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Assessment of Genetic Relationships among Turkish Hazelnut (*Corylus avellana* L.) Cultivars by RAPD Markers

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Abstract

Characterization of Turkish hazelnut cultivars and the genetic relationships among them were determined by Randomly Amplified Polymorphic DNA (RAPD) markers. Forty-three decamer primers produced a total of 241 clear and reproducible bands. The number of bands produced by primers was between 1 and 12 with an average of 5.7. Specific RAPD bands were associated with some cultivars. The high level of polymorphism corresponded to a high degree of genetic variation among hazelnut genotypes with Dice's similarity coefficients ranging from 0.477 to 0.941. The highest similarity coefficient was between 'Uzunmusa' and 'Kan' while the lowest was between 'Yassı Badem' and 'K-24-2'. Cluster analysis of band profiles showed heterogeneity among genotypes from the same area of cultivation. The standard cultivar 'Tombul' and its selections '190' and '260' were not clustered together in the UPGMA dendrogram and their Dice's similarity coefficients with 'Tombul' were 0.745 and 0.696 respectively. The results indicated that 'Tombul' and its selections are different clones, and it appears that 'Tombul' is a commercial name given to a group of clones grown in the Black Sea region with similar appearance but showing minor differences.

Keywords: Hazelnut, genetic relationships, molecular markers, RAPD, UPGMA

Introduction

The European hazelnut, *C. avellana*, is the most widely cultivated species within genus *Corylus* due to its superior quality and large size nuts (Thompson et al., [1]). This species requires special climatic conditions and consequently its production is limited to certain regions in the world. The Black Sea region of Turkey is very much suited for hazelnut cultivation, where hazelnut production has occurred for thousands of years. Shrubby growth habit and shallow roots make hazelnuts an ideal crop, in most cases inevitable on very steep and shallow land in the region. Turkey has been the leading hazelnut producer and exporter accounting for about 70% of the world production.

Turkish hazelnut culture has its own characteristics. The plants are usually defined by weak and shrubby growth habit with numerous suckers. Long tubular husks enclose the nuts but short and slit husks, which allow nuts fall on ground, exist in wild types as we observed. The most of the economically important Turkish hazelnut cultivars were described as selected forms of hybrids between *C. avellana* L. and *C. maxima* Mill. (Ayfer et al., [2]). However Thompson et al., [1] reported that as with of the important commercial cultivars in Europe, Turkish cultivars have been selected from local wild populations of *C. avellana* over many centuries, as supported by the molecular study of Boccacci et al., [3]. Turkish cultivars were suggested as being groups of clones (Islam, [4]; Erdogan et al., [5]), however neither the range of clonal variation or characteristics of these clones are known. Synonyms are common, for example, 'Tombul' is known as 'Yaglı' or 'Giresun Yaglısı' in the east and 'Mehmet Arif' in the west of the region (Ayfer et al., [2]). In addition, some growers keep wild plants called 'yabani' (= wild) in orchards in order to improve pollination.

Accurate and rapid cultivar identification is important for clonally propagated fruit tree species for both practical breeding purposes and for proprietary right protection. The hazelnut is a genetically diverse heterozygous plant species. The traditional methods for identification of hazelnut cultivars were based on phenotypic observations, including pomological and morphological characters such as nut size, nut shape, husk length, kernel blanching ratio, phenology and chilling requirements, etc. (Manzo and Tamponi, [6]; Ayfer et al., [2]; Erdogan and Mehlenbacher, [7]; Germain et al., [8]; Anonymous [9]). However, this can be a slow process, and subject to environmental factors, plant health and epigenetic factors. Furthermore, some phenotypic observations are only possible during particular periods of the year depending on the stage of plant development. In addition, cultivars are becoming increasingly more similar making it difficult to distinguish closely related cultivars and lines (Boccacci et al., [3]; Erdogan, [10]; Galderisi et al., [11]; Wunsch and Hormoza, [12]; Bacchetta et al., [13]). Although isozymes have been used as molecular markers to characterize hazelnut genotypes, they are not reliable since their expression could be influenced by developmental stage and environment in addition to their low level of polymorphism (Cheng et al., [14]; Rovira, [15]; Solar et al., [16]). On the other hand, DNA markers offer a useful and convenient method for accurately identifying and assessing genetic relatedness.

Molecular markers based on PCR amplifications such as RAPDs (Galderisi et al., [11]; Bacchetta et al., [13]; Radicati et al., [17]; Miaja et al., [18]), SSRs (Boccacci et al., [3]; Botta et al., [19]; Gokırmak et al., [20]; Ghanbari et al., [21]), ISSRs (Gurcan et al., [22]; Ferreira et al., [23]) and AFLPs (Ferrari et al., [24]) or combination of these markers (Martins et al., [25]; Kafkas et al., [26]) have been used for the characterization of hazelnut cultivars. RAPD markers offer simplicity, lower cost and speed for cultivar identification and genetic similarity studies in plants (Wunsch and Hormoza, [12]).

The objective of this study was to utilize RAPD markers to characterize Turkish hazelnut cultivars and their selections, and to assess the degree of genetic relatedness among them based on molecular data.

Materials and Methods

Plant material: Commercially produced 18 Turkish hazelnut cultivars together with two selections of ‘Tombul’ and one hybrid of ‘Kargalak’ x ‘Tombul’ located at the hazelnut collection plot maintained at the Hazelnut Research Institute, Giresun were used in the study (Table 1). In addition, an European cultivar, ‘Barcelona’, located in the research plots at the Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara was included in the study as an out-group. Newly expanded fresh leaves were brought to Ankara on ice. After brief washing in distilled water, they were frozen in liquid nitrogen and kept in freezer at -20^{o}C until used.

DNA extraction: Total genomic DNA was extracted using hexadecyltrimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle, [27] and Oliveira et al., [28] with minor modifications. Briefly, 250 mg leaf were grounded in liquid nitrogen and incubated at 60^{o}C for 30-60 min in 600 µl of isolation buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA (pH 8.0), 100 mM Tris-HCl (pH 8.0), 1% polyvinylpyrolidone (PVP-40) and 1% 2-mercaptoetanol), followed by chloroform-isoamyl alcohol (24:1) extraction. The mixture was gently vortexed (~1600 rpm) for 30 sec and inverted several times to form an emulsion. The mixture was centrifuged for 10 min (14.000 rpm at room temperature) and an RNase digestion was performed (10 µg/ml RNase-A at 37^{o}C for 30 min). After second chloroform extraction DNA was precipitated, washed and air-dried overnight. DNA was dissolved in 200µl Tris-EDTA buffer (pH 8.0). Purity of the DNA was determined spectrophotometrically (ND-1000,

NanoDrop Technologies, DE, USA), and DNA concentration was assessed by using fluorometer (TD-360, Turner Design, Sunnyvale, CA, USA). DNA was diluted to10ng/µl for PCR amplifications.

DNA amplification and electrophoresis conditions: PCR amplifications were performed in a volume of 20µl containing 1.25 units of Taq DNA Polymerase (Fermentas), 1X reaction buffer (with NH_4S0_4), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP (Fermentas), 0.4 µM primer and 12 ng genomic DNA. Total of 51 random decamer primers (Operon Technologies Inc., Alemeda, CA, USA) from A, B, J, O and Y series were tested. The amplifications were performed in T1-Thermocycler (Whatman Biometra, Gottingen, Germany) with 40 cycles of 94^{o}C for 30 sec, 36^{o}C for 1 min, 72^{o}C for 1 min with an initial denaturation of 94^{o}C for 90 sec and a final extension at 72^{o}C for 10 min. Amplified DNA fragments were separated on 1.5% agarose gel containing 0.5 µg/ml ethidium bromide. Electrophoresis was carried out at 100V for 4 h. Amplification products were viewed and photographed under UV light using GeneGenius Gel Documentation and Analysis System (Syngene Bioimaging Systems, Cambridge, UK).

Data analysis: Each gel was analyzed by scoring the presence (1) or absence (0) of polymorphic bands in individual lanes with only the clear and reproducible RAPD bands being scored. The NTSYS-pc software version 2.02g (Rohlf, [29]), was used to estimate genetic similarities with the Dice coefficient (Nei and Li, [30]). A dendrogram was constructed based on similarity matrix data by applying unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis using the SAHN clustering module.

Results and Discussions

DNA extraction of leaves yielded 76-193 µg/ml DNA for every 0.25 g of fresh leaf sample with an average $OD_{260/280}$ of 1.88. Galderisi et al., [11] suggested a second treatment of DNA with CTAB buffer to eliminate compounds such as tannins, alkaloids and flavones present in cell vacuoles. However, this process was not necessary in our extractions, since repeating the chloroform isoamyl alcohol treatment was satisfactory for purification of the DNA and for PCR amplifications.

Among the primers tested, five of them did not result in amplification. Two primers produced weak or minor bands, which were excluded from the analysis as Botta et al., [31], suggested that minor bands should be used with caution as markers for cultivar and clonal characterization. While one primer was monomorphic, the rest of the primers gave successful amplifications that produced 241 reproducible polymorphic bands ranging from 293bp to 2680bp. Gel image picture of RAPD bands amplified by the primer A-15 is presented in Figure 1. Number of bands per primer was 1-12 with a mean of 5.7, which was considered high compared to other studies. Miaja et al., [18] obtained 45 polymorphic bands between 500bp and 3000bp using 30 primers while Bacchetta et al., [13] scored 37 polymorphic bands using only six primers. Kafkas et al., [26] obtained 96 polymorphic bands from 25 primers and number of bands ranged from one to six, with a mean of 3.84 polymorphic bands per primer.

Association between certain amplified bands and the genotypes except with ‘Kus’, ‘Uzunmusa’ and ‘190’ (Table 1) was observed. The cultivars ‘Allahverdi’ and ‘Barcelona’ could be identified with a single band while six genotypes including ‘Tombul’ could be identified with more than one band. Kafkas et al., [26] identified six cultivars with RAPD primers, however when ISSR and AFLP primers were added the number increased to 10 cultivars. Similarly, distinct electrophoretic profiles for Italian cultivars ‘Tonda Romana’, ‘Tonda di Giffoni’, ‘Tonda Bianca’, ‘Tonda Rossa’ and ‘Tonda delle Langhe’ were reported (Bacchetta et al., [13]).

Table 1. Commercially and locally grown Turkish hazelnut cultivars and selections used in the study and RAPD bands specific to cultivars.

Code	Genotype	Pomological Group[1]	Production Area[1]	RAPD bands specific to cultivars[5]
1	Acı	Pointed	Ordu	OPA-15_{1908} OPO-1_{1157}
2	Allahverdi	Round	Giresun	OPA-10_{593}
3	Cavcava	Round	Trabzon	OPA-15_{1908} OPA-$16_{926, 1030}$ OPO-1_{1157}
4	Cakıldak	Round	Ordu	OPA-8_{769}
5	Fosa	Round	Trabzon, Akcakoca	OPA-11_{1415}
6	Incekara	Pointed	Giresun	OPA-16_{303}
7	Kalınkara	Round	Giresun, Ordu	OPA-16_{303} OPJ-1_{1500}
8	Kan	Round	Giresun	OPJ-4_{395}
9	Karafındık	Round	Akcakoca	OPA-2_{1526} OPA-18_{1151}
10	Kargalak	Round	Trabzon	OPY-3_{1177}
11	Kus	Pointed	Giresun	-
12	Mincane	Round	Trabzon	OPA-11_{1415} OPB-4_{1392}
13	Palaz	Round	Ordu, Samsun	OPA-11_{1271}
14	Sivri	Pointed	Giresun, Trabzon	OPO-1_{1157}
15	Tombul	Round	Giresun, Samsun	OPA-2_{1526}
16	Uzunmusa	Round	Ordu	-
17	Yassı Badem	Long	Adapazarı, Izmit	OPA-1_{1037}
18	Yuvarlak Badem	Long	Adapazarı, Izmit	OPA-16_{673}
19	190[2]	Round	-[4]	-
20	260[2]	Round	-[4]	OPA-2_{1448}
21	K-24/2[3]	Round	-[4]	OPA-16_{926}
22	Barcelona	Round	USA	OPA-11_{1415} OPA-11_{577}

[1](Ayfer et. Al., 1986 [2]; Calıskan, 1995 [32]). [2]Selections of 'Tombul'. [3]A promising hybrid from 'Kargalak' x 'Tombul'. [4]Genotypes are not under commercial production. [5]Primer names and band sizes (bp).

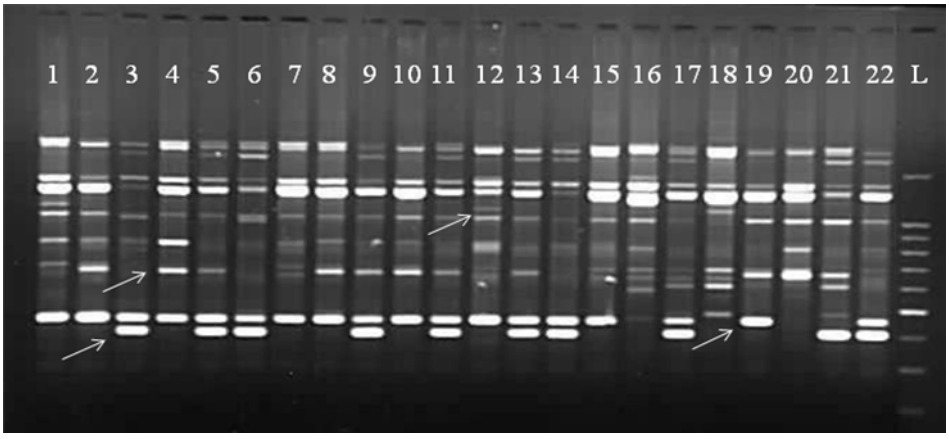

Figure 1. RAPD bands produced by primer OPA-15 for Turkish hazelnut genotypes on 1.5% agarose gel. Arrows show some of the polymorphic bands. Cultivars from left to right 1: Tombul, 2: Incekara, 3: Cavcava, 4: Mincane, 5: Kan, 6: Kargalak, 7: Fosa, 8: Karafındık, 9: Cakıldak, 10: Kus, 11: Uzunmusa, 12: Kalınkara, 13: Palaz, 14: Acı, 15: Sivri, 16: Allahverdi, 17: Yuvarlak Badem, 18: Yassı Badem, 19: 190, 20: 260, 21: Barcelona 22: K-24/2 and L: 100bp ladder (Promega).

Genetic similarity based on Dice's coefficients of 22 hazelnut genotypes with RAPD markers is illustrated in Table 2. The high level of polymorphism corresponded to a high degree of genetic variation among the hazelnut genotypes that Dice's similarity coefficients ranged from 0.477 to 0.941. A narrower genetic variation was found among Turkish hazelnut cultivars as reported by Kafkas et al., [26] such that similarities calculated using Jaccard's coefficients from three PCR based markers ranged from 0.73 to 0.96. The use of different marker systems and/or additional genotypes including 'Barcelona' as an out-group in our study could have caused this difference. The cultivars 'Uzunmusa' and 'Kan' had the highest similarity coefficient while 'Yassı Badem' and 'K-24-2' had the lowest among the genotypes. The cultivars 'Acı' and 'Cavcava', and 'Incekara' and 'Kalınkara' were highly related, on the other hand genetic relationships between several genotypes such as 'Fosa' and 'Mincane' were considered relatively high.

Dendrogram constructed from the Dice's similarity coefficients matrix using the unweighted pair group method with arithmetic average (UPGMA) is shown in Figure 2. The cluster analysis grouped the genotypes into two major clusters. The first one consisted of 19 genotypes with two sub-groups. 'Tombul' and all of the cultivars and selections with round or pointed nuts were grouped in the first sub-group. 'Tombul' clustered with 'Allahverdi' within it. Interestingly, none of the cultivars with pointed nuts such as 'Acı', 'Kus', 'Incekara' and 'Sivri' were clustered together but they were dispersed in both sub-groups. The second major cluster consisted of 'Yuvarlak Badem', 'Yassı Badem', which have long nuts, and 'Barcelona', which has very large and round nuts with thick shells from European gene pool. This cluster was clearly separated from the rest of the cultivars. The UPGMA dendrogram of Kafkas et al., [26] including 18 Turkish hazelnut cultivars generated by the combination of different markers (RAPDs, ISSRs and AFLPs) showed different clustering pattern then ours. In their study, 'Tombul' grouped with 'Sivri', and 'Yassı Badem' and 'Yuvarlak Badem' were placed in different clusters. The use of different marker systems and/or the use of additional genotypes and 'Barcelona' as an out-group in our study could have caused these differences. Bacchetta et al., [13] reported that 'Tombul' formed a single cluster itself while 11 Italian cultivars were clustered together based on RAPD markers in which geographic origin was evident on dendrogram. The comparative analysis of Miaja et al., [18] from RAPD profiles placed cultivars into two groups: 15 Italian and American cultivars in the first group and four cultivars including two Turkish cultivars 'Tombul' and 'Imperiale de Trebizonde', and *C. maxima* 'Fructo rubro' and 'Jeans' in the second group. The authors related the latter grouping to morphological similarities since all four cultivars has a tubular husk.

Combined analysis of Dice's genetic similarity coefficients and UPGMA cluster analysis revealed the relationships better among the cultivars. 'Uzunmusa' and 'Kan' had the highest similarity coefficient (0.941) and this close relationship was supported by results from the cluster analysis (Figure 2). Similarly, Kafkas et al., [26] found high degree of similarity (0.96) between these two cultivars. 'Kan' has very distinct pellicle (testa) color of reddish purple while 'Uzunmusa' has light to dark brown colored pellicle (Ayfer et al., [2]). Although these cultivars have round nuts, they are both low in production and have local importance (Calıskan, [32]). 'Palaz', which is one of the principal cultivars in hazelnut production, grouped with the 'Uzunmusa' - 'Kan' cluster with similarity coefficients of 0.757 and 0.739, respectively. Nuts of 'Palaz' are round and larger than those of these two cultivars, and production is concentrated around Ordu. Kafkas et al., [26] found almost similar results that 'Palaz' formed a group with 'Kan' - 'Uzunmusa' and 'Incekara' - 'Kalınkara' clusters.

Table 2. Genetic similarity matrix between 22 hazelnut genotypes with RAPD markers based on Dice's coefficients.

Genotype*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.000																					
2	0.604	1.000																				
3	0.842	0.529	1.000																			
4	0.682	0.655	0.676	1.000																		
5	0.613	0.683	0.539	0.579	1.000																	
6	0.595	0.616	0.600	0.690	0.638	1.000																
7	0.546	0.595	0.533	0.628	0.604	0.818	1.000															
8	0.673	0.673	0.667	0.689	0.711	0.740	0.657	1.000														
9	0.589	0.695	0.604	0.673	0.716	0.735	0.690	0.743	1.000													
10	0.670	0.576	0.635	0.618	0.603	0.574	0.533	0.637	0.629	1.000												
11	0.585	0.664	0.600	0.650	0.702	0.773	0.727	0.680	0.725	0.585	1.000											
12	0.642	0.752	0.562	0.638	0.780	0.726	0.663	0.715	0.788	0.614	0.746	1.000										
13	0.670	0.631	0.585	0.626	0.612	0.677	0.632	0.739	0.660	0.579	0.635	0.674	1.000									
14	0.644	0.748	0.610	0.651	0.775	0.680	0.647	0.729	0.724	0.647	0.761	0.745	0.604	1.000								
15	0.644	0.766	0.582	0.602	0.681	0.619	0.627	0.700	0.734	0.616	0.650	0.775	0.656	0.710	1.000							
16	0.699	0.688	0.710	0.725	0.729	0.768	0.663	**0.941**	0.780	0.673	0.737	0.732	0.757	0.766	0.716	1.000						
17	0.571	0.593	0.519	0.577	0.602	0.583	0.561	0.586	0.670	0.529	0.531	0.643	0.599	0.574	0.667	0.602	1.000					
18	0.638	0.601	0.594	0.566	0.611	0.541	0.530	0.693	0.626	0.670	0.582	0.631	0.628	0.663	0.653	0.710	0.660	1.000				
19	0.561	0.584	0.517	0.577	0.626	0.714	0.624	0.670	0.663	0.559	0.724	0.719	0.644	0.617	0.745	0.698	0.590	0.588	1.000			
20	0.633	0.679	0.609	0.639	0.647	0.657	0.615	0.667	0.699	0.605	0.677	0.664	0.613	0.676	0.696	0.702	0.555	0.631	0.636	1.000		
21	0.603	0.595	0.577	0.642	0.523	0.652	0.604	0.641	0.578	0.682	0.607	0.584	0.647	0.597	0.630	0.681	**0.477**	0.522	0.580	0.575	1.000	
22	0.569	0.581	0.505	0.646	0.589	0.591	0.558	0.646	0.638	0.588	0.581	0.611	0.630	0.624	0.603	0.674	0.630	0.617	0.542	0.571	0.529	1.000

* See Table 1. for name of the genotypes

High similarity coefficients of 0.842 and 0.818 were obtained between 'Acı' and 'Cavcava', and 'Incekara' and 'Kalınkara' cultivars, respectively, and these relationships were supported by cluster analysis (Figure 2). Kafkas et al., [26] found similar results that 'Acı' clustered with 'Cavcava', and 'Incekara' clustered with 'Kalınkara'. Boccacci et al., [3] also found a close relationship between 'Kalınkara' and 'Incekara' with SSR analysis. 'Acı' is grown in Ordu and 'Incekara' is grown in Giresun, but neither of them is commercially important. Although they have been considered in the pomological group of pointed nuts, we showed that their closest relatives were cultivars with round nuts such as 'Cavcava' (grown in Trabzon) and 'Kalınkara' (grown in Giresun). Bacchetta et al., [13] reported similar results that the phylogenetic analysis of cultivars from the same location did not always cluster together.

Similarity coefficient of 0.722 indicated that the closest relative of 'Cakıldak' was 'Uzunmusa'. However, cluster analysis grouped 'Cakıldak' with the 'Acı' - 'Cavcava' cluster with similarity coefficients of 0.682 and 0.676, respectively. Kafkas et al., [26] reported a different clustering pattern that 'Cakıldak' was grouped with a branch of the 'Kan' - 'Uzunmusa', 'Incekara' - 'Kalınkara' and 'Palaz' clusters. Kernels of 'Cakıldak' (syn. 'Delisava' and 'Gokfındık') are not flavorful as other cultivars, and it requires the highest chilling hours among the Turkish genotypes. Production is concentrated around Ordu, especially at higher altitudes at 500-750m from sea level (Calıskan, [32]).

Although the cultivars 'Fosa' and 'Mincane' had relatively high similarity coefficient (0.780), UPGMA analysis clustered 'Fosa' with 'Sivri' (0.775) which was unexpected (Figure 2). 'Fosa' (syn. 'Yomra' and 'Boyhane') is one of the principal cultivars and is mainly grown around Trabzon. The nuts are round and larger than 'Sivri' with a 92% of blanching ratio. On the other hand, 'Sivri' has pointed nuts with lower kernel blanching ratio (53.3 - 72.3%) (Ayfer et al., [2]; Calıskan, [32]). The nuts are generally sold in shell since they are not suitable for processing. Plants of 'Sivri' are found almost in every orchard in Giresun area. Kafkas et al., [26] found almost similar results that 'Fosa' was clustered with a larger group consisting of 'Tombul'– 'Sivri' and 'Mincane'– 'Kus' clusters.

UPGMA cluster analysis placed the cultivars 'Mincane' and 'Karafındık' together on the dendrogram (Figure 2) and this relationship was supported by relatively high similarity coefficient of 0.788. 'Mincane' (syn. 'Sarıfındık', 'Sarıyaglı' and 'Zango') is grown around Trabzon region (Ayfer et al., [2]; Calıskan [32]). Although nuts of 'Mincane' are similar to that of 'Tombul', it is not favored much by growers since it has shorter husks with slit that nuts fall on ground at maturity thus making harvest difficult. On the other hand, 'Karafındık' is grown around Akcakoca in the Western Black Sea coasts where it is productive, but the number of shriveled kernels is high and it has tendency to alternate bearing (Ayfer et al., [2]).

Similarity coefficient of 0.761 indicated that the closest relative of 'Kus' was 'Sivri' which was congruent with morphological similarity that both cultivars have pointed nuts. These cultivars are produced around Giresun while 'Kus' has only local importance (Calıskan, [32]). However, cluster analysis placed 'Kus' with the 'Incekara' - 'Kalınkara' cluster on the dendrogram (Figure 2). In another study closest relative of 'Kus' was reported 'Mincane' (Kafkas et al., [26]).

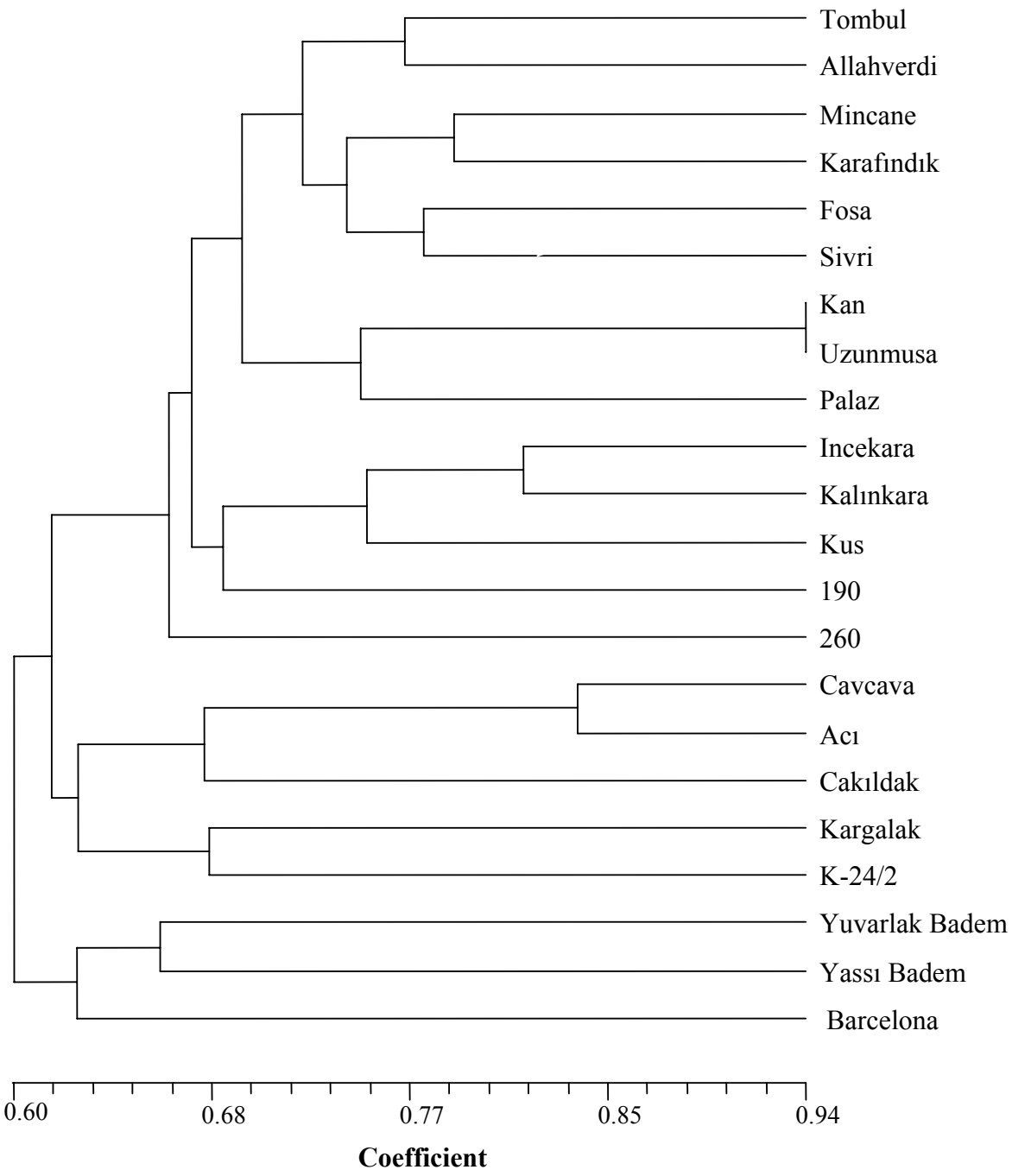

Figure 2. Dendrogram with unweighted pair group method with arithmetic average (UPGMA) of 22 hazelnut genotypes based on RAPD data. The dendrogram was constructed from the Dice's similarity coefficients matrix in Table 2.

Among the cultivars, 'Yassı Badem' and 'K-24-2' had the lowest similarity coefficient (0.477). Kafkas et al., [26] obtained the lowest similarity coefficient (0.73) between 'Yassı Badem' and 'Kalınkara' among Turkish cultivars. 'Yassı Badem' has long but compressed nuts and very similar to 'Yuvarlak Badem', which has long but round nuts. Both cultivars are produced in Adapazarı and Izmit, east of Marmara region. The kernel percentage is considered low about 41 - 45%. They are harvested early in mid-July and sold fresh in husks as soon as their nuts become light brown in color. In our study, these two cultivars clustered together distant from other cultivars on the dendrogram which was expected due to morphological similarities. However, the similarity coefficient between them was relatively

low (0.66). On the other hand, 'K-24/2' is a hybrid between 'Kargalak' x 'Tombul'. 'Kargalak' has largest nuts among Turkish genotypes and it was used as parent to increase the nut size of 'Tombul' (Okay, [33]). As expected, the hybrid 'K-24/2' clustered together with its female parent 'Kargalak' but with a relatively low similarity coefficient of 0.682.

'Tombul' is the leading cultivar in Turkish hazelnut production. Although similarity coefficient of 0.775 indicated that 'Tombul's closest relative was 'Mincane', UPGMA analysis clustered 'Tombul' with 'Allahverdi' (0.766). However, Kafkas et al., [26] found that 'Allahverdi' was a distant genotype from other Turkish cultivars. 'Allahverdi' is a selection found recently in some hazelnut orchards around Giresun, and although it is very productive, it is not a commercial cultivar but is a very promising candidate. 'Tombul' had distant relationship with 'Cavcava' as evidenced with the low similarity coefficient of 0.582. Cluster analysis agreed with this relationship that these two cultivars were placed distantly on the dendrogram (Figure 2). 'Cavcava' (syn. 'Kocakarı fındıgı') nuts are round in shape (Calıskan [32]). This cultivar is characterized by low level of productivity, high amounts of blanks and highly variable nut size. Production is low and limited to Trabzon area (Ali Turan, personal communication).

'Tombul' is a principal cultivar especially in Giresun province. Due to its very superior kernel quality characteristics such as good taste, round shape, medium size (13.1mm), no fiber and twins, high kernel percent (52.4%) and high blanching ratio (96.6%), kernels are suited very much to snack consumption and hazelnut processing industry. Thus, 'Tombul' is one of the most popular levantine hazelnut in international markets. Although 'Tombul' is productive, it has tendency to alternate bearing and suffers from big bud mite (*Phytoptus avellanae*) damage (Ayfer et al., [2]; Calıskan, [32]). There was a common view that trees of 'Tombul' derived from a single clone in the region; however, early field observations indicated that variations exist among Tombul ocaks in orchards. Other authors have made similar observations as well. A selection was performed in grower orchards between 1968 and 1971 (Cetiner, [34]). Ten types including clones '190' and '260' were selected for higher yield and better nut characteristics (Cetiner et al., [35]). These two clones were included in this study; but none of them was clustered together with 'Tombul', and their similarity coefficients with 'Tombul' were 0.745 and 0.696 respectively. In addition, the two selections had relatively low (0.636) similarity coefficient between them. Cetiner, [34] stated that variation in 'Tombul' is not caused by clonal differences but rather due to seedling origin. However, our genetic analysis at molecular level showed that 'Tombul' and its selections are in fact different clones. We therefore suggest that 'Tombul' is a commercial name given to a group of clones grown in the Black Sea region with similar appearance but which show minor differences. Islam, [4] and Erdogan et al., [5] support this finding, and they extend this description to other Turkish cultivars as well.

Conclusions

RAPDs were a useful marker system for characterization and for the determination of genetic relationships among Turkish hazelnut cultivars. However, to get reliable and reproducible results, purity of extracted DNA and PCR protocols should be strictly controlled. The UPGMA dendrogram indicated that the current classification of the cultivars based on morphology do not always coincide with the clustering on the dendrogram. The cluster analysis of RAPD profiles showed heterogeneity among genotypes from the same area of cultivation. Although only two clones were used, we were able to show genetically clonal differences within 'Tombul', but a larger study would be needed to support this finding using many accessions from different production areas for 'Tombul' and other Turkish hazelnut cultivars.

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