 had the highest scores for PC1 and PC2 which indicate that they have the superior nut and kernel properties and may be considered for breeding programs in the future (Fig. 2). With respect to qualitative characters PC1 explained 11.6% of the total variation and was positively associated with tree vigor, branching density, suckering, first male bloom date, under leaf hairiness, involucre length compared to nut length, serration of indentations on the involucre, predominant nut number per cluster, curvature of nut basal scar, and kernel shape. Characters that positively loaded on PC2, including, petiole hairiness, nut maturity date, branching density and suckering, explained 8% of the total variation, (Table 6). Based on qualitative traits, genotypes of numbers 10, 46, 68, 52, 23, 44, 42, 29, 51, 65, 26, 16, 43, 15, 31, 63, 70, 62, 57, and 69 were placed in the first two components (PC1 and PC2) (Fig. 3).

Table 5. Eigenvalues, proportion of total variability as well as eigenvector and correlation between 12 quantitative variables and 11 principal components (PCs) for 70 hazelnut genotypes

Item	PC axis										
Eigenvalue	4.09	2.01	1.71	1.07	0.893	0.783	0.657	0.356	0.195	0.110	0.081
Proportion	0.341	0.168	0.143	0.089	0.074	0.065	0.054	0.029	0.016	0.009	0.007
Cumulative	0.341	0.509	0.652	0.742	0.816	0.881	0.936	0.966	0.982	0.992	0.999
Variable	Eigenvector										
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
LL	-0.320	-0.179	0.843	-0.040	0.155	0.052	0.168	0.154	-0.262	0.011	-0.320
LW	-0.439	-0.197	0.769	-0.061	0.246	-0.039	0.063	-0.178	0.261	-0.012	-0.439
IW	0.235	-0.268	0.183	0.662	-0.351	0.503	0.066	-0.130	0.000	0.024	0.235
NL	0.798	-0.167	-0.011	-0.259	0.325	0.217	-0.208	-0.126	-0.081	0.205	0.798
NT	0.803	-0.258	0.022	-0.164	0.278	0.299	-0.185	-0.033	-0.005	-0.236	0.803
NW	0.704	-0.336	0.066	0.425	0.189	-0.290	-0.065	0.268	0.074	0.0166	0.704
KW	0.724	0.356	0.251	0.385	0.099	-0.331	-0.095	-0.029	-0.028	-0.005	0.724
KL	0.824	-0.188	0.178	-0.275	-0.262	-0.096	0.189	-0.065	0.129	0.073	0.824
KT	0.725	-0.188	0.163	-0.312	-0.434	-0.118	0.26	0.054	-0.059	-0.059	0.725
K%	0.314	0.839	0.273	0.046	-0.077	-0.132	-0.068	-0.280	-0.086	-0.039	0.314
CI	0.142	0.718	0.346	-0.156	-0.178	0.330	-0.230	0.329	0.124	0.028	0.142
TCSA	0.334	0.496	-0.247	0.082	0.400	0.211	0.603	0.050	0.042	0.004	0.334

LL: Leaf length; LW: Leaf width; IW: Involucres weight; NL: Nut length; NT: Nut thickness; NW: Nut weight; KW: Kernel weight; KL: Kernel length; KT: Kernel thickness; K %: Kernel ratio; CI: Chlorophyll index; TCSA: Trunk cross sectional area.

Table 6. Eigenvalues, proportion of total variability as well as eigenvector and correlation between 21 qualitative variables and 11 principal components (PCs) for 70 hazelnut genotypes

Item	PC axis										
Eigenvalue	2.43	1.80	1.65	1.53	1.39	1.32	1.27	1.08	1.01	0.994	0.920
Proportion	0.116	0.086	0.079	0.073	0.066	0.063	0.061	0.051	0.048	0.047	0.044
Cumulative	0.116	0.202	0.281	0.354	0.421	0.483	0.544	0.595	0.643	0.691	0.734
Variable	Eigenvector										
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
TGV	0.256	-0.081	0.248	0.203	-0.188	0.329	0.323	-0.103	-0.176	0.046	-0.119
TGH	-0.194	-0.094	0.067	0.192	0.430	-0.039	-0.065	-0.313	-0.106	-0.161	-0.322
BD	0.405	0.317	0.190	0.177	-0.083	0.062	-0.007	-0.133	0.049	0.043	0.102
S	0.292	0.415	-0.037	0.189	-0.065	0.059	0.101	0.055	-0.035	0.122	0.054
LBD	-0.162	0.046	0.161	-0.200	-0.402	0.158	-0.309	0.125	-0.123	-0.382	-0.206
FMBD	0.231	-0.116	-0.297	-0.179	0.040	0.149	0.131	-0.382	-0.056	-0.447	0.265
FFBD	-0.202	-0.025	0.136	-0.192	0.343	0.278	-0.031	0.179	-0.463	0.175	0.139
LBS	0.014	-0.474	-0.141	0.081	-0.085	-0.119	0.015	0.147	-0.193	0.394	0.229
ULH	0.182	-0.088	-0.300	0.259	-0.059	0.062	-0.236	-0.048	-0.355	0.174	0.156
PH	0.076	0.229	0.121	0.183	0.091	-0.546	0.013	0.038	-0.304	0.072	-0.381
IL/NL	0.201	-0.277	0.222	0.143	0.402	0.008	-0.086	0.203	0.271	-0.121	-0.027
II	-0.257	-0.026	-0.086	0.501	0.027	0.263	0.267	0.089	-0.020	-0.143	-0.052
SII	0.241	-0.248	0.337	-0.165	0.051	-0.311	-0.006	-0.385	0.018	0.068	0.174
ITBI	0.067	-0.122	0.061	-0.314	0.036	-0.008	0.621	-0.017	-0.218	-0.059	-0.242
PN/C	0.172	-0.121	0.075	0.292	0.084	-0.214	-0.013	0.333	-0.224	-0.562	0.284
NS	-0.084	-0.288	-0.205	0.189	-0.406	-0.206	-0.044	-0.254	-0.161	-0.079	-0.250
SNA	0.251	-0.336	-0.075	0.031	-0.103	0.037	-0.015	0.279	0.405	0.055	-0.306
SPS	0.277	-0.004	-0.391	-0.107	0.156	0.115	0.138	0.178	-0.041	0.028	-0.295
CNBS	0.203	0.161	-0.399	-0.262	0.168	-0.176	-0.142	0.138	-0.11	-0.116	-0.092
NMD	-0.218	0.110	-0.314	0.194	0.196	-0.070	0.116	-0.285	0.279	0.043	0.079
KS	0.232	-0.096	0.043	0.043	0.167	0.371	-0.439	-0.262	-0.114	0.065	-0.291

TGV: Tree growth vigor; TGH: Tree growth habit; BD: Branching density; S: Suckering; LBD: Leaf bud break date; FMBD: First male bud bloom date; FFBD: First female bud bloom date; LBS: Leaf blade shape; ULH: Under leaf hairiness; PH: Petiole hairiness; IL/NL: Involucre length compared to nut length; II: Involucre indentation; SII: Serration of indentations on the involucre; ITBI: Involucre thickness at base of involucre; PN/C: Predominant nut number per cluster; NS: Nut shape; SNA: Shape of nut apex; SPS: Size of pistil scar; CNBS: Curvature of nut basal scar; NMD: Nut maturity date; KS: Kernel shape.

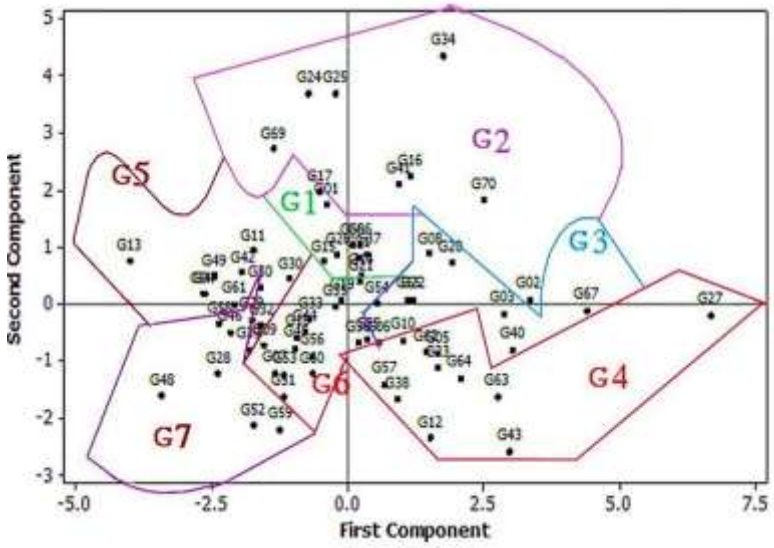

Fig.2. Scatter plot for 70 hazelnut genotypes based on the first two principal components (PC1/PC2). Produced by Ward's cluster analysis, based on 12 quantitative characters

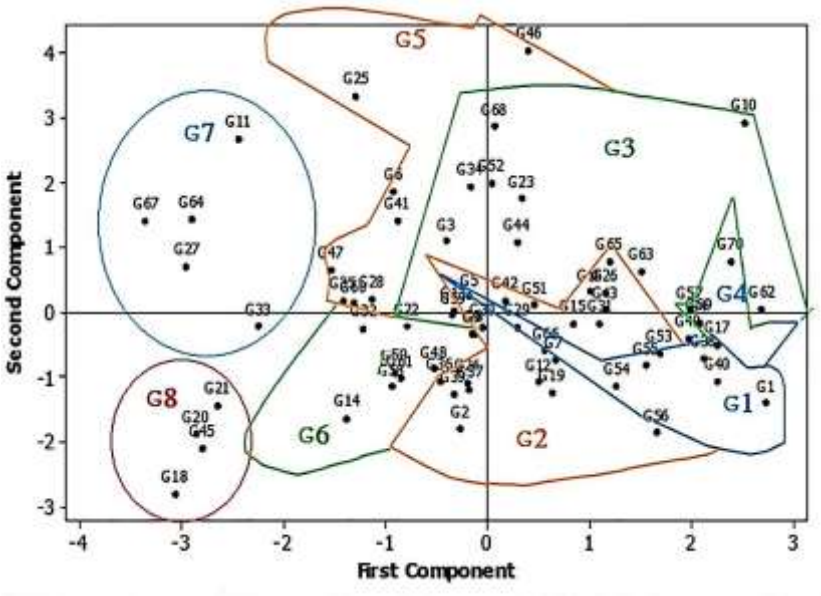

Fig.3. Scatter plot for 70 hazelnut genotypes based on the first two principal components (PC1/PC2). Produced by Ward's cluster analysis, based on 21 qualitative characters

Cluster analysis

According to a dendrogram generated by Ward's method based on squared Euclidean distance, 70 hazelnut genotypes were classified into seven and eight separate groups based on quantitative and qualitative variables, respectively. Regarding the dendrogram generated based on quantitative variables, cluster I, containing 13 genotypes, was the largest one followed by clusters II and III, and each contained 12 genotypes (Fig. 4). According to qualitative traits, clusters III and V, as the largest groups, each contained 11 genotypes (Fig. 5). Some of the genotypes were not grouped according to their geographical distribution.

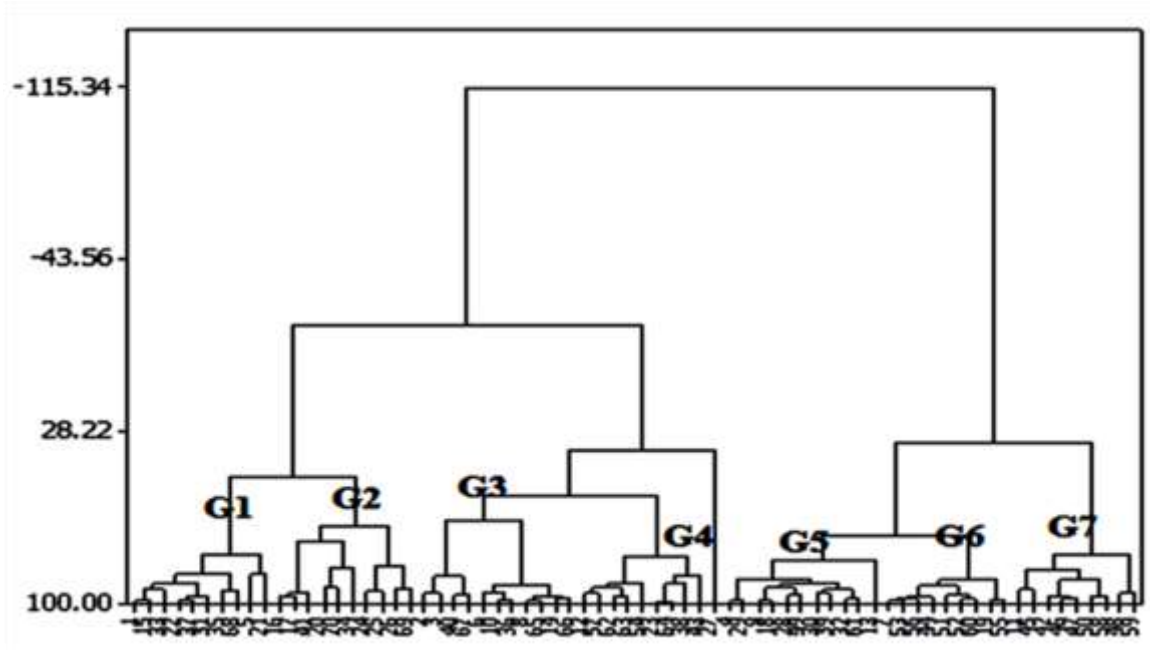

Fig.4. Dendrogram for the 70 hazelnut genotypes collected from Fandogloo jungle produced by Ward's cluster analysis, based on 12 quantitative characters (scale. Squared Euclidean distance)

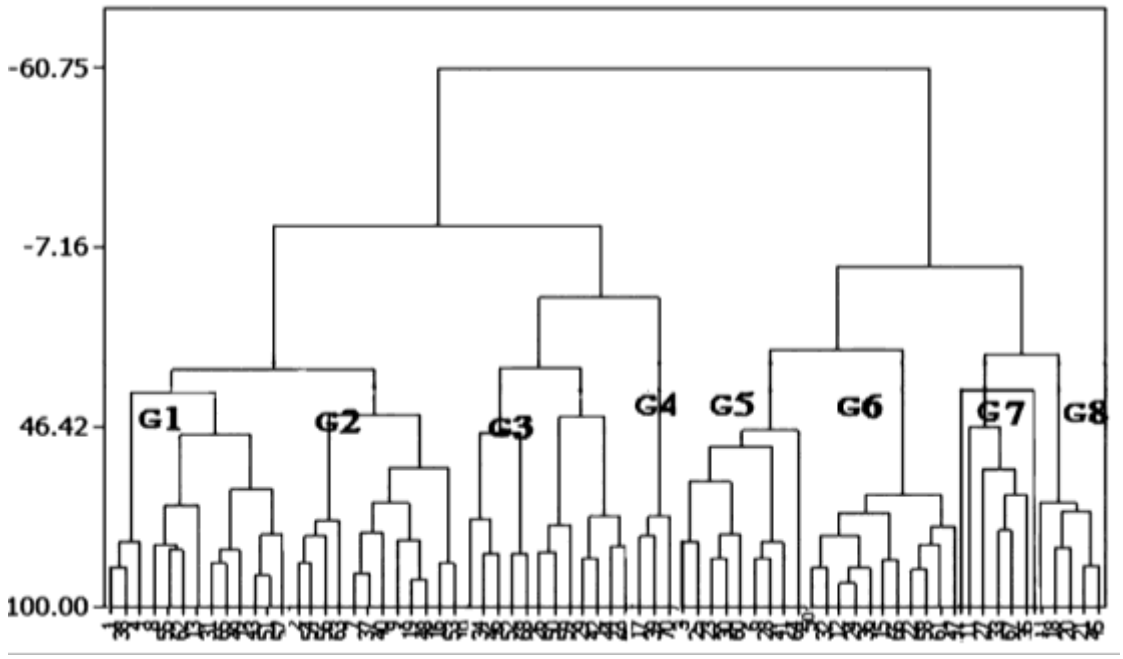

Fig.5. Dendrogram for the 70 hazelnut genotypes collected from Fandogloo jungle produced by Ward's cluster analysis, based on 21 qualitative characters (scale. Squared Euclidean distance).

Discussion

The results revealed that there was a significant variation present across multiple traits which indicates a high level of phenotypic diversity in the hazelnut germplasm of Fandogloo. Generally, genotypes that located in the eastern part were tall shrubs with larger leaf size, compared to genotypes located in the western part of Fandogloo. In the majority of studied genotypes, length of involucres or husk was typically as long as length of nut, and nut and kernel shapes were ovoid and conical, respectively. Diversity in nut and involucre morphology provides opportunity for breeders to select superior genotypes suitable for breeding purposes (Menesatti *et al*., 2008; Boccacci *et al*., 2013; Bacchetta *et al*., 2015). Involucre morphology and suckering habit are important traits used for identification and commercial production of *Corylus* species (Rushforth, 1999). The shorter involucres leads to the easier separation and falling of the nuts at the maturity time and results reduction of harvest cost. Low suckering in terms of saving time and labor costs is desirable characteristics of hazelnuts (Julian *et al*., 2008). Low suckering genotypes are used widely in the hazelnut breeding program (Hosseinova and Pirkhezri 2010).

Mohammadzadeh *et al*. (2014), reported nut weight ranged from 0.90-2.87 g and kernel weight varied from 0.20-1.17 among 35 landraces of Iranian hazelnuts along with six commercial cultivars. In a study on Serbian hazelnuts the nut and kernel weights ranged from 0.42-1.4 and 0.09- 0.50 g, respectively (Miletić *et al*., 2007). Kernel to shell ratio is very important as nuts with higher kernel to shell ratio require less drying time, are easier to crack, and have a higher yield of kernels per volume of in-shell nuts. Thus, genotypes with a high kernel percentage are more desirable as cultivars and as parents in breeding programs (Germain, 1997; Korac *et al*., 1997). Kernel to shell ratio in hazelnuts shows a little variation among genotypes, years and locations and has been reported to be highly heritable (Thompson *et al*., 1996). In this study, the evaluated hazelnut genotypes from Fandogloo had, in general, a lower nut weight, but higher kernel weight, kernel percentage, and also thin shells, compared to populations studied from other regions of Iran (Nejatian *et al*., 2012), Turkey (Bostan and Islam 1999), Spain (Ferriera *et al*., 2010), and Serbia (Miletić *et al*., 2007); however, kernel ratios of 53.2% and 47.5% have been reported for some hazelnut clones from Ordu province, Turkey (Balık and Beyhan, 2014) and (*C. heterophylla* × *C. avellana*) hybrids from northern China (Liang *et al*., 2008).

Native wild genotypes, although very well adapted to local climatic conditions, do not produce commercially valuable crops as the nuts are generally too small and have thick shells (Glen and Holmstrom, 2012). Considerable phenotypic variation observed in the studied genotypes, which indicates a high level of genetic diversity and make them potentially candidates for use in hazelnut breeding in future.

Positive correlations were observed among nut and kernel dimensions in studied hazelnut genotypes. Yao and Mehlenbacher (2000), also reported strong correlations among nut size traits (nut length, nut width, and nut diameter), nut weight and kernel weight in hazelnut. Positive correlations were found between kernel ratio and nut weight (r=0.558), and nut thickness (r=0.415) in some Iranian hazelnut cultivars (Hosseinova and Pirkhezri, 2010). Correlations between nut weight with nut and kernel dimensions were significantly positive within Turkish hazelnut clones (Bostan and İslam, 1999). Positive correlations were also reported among nut and kernel dimensions in other nut crops. For instance, a strong correlation was reported between nut weight and nut thickness (r= 0.71), and nut length (r= 0.55) in persian walnut (*Juglans regia*) (Ebrahimi *et al*., 2015). Branching density was positively correlated with tree vigor and suckering, which was in agreement with previous findings by Monastra and Raparelli (1997). Response to direct selection for these variables may be unpredictable unless there is a good control of environmental variables. Low variance in eigenvectors among qualitative characters may be due to existing low correlations between characters studied (Abdi and Williams, 2010).

The PCA showed that, the first four components among quantitative variables and first nine components of qualitative variables explained 74.2% and 64.3% of the total variation, respectively. The change points of the variance extracted by the 11 PCs in quantitative traits showed that, the variance described by the single main components decreased strongly between the PC1 and PC2, while in terms of qualitative traits, this decreasing was very slow (Tables 5, 6). The reason for the high number of specific vector values of the qualitative variables studied, compared to quantitative traits as well as low variance between the components of qualitative traits is due to the low correlations between qualitative traits, compared to quantitative ones. In addition, the type of measurement of both quantitative and qualitative traits can also affect the number of specific vectors and variances corresponding to them (Abdi and Williams, 2010).

PCA among 21 morphological variables in seven *Corylus avellana* cultivars in Iran, revealed that the seven principal components accounted for 80.3% of total variation from which PC1, PC2, and PC3 accounted for 26.3%, 15%, and 11.2% of total variation, respectively. Loaded variables on PC1 were nut weight, nut length, nut thickness, kernel weight, kernel percentage. PC2 was associated with oil percentage, chlorophyll a and b, and PC3 was related to vegetative growth, and TCSA variables (Hosseinova and Pirkhezri, 2010). PCA among 15 morphological variables in 41 genotypes of *Corylus colurna* in India showed that, the seven principal components were accounted for 77.9% of the total variation. PC1 was accounted for 22.26% of total variation and was correlated to kernel thickness, protein content, kernel weight, nut length and the number of nuts per cluster. PC2 explained 15.8% of the total variation and it was mainly associated with shell thickness and kernel thickness (Srivastava *et al*., 2010). In PCA based on 14 morphological variables for 42 hazelnut genotypes from southern Europe and 11 reference cultivars, the first two components explained 38.7% of the total variation. PC1 accounted for 25.1% and was positively correlated with nut and kernel weight while PC2 accounted for an additional 13.6% and it was mostly associated with nut and kernel shape (Boccacci *et al*., 2013). Based on quantitative traits, genotypes 34, 16, 41, 70, 20, 8, 65, 62, 2, 3, 67, 27, 54, 68, 37 and 41 had positive coefficients in both PC1 and PC2 (Fig.2). These genotypes had higher kernel weight, chlorophyll index, trunk cross-sectional area (TCSA) and kernel percentage. According to qualitative traits, genotypes 10, 46, 68, 52, 23, 44, 42, 29, 51, 65, 26, 16, 43, 15, 31, 63, 70, 62, 57 and 69 had positive coefficients in both PC1 and PC2 (Fig.3). These genotypes had a higher branch density, and the flowering time of their male flower buds was earlier, but the

flowering time of their female flower buds was later than other genotypes. According to PCA, the variables such as: leaf length, leaf width, tree growth vigor, tree growth habit, leaf bud break date, involucre thickness at base of involucre, shape of nut apex. kernel shape, petiole hairiness, nut shape, and size of pistil scar had the lowest value of PCs and it seems that they are less affected by environmental conditions.

Hierarchical cluster analysis was utilized to investigate the similarities and dissimilarities among the genotypes with respect to nut and kernel variables. The high number of generated clusters for both sets of quantitative and qualitative variables showed that there is an intensive in-population diversity in hazelnuts of Fandogloo gene pool which supply desirable potential for using in the next breeding programs. Genotypes with highest inter-cluster distance may be used in hybridization programs. In contrast, lower inter-cluster distance indicates close relationship and similarity among genotypes and selecting parents from these clusters should be avoided (Sirvastava *et al*., 2010). Grouping of genotypes on the basis of both qualitative and quantitative characters showed that, genotypes in the same region could be grouped separately regardless of their geographic origin. Similarly, in a study of 41 genotypes of *C. colurna*, 9 clusters were generated and the pattern of clustering was also independent from the pattern of their geographical distribution (Sirivastava *et al*., 2010), and provides an example of the wide phenotypic variation found in *Corylus avellana* (Mehlenbacher, 1991).

Conclusions

The present research was conducted in the Fandogloo jungle, which holds one of the most important hazelnut (*Corylus avellana*) gene pools in Iran. Thus, these native genotypes may be useful in the breeding of improved plants exhibiting enhanced adaptation to harsh (cold/semi-dry) environments. Cold tolerance of the promising genotypes of this gene pool need to get more carefully assayed in the lab in future studies (Aslamarz et al., 2010). Our results revealed a high diversity in morphological and phenological characteristics in the 70 genotypes studied, supporting the presence of a high level of genetic diversity in the region. Hazelnut genotypes studied had lower nut weight, but higher kernel weight and kernel to shell ratio, compared to genotypes evaluated from other regions of Iran. PC1 in quantitative and qualitative variables was associated with nut and kernel traits, and vegetative and reproductive properties, respectively. The pattern of genotype grouping was independent of their geographical distances.

In summary, the analysis of multiple phenological and pomological traits of the hazelnut genotypes of Fandogloo documented the significant variation present in the *in situ* hazelnut gene pool found in the region and supports the needs to conserve this valuable resource. The results may also help breeders choosing the appropriate individual genotypes and utilize them as parents in a breeding program for improving future generations of commercial cultivars with improved adaptation to harsh climatic condition.

Acknowledgements

This work is a part of the PhD thesis of Javad Farrokhi Toolir, which was carried out in Bu-Ali Sina university. Hence The authors respectfully thank ACECR, Ardabil branch for providing financial support for this study, and Bu-Ali Sina University for providing laboratory facilities. The authors also thank Dr. Hamid Hatami Maleki, university of Maragheh, for generously sharing his knowledge and experience.

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