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Research
Araştırma

MORPHOLOGICAL AND MOLECULAR COMPARISON OF SELECTED CHESTNUT (*Castanea sativa* Mill.) GENOTYPES FROM BLACK SEA REGION OF TURKEY

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ABSTRACT : Here we report phenotypic and genotypic differences among 14 Turkish chestnut genotypes. The genotypes were analyzed both genetically and for 30 morphological criteria comprising 12 qualitative and 18 quantitative characteristics. The phylogenetic relationships were determined between different chestnut genotypes selected from Sinop, Samsun, Artvin and Bartin provinces of Black Sea Region in Turkey. Morphological criteria were investigated biometrically using multivariate analysis. Ten morphological criteria were found to be effective for discrimination between the genotypes. Five morphological criteria accounts for 64.1% of the variability. These morphological criteria were the ratio of length of teeth to width of teeth, the ratio of length of hilum to length of fruit, cross section of leaves, the ratio of height of fruit to length of fruit and peeling of testa. The discrimination of morphological criteria was shown using cluster analysis, which created four main groups. Dice's coefficient was used to evaluate the genetic similarity by RAPD analysis, which created three main groups. The UPGMA method was used for the determination of phylogenetic trees. The genetic and morphological dendrograms were compared using the Mantel test, which gave a correlation of r=-0.33. The discrimination of morphological criteria was shown using cluster analysis, which created four main groups. Dice's coefficient was used to evaluate the genetic similarity by RAPD analysis, which created three main groups. The UPGMA method was used for the determination of phylogenetic trees. The genetic and morphological dendrograms were compared using the Mantel test, which gave a correlation of r=-0.33. This study illustrates that the selected chestnut genotypes might be valuable genetic resources for future chestnut breeding programs.
Keywords: Genetic similarity, morphological criteria, phylogenetic tree, RAPD

TÜRKİYE'NİN KARADENİZ BÖLGESİNDEN SEÇİLEN KESTANE (*Castanea sativa* Mill.) GENOTİPLERİNİN MORFOLOJİK VE MOLEKÜLER KARŞILAŞTIRMASI

ÖZET: Bu çalışmada 14 Türk kestane genotipinde fenotipik ve genotipik farklılıklar incelenmiştir. Genotipler, hem genetik olarak hem de 12'si kalitatif ve 18'i kantitatif olmak üzere toplam 30 morfolojik kritere göre analiz edilmiştir. Çalışmada, Sinop, Samsun, Artvin ve Bartın illerinden seçilen farklı kestane genotipleri arasındaki filogenetik ilişkiler belirlenmiştir. Morfolojik kriterler çok değişkenli analizler kullanılarak biometrik olarak araştırılmıştır. Genotiplerin ayırt edilmesinde 10 morfolojik kriterin etkili olduğu tespit edilmiştir. Bunlardan beşinin genotipler arasındaki farklılığın %64,1'ini açıkladığı saptanmıştır. Bu kriterler: diş uzunluğu/diş genişliği oranı, hilum uzunluğu/meyve uzunluğu oranı, yaprakta enine kesit, meyve yüksekliği/meyve uzunluğu oranı ve tohum zarının soyulabilirliği'dir. Morfolojik kriterlere göre yapılan kümeleme analizinde dört ana grubun oluştuğu görülmüştür. "Dice" katsayısı RAPD analizi ile sonuçlarına gore hesaplanmış ve genetik benzerlik durumunun değerlendirilmesinde kullanılmıştır. Buna göre de 3 ana grubun oluştuğu görülmüştür. Filogenetik ağaçlar UPGMA metoduna göre oluşturulmuştur. Genetik ve morfolojik dendogramlar Mantel testi yardımıyla karşılaştırılmış ve r=-0.33 korelasyon katsayısı hesaplanmıştır. Bu çalışma seçilen genotiplerin gelecekte yapılacak ıslah çalışmaları için değerli genetik kaynaklar olduğunu göstermiştir.
Anahtar Sözcükler: Genetik benzerlik, morfolojik kriterler, filogenetik ağaç, RAPD

1. INTRODUCTION

The chestnut (*Castanea* Miller) belongs to the beech family (*Fagaceae*), which also includes the beech (*Fagus*), the oak (*Quercus*), and the chinquapin (*Castanopsis).* The thirteen *Castanea* species are native to the temperate zone of the Northern Hemisphere: five to East Asia, seven to North America and one to Europe (Burnham et al., 1986). The European chestnut (*Castanea sativa* Mill.) is widespread throughout Europe and southwest Asia. Anatolia is one of the places chestnuts originated and were first cultivated (Soylu, 2004). The first scientific selection studies on the chestnut started in 1975 in the Marmara region, while other regions later followed with selection studies at different institutes (Ayfer et

al., 1977; Ozkarakas et al., 1995; Serdar, 1999; Serdar and Soylu, 1999; Serdar, 2002; Ertan et al., 2007; Koyuncu et al., 2008). Some isozyme studies on the European chestnut indicate that Turkey has a range of potential of chestnut genotypes and is considered to have different subspecies of *C. sativa* Mill. (Pigluici et al., 1990; Villani et al., 1991; Villani et al., 1992; Lauteri et al., 1999). Although Turkey is the world's third largest producer of chestnuts, the production has decreased due to chestnut blight disease in recent decades (caused by *Cryphonectria parasitica* [Murrill] Barr) (Anonymous, 2008). It is believed that the best way of controlling the disease is to use resistant genotypes and biological control using hypovirulent types of chestnut blight disease. Successful control of disease have been obtained in France, Italy, Portugal and other countries, some studies were performed in Turkey to find genotypes of chestnut resistant to chestnut blight (Baykal et al., 2000; Erper et al., 2004). However, these studies did not include tests of genetic similarity for discrimination of genotypes. It is thought that genetic diversity among the species can influence blight resistance. Although it is known that American and European species are susceptible to blight, Asian species are resistant (Huang et al., 1996; Huang et al., 1998). Several studies on allozyme diversity propose that the American chestnut has the lowest level of genetic diversity among species in the genus *Castanea* (Villani et al., 1991; Huang et al., 1998; Huang et al., 1994).

RAPD markers were used in plants for diversity studies widely (Bojović et al., 2000; Ding et al., 2009; Ikegami et al., 2009; Okumus and Balkaya, 2007; Arslan and Okumus, 2006; Okumus, 2007; Sivolap, 1995). Many PCR-based marker studies have been performed on chestnut. Some studies with ISSRs (inter-simple sequence repeat markers) have determined a high level of genetic diversity among Shandong natural trees (Ai et al., 2007) and Chinese chestnuts (*Castanea mollissima*) (Han et al., 2007). In addition, there are some other examples of genetic diversity and genetic characterization studies on chestnut using RAPD (Random Amplified Polymorphic DNA), SSRs (Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) and RFLP (Restriction Fragment Length Polymorphism) (Yamamoto et al., 1998; Fineschi et al., 2000; Solar et al., 2005; Martin et al., 2008).

The purpose of the present study was to discriminate between selected *C. sativa* genotypes using RAPD and morphological characteristics and to ascertain whether the similarity matrix of both characterization methods could provide guidelines for use in further breeding programs.

2. MATERIAL AND METHODS

2.1. Material

The study was carried out on chestnut genotypes (*Castanea sativa* Mill.) selected from the Black Sea Region of Turkey, in 2004-2005. The genotypes studied were SE 3-12, SA 5-1, SE 18-2, SE 21-2 and SE 21-9 from the province of Sinop (Serdar, 1999), 552-8, 552-10, 554-14 and 556-8 from Samsun (Serdar and Soylu, 1999), 08-Camili-8, 08-Camili-13 and 08-Camili-14 from Artvin (Serdar, 2002), and 74-Ulus-1 and 74-Ulus-5 from Bartin (Figure 1). The samples were collected from trees in trial orchards in Samsun and Ordu for the Sinop and Samsun genotypes. The samples were collected from original native trees for the other genotypes (Artvin and Bartin). Molecular analyses were performed at the Genetics and Biotechnology Laboratory of Agriculture Faculty of Ondokuz, Mayis University.

2.2. Methods

2.2.1. Morphological Analysis

In the study, thirty criteria, consisting generally of ratios and shapes, were used. Twelve of the characteristics were qualitative, while eighteen were quantitative.

The following morphological traits were determined for every genotype according to (Pigliucci et al., 1990; Kotobuki, 1996; Oraguzie et al., 1998; UPOV, 1988).

1. Shoot: Density of shoots and anthocyanin coloration on the shoots were investigated.

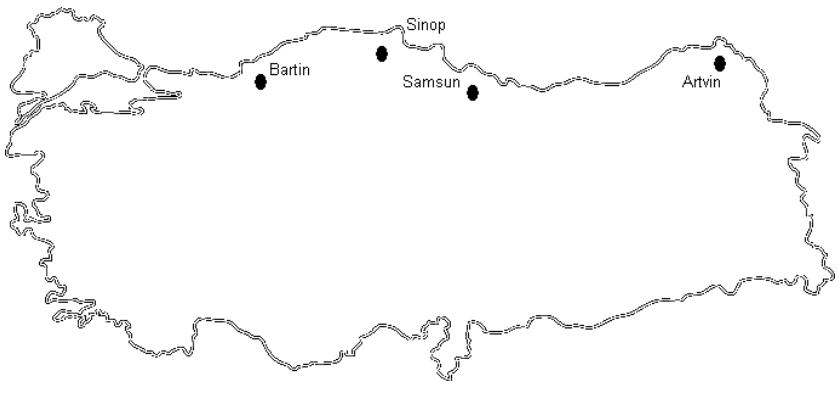

Figure 1. Map of the sample locations for chestnut genotypes in this study

2. Bud: Length and width measurements of buds were done in the in the middle parts of shoots. The ratio of the width of bud to the length of bud was used.

3. Leaf: Shape of leaf tip, cross section in leaf, shape of teeth, number of teeth and number of lateral veins in leaf were investigated, as well as the ratios of length of lamina to width of lamina, length of lamina to length of petiole, number of teeth to number of lateral veins in leaf and length of teeth to width of teeth.

4. Flower: Length of male flower (cm), length of mixed catkin (cm), length of stamen (mm), number of pistil clusters on the mixed catkin and habit of male catkin were investigated.

5. Bur: Shape of the bur, length of the bur and density of spines per cm^2 on the outer portion of the bur were investigated.

6. Fruit: Weight of the fruit (g), the ratio of height of fruit to length of fruit, cracking ratio on the shell (%), the ratio of length of hilum to length of fruit, the ratio of length of hilum to width of hilum, shape of fruit, shape of pericarp lines on the outside of the fruit, shape of separation line in hilum, peeling of testa and color of kernel were investigated.

Biometric analyses were done to establish the best criteria for identifying chestnut genotypes for discrimination. The results were shown as cluster analysis and principal component analysis of chestnut genotypes using SPSS statistical software.

2.2.2. RAPD Analysis

The DNA samples for RAPD-PCR analysis were collected from young buds of original trees during visits to the region and saved in -196 ^oC liquid nitrogen. DNA extraction of samples was done according to the method of Doyle and Doyle (1990). The total genomic DNAs of samples were prepared in 0,1 M TE pH 8.0 at 10 ng/µl and saved at -20 ^oC for further analysis. Five sets of Operon ten-mer RAPD primers (100 primers in total, OPA, OPB OPC, OPD, OPE series-Operon Biotechnologies GmbH, Köln, Germany) were tested for analysis of genotypes (Sambrook et al., 1989; Williams et al., 1990; Steward and Via, 199; Kubisiak, 1999).

Optimized RAPD-PCR reactions were performed in volumes of 25.0 µl containing 0.5 U Taq polymerase (RedTaq, SIGMA) 0.4 µM primer, 0.64 mM each dNTP, 2.5 µl 10X Reaction Buffer (500 mM KCl, 15 mM $MgCl_2$ and 100 mM Tris-HCl (pH:9.0)) and 30 ng template DNA. Reactions were then placed into the thermal cycler and run 1 cycle of 94 ^oC for 5 minutes for initial denaturation, followed by 45 cycles of 94 ^oC for 30 seconds for denaturation, 36 ^oC for 30 seconds for primer annealing and 72 ^oC for 1 minute for extension. The final extension step was at 74^oC for 4 minutes.

In the study, 9 polymorphic RAPD primers were selected as seen in Table 1. The RAPD-PCR products were run on a 1.5% agarose gel and imaged using the gel documentation system (SYNGENE, Cambridge, UK). A 100-bp DNA ladder (Amresco) was used as a molecular size marker to compare DNA fragments. The bands established were summarized and analyzed by UPGMA for cluster analysis and by the Mantel test for comparison of genotypes using NTSYS-PC v2.1 software (Numerical Taxonomy System, Exeter Software, NY, USA) (Rohlf, 1989).

Table 1. The polymorphic primers selected in this study

Primer	Sequence 5´— 3'
OPA02	5'-TGCCGAGCTG-3'
OPA04	5'-AATCGGGCTG-3'
OPA18	5'-AGGTGACCGT-3'
OPB01	5'-GTTTCGCTCC-3'
OPB08	5'-GTCCACACGG-3'
OPB11	5'-GTAGACCCGT-3'
OPB17	5'-AGGGAACGAG-3'
OPE07	5'-AGATGCAGCC-3'
OPE15	5'-ACGCACAACC-3'

3. RESULTS AND DISCUSSION

3.1. Morphological Data Analysis

Morphological analysis is one of the criteria used for discrimination of genotypes. Table 2 shows the Eigen values for different components. The first component shows 17.0% success if considered alone, while together with the second component there is a cumulative 32.3% success at discrimination, and with the third component there is a 43.9% success at discrimination between genotypes according to these morphological criteria. Ten morphological criteria were found to be the most effective for discrimination of the genotypes. The five most effective morphological criteria accounts for 64.1% of the variability. These morphological criteria are the ratio of length of teeth to width of teeth, the ratio of length of the hilum to length of the fruit, cross section in leaf, the ratio of height of fruit to length of fruit and peeling of testa.

The Pearson correlation matrix for all the morphological criteria was calculated, as seen in Table 3. The lowest similarity was seen between SE 3-12 and 08-Camili-14 with a value of 0.138. The highest correlation coefficient was seen between SE 18-2 and SE 21-2 with a value of 0.724 in the Sinop genotypes.

The dendrogram related to this matrix is shown in Figure 2. This classification established four main groups; the first group: 08-Camili-8, 552-8 and 552-10; the second group: 08-Camili-13, 74-Ulus-1, SE 18-2, SE 21-2, SE 21-9, 554-14, SE 3-12, 556-8; the third group: 08-Camili-14, 74-Ulus-5; and the fourth, most recent group: SA 5-1. No geographical grouping between genotypes was seen due to the selection of genotypes for good yield.

The Artvin genotypes were separated into different groups although Samsun and Sinop were mostly in the

Table 2. Eigen value and variation of quantitative and qualitative criteria for selection success

Component Matrix	Total	Variance (%)	Cumulative (%)
Length of teeth to width of teeth	5.110	17.035	17.035
Length of the hilum to length of the fruit	4.577	15.256	32.291
Cross section in leaf	3.479	11.598	43.889
Height of fruit to length of fruit	3.130	10.435	54.323
Peeling of testa	2.946	9.818	64.142
Length of stamen	2.376	7.920	72.061
Number of spines at outer of the bur	2.140	7.135	79.196
Habit of male catkin	1.932	6.440	85.636
Number of pistil cluster on the mixed catkin	1.540	5.133	90.769
Length of lamina to length of petiole	1.059	3.530	94.299

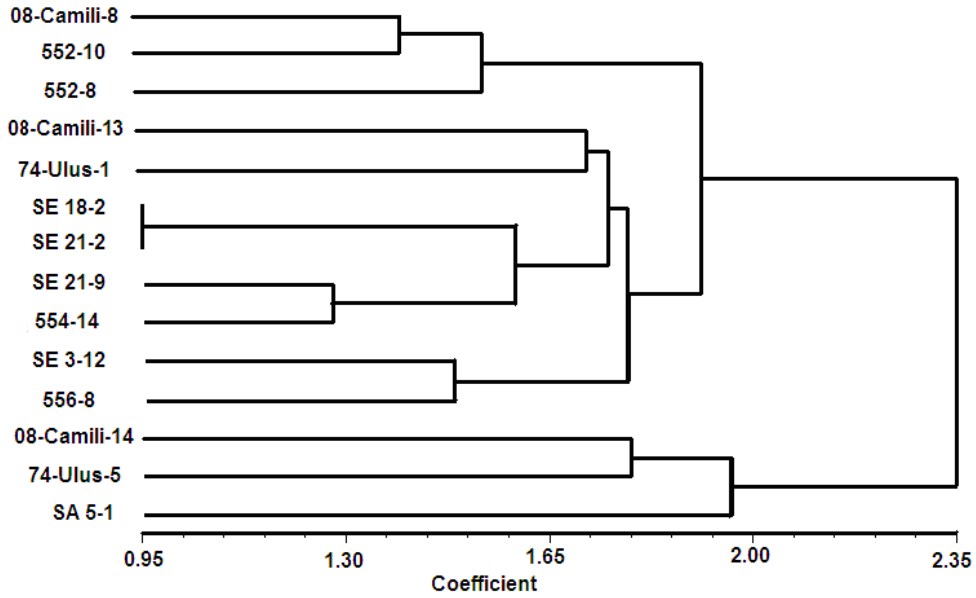

Figure 2. A dendrogram based on the morphological criteria

second group. It is interesting that 08-Camili-14, 74-Ulus-5 and SA 5-1 appear to be more morphologically similar than other genotypes because they are placed in different groups genetically and geographically.

3.2. RAPD Data Analysis

In total, 100 RAPD primers were scanned for polymorphic bands but, unlike the 9 selected polymorphic primers, the others showed monomorphic band patterns or no amplification. It is seen in similarity matrix of tree values varies between 84.4 and 34.8 percent with higher similarities seen between genotypes from the same region, as seen in Table 4.

The similarity was seen higher in the Artvin genotypes compared to other genotypes as shown in Figure 3. In the cluster analysis, three main groups were separated from each other; the first group consisted of the Samsun-Bartin genotypes, the second one was the Sinop genotypes and the last ones included a mix of the rest of genotypes divided into subgroups including the Artvin genotypes.

The genotypes show a range of variation with different groupings and similarity matrix values. These results will help to develop better chestnut cultivars in breeding programs. Two genotypes of the Sinop group clustered together in one group, and these are considered to be more closely related to each other than to other genotypes. However, these genotypes in the same group in morphological cluster analysis moved to third group divided into different groups' subgroups in RAPD cluster analysis. The 556-8 genotype did not show similarities with or group with the genotypes with which it formed a group in the morphologic analysis. Serdar and Soylu (2004) reported that this genotype blooms twice in a year: first in June and then in September. This genotype was specialized for use in chestnut honey production.

Table 3. Similarity matrix of chestnut genotypes due to morphological data analysis

GENOTYPES	08-Ca.-8	08-Ca.-13	08-Ca.-14	74-Uhs-1	74-Uhs-5	SE 3-12	SA 5-1	SE 18-2	SE 21-2	SE219	552-8	552-10	554-14	556-8
08-Camili-8		0.483	0.483	0.414	0.517	0.448	0.448	0.379	0.448	0.414	0.517	0.517	0.414	0.517
08-Camili-13	0.483		0.552	0.552	0.31	0.31	0.345	0.449	0.483	0.512	0.586	0.414	0.552	0.517
08-Camili-14	0.483	0.552		0.517	0.552	0.138	0.517	0.414	0.449	0.414	0.552	0.414	0.448	0.448
74-Uhs-1	0.414	0.552	0.517		0.517	0.379	0.448	0.379	0.345	0.552	0.414	0.345	0.483	0.586
74-Uhs-5	0.517	0.31	0.552	0.517		0.241	0.483	0.414	0.31	0.448	0.448	0.379	0.586	0.379
SE 3-12	0.448	0.31	0.138	0.379	0.241		0.448	0.276	0.276	0.414	0.379	0.31	0.379	0.448
SA 5-1	0.448	0.345	0.517	0.448	0.483	0.448		0.448	0.379	0.414	0.345	0.414	0.379	0.483
SE 18-2	0.379	0.449	0.414	0.379	0.414	0.276	0.448		0.724	0.448	0.414	0.448	0.414	0.517
SE 21-2	0.448	0.483	0.449	0.345	0.31	0.276	0.379	0.724		0.517	0.552	0.586	0.379	0.517
SE 21-9	0.414	0.512	0.414	0.552	0.448	0.414	0.414	0.448	0.517		0.448	0.414	0.517	0.586
552-8	0.517	0.586	0.552	0.414	0.448	0.379	0.345	0.414	0.552	0.448		0.62	0.552	0.483
552-10	0.517	0.414	0.414	0.345	0.379	0.31	0.414	0.448	0.586	0.414	0.62		0.379	0.448
554-14	0.414	0.552	0.448	0.483	0.586	0.379	0.379	0.414	0.379	0.517	0.552	0.379		0.414
556-8	0.517	0.517	0.448	0.586	0.379	0.448	0.483	0.517	0.517	0.586	0.483	0.448	0.414	

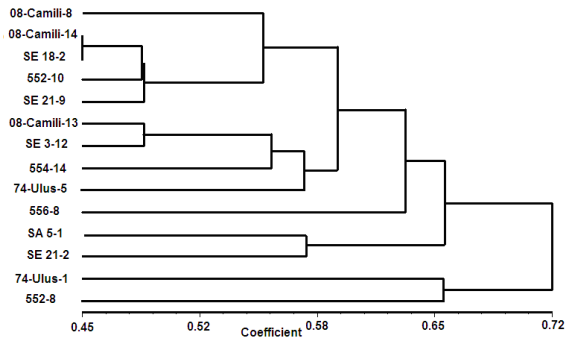

Figure 3. A dendrogram of RAPD-PCR results

Table 4. Similarity matrix of chestnut genotypes due to RAPD-PCR analysis

GENOTYPES	08-Ca.-8	08-Ca.-13	08-Ca.-14	74-Ulus-1	74-Ulus-5	SE 3-12	SA 5-1	SE 18-2	SE 21-2	SE219	552-8	552-10	554-14	556-8
08-Camili-8		0.7	0.811	0.438	0.666	0.791	0.718	0.769	0.621	0.666	0.457	0.757	0.556	0.606
08-Camili-13	0.7		0.718	0.588	0.78	0.844	0.683	0.732	0.452	0.737	0.541	0.821	0.684	0.629
08-Camili-14	0.811	0.718		0.516	0.632	0.81	0.684	0.842	0.571	0.8	0.588	0.777	0.686	0.625
74-Ulus-1	0.438	0.588	0.516		0.485	0.649	0.545	0.545	0.348	0.467	0.552	0.452	0.467	0.37
74-Ulus-5	0.666	0.78	0.632	0.485		0.773	0.6	0.7	0.467	0.595	0.611	0.789	0.703	0.647
SE 3-12	0.791	0.844	0.81	0.649	0.773		0.773	0.818	0.529	0.732	0.65	0.81	0.829	0.579
SA 5-1	0.718	0.683	0.684	0.545	0.6	0.773		0.7	0.667	0.649	0.5	0.684	0.649	0.529
SE 18-2	0.769	0.732	0.842	0.545	0.7	0.818	0.7		0.6	0.811	0.667	0.842	0.703	0.647
SE 21-2	0.621	0.452	0.571	0.348	0.467	0.529	0.667	0.6		0.519	0.385	0.571	0.519	0.5
SE 21-9	0.666	0.737	0.8	0.467	0.595	0.732	0.649	0.811	0.519		0.545	0.8	0.647	0.581
552-8	0.457	0.541	0.588	0.552	0.611	0.65	0.5	0.667	0.385	0.545		0.471	0.606	0.4
552-10	0.757	0.821	0.777	0.452	0.789	0.81	0.684	0.842	0.571	0.8	0.471		0.379	0.448
554-14	0.556	0.684	0.686	0.467	0.703	0.829	0.649	0.703	0.519	0.647	0.606	0.379		0.414
556-8	0.606	0.629	0.625	0.37	0.647	0.579	0.529	0.647	0.5	0.581	0.4	0.448	0.414	

However, it is sensitive to graft incompatibility. Hence, determining compatible rootstock/s for this genotype was necessary. As a result of preliminary studies, the 554-14 genotype was suggested as a compatible stock. Additionally, Erper et. al. (2004) reported that 556-8 is a promising genotype for use in control against chestnut blight. According to our dendrogram, SA5-1 and 554-14 look to be quite different genotypes, although they show 0.649 similarity. Serdar and Soylu (2005) reported that there is a graft incompatibility between these genotypes. In cluster analysis, the 74-Ulus-1 and 552-8 genotypes were placed in the same group, while others separated into two groups with more similarity, and the 556-8 genotype was placed in a different group. The SE 21-2 and 552-8 genotypes also showed different grouping.Although both of the genotypes produce an early harvest, SE 21-2 was determined to be more resistant to chestnut blight disease than 552-8 (Aksoy et al., 2005). The Artvin genotypes were placed in the same subgroups, but some differences in groupings were seen at a finer level of details. In addition, the 08-Camili-8 genotype displayed different RAPD patterns than others, but we have no specific information about this genotype.

Principal Component Analysis (PCA) showed that all groups show genetic diversity either in the morphologic data analysis or in the RAPD data analysis. It seems that the morphologic data showed closer relationships between genotypes compared to the RAPD data, as seen in Figure 4a and Figure 4b. The Artvin genotypes spread to larger area than the Bartin genotypes. The Bartin genotypes showed a very clear separation in RAPD data by the PCA test. Some genotypes also separated as well as Sinop in RAPD data test of PCA.

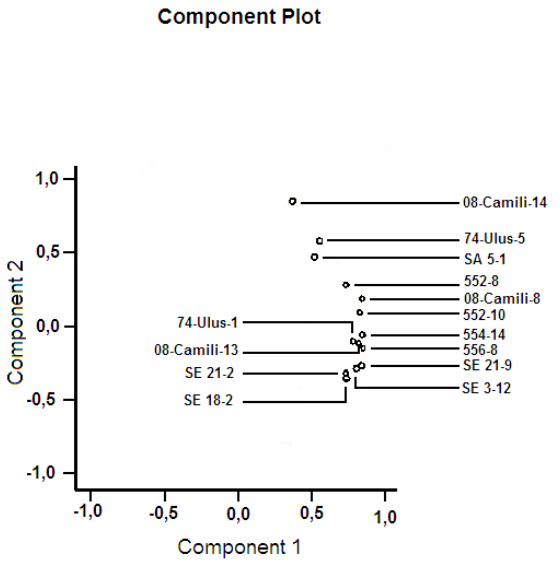

Figure 4a. Principal component analysis of morphological data

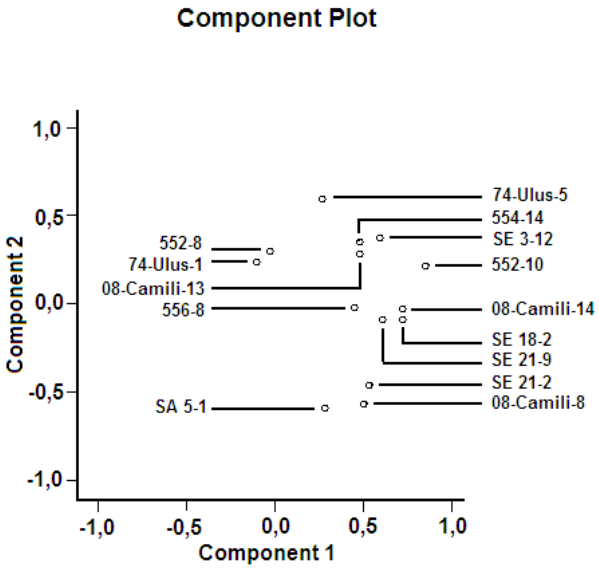

Figure 4b. Principal component analysis of RAPD-PCR data

The Mantel test was used to compare morphological and RAPD-PCR clusters to see the association between the two results. Correlation between morphological and genetic analysis done by RAPD-PCR was found to be significantly different negatively (r=-0.33) as matrix correlation (=normalized Mantel statistic Z) as applied by Mantel (1967) ($p<0.05$). This implies that morphological data can give very limited information compared to the RAPD data analysis. Distinguishing the data in PCA analysis gives parallel results for the Mantel analysis.

4. CONCLUSION

The study covered fourteen genotypes from the four geographic areas of Artvin, Bartin, Samsun and Sinop. The 30 morphological criteria, including 12 qualitative and 18 quantitative characteristics, were biometrically analysed for selection success using multivariate statistics. The results show that ten morphological criteria were very important for discrimination. The five most important morphological criteria accounted for 64.1% of the variability. These morphological criteria were the ratio of length of teeth to width of teeth, the ratio of length of hilum to length of fruit, cross section of leaves, the ratio of height of fruit to length of fruit and peeling of testa. These results also displayed a difference from those of Pereira-Lorenzo et al. (1996) who performed a study on Spanish chestnuts where nut size, fruit shape, male flower type and length of bur spines were the best criteria for discrimination between genotypes. However, in that study, morphological criteria were selected to minimize the effect of ecological conditions in selected genotypes. Explanation of the similarity; some genotypes like 08-Camili-14 and SE 18-2 settled in very close areas geographically due to the morphological analysis, and Samsun, Bartin and Sinop chestnuts were found to be quite different from each other in the PCA analysis shown in Figure 4. The correlation between RAPD and morphological analyses was found to be quite low, at negative (r=0.33). This comparison was done by Solar et al. (2005) who reported the level of accordance between the pomological and RAPD clusters as having 60.0 to 83.0 percent similarity.

The cumulative Eigen value of three components was found to be 43.9%, which is very similar to that of European genotypes (45%), but lower than that of American genotypes (60%) Huang et al. (1998). Spanish genotypes have a 26% Eigen value and New Zealand genotypes show 21.6% (Oraguzie et al., 1998).

On the other hand, none of the cluster analyses showed any differences in terms of geographical grouping. In addition, different results were obtained from the morphological and RAPD analyses. Both types of analysis produced groupings but the groups were different. Oraguzie et al. (1998) discussed similar results with *C. sativa* and *C. crenata* for unexpected results in grouping. They reported that morphological characteristics were not able to separate the accessions of chestnuts and suggested that combining cluster analysis with PCA can give more useful results. In the present study, RAPD and PCA analyses were done together to compare to morphological characteristics. It was suggested that all the analyses should be considered together for discrimination because of the high variation. The situation also was been exhaustively explained by (Cross, 1996; Hanboonsong, 1994; Ahmed and McNeil, 1973). These studies reported that morphological and molecular analysis can give different results. In the present study, the reason for this may be the limitations of the selected genotypes, which have different ages and were collected from different ecological regions. Also, some of the genotypes were picked up from the experiment orchard prepared from the original trees, such as Bartin, Artvin and Samsun-Sinop. However, the multivariate analysis for discrimination criteria of morphological traits showed a difference but, it is expected that the results had conform with the RAPD-PCR analysis. Future selection based on the morphological criteria determined in this study together with molecular analysis will help to further chestnut breeding programs.

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