The Journal of Animal & Plant Sciences, 24(3): 2014, Page: 820-828
ISSN: 1018-7081

MOLECULAR CHARACTERIZATION OF TURKISH HAZELNUT CULTIVARS AND ACCESSIONS

T. Demir

Sakarya University, Vocational School of Pamukova, Sakarya-TURKEY
Corresponding Author E-mail: tdemir@sakarya.edu.tr

ABSTRACT

The present study was conducted to determine the molecular profile of hazelnut cultivars and accessions grown in Turkey, and to assess their genetic relationships. Fifteen Turkish hazelnut cultivars and twelve hazelnut accessions were used as plant materials. Genetic relationships of 27 hazelnut cultivars and accessions were assessed using 22 Random Amplified Polymorphic DNA (RAPD) primers. Thirteen of the 22 primers produced polymorphic patterns resulting in 29 informative alleles. The best polymorphism was obtained from OPAD-02 (5 polymorphic bands) primer. Reproducible and clear 29 polymorphic fragments were used calculating similarity matrix and constructing dendrogram with UPGMA cluster analysis of MVSP 3.13 software. The mean number of alleles per locus was 2.23 while the similarity over the 13 polymorphic loci averaged 0.697. UPGMA cluster analysis of the data separated the 27 genotypes into two main groups. Most of the hazelnut cultivars were settled on the first group while 'Kalınkara', 'İncekara' and 'Mincane' cultivars and 'FAE-190' accession were placed on the second group. Depending on the genotypes, similarity ratios ranged from 0.364 to 0.974, with a mean value of 0.697. Overall, the results demonstrate a high level of polymorphism among hazelnut cultivars and accessions in Turkey.

Key words: Hazelnut (*Corylus*), RAPD, Molecular marker, Germplasm.

INTRODUCTION

The genus *Corylus* includes a large array of species, originated mainly in the Northern hemisphere and widely represented in Anatolia, Northern Caucasia, Chines, Himalayas and some parts of Europe (Arıkan, 1960; Kasaplıgil, 1972; Özbek, 1978). Although *Corylus* genus includes many species, cultivated hazelnut varieties are placed at *Corylus avellana* L. which is one of the great *Corylus* species (Thompson *et al.,* 1996; Rovira, 1997; Erdogan and Mehlenbacher, 2000; Köksal, 2002).

Turkey potentially has a very rich source of hazelnut germplasm (*Corylus avellana* L.). Hazelnut is clearly native to Turkey. Turkey is the leading country in the production [600 million tons per year (70% of the total world production)] and export (75% of the total world production) of hazelnuts (FAO, 2012).The Black Sea Region has the appropriate climatic conditions for the cultivation of hazelnuts. This region is the most important hazelnut production center. Several different cultivars and varieties of hazelnut are grown at this region in Turkey (Demir and Beyhan, 2000).

Traditionally, cultivar identification has relied on morphological, pomological and agronomic characteristics of plant materials. It is difficult to distinguish cultivars on their external morphology alone. Since this requires a very long period, it is difficult to determine morphologic, pomologic and agronomic characteristics in nuts and fruit trees. Further, these phenotypic characters are generally influenced by environmental factors and the growth stage of the plants. In fruit trees, this requires a lengthy and expensive evaluation during the whole vegetative growth. On the other hand, the relatively narrow range of variation of morphological traits limits cultivar identification, and thus methods based on molecular markers should be used. Identification of the hazelnut genotypes will help in choosing appropriate cultivars and the preservation of natural resources required for breeding studies in Turkey.

Initial molecular studies in hazelnut were carried out using isozymes to characterize and determine genetic variability (Ahmad *et al.,* 1987; Rovira, 1997; Solar *et al.,* 1997) and using restriction fragment length polymorphism (RFLP) markers (Malusà, 1994). Successively, random amplified polymorphic DNA (RAPD) markers have been used to identify hazelnut cultivars (Radicati *et al.,* 1997; Galderisi *et al.,* 1999; Miaja *et al.,* 2001; Valentini *et al.,* 2001; Kafkas *et al.,* 2009; Erdoğan *et al.,* 2010), determine self-incompatibility alleles (Pomper *et al.,* 1996; Pomper *et al.,* 1998; Bassil and Azarenko, 2001), identify a marker linked to Eastern Filbert Blight (Davis and Mehlenbacher, 1997; Lunde *et al.,* 2000), and construct a linkage map (Mehlenbacher *et al.,* 2006a; Gökırmak, 2005). Recently SSRs and SNPs are used for both cultivar identification and genetic map construction (Gurcan *et al*. 2010).

The aims of our study were to determine the molecular profile and genetic relationships of hazelnut cultivars and accessions grown in Turkey.

MATERIALS AND METHODS

Plant material: In this study, fifteen Turkish hazelnut cultivars and twelve hazelnut accessions [four hybrids ('Tombul' x 'Kargalak') and eight selections] were used as plant material. Plant materials and obtained locations and their pedigree are listed in Table 1.

DNA extraction: For DNA isolation, young leaves were collected from a single plant for each accession; they were frozen in liquid nitrogen and stored at -80 °C. Genomic DNA was isolated from the leaves using the DNeasy Plant Mini Kit (QIAGEN, GmbH, Germany) according to the manufacturer's instructions. DNA concentrations were measured using a spectrophotometer. DNA was diluted in water to a final concentration of 50 ng/µl and stored at -20 °C.

RAPD analysis: RAPD analysis was performed according to (Williams *et al.,* 1990) with minor modifications. Totally, 22 decamer oligonucleotide primer (Operon Technologies) were used for PCR amplification. DNA amplification was performed in a volume of 25 µl, containing 20 ng of template DNA, 15 pmol dekamer RAPD primer (Operon Technologies), 12.5 µl Promega M7502 PCR Mix 2X (50 mM Tris-HCl pH 9.0, 50 mM NaCl, 3 mM $MgCl_2$, 400 µM dATP, dGTP, dCTP, dTTP, 50 unit/ml Taq DNA polymerase), 1.0 µl 25 mM $MgCl_2$, 0.5 unit Taq DNA polymerase (Promega), and 4.9 µl ddH_2O (nuclease-free). Amplifications were performed at Eppendorf Mastercycler Gradient (Eppendorf Scientific, Inc. Westbury, NY, USA) programmed for 4 min at 94 ºC initial denaturation, then 45 cycles of 1 min at 94 ºC denaturation, 1 min at 36 ºC annealing and 1 min at 72 ºC extension, and then 7 min at 72 ºC final extension. Amplified products were stored at 4 °C for electrophoresis. The amplified products were separated in a 1.5% agarose gel electrophoresis using 1X TAE buffer, and stained with ethidium bromide. The stained gels were photographed under UV light. The molecular sizes of the amplification products were estimated using 1 kb DNA Ladder (Promega G5711). PCR reactions were repeated two times to control reproducibility.

Table 1. Hazelnut cultivars used in RAPD analysis, production area, pomological group and husk aspect[1]

No*	Genotype*	Production Area	Pomological Group	Husk Aspect
1	Tombul	Giresun, Ordu, Samsun	Round	Long tubular
2	Palaz	Ordu, Samsun	Flattened Round	Medium Tubular
3	Çakıldak	Ordu, Samsun, Sakarya	Round	Long Tubular
4	Foşa	Trabzon, Düzce	Round	Medium Tubular
5	Mincane	Trabzon	Round	Medium Tubular
6	Uzunmusa	Ordu	Round	Short Tubular
7	Kargalak	Trabzon	Flattened Round	Long Tubular
8	Kan	Giresun	Round	Medium Tubular
9	Kalınkara	Giresun, Ordu	Round	Long Tubular
10	İncekara	Giresun	Pointed	Long Tubular
11	Sivri	Giresun, Trabzon	Pointed	Long Tubular
12	Acı	Ordu	Pointed	Short Vase
13	Yuvarlak Badem	Sakarya, İzmit	Tubular Long	Long Vase
14	Yassı Badem	Sakarya, İzmit	Flattened Long	Long Vase
15	Allahverdi	Giresun	Round	Medium Tubular
16	K1/1	Hazelnut Res. Inst. Giresun	Round	Medium Tubular
17	K19/6	Hazelnut Res. Inst. Giresun	Round	Medium Tubular
18	K24/2	Hazelnut Res. Inst. Giresun	Round	Medium Tubular
19	K26/3	Hazelnut Res. Inst. Giresun	Round	Long Tubular
20	FAE-190	Hazelnut Res. Inst. Giresun	Round	Medium Tubular
21	FAE-260	Hazelnut Res. Inst. Giresun	Round	Medium Tubular
22	FAE-580	Hazelnut Res. Inst. Giresun	Round	Long Tubular
23	Yerli Azmanı	Selected from Ordu	Round	Medium Tubular
24	Yerli	Selected from Samsun	Round	Medium Tubular
25	Sandık Fındığı	Selected from Ordu	Round	Long Tubular
26	Erkenci	Selected from Ordu	Round	Long Tubular
27	Hanımfındığı	Selected from Samsun	Flattened Long	Short Vase

* 1-15 Turkish standard cultivars; 16-19 Promising hybrids from Kargalak x Tombul; 20-22 Selections of Tombul; (Plant materials were taken from collection orchard at Hazelnut Research Institute, Giresun-Turkey); 23-27 Promising selections from Turkey germplasm.

[1] (Ayfer, *et al.,* 1986; Çalışkan, 1995; Beyhan & Demir, 2001; Köksal, 2002; Demir, 2004)

Data analysis: For each primer, RAPD fragments were scored as present (1) or absent (0). Genetic similarity values were calculated by Dice coefficients (Nei and Li, 1979). Unweighted Pair Group Method Analysis (UPGMA) was performed to generate a dendrogram with MVSP 3.13 software (MVSP 3.13p 2007).

RESULTS AND DISCUSSION

RAPD analysis: In the present study, two sets of plant material covering 15 hazelnut cultivars and 12 accessions were characterized with 22 RAPD primers. All of the primers produced amplification. Thirteen of 22 primers (OPA08, OPA10, OPA17, OPAD02, OPH17, OPM05, OPM11, OPO18, OPS07, OPU09, OPU11 and OPU12) produced reproducible bands and it was possible to clearly discriminate the hazelnut cultivars and accessions studied. These primers have been reported polymorphic in a wider sample of *Corylus* species in previous studies (Demir, 2004; Galderisi *et al.*, 1999; Pomper *et al.*, 1996; Pomper *et al.*, 1998; Radicati *et al.*, 1997; Valentini, *et al.*, 2001; Miaja, *et al.*, 2001). The OPAD02 was the most informative primer (5 polymorphic bands) used in this study. Six putative alleles were previously identified with the same primer in 18 hazelnut cultivars and 22 clones (Demir, 2004). The OPU12 was the least informative primer in this study. Two primers (OPAD17 and OPU07) produced only monomorphic bands in this study. However, OPAD17 primer has been reported polymorphic in hazelnut cultivars and clones in previous studies (Demir, 2004). In total 115 bands were obtained of which 100 bands (87.0%) were polymorphic and 15 bands (13.0%) were monomorphic. Twenty-nine (25.2%) reproducible and clear polymorphic bands (markers) were used for analysis (Table 2). Band sizes varied from 90 to 1860 bp, and band numbers varied from 4 to 14. We observed band sizes ranging from 200 to 2700 bp and band numbers ranging from 4 to 15 with the same primers in a previous research (Demir, 2004). Average markers were 2.23 per primer in this research. Similarly, in previous studies, it had been stated as 2.17 markers per primer with 12 primers in 19 hazelnut genotypes (Radicati *et al.*, 1997), 1.5 markers per primer with 30 primers in 19 hazelnut cultivars (Miaja *et al.*, 2001), 2.91 markers per primer with 33 primers in 18 standard Turkish cultivars and 22 clones (Demir, 2004), 3.84 markers per primer with 25 primers in 18 hazelnut cultivars (Kafkas *et al.*, 2009), 5.7 markers per primer with 43 primers in 19 hazelnut cultivars (Erdoğan *et al.*, 2010). In the studies stated above, the difference in the number of markers is due to the number of genotypes or primers.

Table 2. Band profiles obtained from primers

Primers	5'……3'	Polymorphic bands (Markers) (bp)	Monomorphic bands (bp)
OPA-08	GTG ACG TAG G	90, 215, 260, 330, 410, 450, 540, 620, 700, 750, 850, 950, 1100, 1350	-
OPA-10	GTG ATC GCA G	330, 375, 480, 590, 710, 870, 1110, 1500	420
OPA-17	GAC CGC TTG T	340, 420, 460, 550, 600, 850, 980, 1200, 1300, 1450, 1500, 1600	700
OPAD-02	CTG AAC CGC T	670, 770, 870, 1100, 1300	-
OPH-17	CAC TCT CCT C	270, 325, 460, 550, 575, 620, 1550	400, 900, 1250
OPH-19	CTG ACC AGC C	270, 540, 630, 720, 950, 1350	800, 900
OPM-05	GGG AAC GTG T	300, 480, 550, 600, 650, 700, 1300	200, 380, 450, 1200
OPM-11	GTC CAC TGT G	270, 330, 390, 525, 600, 680, 850, 1090, 1480, 1660, 1860	450
OPO-18	CTC GCT ATC C	400, 500, 670, 900	-
OPS-07	TCC GAT GCT G	200, 295, 310, 660, 1200	-
OPU-09	CCA CAT CGG T	130, 350, 480, 560, 650, 1000, 1200, 1400	-
OPU-11	AGA CCC AGA G	210, 280, 355, 420, 470, 525, 825, 930, 1500	620
OPU-12	TCA CCA GCC A	350, 1300	550, 900

Underlined bands are clear and reproducible polymorphic markers used for construction dendrogram.

Genetic similarity relationships among hazelnut cultivars and accessions: A similarity matrix (Table 3) was generated for 29 fragments using Dice coefficients of Nei and Li (1979). All of the studied hazelnut cultivars were differentiated by RAPD markers, and genetic similarity relationships among the cultivars were assessed. The dendrogram constructed by UPGMA cluster analysis is illustrated in Figure 1.

Table 3. Genetic similarity ratios among the 27 hazelnut genotypes with 29 markers using by Dice measurements (Nei and Li, 1979)

	Tombul	**Palaz**	**Çakıldak**	**Foşa**	**Mincane**	**Uzunmusa**	**Kargalak**	**Kan**	**Kalınkara**	**İncekara**	**Sivri**	**Acı**	**Yuvarlak Badem**	**Yassı Badem**	**Allahverdi**	**K1/1**	**K19/6**	**K24/2**	**K26/3**	**FAE-190**	**FAE-260**	**FAE-580**	**Yerli Azmanı**	**Yerli**	**Sandık Fındığı**	**Erkenci**	**Hanımfındığı**
Tombul	1,000																										
Palaz	0,923	1,000																									
Çakıldak	0,811	0,778	1,000																								
Foşa	0,842	0,811	0,800	1,000																							
Mincane	0,645	0,733	0,714	0,690	1,000																						
Uzunmusa	0,872	0,842	0,778	0,811	0,667	1,000																					
Kargalak	0,842	0,811	0,686	0,722	0,552	0,703	1,000																				
Kan	0,850	0,821	0,811	0,789	0,645	**0,974**	0,684	1,000																			
Kalınkara	0,667	0,688	0,667	0,774	0,583	0,688	0,581	0,667	1,000																		
İncekara	0,483	0,571	0,538	0,593	0,600	0,500	0,444	0,483	0,636	1,000																	
Sivri	0,688	0,581	0,621	0,667	0,522	0,710	0,600	0,688	0,480	0,476	1,000																
Acı	0,706	0,667	0,645	0,625	0,480	0,848	0,625	0,824	0,667	0,435	0,615	1,000															
Yuvarlak Badem	0,778	0,743	0,727	0,647	0,593	0,743	0,706	0,722	0,483	0,480	0,714	0,667	1,000														
Yassı Badem	0,811	0,778	0,824	0,800	0,714	0,778	0,743	0,757	0,600	0,462	0,552	0,645	0,727	1,000													
Allahverdi	0,667	0,625	0,600	0,645	0,583	0,688	0,581	0,667	0,615	**0,364**	0,560	0,593	0,552	0,667	1,000												
K1/1	0,800	0,718	0,757	0,737	0,516	0,667	0,737	0,700	0,606	0,414	0,563	0,588	0,667	0,703	0,788	1,000											
K19/6	0,872	0,842	0,722	0,757	0,600	0,737	0,865	0,718	0,688	0,500	0,581	0,667	0,686	0,722	0,750	0,872	1,000										
K24/2	0,895	0,811	0,686	0,778	0,483	0,811	0,778	0,789	0,581	0,370	0,733	0,625	0,706	0,686	0,645	0,789	0,811	1,000									

K26/3	0,8	0,8	0,5	0,6	0,5	0,7	0,7	0,7	0,6	0,5	0,6	0,6	0,6	0,5	0,5	0,5	0,7	0,7	1,0									
	24	48	81	88	60	88	50	65	67	22	15	43	67	81	93	88	27	50	00									
FAE-190	0,6	0,6	0,7	0,7	0,7	0,6	0,5	0,6	0,8	0,7	0,5	0,5	0,5	0,6	0,6	0,6	0,6	0,5	0,5	1,0								
	67	88	33	74	50	88	81	67	46	27	60	93	52	67	15	06	88	16	93	00								
FAE-260	0,8	0,7	0,7	0,8	0,6	0,8	0,6	0,8	0,7	0,5	0,7	0,7	0,6	0,7	0,7	0,7	0,7	0,7	0,7	0,7	1,0							
	21	89	78	65	67	95	49	72	50	71	10	27	29	78	50	18	37	57	27	50	00							
FAE-580	0,9	0,8	0,7	0,8	0,6	0,8	0,7	0,7	0,5	0,4	0,6	0,6	0,7	0,7	0,5	0,7	0,8	0,8	0,7	0,6	0,7	1,0						
	47	65	43	33	90	11	78	89	81	44	67	25	06	43	81	37	11	33	50	45	57	00						
Yerli	0,8	0,7	0,7	0,7	0,5	0,8	0,6	0,8	0,6	0,4	0,6	0,7	0,7	0,7	0,6	0,7	0,7	0,7	0,7	0,6	0,8	0,7	1,0					
Azmanı	33	43	88	65	93	57	47	33	90	80	43	33	50	27	90	22	43	65	33	90	00	65	00					
Yerli	0,8	0,8	0,7	0,8	0,5	0,8	0,6	0,8	0,7	0,4	0,5	0,7	0,6	0,7	0,6	0,6	0,6	0,7	0,7	0,7	0,8	0,7	0,8	1,0				
	33	00	88	24	93	57	47	33	59	80	71	33	25	88	21	67	86	65	33	59	57	65	75	00				
Sandık	0,7	0,7	0,6	0,7	0,5	0,7	0,7	0,6	0,5	0,4	0,6	0,5	0,6	0,6	0,4	0,5	0,6	0,6	0,7	0,6	0,6	0,7	0,7	0,7	1,0			
Fındığı	50	10	90	33	22	10	33	88	60	76	67	38	43	21	80	00	45	67	69	40	45	33	14	14	00			
Erkenci	0,7	0,7	0,6	0,8	0,6	0,7	0,6	0,7	0,7	0,5	0,5	0,6	0,5	0,6	0,6	0,6	0,7	0,6	0,6	0,8	0,8	0,7	0,7	0,9	0,7	1,0		
	43	65	88	48	15	65	06	43	86	83	19	21	16	88	43	29	06	67	90	57	24	27	74	03	41	00		
Hanımfındığı	0,8	0,8	0,7	0,7	0,6	0,9	0,6	0,9	0,6	0,5	0,7	0,8	0,7	0,7	0,6	0,6	0,7	0,8	0,7	0,6	0,8	0,7	0,8	0,8	0,6	0,7	1,0	
	42	11	43	78	21	73	67	47	45	19	33	13	06	43	45	32	03	33	50	45	65	78	24	24	67	27	00	

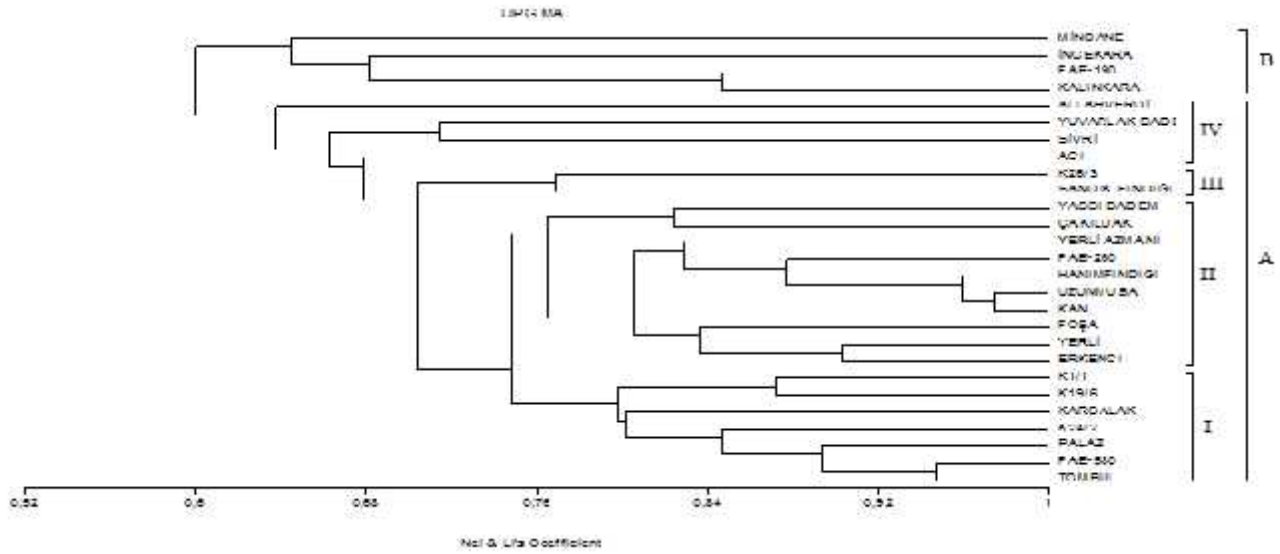

Figure 1. Constructed dendrogram among the 27 hazelnut genotypes with 29 markers using by Dice measurements (Nei and Li, 1979).

In dendrogram, the genotypes were separated into two main clusters with a similarity value of 0.600. The majority of the cultivars and accessions were included in the first cluster (A), while the 'Kalınkara', 'İncekara', 'Mincane' and 'FAE-190' were included in the second cluster (B).

The genotypes in the first cluster (A) were divided into four sub-groups. 'Tombul', 'FAE-580', 'Palaz', 'K24/2', 'Kargalak', 'K19/6' and 'K1/1' have taken place in the first sub-group (I); 'Erkenci', 'Yerli', 'Foşa', 'Kan', 'Uzunmusa', 'Hanımfındığı', 'FAE-260', 'Yerli Azmanı', 'Çakıldak', 'Yassı Badem' in the second sub-group (II); 'Sandık Fındığı' and 'K26/3' in the third sub-group (III); 'Acı', 'Sivri', 'Yuvarlak Badem' and 'Allahverdi' in the fourth sub-group (IV).

As expected, the 'K1/1', 'K19/6' and 'K24/2' hybrids, with the exception of 'K26/3', were included in the same sub-group (I) with their parents: 'Tombul' and 'Kargalak'. Similarity between the four hybrids and the male parent 'Tombul' were higher (0.800, 0.872, 0.895 and 0.824 respectively) than female parent 'Kargalak' (0.737, 0.865, 0.778 and 0.750 respectively). In a previous research, it was reported that 'K24/2' has relatively low similarity ratio to 'Tombul' (0.630) and 'Kargalak' (0.682) (Erdoğan *et al.*, 2010). 'Palaz' and 'FAE-580' accessions were placed in this sub-group with higher similarities. The accession 'K26/3' was placed together with 'Sandık Fındığı' in the sub-group III, showing a similarity of 0.769. Their similarity to first sub-group was 0.704. The cultivars and accessions placed in these sub-groups have similar pomological characteristics, with the exception of 'Kargalak' and 'Palaz'. These two cultivars have flattened round nut shape and similar husk traits. But, 'Kargalak' has the biggest nuts among the Turkish hazelnut cultivars (Çalışkan, 1995; Demir, 2004).

It was stated that 'Tombul', 'Sivri' and 'Palaz' cultivars are located in the *C. pontica* botanical group (Mehlenbacher, 1991). In this study, 'Tombul' and 'Palaz' cultivars are placed in same sub-group (I) with higher (0.923) similarity ratios. In a previous study, it was stated that similarity ratio is 0.656 between 'Tombul' and 'Palaz' (Erdoğan *et al.*, 2010). On the contrary, 'Sivri' is placed in the sub-group IV with mean similarity of 0.617. This cultivar shows relatively moderate genetic relationships to investigated cultivars and accessions. There is 0.688 similarity ratio between 'Tombul' and 'Sivri' that are compatible with that reported by Erdoğan *et al.* (2010). On the other hand, it was stated that 'Tombul' is closely related (0.85) to 'Sivri', in another research (Kafkas *et al.*, 2009).

In this study, the cultivar 'Kan' was placed in the sub-group II with mean similarity of 0.759. Among the investigated genotypes, this cultivar has the highest similarity ratio (0.974) with 'Uzunmusa'. Similarly, it was reported that these two cultivars were closely related in previous studies (Kafkas *et al.*, 2009; Erdoğan *et al.*, 2010; Gurcan *et al.*, 2010). 'Kan' also has a high similarity ratio (0.850) with the leading Turkish cultivar 'Tombul'. It was stated that 'Kan' is located in the *C. maxima* Gill. (*Corylus tubulosa* Will) species (Özbek, 1978). On the other hand, in another study, it is claimed that 'Kan' cultivar (leaves and kernel color is purple red) is located in the *C. avellana* var. *pontica* group (Ayfer *et al.*, 1986). Özbek (1978) suggested that Turkish hazelnut cultivars are hybrids of *C. avellana* and *C. maxima*. In our opinion, the results show that 'Kan' and other Turkish cultivars are placed in the same species as

reported in a previous research (Rovira, 1997). Also 'Hanımfındığı', 'FAE-260' and 'Yerli Azmanı' accessions were placed in this group with high similarity ratios (0.960, 0.877 and 0.829 respectively). 'Hanımfındığı' is different from these cultivars and accession in terms of pomological traits (Table 1).

In the first main cluster, the similarity values among some cultivars ('Uzunmusa' and 'Kan', 0.974 similarity ratio; 'Hanımfındığı' and 'Uzunmusa', 0.973 similarity ratio; 'Tombul' and 'FAE-580', 0.947 similarity ratio) were extremely high. However, in terms of morphological and pomological characteristics (nut, leaf and husk), these cultivars are quite different (Ayfer *et al.* 1986; Köksal, 2002; Beyhan and Demir, 2001; Demir, 2004). Only 'FAE-580' that was collected from Turkey germplasm can be a clone of 'Tombul' cultivar. They were highly similar to the cultivars and accessions in the first sub-cluster. Interestingly alleged the selection of 'Tombul', 'FAE-190' was placed in the second cluster (B). So this accession has low similarity (0.667) with 'Tombul'. This study revealed the results which are comparable with the previous reports (Erdoğan *et al.* 2010).

The accessions 'Yerli' and 'Erkenci' were placed together in the sub-group II of the cluster A with a similarity of 0.903. Also 'Foşa' is placed in this group with a similarity of 0.836. Although these accessions were grouped together, they were similar in terms of pomological characteristics and not in terms of nut maturation time. 'Erkenci' is a promising selection from Turkey germplasm (Demir, unpublished results) that is absolutely different from 'Yerli', which is a local cultivar (Beyhan and Demir, 2001), and other investigated cultivars and accessions in terms of nut maturation time. 'Erkenci' has the earliest nut maturation time**.** 'Erkenci' and 'Yerli' may be more closely related accessions than anticipated.

Interestingly, 'Çakıldak' and 'Yassı Badem' are connected each other with a high similarity ratio (0.824). These two cultivars were different in terms of pomological traits such as nut shape and husk properties (Table 1). Besides, while 'Çakıldak' is a cultivar for late season, 'Yassı Badem' is for early season.

'Acı', 'Sivri', 'Yuvarlak Badem' and 'Allahverdi' were placed in the same cluster with lower similarities (Figure 1, Table 3). Similar results were reported in previous researches (Kafkas *et al.* 2009; Erdoğan *et al.* 2010; Demir, 2004). These cultivars were different from each other in terms of pomological traits (Table 1). In contrast to our results, 'Allahverdi' and 'Tombul' are much more similar in previous researches (Demir, 2004; Erdoğan *et al.* 2010).

In the second main cluster (B), the 'FAE-190' and 'Kalınkara' are connected to each other with a similarity of 0.846. 'İncekara' is connected to this group with a similarity of 0.682, and then 'Mincane' is connected to this group with 0.644 similarity ratio. 'Kalınkara' and 'İncekara' cultivars have both long tubular husk and higher double kernel ratio (Ayfer *et al.* 1986; Demir, 2004) which has a high heritability (84%), according to previous studies (Mehlenbacher *et al.* 2006b). Similarly, it was reported that 'Kalınkara' and 'İncekara' were closely related in previous researches (Demir, 2004; Kafkas *et al.* 2009; Erdoğan *et al.* 2010; Gurcan *et al.*, 2010). 'Mincane' was placed in this cluster with a similarity of 0.644. This result was compatible with a previous study (Demir, 2004) and not compatible with some other studies (Kafkas *et al.* 2009; Erdoğan *et al.* 2010). Interestingly, 'FAE-190' has higher similarity ratio with 'Kalınkara' in this cluster. It was reported that this accession is a clone of 'Tombul' (Çetiner *et al.* 1984). 'İncekara' is the most distant cultivar among the investigated genotypes in this research. This cultivar has the lowest similarity ratio (0.364) with 'Allahverdi' and the highest similarity ratio (0.727) with 'FAE-190'.

In general, the mean genetic similarity among the investigated hazelnut cultivars and accessions differ from 0.506 ('İncekara') to 0.784 ('Tombul') with a mean value of 0.697. The highest genetic similarity was 0.974 (between 'Uzunmusa' and 'Kan'), the lowest genetic similarity was determined as 0.364 (between 'İncekara' and 'Allahverdi') (Table 3). Then, the results obtained show that an obvious inter-varietal variations among the investigated hazelnut cultivars and accessions exists, to exception of a few cultivars and accessions. Suitably it was reported that all of the deciduous hazelnut cultivars belong to *C. avellana* L. which is very big and polymorphic species (Rovira, 1997). However, it is emphasized that there is a significant variation among the clones of some important hazelnut cultivars (Çalışkan 1995; Demir and Beyhan, 2000).

Conclusion: In conclusion, the results obtained demonstrate a high level of polymorphism among hazelnut cultivars and accessions in Turkey. In general, The Turkish hazelnut cultivars and accessions were clearly segregated to each other with high genetic variations. Moreover, these cultivars were also observed to be Turkish germplasm. We verified that the genetic information, observed will assist the effective protection and sustainable utilization of hazelnut resources in Turkey.

Acknowledgements: DNA extraction was performed at the Biotechnological Laboratory at Department of Horticulture (Ondokuz Mayıs University). The author thanks the Department of Horticulture, Ondokuz Mayıs University for providing the opportunity to work at the laboratory. Also the author expresses gratitude to Hazelnut Research Institute, Giresun-Turkey for providing the leaf samples.

REFERENCES

Ahmad, Z., L. S. Daley, R. A. Menendez and H. B. Lagerstedt (1987). Characterisation of filbert (*Corylus*) species and cultivars using gradient polyacrylamide gel electrophoresis. J. Environ. Hort., 5(1):11-16.

Arıkan, F. (1960). The Fertilization Biology Properties of Important Hazelnut Cultivars Grown in Giresun (Giresun'da Yetisen Önemli Fındık Çesitlerinin Döllenme Biyolojisi Bakımından Hususiyetleri). Giresun Horticultural Station Printings: 2, Yeşil Giresun Press.

Ayfer, M., A.Uzun and F. Baş (1986). *Turkish Hazelnut Cultivars (Türk Fındık Çeşitleri).* Ankara: Blacksea Region Hazelnut Exportes Association.

Bassil, N. V. and A. N. Azarenko (2001). RAPD markers for self-incompatibility in *Corylus avellana* L. Acta Horticulturae,556:537-543.

Beyhan, N. and T. Demir (2001). Performance of the local and standard hazelnut cultivars grown in Samsun province, Turkey. Acta Horticulturae, 556:227-234.

Çalışkan, T. (1995). *Hazelnut Cultivar Catalogue (Fındık Çeşit Katalogu).* Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Production and Development.

Çetiner, E., N. Okay and F. Baş (1984). Final report of pre-selection of pollinator variety for round pomological group hazelnuts (Yuvarlak Pomolojik Fındık Grubunda Çeşit ve Tozlayıcı ön Seçimi Sonuç Raporu), Giresun: Hazelnut Research Institute.

Davis, J. W. and S. A. Mehlenbacher (1997). Identıfıcatıon of RAPD markers linked to eastern filbert blight resistance in hazelnut. Acta Horticulturae,445:553-556.

Demir, T. (2004). Determination of Relationships among the Turkish Hazelnut Cultivars and Clones by RAPD Markers and Pomological Characteristics (Türk Fındık Çesitlerinin RAPD Markörleri ve Pomolojik Özellikleriile Tanımlanarak Çesitler Arasında Akrabalık İliskilerinin Belirlenmesi) PhD Thesis (unpublished), Department of Horticulture, Ondokuz Mayıs University, Samsun (Turkey).

Demir, T. and N. Beyhan (2000). A research on the selection of hazelnuts grown in Samsun (Samsun ilinde yetiştirilen fındıkların seleksiyonu üzerine bir araştırma). Turk J. Agric For., 24:173-183.

Erdogan, V. and S. A. Mehlenbacher (2000). Phylogenetic relationships of *Corylus* species (*Betulaceae*) based on nuclear ribosomal DNA ITS region and chloroplast matK gene sequences. Systematic Botany, 4:727-737.

Erdoğan, V., A. İ. Köksal and A. Aygün (2010). Assessment of Genetic Relationships among Turkish Hazelnut (*Corylus avellana* L.) cultivars by RAPD Markers. Romanian Biotechnological Letters, 15(5):5591-5601.

FAO (2012). FAOSTAT Database. FAO, Rome. Retrieved from http://faostat.fao.org/faostat/ cited on 2012.

Galderisi, U., M. Cipollaro, G. Di Bernardo, L. De Masi, G. Galano and A. Cascino (1999). Identification of hazelnut (*Corylus avellana*) cultivars by RAPD analysis. Plant Cell Reports, 18:652-655.

Gökırmak, T. (2005). *Characterization of European Hazelnut (Corylus avellana L.) Cultivars Using SSR Markers.* M.Sc. Thesis (unpublished), Departmen of Horticulture, Oregon State University, Oregon (USA).

Gurcan, K., S. A. Mehlenbacher and V. Erdoğan (2010). Genetic diversity in hazelnut (*Corylus avellana* L.) cultivars from Black Sea countries assessed using SSR markers. Plant Breed, 129: 422-434.

Kafkas, S., Y. Doğan, A. Sabır, A. Turan and H. Şeker (2009). Genetic Characterization of Hazelnut (*Corylus avellana* L.) Cultivars from Turkey Using Molecular Markers. *HortScience*, 44(6):1557–1561.

Kasaplıgil, B. (1972). *A bibliography on Corylus (Betulaceae) with annotations,* Northern Nut Growers Ann. Rept. 63.

Köksal, A. İ. (2002). *Turkish Hazelnut Cultivars (Türk Fındık Çesitleri).* Ankara: Blacksea and İstanbul Hazelnut and Production Exporters' Associations.

Lunde, C. F., S. A. Mehlenbacher and D. C. Smith (2000). Survey of hazelnut cultivars for response to Eastern Filbert Blight Inoculation. HortScience, 35(4):729-731.

Malusà, E. (1994). Interspesific relationships among *Corylus* species. Acta Horticulturae, 351:335-339.

Mehlenbacher, S. A. (1991). Hazelnuts (*Corylus*). Genetic resources of temperate fruit and nut crops. Acta Horticulturae, 290:791-836.

Mehlenbacher, S. A., R. N. Brown, E. R. Nouhra, T. Gökirmak, N. V. Bassil and T. L. Kubisiak (2006a). A genetic linkage map for hazelnut (*Corylus avellana* L.) based on RAPD and SSR markers. Genome,49(2):122-33.

Mehlenbacher, S. A., D. C. Smith and L. K. Brenner (2006b). Variance Components and Heritability of Nut and Kernel Defects in Hazelnut. Plant Breeding, 110(2):144-152.

Miaja, M. L., R. Vallania, C. Me, A. Akkak, O. Nassi and G. Lepori (2001). Varietal characterization in hazelnut by RAPD markers. Acta Horticulturae, 445:247-250.

MVSP 3.13p, (2007). *Multi Variate Statistical Package.* http://www.kovcomp.com/.

Nei, M. and W. Li (1979). Mathematical model for studying genetic variance in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, 76:5256-5273.

Özbek, S. (1978). *Special Fruit Growing.* Ankara: Çukurova University, Publication of Agricultural Faculty:128, Textbook:11. Ankara University Press.

Pomper, K. W., A. N. Azarenko, J. W. Davis and S. A. Mehlenbacher (1996). Identification of random amplified polymorphic DNA (RAPD) markers for self-incompatibility alleles in hazelnut. HortScience, 31(4):591

Pomper, K. W., A. N. Azarenko, N. Bassil, J. W. Davis and S. A. Mehlenbacher (1998). Identification of random amplified polymorphic DNA (RAPD) markers for self-incompatibility alleles in *Corylus avellana* L. Theor. Appl. Genet., 97:479-487.

Radicati, L. R., R. Botta, G. Vergano and A. Akkak (1997). DNA characterisation of *Corylus* seedlings and their evaluation as rootstocks for hazelnut. Acta Horticulturae, 445:423-432.

Rovira, M. (1997). Genetic variability among hazelnut (*Corylus avellana* L.) cultivars. Acta Hoticulturae, 445:45-50.

Solar, A., F. Stampar and V. Usenik (1997). Identification of some hazelnut cultivars (*Corylus avellana* L.) with isozyme analysis. Acta Horticulturae, 445:21-29.

Thompson, M. M., H. B. Lagerstedt and S. A. Mehlenbacher (1996). Hazelnut. Fruit Breeding, 3:125-84.

Valentini, N., D. Marinomi, G. Me and R. Botta (2001). Evaluation of 'Tonda Gentile Delle Langhe' clones. Acta Horticulturae, 556:209-218.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafaelski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18(22):6531-6535.