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Original Article

Morphophysiological and Phytochemical Diversity of Hazelnut (*Corylus avellana* L.) Populations in Northwestern Iran

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ABSTRACT

Due to the nutritional and pharmaceutical importance of hazelnut (*Corylus avellana* L.), the morphophysiological and phytochemical diversity of 15 Iranian hazelnut populations with two commercial cultivars were studied. The genotypes had significant differences in morphophysiological and phytochemical characteristics. The significant variance among genotypes ($p < 0.01$) and high genetic variance ($\sigma^2 g$) indicated the divearsity in most traits. The highest coefficient of genetic changes was related to the percentage of fruit kernel, kernel width, calcium, phosphorus, total phenol and taxol. The main components did not correspond to different traits. Mantel test showed that the correlation between the groupings based on morphological traits and climatic variability was 0.67%, while it was 0.51% for the phytochemical traits and 0.41% for taxol. The significant diversity among hazelnut genotypes and also the mismatch with climatic diversity indicated the role of genetic factors on traits, which can be used in future breeding programs to produce superior hazelnut cultivars.

INTRODUCTION

Corylus avellana L., with the common name Hazelnut, belongs to the Betulaceae family. Hazelnut is a dicotyledonous plant with genome formula (2x = 2n = 22). It's the sporophytic self-incompatible plant. The Corylus genus includes 25 species, nine of which are economically and racially important [1].

Most hazelnut species have shrub shapes and are rarely seen as tree. The geographical distribution and main habitats of hazelnuts are concentrated in the northern hemisphere and on the coastal areas of seas and oceans with mild winters and cool summers [2]. Hazelnut is grown in a few areas of Iran with high rainfall and high relative humidity, as high relative humidity is a major factor for successful hazelnut production [3]. The natural habitats of Iranian Hazelnut are mainly located in the Eshkevarat, Navan, Dinochal, Goli Dagh, Alamut, Tarom, and Ardabil. Based on International and regional data, Turkey is the first (420000 tons/year), and Iran is the seventh hazelnut world producer (16,327 tons/year) (https://www.atlasbig.com/en-us/countries-hazelnut-production). The global area under hazelnut cultivation is estimated to be 660,000 hectares, with a total production of about 730,000 tons. Hazelnut yield in Iran is low (912.2 Kg/hectare), while in major hazelnut-producing countries, this amount is 4.5 to 5 tons per hectare [4]. Iran is one of the main hazelnut producers in the world, with an annual production of 14,299 tons. Hazelnut is grown in a few areas of Iran with high rainfall and high relative humidity as high relative humidity is a major factor for successful hazelnut production [3,5-8].

Hazelnut kernels have an important role in human nutrition and health due to having about 60% oil, 17.5% carbohydrates, 13 to 17% protein and large amounts of essential fatty acids, minerals, and vitamins [9]. Hazelnut is the only fruit that has all 20 essential amino acids of the human body and, due to its high amounts of phosphorus, can strengthen and improve brain functions [10]. Hazelnuts are very important in terms of phytochemicals. The presence of secondary metabolic compounds such as phytosterols, antioxidants, phenol, and the anti-cancer diterpenoid substance called paclitaxel has also made this product medically important. The important point is the extraction of Taxol from the excess and mostly unused parts of different parts of this plant, such as green bark (leaf), tree bark, and old fallen leaves of the tree. With further study, hazelnuts can be introduced as an alternative to yew in taxol extraction [11]. Hazelnut secondary metabolites are also recommended for the treatment of diseases such as MS, cancer, chronic kidney disease, Alzheimer's disease and psoriasis [12]. Genetic diversity analysis serves as a key strategy in plant improvement and is the first step in identifying and preserving hereditary reserves [13]. Studying the diversity of existing germplasms and recognizing their genetic relationships are valuable goals in improving species conservation and breeding strategies [14]. The study of genetic diversity can also be used as an effective aid in selecting individuals for the entry of desirable genes from diverse germplasms into the existing genetic base [15]. Because with the entry of desirable genes into the population, the quantity and quality of products can be increased [14]. However, due to the expansion of the population habitation in forested areas, many wild hazelnut trees, which can be considered valuable genetic resources, are endangered. Considering the economic importance of hazelnuts and their high processing capability, as well as the uncertainty of the genotypes in the Gilan and Ardabil hazelnut regions, it seems absolutely necessary to prepare a complete identity card of the available hazelnuts [16]. The biodiversity of diploid hazelnuts is influenced by diversity in the structure of the chromosome karyotype. Some of the genotypes have reported a total of 22 metacentric chromosomes, some 20 metacentric chromosomes and two sub-metacentric chromosomes, others 16 metacentric chromosomes and six sub-metacentric chromosomes and the second factor that influenced biodiversity is the presence of the wide range of self-incompatible genes in hazelnuts, so far 25 alleles of self-incompatibility have been reported in hazelnuts [17]. The third-factor determination of genetic diversity in hazelnuts is intraspecific and interspecies intersections [18].

Evaluation of morphophysiological aspects and phytochemical composition of fruit is one of the reliable tools to identify and describe the biodiversity of hazelnut genotypes [16]. Given that the first step in any breeding program is to have high genetic diversity to achieve the goals, so germplasm resources should be collected and evaluated. By studying the genetic diversity in a plant species, in addition to identifying existing genotypes and creating a collection to preserve genetic resources, it is possible to better organize and manage the existing germplasm and provide a basis for the cultivation of promising genotypes [19]. The availability of reliable data from the assessment of plant masses and cultivars, climatic conditions, topography, and density of natural habitats is a primary and important prerequisite for the adoption of policies for rehabilitation, exploitation, cultivation, and economic development of hazelnuts in each region [20]. For data classifying, an appropriate statistical method is needed that can simultaneously analyze multiple data for each genotype. Multivariate analysis, such as principal component analysis, has been used in determining the genetic diversity of several fruit species, including olive [21], pomegranate [22], and hazelnut [23]. Principal component analysis was used to determine some fruit chemical compounds in hazelnut genotypes [24]. Examination of 15 morphological traits among 41 hazelnut genotypes in India by multivariate analysis led to the creation of nine distinct groups between genotypes [25]. Multivariate statistical analysis was used to determine characteristics such as the shape and size of Hazelnut [24]. The purpose of this study was to investigate the biodiversity analysis of hazelnut genotypes in habitats in northwestern Iran using multivariate methods such as principal component analysis and cluster analysis as well as correlation analysis of fruit characteristics of each genotype by environmental conditions of their growth place.

MATERIALS AND METHODS

This study was performed from 2020 to 2021 on 17 hazelnut genotypes (15 Iranian genotypes and two commercial cultivars) in Fandoghlou frosts (Mehdi Posti, Asigran, Abibeyghloo, Arsabaran, Miyaneh, and Astara) (Table-1).

These genotypes were selected from the habitat of this plant in Iran, including the forest areas of Ardabil, Ahar, and Miyaneh. The geographical location of each tree was recorded by a GPS device (model). Using ArcGIS software, information about geographical characteristics such as average annual rainfall, annual temperature, altitude, number of frost days, and soil acidity of the sampled sites were recorded and matched with previous information available at the meteorological station and the General Department of Natural Resources.

The morphophysiological traits such as tree height, number of offshoots, leaf length and width, Spad value, trunk cross-sectional area, and fruit characteristics, including kernel thickness, kernel length and width, fruit thickness, length and width, glume weight, and percentage of the kernel to fruit were assessed. Also, kernel percentage (ratio of dried kernel weight to fruit weight), total carbohydrate, oil percentage, protein and taxol content were evaluated. The environmental traits, including average annual rainfall and temperature, altitude, number of frosty days and soil acidity, were measured (Table- 2).

The oil percentage was determined by the Soxhlet method with oil ether solvent [26]. The protein and carbohydrate amounts were measured by Kjeldahl [27] and Shlighel [28] methods, respectively. HPLC device (Knauer, Germany) equipped with c-18 column (µm, 250 c 4.6mm Teknokrome, Germany Perfectsil Target ODS3,5) and taxol standard (SIGMA, CAS Number: 33069-62-4) were used to determine the taxol concentration. A gas chromatography device (GC-7890A-USA) was used to determine fatty acid compositions. The amount of total phenol was measured by the Folin-Ciocalteu method [29] and the total flavonoid content was measured by the method proposed by Kaijv (Kaijv *et al*., 2006) (30) through a spectrophotometer device (Specorp250 Jena-History). Antioxidant capacity was measured by DPPH (RSA) [31,32].

The phosphorus content was measured by the calorimetric method using a spectrophotometer device. The potassium content was measured by a flame photometer device (model Jenway PFP7 made in England). The content of calcium, magnesium, iron and zinc was measured using an atomic absorption device (AS. A 2000-CTA size). The amounts of hazelnut kernel elements were expressed in mg/kg of dry matter.

Descriptive statistics, including mean, standard error and coefficient of variation, were calculated for the study traits. For estimation of genetic and coefficient of variation, index Expected Mean Squares in Nested design analysis were used.

Relationships between traits were calculated by the Pearson correlation coefficient. To identify the pattern of changes, the principal component analysis was used. The clustering of genotypes into similar groups was performed using the Ward method and based on the Euclidean square distance. The Mantel test was used to adapt the groupings obtained from different traits in the study of genetic diversity. First, a matrix similarity table was extracted from each test, and the soft correlation matrix between the correlation matrices was calculated using the SAS software. All statistical analyses were run using SPSS software, version 16.

Table 1 Names of studied genotypes

G16	1	Ardabil, Asighran
G24	2	Ardabil, Asighran
G28	3	Ardabil, Asighran
G29	4	Ardabil, Fandoghloo
G34	5	Ardabil, Fandoghloo
G41	6	Ardabil, Fandoghloo
G14	7	Ardabil, Mehdiposti
G4	8	Ardabil, Mehdiposti
G8	9	Ardabil, Mehdiposti
G47	10	East Azarbaijan, Arasbaran
G53	11	East Azarbaijan, Arasbaran
G59	12	Eas Azarbaijan, Arasbaran
G64	13	East Azarbaijan, Mianeh
G69	14	East Azarbaijan, Mianeh
G74	15	East Azarbaijan, Mianeh
16	16	Fertile, Astara, Gilan
17	17	Daviana, Astara, Gilan

RESULTS

Results of Analysis of Variance Between Populations for the Study Traits

Significant differences were observed between populations in leaf width and length, kernel weight, fruit weight, fruit thickness, trunk cross-sectional area, moisture, protein, fat, fibre, and carbohydrate percentage, total phenol and palmitoleic acid ($p< 0.01$). Populations were significantly different in terms of kernel width, fruit length and width, glume weight, percentage of the kernel to fruit, percentage of magnesium, palmitic acid, and percentage of ash at 0.05 probability level ($p< 0.05$), but there was no significant difference for other traits among the populations.

Therefore, for significant traits, the existence of genetic diversity between populations can be understood. The presence of germplasm richness in the study of hazelnut populations for significant traits indicates the potential of these populations in hazelnut breeding programs (Table-3).

While parameters such as kernel length, fruit length and width, fruit thickness and glume weight, percentages of phosphorus and potassium, antioxidant properties and ash content, fat percentage and tree height were also greater than the mean. In terms of the percentage of iron, calcium and zinc, the study populations were at the mean level. In Arasbaran genotypes, traits of kernel thickness, length, and width, length, width and thickness of the fruit as well as glume weight and percentage of phosphorus, potassium, calcium and iron and percentage of ash, fibre and cross-sectional area of the trunk had a mean lower than the total mean. Antioxidant properties, taxol percentage, stearic acid percentage, kernel to fruit percentage and fat percentage were also above the mean in the Arasbaran population. In the middle population, the traits of thickness, length, width and weight of the kernel and the weight of glume, the percentage of Taxol, stearic and oleic acid, the percentage of the kernel to fruit, the cross-sectional area of the trunk and the percentage of fat were above the mean.

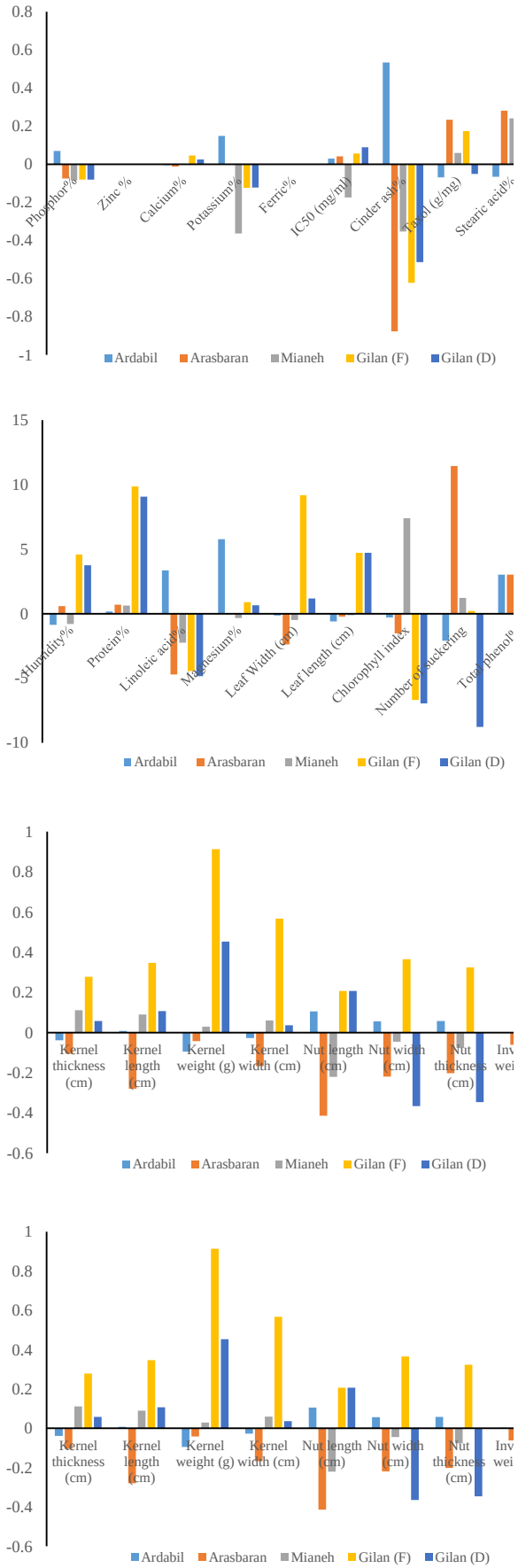

Fig. 1 Comparison of the deviation of the traits in the studied populations from the average of the total population

The percentage of phosphorus, zinc, potassium and iron, the level of antioxidant properties, the percentage of ash and tree height and the percentage of fibre were lower than the mean. The percentage of iron and calcium did not differ from the mean in this population. In the fertile cultivar, traits of thickness, length, and width of the kernel, length, width and thickness of the fruit, glume weight, amount of antioxidant properties and percentage of Taxol, carbohydrate, oleic acid, percentage of the kernel to fruit, cross-sectional area of the trunk, percentage of fat and fibre were greater than the mean. In the Daviana cultivar, kernel weight, fruit width and length, fat percentage, and trunk cross-sectional area were significantly mean than the mean. Fruit weight and thickness, taxol percentage, stearic acid percentage and tree height were also significantly lower than the mean in this cultivar. The lower value than the mean for Taxol in the modified Daviana cultivar was interesting.

Results of Analysis of Variance Between Genotypes

The existence of genetic diversity of traits between populations and within populations caused every single tree to be considered as a genotype, and analyses were performed based on genotypes. Among the genotypes, in terms of the number of offshoots, Spad Value, percentage of kernel to fruit, phosphorus, calcium, potassium, moisture, percentage of protein, fat, fiber, carbohydrates, percentage of total phenol, flavonoids, amount of antioxidant properties, taxol percentage, palmitic acid percentage, palmitoleic and stearic acid percentage, oleic and linoleic acid percentage were significantly different ($p< 0.01$) (Table-4). Genotypes had significant differences for ash ($p< 0.01$), but no significant difference was observed for other traits among genotypes ($p> 0.05$). Calculation of genetic variance and genetic CV by removing the effect of environment on diversity revealed changes due to genetic effect on diversity. The amount of genetic variance for significant traits ranged from 18792.63 for zinc to 408.82 for flavonoids. Comparing the genetic diversity without considering (CVg) unit showed that the highest coefficient of genetic variation was related to the maximum of 100% for the traits of phosphorus and flavonoid percentage. Investigation of appropriate and significant diversity for phytochemical and medicinal properties such as total phenol content, percentage of flavonoids, antioxidant properties, taxol and fatty acids percentage indicates the potential for medicinal and economical use of hazelnut genotypes in the study areas. Therefore, for significant traits, the existence of genetic diversity between genotypes can be understood. The presence of germplasm richness in the study of hazelnut genotypes for significant traits indicates the potential of these populations in hazelnut breeding programs.

Radar plots for offshoots in G16, G39, G53 genotypes, for leaf width traits in Fertile genotypes of G28, G41, G69, G16, G24 had the highest mean, while for these morphological traits, the Daviana cultivar had the lowest mean (Fig. 2). In terms of fatty acid composition and contents of Taxol and active ingredients, cultivars were also compared, and the results are shown in Figure 2.

Principal Component Analysis

The results of principal component analysis for morphological traits are given in (Tables 5 and 6).

The five principle components showed 82.9% of the total genotype variations meaning that the first five components were able to show about 83% of the total genotype variations (Table-6A). The most effective coefficients of traits in PC2 were kernel length, kernel width and thickness, all of which were related to the functional components of Hazelnut. The highest coefficients of change were related to leaf length, leaf weight, kernel weight, and fruit weight. Principal component analysis based on phytochemical variables is listed in Table-6B.

The four principle components showed 80.20% of the variations in the subtypes based on phytochemical traits. PC1, which allocated 36% of the changes to itself, was positive for carbohydrates and protein content based on the Kjeldahl method. PC2, with 25% of the variation's changes, had the highest impact coefficients inversely from stearic, palmitic and oleic acid and the highest positive coefficient was related to the iron percentage. Principal component analysis was performed separately based on fatty acid and taxol traits (Table-6C). The results revealed that based on these traits, genotypes with three principle components allocated 81.88% of the total variability to themselves. So, PC1, with 39.84% changes for stearic and oleic acid, Taxol and palmitic acid traits had the highest coefficients. PC2 had the highest coefficients for flavonoid and linoleic acid traits.

Table 2 Average geographical and climatic characteristics of distribution areas in the study hazelnut populations

No.	Province	Location name	Latitude (N)	Longitude (E)	Altitude (m)	Mean Temp. (°C)	Rainfall (mm/year)	Mean Annual frosty days	Solar radiation (W/m^2)	Soil PH
1	Ardabil	Asighran	38° 24'.508 N	48° 32'.966 E	1515	9.12	356.6310	134.38	750.934	6.51
2	Ardabil	Asighran	38° 23'.997 N	48° 32'.488 E	1448	9.19	356.5410	134.6	690.902	6.42
3	Ardabil	Asighran	38° 23'.760 N	48° 33'.493 E	1455	9.03	356.6850	134.5	821.885	6.47
4	Ardabil	Fandoghloo	38° 17'.721 N	48° 38'.009 E	1405	8.24	371.1580	134.4	792.958	7
5	Ardabil	Fandoghloo	38° 17'.790 N	48° 37'.935 E	1371	8.25	371.1580	134.4	792.958	6.13
6	Ardabil	Fandoghloo	38° 17'.645 N	45° 38'.005 E	1399	8.23	371.1580	134.4	776.503	6.42
7	Ardabil	Mehdi Posti	38° 20'.109 N	48° 35'.547 E	1391	8.39	356.0410	134.6	777.213	6.52
8	Ardabil	Mehdi Posti	38 ° 20'.236 N	48° 35.'669 E	1422	8.42	356.0410	134.6	792.958	6.62
9	Ardabil	Mehdi Posti	38° 20'.215 N	48° 35'.700 E	1412	8.44	356.0410	134.5	792.958	6.94
10	East Azerbaijan	Miyaneh	37° 65'.933 N	47° 50'.340 E	1894	8.13	318.02	135.6	732.1	6.32
11	East Azerbaijan	Miyaneh	37° 46'.081N	47° 50'.376 E	1923	8.35	323.16	135.1	733.1	6.39
12	East Azerbaijan	Miyaneh	37° 46'.145N	47° 50'.409 E	1960	8.22	307.2	135.2	735.6	6.72
13	East Azerbaijan	Arasbaran	38° 21'.755 N	47° 16'.338 E	1779	11.25	427.6040	101	673.565	6
14	East Azerbaijan	Arasbaran	38° 21'.793 N	47° 16'.546 E	1730	11.25	308.6780	98.7	646.062	6.1
15	East Azerbaijan	Arasbaran	38° 21'.965 N	47° 16'.490 E	1652	11.28	308.6780	97.98	662.790	6.4
16	Gilan	Astara	38° 42'.271 N	48° 86'.892 E	-21.9	16.68	1630.5	0.3	4820.9	6.9
17	Gilan	Astara	38° 42'.278 N	48° 86'.902 E	-21.2	16.68	1630.8	0.3	4821.2	6.97

Table 3 Analysis of variance for some morphological and phytochemical traits of *C. avelana*

Source of variance	df	Mean of square															
		Suckering	Tree size	Leaf width	Leaf length	Chlorophyll Index	Kernel thickness	Kernel length	Kernel weight	kernel width	Nut length	Nut weight	Nut width	Nut thickness	Involucre weight	Kernel%	TCSA
Population	4	192.611^{ns}	9006.349^{ns}	62.057**	23.375**	105.975^{ns}	0.138^{ns}	0.159^{ns}	0.557**	0.221*	0.292*	2.398**	0.247*	0.208**	0.011*	337.623*	3172.003**
Error	16	83.639	16734.722	1.466	2.215	117.454	0.051	0.089	0.020	0.069	0.088	0.089	0.066	0.037	0.003	92.978	405.323

Source of variance	df	Mean of square												
		Phosphor %	Zinc	Calcium %	Magnesium%	Potassium %	Ferric %	Humidity %	Protein %	Fat%	Fiber%	Carbohydrate %	Total phenol%	Flavonoid %	IC50 (mg/)
Population	4	0.029^{ns}	0.000^{ns}	0.02^{ns}	0.902*	0.168^{ns}	0.000^{ns}	25.428**	89.372**	794.519**	1811.486**	220.579**	0.333**	7.292^{ns}	0.33^{ns}
Error	16	0.015	0.000	0.02	0.219	0.084	0.000	0.452	0.040	36.5000	11.692	0.305	0.033	7.952	0.089

Source of variance	df	Mean of square						
		Cinder ash%	Taxol (mg/g)	Palmitic acid%	Palmitoleic acid%	Stearic acid%	Oleic acid%	Linoleic acid%
Population	4	1.739*	0.074^{ns}	15.017*	13.765**	0.441^{ns}	532.602^{ns}	74.272^{ns}
Error	16	0.562	0.049	4.409	0.009	0.412	243.871	54.771

ns= No Significant, *P< 0.05, **P< 0.01

Table 4 Analysis of variance for some morphological and phytochemical traits of *C. avellana*

Source of variance	df	Mean square												
		Suckering	Tree size	Leaf width	Leaf length	Chlorophyll Index	kernel width	Kernel%	TCSA	Phosphor%	Zinc	Calcium%	Magnesium%	Potassium%
Genotype	16	236.971**	37586.029**	15.603**	9.275**	267.453**	0.182^{ns}	273.688**	21.915**	0.042^{ns}	0.000^{ns}	0.005^{ns}	0.639^{ns}	0.247**
Error	17	0.500	0.769	0.769	0.451	1.901	0.020	0.146	0.281	0.000	0.000	0.000	0.5	0.005
σ2g		118.235	18792.63	7.417	4.412	132.776	0.081	136.771	10.817	0.021	0.000	0.0025	0.695	0.121
CV% g		0.286	0.0227	1.680	1.345	0.267	1.937	0.266	10.817	21.596	0	65.789	1.407	8.629

Source of variance	df	Mean square												
		Ferric%	Protein%	Fiber%	Carbohydrate%	Total phenol%	Flavonoid%	IC50 (mg/ml)	Cinder ash%	Taxol (mg/g)	Palmitic acid%	Stearic acid%	Palmitoleic acid%	Oleic acid%	Linoleic acid%
Genotype	16	0.000^{ns}	18.536**	405.482**	46.482**	0.136**	817.669**	0.190**	0.861*	0.124**	11.922**	0.943**	2.657**	701.368**	137.891**
Error	17	0.005	0.022	0.101	0.101	0.005	0.022	0.022	0.022	0.02	0.022	0.007	0.007	0.007	0.007
σ2g	-		9.257	202.54	23.1905	0.0655	408.823	0.084	0.4195	0.052	5.95	0.468	1.325	350.680	66.442
CV% g	-		1.024	0.219	0.646	11.39	6.999	8.48	2.148	0.052	1.276	4.497	2.699	350.680	0.369

σ^2g= Genetic Variance, CV= Genetic Coefficient, ns= No Significant, *p< 0.05, **p< 0.01

Table 5 Results of comparing the mean of genotypes evaluated with principal component analysis

Traits Genotype	Number of suckering	Tree height (cm)	Leaf Width (cm)	Leaf length (cm)	Spad Value	Kernel thickness (cm)	Kernel length (cm)	Kernel weight (g)	Kernel width (cm)	Nut length (cm)	Nut weight (g)	Nut width (cm)	Nut thickness (cm)	Involucre weight (g)	Kernel%	TCSA
1	21 ef	425 e	12.5 de	13 d	10.7 g	0.6 a	0.7 a	0.36 a	0.63 cd	1.9 a	2.01 a	1.93 a	1.93 a	0.26 a	17.8 l	57.46 e
2	22 fg	670 b	12 cde	13.5 de	24.66 d	0.6 a	0.93 a	0.37 a	0.7 cd	1.76 a	1.86 a	1.63 a	1.63 a	0.22 a	20.2 j	43.96 g
3	18 d	600 c	10.5 bcd	13 d	4.05 h	0.46 a	0.66 a	0.18 a	0.53 cd	1.8 a	1.97 a	1.76 a	1.76 a	0.18 a	9.3 m	32.97 k
4	48 j	450 d	10 abc	10.1 a	34.99 b	0.56 a	1 a	0.25 a	0.6 cd	1.16 a	1.06 a	1.03 a	1.03 a	0.3 a	23.3 h	47.1 f
5	12 b	300 k	9 ab	10.5 ab	33 b	0.8 a	1 a	0.41 a	0.7 cd	1.36 a	1.4 a	1.3 a	1.3 a	0.2 a	29.4 e	57.14 h
6	15 c	750 a	12 cde	15 e	10.75 g	0.8 a	0.83 a	0.5 a	0.83 c	2.03 a	2.24 a	1.04 a	1.04 a	0.26 a	22.2 i	31.4l
7	17 d	310 j	8.3 a	10 a	10 g	1.4 a	1.96 a	0.63 a	1.56 a	2.43 a	2.23 a	1.93 a	1.93 a	0.27 a	28.2 f	37.68 i
8	10 a	380 g	10 bc	12 bcd	39.93 abc	0.9 a	0.8 a	0.5 a	0.73 cd	1.6 a	1.93 a	1.5 a	1.5 a	0.22 a	25.9 g	97.34 b
9	15 c	320 i	13 e	13.5 de	39.53 a	0.56 a	0.7 a	0.28 a	0.56 cd	1.2 a	1.4 a	1.16 a	1.16 a	0.14 a	20.33 j	31.4 l
10	20 e	600 c	8.2 a	12 bcd	30 c	0.5 a	0.5 a	0.76 a	0.4 d	1.13 a	1.26 a	1.1 a	1.1 a	0.14 a	60.2 a	34.54 j
11	40 i	300 k	8 a	11 abc	19.58 e	0.8 a	0.8 a	0.31 a	0.8 c	1.33 a	1.63 a	1.3 a	1.3 a	0.21 a	19.03 k	47.1 f
12	40 i	450 d	9.5 ab	15 e	15.89 f	0.73 a	0.7 a	0.25 a	0.76 c	1.06 a	1.34 a	1.2 a	1.2 a	0.14 a	18.6 kl	42.39 h
13	23 g	350 h	10 abc	13 d	25.96 d	0.7 a	0.9 a	0.36 a	0.67 cd	1.49 a	1.61 a	1.4 a	1.4 a	0.16 a	22.6 hi	84.78 d
14	15 c	350 h	11b cde	11 abc	39.99 a	1.13 a	1.3 a	0.38 a	1.2 b	1.4 a	1.75 a	1.43 a	1.43 a	0.18 a	22.02 i	84.78 d
15	25 h	350 h	10 abc	12.5 cd	25.43 d	0.73 a	0.93 a	0.51 a	0.6 cd	1.53 a	1.63 a	1.46 a	1.46 a	0.18 a	31.03 d	150.72 a
16	11 e	450 f	12f	17f	16.11 f	0.8 a	1.06 a	0.84 a	0.8 b	1.9 a	1.9 a	1.84 a	1.84 a	0.18 a	35.66 c	92.0 c
17	20 a	400 d	20 cde	17f	16.38 f	1.2 a	1.3 a	1.3 a	1.33 c	1.9 a	3.61a	1.11 a	1.11 a	0.18 a	44 b	85. d

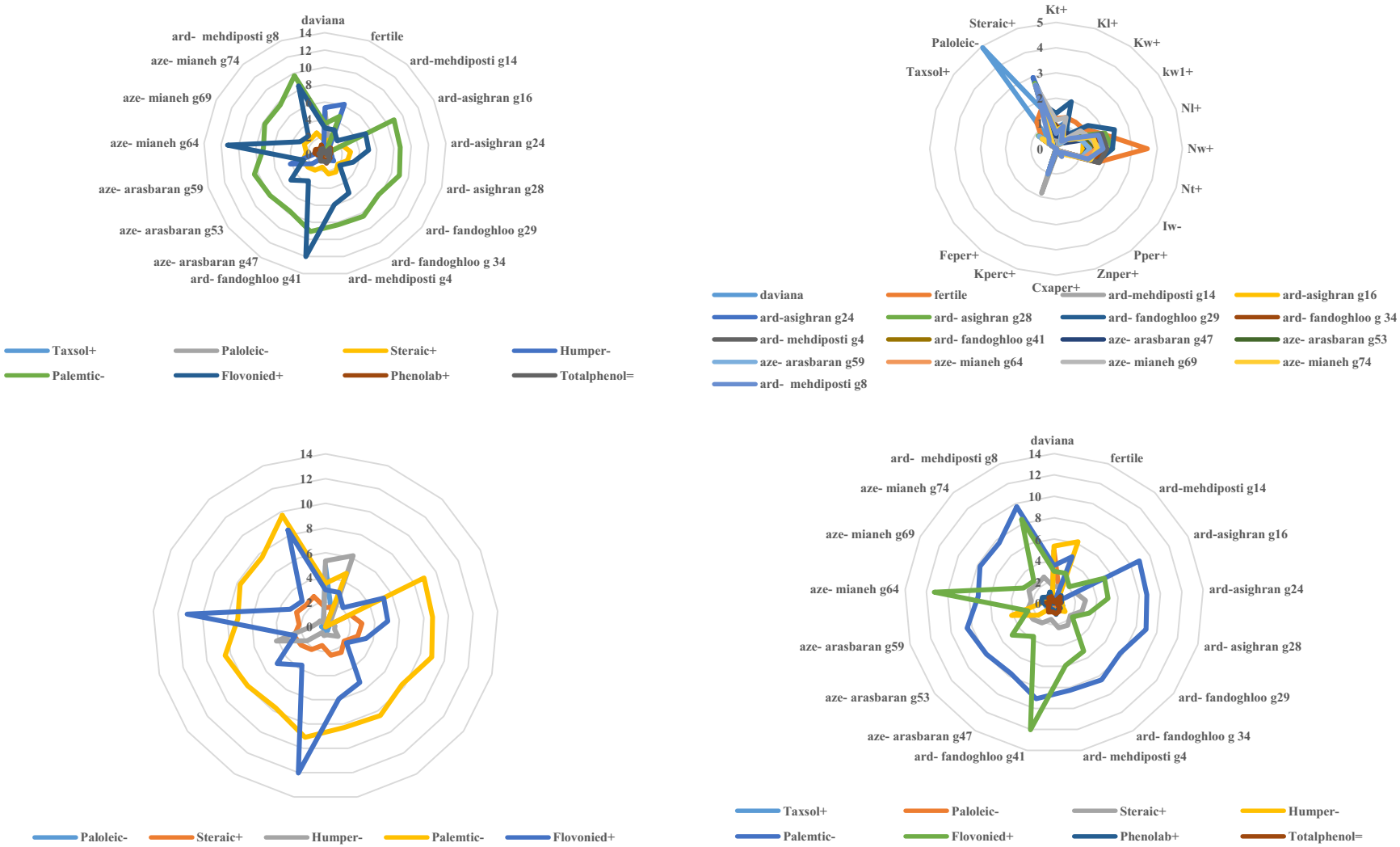

Fig. 2 Radar model results of comparing the mean of genotypes evaluated with principal component analysis.

In terms of phenolic contents, G64 and G69 (middle origin) and Fertile genotypes had the highest levels. Palmitoleic acid had the highest mean percentage in G8, G64 and G41 genotypes.

Clustering based on principal component coefficients related to climatic traits revealed that the first cluster included genotypes of G34, G53, G59, G64, G4, G29, G28, G8, G24, G47, G16, G74, G53, 641, and Fertile. The second cluster included only the Daviana, and the third cluster included native G14 (genotype). Cluster-based on principal component coefficients of climatic characteristics revealed that the first cluster included genotypes of G69, G74, G64, G29, G41, G34, G4, G8, G16, G28, G24, G14.

The second cluster includes fertile and Daviana and the third cluster includes three genotypes of G47, G53 and G59.The cluster-based on principal component coefficients for elemental analysis revealed four cluster groups. The first cluster included genotypes of G14, G8, Daviana, G69. The second cluster included genotypes of G4, G64, G47, and the third cluster included genotypes of G24, G28, G29, G59 and G41. Genotypes of G16, Fertile, G74, G53, G34 were also included in the fourth cluster.

The cluster-based on principal component coefficients for elemental analysis showed 3 cluster groups. The first cluster included G69, G74, G53, G14, G47, Fertile, Daviana, G41, G59, G29, G34, G16, G24, G64, G4 genotypes, and the second and third clusters included G8 and G28 genotypes (Table-6).

DISCUSSION

Hazelnut breeding programs started in 1967 in the United States, 1970 in Spain and Europe to breed against diseases, pests and colds, and several types of these projects have been introduced. This program was started in Iran in 2000, which has resulted in the introduction of commercial types of walnut and Pashmineh [32] .

Natural forest veins of the Fandoghlou region (Ardabil and Gilan provinces), Hatam Meshasi (Ardabil province), Middle Fandoghlou (East Azerbaijan) and genetically diverse region of Arasbaran (East Azerbaijan) justify the presence of valuable germplasm in this region. The vast expanse of Ardabil and Miyaneh hazelnut forests along the hazelnut forests of Azerbaijan and Turkey doubles the importance of studying the genetic diversity of hazelnuts in these areas. Numerous reports of biodiversity in the Arasbaran Protected Area as a plant reserve and cradle of flora make the evaluation of hazelnut trees in this area important in the study of hazelnuts in northwestern Iran.

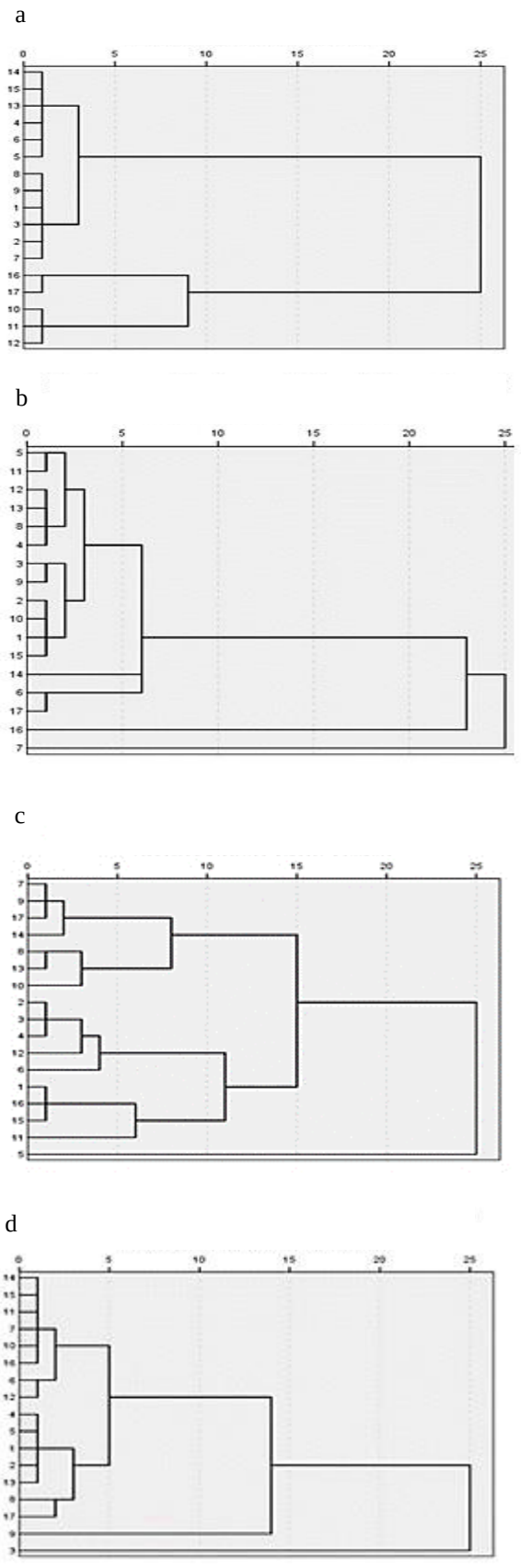

Fig. 3 Results of grouping genotypes based on principle components according to morphological traits (a) climate, (b) phytochemical, (c) fatty acid and (d) taxol pharmaceutical (Dendrogram using Average Linkage (between group) Rescaled Distance Cluster Combine)

Climatic differences in the spread areas of Hazelnut in the study areas are listed in Table-2. The natural range of hazelnuts in these areas has been recorded with a temperature difference between 8.23 and 14.56, rainfall of 308 and 1347.2 and altitude between -13 and 196 meters. Due to the ecological needs of hazelnuts, these areas are significantly different from the intensive cultivation areas of hazelnut trees.

For example, high humidity in Fandoghlou of Ardabil and consequently good sources of pathogen resistance for the initial screen and also low-temperature conditions (number of frosty days) in this area and not damaging some trees and even having acceptable fruit in them can refer to the presence of cold-resistant genes in these genotypes or ecotypes. This is in consistent with hazelnut breeding programs in most countries, including the USA, Europe, and Turkey [33], and natural selections over the years in these areas that have experienced temperatures below -30 degrees Celsius acted in favour of this trait. Genetic diversity for existing germplasms should be evaluated based on various morphological, physiological, chemical compositions, and genetic and biochemical markers. The study populations in three hazelnut growing areas in the northwest had significant diversity in terms of morphological traits. High genetic CV Table for protein, flavonoid, Spad Value, palmitic acid percentage traits among populations makes it possible to use these traits in breeding programs. Numerous reports on the diversity of quantitative and qualitative traits for the world's hazelnuts have been presented so far [33]. Based on a study of 29 hazelnut genotypes in Slovenia, the trait of kernel thickness was identified as the most diverse trait among the genotypes [34]. The study of the genetic diversity of hazelnut genotypes and the selection of genotypes with high kernel percentages constitute a significant part of hazelnut breeding programs [35].

In a study of 29 Iranian hazelnut genotypes from Kalibar, Eshkorat and Fandoghlou regions, along with six commercial hazelnut types, the mean for fruit length, fruit thickness, kernel length, and kernel percentage was reported to be 2.89, 1.63, 1.25 cm and 20.86% for Kalibar genotypes, 1.72, 1.68, 1.34 cm and 69.45% for Eshkorat genotypes, and 1.31, 1. 04, 1.68, 0.97 cm and 40.39% for Fandoghlou genotypes [36].

The obtained results for Fandoghlou genotypes were consistent with the results of this study. The above traits for phenolic contents, fatty acids, fruit weight and percentage of the kernel to whole fruit, which are important quantitative and qualitative traits of hazelnut yield, were improved compared to the total and average types. Figure 2 indicates that modification by the Means method (mass) is also possible among these genotypes for the study areas, although hybridization between them and commercial types can also be used for describing grain weight traits, chemical, and medicinal contents. The presence of large trees among the populations with acceptable offshoot power makes the practical activity of this breeding method more visible than before. In the study populations, Ardabil population for kernel percentage and traits kernel weight, and protein percentage had a higher potential than the average of total populations and breeding types significantly. The existence of genetic diversity in terms of morphological traits for hazelnuts was published in 2010 by Hosseinova. High diversity in a limited number of hazelnut trees in the Fandoghlou region of Ardabil was reported [23]. In twenty American hazelnut hybrids in Nebraska, the amount of kernel oil (58.2%), protein (17.1%) and carbohydrates (21.7%) were reported over two consecutive years [37]. Genotypes within populations were classified into three groups based on cluster grouping using morphological traits (Fig. 3).

G14 genotypes from the Fandoghlou region were located in a separate cluster with desirable traits of kernel weight, percentage of potassium, calcium, and phosphorus in one cluster, and the Daviana cultivar was in the second cluster and in a separate cluster. The grouping of different selected genotypes from different regions and populations with the modified Fertile cultivar showed the high potential of native genotypes of Fertile percentage for morphological traits. In present, the differences between populations were evaluated in terms of phytochemical traits, which were significantly different between populations in terms of all traits except calcium, potassium, moisture, and iron content. However, there was a significant difference between the study genotypes in terms of all traits (except iron and zinc). Genetic CV as the purest indicator of genetic diversity for the study traits varied between genotypes from 100% to 14% for significant traits. In general, for traits such as phosphorus, moisture, flavonoids percentage, the genetic variation coefficient was estimated 100%, and for traits such as fibre, carbohydrate, and CV protein, it was above 80%. This potential showed the enrichment of germplasm for breeding programs in these genotypes.

Table 6 Coefficients of each trait in the principle components based on the traits of fatty acids and taxol, A) Cumulative variance for Component, Cumultative variance for Component and C) Cumulative variance for Component
A) Cumulative variance for Component=82.9%, B) Cumultative variance for Component=80.2%, C) Cumulative variance for Component=81/88%

Traits	Component				
	1	2	3	4	5
Kernel Length	0.933	-0.020	0.099	0.099	-0.024
Kernel width	0.930	0.121	0.002	0.002	0.022
Kernel Thickness	0.925	0.103	0.181	0.181	0.100
Leaf length	-0.170	0.840	-0.223	-0.223	0.069
Leaf width	0.055	0.798	0.91	0.091	0.056
Kernel weight	0.488	.0781	0.127	0.127	0.173
Nut weight	0.448	0.711	-0.050	-0.050	0.134
Involucre weight	0.315	0.459	0.356	0.356	-0.447
Nut width	0.325	0.116	0.060	0.060	0.157
Nut thickness	0.453	0.241	0.129	0.129	0.120
Kernel percent	0.213	0.384	0.127	0.127	0.235
Nut length	0.517	0.349	-0.283	-0.283	0.215
TCSA	0.051	0.338	0.827	0.827	-0.032
Tree height	-0.352	0.340	-0.674	-0.674	0.031
Spad Value	-0.145	-0.334	0.629	0.629	0.207
Suckering	-0.130	-0.223	-0.51	-0.051	-0.918

Traits	Component		
	1	2	3
Taxol	0.781	-0.330	0.269
Palmitic acid%	0.761	0.545	0.269
Palmitoleic acid%	-0.234	0.002	-0.922
Stearic acid%	0.888	0.228	-0.003
Oleic acid%	0.832	0.125	-0.353
Linoleic acid%	0.318	0.735	0.032

Significant variation was also observed in terms of the valuable biochemical medicinal properties of this fruit. This means that the coefficient of genetic variation for the traits of Taxol, palmitic acid, stearic and oleic acid and linoleic acid, despite being significant in the variance analysis, varied between 100% for palmitic acid and 27.7% for oleic acid. Therefore, these genotypes justify the medicinal use of hazelnuts. In general, the amount of Taxol was the highest among genotypes 1, 4, 6, and 7, and this amount was very little for commercial types such as Daviana (Fig. 2). One of the most important phytochemical compounds examined in this study is the active ingredient of Pakli Taxel by the brand name Taxol. The mean concentration of Taxol was 1.31 µg/g in dry weight. The presence of Taxol and its related taxa in latent seed plants was first reported in the stem and leaf branches of European hazelnut trees and their symbiotic fungi [38]. The highest amount of Taxol was reported in older leaves, twigs and finally, very little amounts were related to the raw kernel and hazelnut shell [39]. Based on our data, the concentration of Taxol reported in older leaf samples of hazelnut genotypes 13 and 6 of this study is higher than the values reported by Shirazi *et al.* (2020) for commercial types of Ennis with 1.05 and Nonpareil with 1.01 µg [10]. Although the amount of Taxol in hazelnut genotypes of this study is very low compared to yew, the high economic value of Taxol, the shortage of yew trees, the high cost of its synthesis and the growing demand for this drug, other natural resources such as hazelnuts without the limitations of yew types, can be considered for its production [41]. The major phytochemical compounds in hazelnuts are fat and protein, so one of the goals of hazelnut breeding is to create cultivars with a higher percentage of protein [42].

PC-based Cluster Analysis

Analysis of principal components of genotypes was performed based on morphological traits.

Table 7 Correlation results of different groupings of genotypes by Mantel method

Medicinal contents	Chemical contents	Morphology	Climatic indicators	Mantel correlation index
			1	Climatic indicators
	1	1	0.67	Morphology
1	0.31	0.36	0.51	Chemical contents
		0.41	0.41	Medicinal contents

Then, clustering was done based on the main components of PC1, PC2 and PC3. We classified the genotypes into three groups by setting the cluster cutting at a distance of 10 units. The grouping of modified Fertile cultivar among most of the genotypes in the first group refers to the high potential of the genotypes in terms of morphological traits for its breeding, at least at the level of improved cultivar. Genotype G14 in the region of Mehdi Posti of Fandoghlou in Ardabil was located in a single cluster. This genotype had the highest mean for other morphological traits with the average tree height, number of offshoots, percentage of the kernel to fruit, the cross-sectional area of the trunk, and lower spad value. This genotype, in particular, had the highest mean traits of hazelnut fruit, which even had significantly higher mean fruit traits than the improved Daviana and Fertile cultivars. Due to the fact that this genotype has been subjected to natural screening for many years and has low amounts of offshoots, it can be used as a mass selection in a program with asexual reproduction. Classification of genotypes based on PC use related to climatic conditions revealed that genotypes were classified Another study based on multivariate analysis of the effects of different geographical areas on the mineral composition of the kernel in 53 hazelnut masses stands from six European regions, including France, southern and northern Italy, Portugal, Spain and Slovenia. Major differences were observed in the kernel composition of hazelnut genotypes of natural habitats in France and Italy, which had higher altitude differences than the other areas [44]. Another grouping was performed based on the contents of fatty acid and valuable substance of Taxol. The three subgroups obtained from this classification and the placement of modified cultivars with native masses and their lack of differences based on the relevant traits can indicate two points. First, the modified cultivars were not changed in terms of fatty acid and taxol traits, and second, the native genotypes were so illegible in terms of fatty acids that they were included in the range of modified cultivars. However, into three sub-clusters. The presence of Fertile and Daviana genotypes in one class indicates a suitable climatic classification because the climatic conditions of the two improved cultivars were different with the change of genotypes. The grouping of genotypes 47, 53 and 59 in a sub-cluster, all of which originated in Arasbaran, also reinforces the hypothesis of appropriate grouping based on climatic conditions. The main components analysis for the chemical contents of Hazelnut was also calculated, and grouping was done according to the cluster method. The four sub-clusters obtained and the placement of cultivars of Daviana with genotypes of G8, G14 and G69 under the first cluster and Fertile, G16, G74, G53 and G34 under the fourth cluster revealed the difference and diversity of chemical contents.

The results obtained from cluster analysis showed that the location of hazelnut genotypes in different clusters indicates the existence of high diversity among the study genotypes. In the study of 41 genotypes of the hazelnut species of Colurna in India, 9 clusters were created, which showed a clustering pattern independent of the eco-geographical distribution pattern of the genotypes [43].

the G8 genotype with the origin of Mehdi Posti hazelnut in one cluster and G28 with the origin of Esigaran in a separate cluster showed the difference between these genotypes and all study genotypes. G8 had the minimum number for Taxol and fatty acids other than linoleic acid, and G28 had the lowest mean for all fatty acids and taxol traits except for unsaturated fatty palmitic acid. In other words, 15 genotypes of the same genus as the modified Fertile and Daviana cultivars can be used in harvesting taxol, fatty acids and breeding for medicinal purposes. The cold and semi-arid climate in this habitat, which leads to a reduction in the growing season, likely affects the reduction of growth and small size of the fruit and kernel compared to other native hazelnut habitats. Also, a semi-arid condition in the Fandoghlou habitat is another factor in increasing the amount of Taxol in plant tissues. The effect of drought conditions on increasing taxol concentration in older leaves of yew

shrubs has also been reported [45]. Humid conditions, especially foggy weather, is an important environmental factor in increasing the amount of hazelnut oil as well as filling the kernel [46]. The results of previous studies show that one of the factors reducing the amount of hazelnut kernel oil is cold and dry weather during the growing period [47]. The results of the Mantel test based on the matrix of correlation coefficients between genotypes in the groupings are listed in Table-7. This index shows the degree of compatibility of different groupings. The existence of relatively similar conditions about the width spread of Hazelnut on the one hand and the genetic screen of the genotypes over the years, on the other hand, justifies this discrepancy. In other words, morphological traits and other traits that were less grouped by climatic conditions were the results of grouping the environment at a lower level. In other words, most traits had significant genetic changes. Fruit weight is an important attribute of functional components. Fruit weight had a positive and significant correlation with leaf width and length, kernel thickness, fruit length and width. The existence of significant and acceptable variation between genotypes and their positive correlation with fruit weight (Table-7) makes it possible to use these genotypes to modify higher fruit weights.

Mantel coefficients showed that the highest agreement between the grouping of genotypes in terms of morphological traits was with climatic conditions (v = 0.67), while the correlation for chemical characteristics with climatic conditions was 0.51 (Table-7). But the rest of the correlations was less than 0.50. The coefficient of explanation of concordance between morphological traits with climatic conditions was 45%. This means that 45% of morphological changes were justified by climate change.

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