Geliş Tarihi (date of arrival): 2024-12-30
Kabul Tarihi (date of acceptance): 2025-02-03

MEYVECİLİK ARAŞTIRMA ENSTİTÜSÜ MÜDÜRLÜĞÜ
FRUIT RESEARCH INSTITUTE

e-ISSN: 2148-8770
Yıl /Year: 2025
Cilt(Sayı)/Vol.(Issue): 12 (1)
Sayfa/Page: 18-30

Araştırma Makalesi/ Research Article

DOI: 10.51532/meyve.1609797

Meyve Bilimi
Fruit Science

Phenotypic Diversity of The Chestnut Genotypes in Yağlıdere District (Giresun, Türkiye)

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Abstract

Phenotypic diversity and population relationships of chestnut genotypes (*Castanea sativa* Mill.) growing spontaneously in forested areas of Yağlıdere district (Giresun, Türkiye) were investigated according to fruit traits. In 7 different populations where chestnut trees are dense in the region, 15 healthy genotypes and 105 genotypes in total were evaluated for 14 fruit traits. It was determined that the coefficient of variation was less than 20% except for nut and kernel weight. Correlation analysis revealed that 56 out of 91 relationships were significant and 51 of them were positive. The highest positive relationships were found between nut weight, kernel weight, kernel percentage, nut height and nut length. Only nut height/nut length ratio and stalk base length showed significant variation among populations. In within-population principal component analysis, the first four principal components explained 77.38% of the total variance. The relationships between the first principal component and nut width, nut length, nut height, the distance from the base to the largest section of the nut, nut weight and kernel weight were found to be high and positive. In the principal component analysis between populations, the first four principal components explained 88.98% of the total variance. The highest positive correlation was shown by scar length/scar width and nut height in the first principal component, nut length and distance from the base to the largest section of the nut in the second principal component, scar length and scar length/nut length in the third component and nut width in the fourth component, respectively. Cluster analysis divided genotypes into 12 clusters and populations into 2 clusters. In conclusion, principal component and clustering analysis explained the phenotypic diversity of 105 chestnut genotypes in the natural population in Yağlıdere district according to fruit traits and population relationships explained the whole phenotypic variation among genotypes.

Keywords: *Castanea sativa*, Clustering, Correlation, Population, Principal component, Variation.

Yağlıdere İlçesindeki (Giresun, Türkiye) Kestane Genotiplerinin Fenotipik Çeşitliliği

Özet

Yağlıdere ilçesi (Giresun, Türkiye) ormanlık alanlarda kendiliğinden yetişen kestane genotiplerinin (*Castanea sativa* Mill.) meyve özelliklerine göre fenotipik çeşitliliği ve populasyon ilişkileri araştırılmıştır. Yörede kestane ağaçlarının yoğun olduğu 7 farklı populasyonda sağlıklı durumda olan 15'er genotip ve toplamda 105 genotip 14 meyve özelliği yönünden değerlendirilmiştir. Kabuklu ve iç meyve ağırlığı dışındakilerin %20'nin altında varyasyon katsayısına sahip olduğu belirlenmiştir. Korelasyon analizi toplam 91 ilişkiden 56 tanesinin önemli olduğunu ve bunların da 51 tanesinin pozitif yönlü olduğunu ortaya koymuştur. En yüksek pozitif ilişkiler meyve ağırlığı, iç ağırlığı, iç oranı, meyve yüksekliği ve boyu arasında ortaya çıkmıştır. Sadece meyve yüksekliği/meyve boyu oranı ve sap tabanı uzunluğu populasyonlar arasında önemli değişim göstermiştir. Populasyon içi temel bileşen analizinde ilk dört temel bileşen toplam varyansın %77.38'ini açıklamıştır. Birinci temel bileşenle meyve eni, meyve boyu, meyve yüksekliği, meyve tabanı ile en geniş yeri arasındaki mesafe, meyve ağırlığı ve iç ağırlığı arasındaki ilişkilerin yüksek ve pozitif yönde bulunmuştur. Populasyonlar arası temel bileşen analizinde ilk dört temel bileşen toplam varyansın %88.98'ini açıklamıştır. En yüksek pozitif ilişkiyi, sırasıyla, birinci temel bileşende meyve tabanı boyu/meyve tabanı eni ve meyve yüksekliği, 2. temel bileşende meyve boyu ve meyve tabanı ile en geniş yeri arasındaki mesafe, üçüncü bileşende meyve tabanı boyu ve meyve tabanı boyu/meyve boyu ve dördüncü bileşende meyve eni göstermiştir. Kümeleme analizi genotipleri 12 kümeye, popülasyonları da 2 kümeye ayırmıştır. Sonuç olarak, temel bileşen ve kümeleme analizi ile Yağlıdere ilçesindeki doğal populasyondaki 105 kestane genotipinin meyve özelliklerine göre fenotipik çeşitliliği ve populasyon ilişkileri genotipler arasındaki fenotipik varyasyonun tamamını açıklamıştır.

Anahtar Kelimeler: *Castanea sativa*, Kümeleme, Korelasyon, Populasyon, Temel bileşen, Varyasyon.

Introduction

Chestnuts belong to the same order (*Fagales*) as hazelnuts and to the same family (*Fagaceae*) as oaks and beeches. Within the genus Castanea, several culturally important species have emerged in different parts of the world. The most widely distributed species, *Castanea sativa*, known as European chestnuts, is native to the Mediterranean countries and its homeland is not known for certain, but it is highly probable that it is Anatolia. According to some authors, this species is named after the city of Kastanis (Kastamonu, Northern Türkiye), its first distribution center. Although it is found at elevations up to 1800 m in the Caucasus, it can reach up to 1200 m in the region starting from the entire Black Sea coast in Anatolia, from Marmara and Western Anatolia to the Mediterranean coast (Soylu, 1984; Özçağıran et al. 2014).

Chestnut is a temperate climate fruit species that generally prefers acidic, deep and well-drained soils and does not grow in lowlands where it is too humid and cold, or in high mountainous areas with large differences in daily and annual temperatures (Poljak et al. 2022). *Castanea sativa* has long been recognized as a multipurpose species (Aravanopoulos, 2005), as it is widely cultivated for timber and nut production and represents an integral part of the economy in many areas, especially in rural areas (Diamandis and Perlerou, 1996).

European chestnut has been cultivated in Anatolia since ancient times, many chestnut genotypes with different fruit quality and tree characteristics have emerged. The nearly 2.5 million chestnut trees in Anatolia are highly variable. Within these rich European chestnut populations, there are species

This article is prepared from a part of Nesrin PUL's Master's Thesis.

with large fruits with bright and striking colors, as well as species with low quality and small fruits. Although plant breeders in Türkiye have made chestnut selections from natural populations, European chestnut varieties are still restricted to individual chestnut regions. Genotypes with important characteristics such as good fruit quality, earliness and high yield capacity were selected by local breeders and propagated by grafting. There are significant differences in each region in terms of the mentioned characteristics (Ertan, 2007).

Natural populations of *Castanea sativa* are facing extreme degradation for timber utilization. Therefore, conservation and management of these natural areas is necessary (Aravanopoulos et al. 2001). Assessment of the genetic diversity and population structure of natural Chestnut areas is crucial for good management strategies and conservation strategy and sustainable use of this natural resource (Lang and Huang, 1999). The magnitude and structure of genetic variation in natural populations should also be known when determining gene conservation strategies (Zarafshar et al. 2010).

Varieties belonging to species of the genus *Castanea* possess many traits that are desirable for breeding (Huang et al. 1995; Huang, 1998). Morphological traits have often served as tools for studying genetic diversity (Neophytou et al. 2007), especially because they are easy to use and are clear traits (Cousens, 1963; Olsson, 1975; Kremer et al. 2002). In general, the study of morphological traits constitutes an important component in the study of species with population relationships and diversity (Aravanopoulos, 2005). Morphological and phenological traits are used to develop quantitative estimates of genetic similarities and relationships. MacKey (1988) emphasized the importance of morphological traits in taxonomic studies of cultivated plants. Morphological characterization is an accepted formal method for the registration and conservation of new varieties (Pereira-Lorenzo et al. 1996). Varieties have traditionally been characterized by morphological traits, which in turn are influenced by developmental status and environmental and cultural factors. In order to reduce the influence of environmental factors, these studies should be repeated in different years and regions (Pereira-Lorenzo et al. 1996; Oraguzie et al. 1998).

On the other hand, multivariate analysis, and in particular principal component and cluster analysis, has been used for the evaluation of germplasm when studying several traits and many genotypes (Cruz and Regazzi, 1994). The use of multivariate methods is an important strategy for the characterization, evaluation and classification of plant genetic resources when many genotypes are to be evaluated for several characters (Peeters and Martinelli, 1989).

Traditionally, leaf morphological traits have been used by scientists to study phenotypic diversity. It is widely accepted that leaves are the most important organs for photosynthesis and transpiration in plants and the arrangement, size, shape and anatomy of leaves vary greatly in different environments (Bruschi et al. 2003) and are also easy to measure (Neophytou et al. 2007).

In this study, statistical analyses were carried out using morphological traits with multivariate methods to determine phenotypic diversity and population relationships according to fruit traits in seven natural chestnut populations in Yağlıdere district of Giresun province (Türkiye).

Material and Methods

Experimental Site and Plant Material

This research was conducted in 2020 and 2021 in Yağlıdere district of Giresun province (Türkiye). Yağlıdere district is located between 40 ° 51′ 43″ North latitude and 38 ° 37′ 24″ East longitude, 50 meters above sea level and 14 km from the coast (Figure 1). There is a dense chestnut population in the forest area in the district, and all trees in the chestnut population were grown from seed and each of them is a separate gene source.

The climate of the district is typical Black Sea climate with cool summers and mild and rainy winters. Precipitation is distributed over four seasons. The average annual precipitation is 1300 m^3. The coldest month is february and the lowest air temperature is -3 °C. The hottest month is august, and the average temperature is 24 °C. The average annual temperature is 14 °C. The average humidity is 70% (Anonymous, 2022).

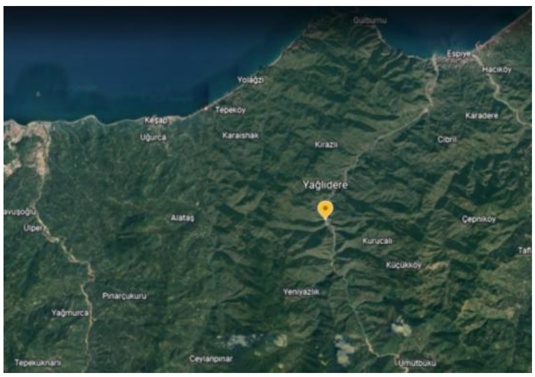

Figure 1. Google Earth view of Yağlıdere district
Şekil 1. Yağlıdere ilçesinin Google Earth görünümü

Chestnut genotypes are found in the population together with alder, beech, hornbeam, oak species and spruce forest species. According to the stand map, seven populations with a high density of chestnut trees and in different locations were identified (Figure 2).

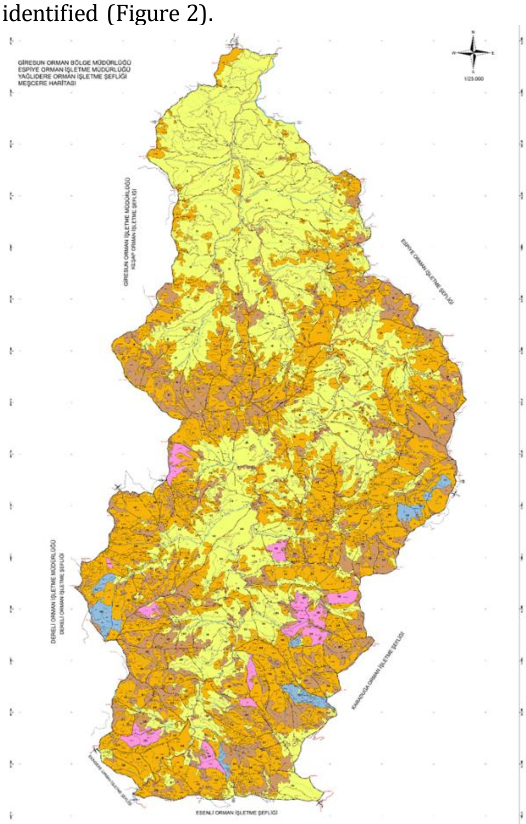

Figure 2. Stand Map of Yağlıdere District
Şekil 2. Yağlıdere İlçesi Meşcere Haritası

Nut Parameters

Fifteen genotypes were selected from each population and the genotypes were selected since they were at least 50 m away from each other to ensure that the genotypes were of productive age, healthy and unaffected by pollination and fertilization. When taking nut samples, care was taken to take them from the middle part of the shoots and from all four sides of the tree. Ten burs were sampled from 105 genotypes and brought to the measurement site on the same day in bags with air inlets.

Nut height, nut length, nut width, nut height/ nut length, width of the scar, length of the scar, length of the scar/width of the scar, length of the scar/ nut length, distance from the base to the largest section of the nut, length of the stalk's base, nut weight, kernel weight, kernel percentage and shell thickness were measured in ten nut samples (Figure 3).

Şahin (1989), Pigliucci et al. (1991), Pereira-Lorenzo et al. (1996), Oraguzie et al. (1998), Bolvansky et al. (2001), Alizoti and Aravanopoulos (2005), Aravanopoulos (2005), Solar et al. (2005), Ertan (2007), Soylu and Serdar (2009), Zarafshar et al, (2010), Serdar and Kurt (2011), Mujagić-Pašić and Ballian (2012), Serdar et al. (2014), Grygorieva et al. (2017), UPOV (International Union for the Protection of New Varieties of Plants) (2017), Atar and Turna (2018), Bostan et al. (2018), Grygorieva et al. (2018) and Serdar et al. (2018) were used to determine fruit parameters.

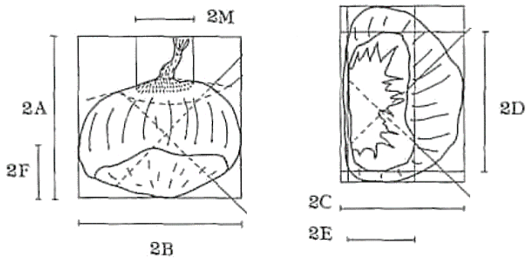

Figure 3. Nut height (2A), length (2B), width (2C), length of the scar (2D), width of the scar (2E), distance from the base to the largest section of the nut (2F) and length of the stalk's base (2M) measurements (Pigliucci et al., 1991)
Şekil 3. Meyvede yükseklik (2A), uzunluk (2B), en (2C), meyve tabanı uzunluğu (2D), meyve taban genişliği (2E), meyve tabanı ile en geniş yeri arası mesafe (2F) ve meyve sap tabanı uzunluğu (2M) ölçümleri (Pigliucci ve ark., 1991).

Statistical analysis

Statistical analyses were performed on 2-year average data. Descriptive statistics, correlation analysis, analysis of variance, principal component analysis and cluster analysis of fruit traits of the genotypes were performed using SAS JMP 13.2.0 statistical program.

Quantitative data were analyzed by ANOVA and means were compared using LSD (0.05) significance test.

Results and Discussion

Descriptive statistics

Among 105 chestnut genotypes, intrapopulational coefficient of variation was highest in kernel weight (27.30%) and nut weight (21.03%) and lowest in nut height/nut length ratio (6.81%) (Table 1).

The coefficients of variation of 10 genotypes from 10 different locations in Nazilli district (Aydın, Türkiye) were determined as 1.23-14.42% for nut weight, 2.94-8.90% for shell thickness, 0.84-7.74% for nut width, 0.28-4.55% for nut height, 0.47-3.39% for nut length and 0.14-2.85% for kernel percentage (Ertan, 2007). Although the coefficients of variation determined in our study were slightly higher, the order from high to low was the same as in the previous study. The high coefficients in our study may be due to the high number of genotypes.

Table 1. Minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) values of 14 nut parameters of 105 chestnut genotypes intrapopulational
Çizelge 1. 105 Kestane genotipinin 14 meyve özelliğine ait populasyon içindeki minimum, maksimum, ortalama, standart sapma (SD) ve varyasyon katsayısı (CV) değerleri

Fruit parameters		Abbreviation	Min.	Max.	Mean	SD	CV (%)
1.	Nut width (mm)	NWI	10.56	18.97	13.35	1.75	13.12
2.	Nut length (mm)	NL	15.91	23.90	20.18	1.94	9.59
3.	Nut height (mm)	NH	14.34	23.55	19.21	1.91	9.92
4.	NH/NL	-	0.80	1.15	0.96	0.07	6.81
5.	Scar width (mm)	SW	6.69	13.30	9.53	1.43	15.02
6.	Scar length (mm)	SL	10.44	20.23	15.57	2.04	13.08
7.	SL/SW	-	1.05	2.41	1.71	0.23	13.18
8.	SL/NL	-	0.59	0.92	0.77	0.08	10.11
9.	Distance from the base to the largest section of the nut (mm)	DBLS	6.15	12.92	10.09	1.27	12.54
10.	Length of the stalk's base (mm)	LSB	3.28	9.65	6.01	1.03	17.06
11.	Nut weight (g)	NW	2.25	7.00	4.13	0.87	21.03
12.	Kernel weight (g)	KW	1.33	6.00	3.06	0.84	27.30
13.	Kernel percentage (%)	KP	55.00	85.71	72.23	5.95	8.23
14.	Shell thickness (mm)	ST	0.61	1.64	1.00	0.19	18.66

In many previous studies, the rate of within-population variation was found to be very significant for many parameters (Pigliucci et al., 1991; Pereira-Lorenzo et al., 1996; Oraguzie et al., 1998; Lang and Huang, 1999; Aravanopoulos et al., 2001; Alizoti and Aravanopoulos, 2005; Qin et al., 2005; Queijeiro et al., 2005; Beccaro et al., 2005; Ormeci et al., 2016; Grygorieva et al., 2017; Bilgen and Bostan, 2018; Bostan et al., 2018; Zenginbal et al., 2018; Poljak et al., 2021; Poljak et al., 2022). In addition, it has been reported that intrapopulational variation is moderate compared to interpopulational variation (Peterson et al., 1992); the most variation is observed between populations, within populations and within genotypes (individuals), respectively (Glushkova, 2007), and similarities within populations can also be seen (Solar et al., 2001). As can be understood from the studies, the variation within the population may vary according to the size of the population, heterogeneity of the genotypes, parameters studied and environmental conditions.

Among the 7 chestnut populations, the coefficient of variation was 9.32% (5th population)-17.38% (3rd population) for NWI, 6.81% (6th population)-11.16% (4th population) for NL, 6.40% (5th population)-11.69% (3rd population) for NH, 4.51% (7th population)-8.68% (1st population) for NH/NL, 9.80% (1st population)-9.80% (SW) for SW. population), 4.51% (7th population)-8.68% (1st population) for NH/NL, 9.80% (1st population)-18.46% (6th population) for SW, 10.17% (5th population)-16.34% (7th population) for SL, 7.70% (4th population)-19.21% (6th population), 7.11% (3rd population)-13.94% (1st population) for SL/NL, 9.84% (6th population)-15.82% (1st population) for DBLS, 9.13% (6th population)-21.31% (2nd population) for LSB, 18.33% (5th population)-26. 71% (3rd population), 22.07% (1st population)-35.73% (3rd population) for KW, 4.40% (1st population)-9.88% (4th population) for KP and 12.21% (3rd population)-24.39% (5th population) for ST (Table 2).

On the other hand, within the populations, the coefficient of variation was 4.40% (KP)-22.07% (KW) in population 1, 5.09% (NH/NL)-25.59% (KW) in population 2, 7.11% (NH/NL)-35.73% (KW) in population 3, 5. 61% (NH/NL)-26.12% (KW), 6.40% (NH)-24.87% (KW) in population 5, 5.77% (KP)-26.48% (KW) in population 6, 4.51% (NH/NL)-26.77% (KW) in population 7 (Table 2).

In previous studies, the coefficient of variation was 5.1-9.6% for nut height, 5.4-10.9% for nut width, 6.6-12.2% for nut thickness, 11.2-32.4% for nut weight, 9.5-20.3% for scar length and 9.6-18.1% for scar width in 6 subpopulations in Slovenia (Solar et al, 2001); 7.4-12.5% for nut height, 6.8-12.4% for nut width, 7.7-14.4% for nut thickness, 17.3-38.6% for nut weight, 12.7-17.8% for scar length and 10.0-17.9% for scar width in a population of 3 locations in the same country (Solar et al., 2005); 32.18% for nut weight and 10.14% for nut size in 3 different locations in Srinagar district (Kashmir) (Pandit et al., 2013); 10.42% for nut height, 10.82% for nut width, 16.68% for nut thickness, 25.71% for nut weight, 17.76% for scar length and 19.65% for scar width at 3 locations in Bosnia and Herzegovina (Skender et al., 2013); 13.74% in nut height, 14.98%

in nut width, 20.57% in fruit thickness, 45.92% in nut weight, 19.58% in scar length and 20.66% in scar width in Forest-Steppe regions of Ukraine (Grygorieva et al., 2017); 24.59-35.85% in nut weight, 7.97-13.03% in nut height, 10.34-14.40% in nut width, 11.22-15.82% in DBLS, 13.78-17.37% in nut thickness, 13.69-21.07% in scar length, 16.66-24.78% in scar width, 7.25-10.28% in NH/NL, 6.15-10.50% in SL/FL in eight populations in Italy (Poljak et al., 2022). It has also been reported that there are significant differences in fruit characteristics among 8 chestnut populations in Türkiye (Atar and Turna, 2018) and that the nuts of different plantations in Sicily vary most in height, width, thickness and weight (Cutino et al., 2010). As in previous studies, the highest coefficient of variation among populations in our study was determined in nut weight, and it can be said that nut and scar sizes also have significant coefficients.

Table 2. Interpopulations coefficients of variation (%) of 14 nut parameters of 105 chestnut genotypes
Çizelge 2. 105 kestane genotipinin 14 meyve özelliğinin populasyonlararası varyasyon katsayıları (%)

Fruit parameters	Populations						
	1	2	3	4	5	6	7
Nut width	11.84	9.58	17.38	12.57	9.32	9.84	14.15
Nut length	7.57	9.90	10.44	11.16	9.81	6.81	10.80
Nut height	6.48	9.77	11.69	10.58	6.40	6.63	11.66
NH/NL	8.68	5.09	7.44	5.61	7.83	4.84	4.51
Scar width	9.80	17.76	11.71	13.41	14.20	18.46	15.97
Scar length	11.09	11.73	10.38	14.17	10.17	13.06	16.34
SL/SW	11.34	10.67	10.21	7.70	9.38	19.21	14.71
SL/NL	13.94	8.08	7.11	11.78	8.74	9.26	9.65
DBLS	15.82	11.91	13.32	15.64	12.36	9.84	10.29
LSB	17.70	21.31	14.90	13.12	12.60	9.13	13.43
Nut weight	19.74	19.51	26.71	18.67	18.33	22.45	20.33
Kernel weight	22.07	25.59	35.73	26.12	24.87	26.48	26.77
Kernel percentage	4.40	8.60	9.53	9.88	9.49	5.77	8.51
Shell thickness	22.01	20.52	12.21	19.82	24.39	18.33	15.63

In previous studies, the coefficient of variation was 5.1-9.6% for nut height, 5.4-10.9% for nut width, 6.6-12.2% for nut thickness, 11.2-32.4% for nut weight, 9.5-20.3% for scar length and 9.6-18.1% for scar width in 6 subpopulations in Slovenia (Solar et al, 2001); 7.4-12.5% for nut height, 6.8-12.4% for nut width, 7.7-14.4% for nut thickness, 17.3-38.6% for nut weight, 12.7-17.8% for scar length and 10.0-17.9% for scar width in a population of 3 locations in the same country (Solar et al., 2005); 32.18% for nut weight and 10.14% for nut size in 3 different locations in Srinagar district (Kashmir) (Pandit et al., 2013); 10.42% for nut height, 10.82% for nut width, 16.68% for nut thickness, 25.71% for nut weight, 17.76% for scar length and 19.65% for scar width at 3 locations in Bosnia and Herzegovina (Skender et al., 2013); 13.74% in nut height, 14.98% in nut width, 20.57% in fruit thickness, 45.92% in nut weight, 19.58% in scar length and 20.66% in scar width in Forest-Steppe regions of Ukraine (Grygorieva et al., 2017); 24.59-35.85% in nut weight, 7.97-13.03% in nut height, 10.34-14.40% in nut width, 11.22-15.82% in DBLS, 13.78-17.37% in nut thickness, 13.69-21.07% in scar length, 16.66-24.78% in scar width, 7.25-10.28% in NH/NL, 6.15-10.50% in SL/FL in eight populations in Italy (Poljak et al., 2022). It has also been reported that there are significant differences in fruit characteristics among 8 chestnut populations in Türkiye (Atar and Turna, 2018) and that the nuts of different plantations in Sicily vary most in height, width, thickness and weight (Cutino et al., 2010). As in previous studies, the highest coefficient of variation among populations in our study was determined in nut weight, and it can be said that nut and scar sizes also have significant coefficients.

Correlations

Correlation analysis revealed that there were many significant relationships among the fruit traits of chestnut genotypes (Table 3).

The 14 parameters had correlation coefficient values ranging from (-) 0.001 (NW-SL/SF) to 0.982 (KW-NW). Out of a total of 91 relationships, 56 (61.54%) were significant, of which 51 were positive (91.07%) and 5 were negative (8.93%). Out of 56 significant relationships, 75% (42) were significant at 1‰ ($P<0.001$), 17.86% (10) at 1% ($P<0.01$) and 7.14% (4) at 5% level. The highest positive relationship was found between kernel weight and nut weight (0.982). This was followed by kernel percentage-kernel weight (0.868), kernel percentage-nut weight (0.780), nut height-nut length (0.773), DBLS- nut length (0.706).

Table 3. Pairwise correlation coefficients between fruit traits
Çizelge 3. Meyve özellikleri arasındaki Pairwise korelasyon katsayıları

Variable	by Variable	Corr.	Sign Prob	Variable	by Variable	Corr.	Sign Prob
KW	NW	0.982	<.0001*	SL/SW	NWI	-0.305	0.0015*
KP	KW	0.868	<.0001*	LSB	NH	0.291	0.0026*
KP	NW	0.780	<.0001*	ST	SL	0.287	0.0030*
NH	NL	0.773	<.0001*	ST	NWI	0.257	0.0080*
DBLS	NH	0.706	<.0001*	DBLS	SL/NL	0.255	0.0086*
NL	NWI	0.687	<.0001*	ST	NW	0.251	0.0098*
SL/NL	SL	0.677	<.0001*	ST	DBLS	0.244	0.0122*
NW	NL	0.674	<.0001*	ST	SW	0.242	0.0128*
KW	NL	0.665	<.0001*	KP	NH/NL	-0.233	0.0170*
DBLS	SL	0.648	<.0001*	LSB	NL	0.211	0.0308*
SL	NL	0.647	<.0001*	ST	KW	0.188	0.0542
SL	NH	0.642	<.0001*	ST	LSB	0.179	0.0671
KW	NWI	0.631	<.0001*	NW	LSB	0.169	0.0857
SW	NL	0.628	<.0001*	DBLS	NH/NL	0.167	0.0885
NW	NWI	0.623	<.0001*	KW	LSB	0.166	0.0910
SW	NWI	0.618	<.0001*	LSB	DBLS	0.165	0.0917
SL/NL	SL/SW	0.618	<.0001*	KP	LSB	0.164	0.0946
DBLS	NL	0.591	<.0001*	LSB	SW	0.146	0.1381
KP	NL	0.590	<.0001*	LSB	NWI	0.130	0.1878
SL/SW	SW	-0.574	<.0001*	LSB	NH/NL	0.119	0.2286
SW	NH	0.585	<.0001*	ST	SL/NL	0.116	0.2389
DBLS	SW	0.564	<.0001*	SL/SW	NH/NL	0.109	0.2688
NW	SW	0.553	<.0001*	SL/NL	NH	0.105	0.2846
NW	NH	0.551	<.0001*	ST	KP	0.078	0.4285
KW	SW	0.534	<.0001*	ST	NH/NL	0.068	0.4932
KW	NH	0.527	<.0001*	SL	NH/NL	0.014	0.8894
KP	NWI	0.522	<.0001*	LSB	SL	0.011	0.9137
NW	SL	0.495	<.0001*	NW	SL/NL	-0.001	0.9953
SL	SW	0.473	<.0001*	SL/NL	SW	-0.003	0.9738
NH	NWI	0.462	<.0001*	ST	SL/SW	-0.014	0.8880
NW	DBLS	0.448	<.0001*	DBLS	SL/SW	-0.014	0.8849
KW	SL	0.448	<.0001*	SL/SW	NH	-0.036	0.7156
KP	NH	0.430	<.0001*	KW	SL/NL	-0.053	0.5908
KW	DBLS	0.425	<.0001*	SL/SW	NL	-0.082	0.4048
SL	NWI	0.424	<.0001*	SW	NH/NL	-0.088	0.3741
KP	SW	0.418	<.0001*	SL/NL	NWI	-0.099	0.3166
DBLS	NWI	0.411	<.0001*	SL/NL	NL	-0.116	0.2384
SL/SW	SL	0.401	<.0001*	LSB	SL/SW	-0.145	0.1399
SL/NL	NH/NL	0.349	0.0003*	KP	SL/SW	-0.161	0.1013
KP	DBLS	0.344	0.0003*	NW	SL/SW	-0.164	0.0955
NH/NL	NH	0.343	0.0003*	KP	SL/NL	-0.165	0.0922
NH/NL	NL	-0.323	0.0008*	NW	NH/NL	-0.169	0.0849
NH/NL	NWI	-0.317	0.0010*	KW	SL/SW	-0.180	0.0662
KP	SL	0.310	0.0013*	LSB	SL/NL	-0.182	0.0625
ST	NH	0.308	0.0014*	KW	NH/NL	-0.191	0.0514
ST	NL	0.256	0.0084*				

In previous studies, Alizoti and Aravanopoulos (2005) found that the correlation coefficients between 7 nut traits ranged between 0.000-0.874. In the study, high positive correlations were found between nut weight- nut width and nut width- nut length; moderate positive correlations were found between nut weight- nut thickness, nut width-scar length, nut weight- nut height, nut weight- nut length, nut width- nut height and nut weight-scar length. Ertan (2007) examined the correlations among 11 nut traits. The correlation coefficients between nut weight, nut width, nut length, nut height, kernel percentage and shell thickness ranged between 0.026-0.947; the highest correlations were found between nut weight-fruit width (0.947), nut weight- nut width (0.910), nut length- nut height (0.910), nut length- nut height (0.871), nut width- nut height (0.871), nut width- nut thickness (0.947) and nut weight- nut width (0.947), respectively. 947), nut weight- nut height (0.910), nut length- nut height (0.871), nut width- nut height (0.855), nut weight- nut height (0.832) and nut width- nut height (0.828), respectively. Soylu and Serdar (2009) reported that the highest relationships between nut width, nut length, nut height and nut weight were nut weight- nut length (0.961), nut width- nut length (0.925), nut width-nut weight (0.890), nut width- nut height (0.865), nut height- nut height (0.825) and nut height-nut weight (0.824), respectively. Pandit et al. (2013) reported that nut size and nut weight were highly correlated (0.855). Bostan et al. (2018) found that the relationship between nut weight and nut size was positive and significant (0.860). Tuğ et al. (2022) found that the correlation coefficients between 11 nut traits ranged between (-) 0.006-0.923 and 43 (78.18%) out of 55 correlations were significant. The highest correlations between nut weight, nut length, nut height, nut width, nut height, scar width and scar length were found between nut width and nut weight (0.877), nut height and nut weight (0.796), nut height and nut weight (0.794) and nut height and nut width (0.766), respectively. Atar and Turna (2018) determined that the correlation coefficients between nut height, nut width, nut thickness and 1000 nut weight varied between 0.914-0.965. As in previous studies, especially NW-NWI, NW-NH, NW-NL, NW-NL, NW-SL, NWI-FL, NWI-SL, NWI-NH and NH-NL correlations were positive and high in our study.

Variance Analysis

Analysis of variance revealed that only NH/NL ratio and LSB were significant among the populations. The highest NH/NL ratio (1.00) was observed in population 2, while the lowest was observed in populations 3 (0.91) and 6 (0.93). The highest LSB was observed in population 1 (6.89), while the lowest was observed in populations 3 (5.44) and 2 (5.50) (Table 4).

Table 4. Mean and standard deviation (second rows) values of 14 fruit traits of 105 chestnut genotypes interpopulations
Çizelge 4. 105 kestane genotipinin 14 meyve özelliğinin populasyonlararası ortalama ve standart sapma (ikinci satırlar) değerleri

Nut traits	Populations							Prob > F
	1	2	3	4	5	6	7	
NWI	13.77	12.48	14.03	14.11	13.16	13.13	13.17	ns
	1.63	*1.19*	*2.44*	*1.77*	*1.23*	*1.29*	*1.86*	
NL	20.33	19.96	20.14	20.52	20.58	20.46	19.84	
	1.54	*1.97*	*2.10*	*2.29*	*2.02*	*1.39*	*2.14*	ns
NH	19.69	19.82	18.32	19.27	20.22	18.90	18.71	ns
	1.28	*1.94*	*2.14*	*2.04*	*1.30*	*1.25*	*2.18*	
NH/NL	0.98 abc	1.00 a	0.91 d	0.95 bcd	0.99 ab	0.93 d	0.95 cd	0.0004***
	0.09	*0.05*	*0.07*	*0.05*	*0.08*	*0.04*	*0.04*	
SW	9.63	9.32	9.99	9.07	9.68	9.77	9.39	ns
	0.94	*1.65*	*1.17*	*1.22*	*1.38*	*1.80*	*1.50*	
SH	15.73	16.01	15.69	15.96	15.67	15.91	14.68	ns
	1.75	*1.88*	*1.63*	*2.26*	*1.59*	*2.08*	*2.40*	
SL/SW	1.68	1.81	1.63	1.80	1.73	1.74	1.63	ns
	0.19	*0.19*	*0.17*	*0.14*	*0.16*	*0.33*	*0.24*	
SL/NL	0.78	0.80	0.78	0.78	0.77	0.78	0.74	ns
	0.11	*0.07*	*0.05*	*0.09*	*0.07*	*0.07*	*0.07*	
DBLS	10.08	10.23	9.87	10.10	10.46	10.47	9.73	ns
	1.60	*1.22*	*1.32*	*1.58*	*1.29*	*1.03*	*1.00*	
LSB	6.89 a	5.50 c	5.44 c	5.74 c	6.16 bc	6.62 ab	5.96 c	<.0001***
	1.17	*1.16*	*0.81*	*0.75*	*0.78*	*0.60*	*0.80*	
NW	4.13	3.92	4.41	3.95	4.09	4.12	4.21	ns
	0.82	*0.76*	*1.18*	*0.74*	*0.75*	*0.93*	*0.86*	
KW	2.99	2.84	3.36	2.95	3.03	3.01	3.17	ns
	0.66	*0.73*	*1.20*	*0.77*	*0.75*	*0.80*	*0.85*	
KP	70.61	70.58	73.90	72.79	72.63	71.29	73.50	ns
	3.11	*6.07*	*7.05*	*7.19*	*6.89*	*4.11*	*6.25*	
ST	0.96	1.04	0.96	1.01	0.99	1.01	0.99	ns
	0.21	*0.21*	*0.12*	*0.20*	*0.24*	*0.19*	*0.15*	

Significant level, ***: 1‰, ns: nonsignificant, Önemlilik düzeyi: ***: ‰1, ns: önemli değil

Previous studies have shown that although intra-population variation in chestnut is moderate, inter-population variation, including fixed differences between populations, is high (Peterson et al. 1992); chestnut species and genotypes can be separated along geographical lines (Oraguzie et al., 1998); the level of variability can vary significantly between species and regions (Lang and Huang, 1999); the studied nut traits vary more between regions than between populations (Aravanopoulos et al., 2001); that the variance between populations was greater than that within a population and within a genotype, respectively (Glushkova, 2007); and that all nut traits examined varied significantly among populations (Skender et al., 2013; Atefe et al., 2015; Atar and Turna, 2018; Poljak et al., 2022). As can be seen from previous studies, the differences among populations may vary according to the degree of heterozygosity in genotypes, parameters studied, size and density of populations and geographical distance.

Principal Component Analysis

As a result of the principal components analysis for intrapopulational relationships, the first four components with eigenvalues above 1 explained 77.38% of the variation. Nut size and weight were the most important variables in component 1, which accounted for 41.69% of the total variance in terms of the traits analyzed. In the second component, which accounted for 17.80% of the variation, scar traits (except scar width) were the most important variables. All 14 fruit traits explained 100% of the phenotypic variation among genotypes (Table 5).

Table 5. Eigenvalues, total variability and correlation between the original variables and the three principal components intrapopulational

Çizelge 5. Populasyon içindeki özdeğerler, toplam değişkenlik oranı ve orijinal değişkenler ile incelenen kestane genotiplerindeki üç temel bileşen arasındaki korelasyon

Fruit traits	**PCA1**	**PCA2**	**PCA3**	**PCA4**
Nut width	0.318	-0.144	-0.084	-0.232
Nut length	0.366	-0.026	-0.074	-0.066
Nut height	0.327	0.185	0.295	0.072
NH/NL	-0.054	0.329	0.528	0.229
Scar width	0.317	-0.091	0.211	-0.432
Scar length	0.284	0.410	-0.170	-0.142
SL/SW	-0.082	0.474	-0.371	0.314
SL/FL	0.018	0.564	-0.151	-0.101
DBLS	0.291	0.226	0.190	-0.144
LSB	0.105	-0.076	0.437	0.518
Nut weight	0.359	-0.076	-0.165	0.232
Kernel weight	0.357	-0.115	-0.192	0.280
Kernel percentage	0.307	-0.170	-0.218	0.366
Shell thickness	0.146	0.118	0.223	-0.112
Eigenvalue	5.84	2.49	1.48	1.03
Variance (%)	41.69	17.80	10.55	7.34
Total variance (%)	41.69	59.49	70.04	77.38

As a result of the principal component analysis for interpopulational relationships, 48.93% of the total variance for the traits examined could be explained by the first component, 66.88% by the first two components, 79.83% by the first three components and 88.98% by the first four components. All 14 nut traits explained 100% of the phenotypic variation among the populations (Table 6).

SL/SW and NH in the first principal component, NL and DBLS in the second principal component, SL and SL/NL in the third component and NWI in the fourth component showed the highest positive relationship, respectively.

Table 6. Eigenvalues, total variability and correlation between the original variables and the three principal components interpopulations
Çizelge 6. Populasyonlar arasındaki özdeğerler, toplam değişkenlik oranı ve orijinal değişkenler ile üç temel bileşen arasındaki korelasyon

Fruit traits	PCA1	PCA2	PCA3	PCA4
Nut width	-0.192	0.285	0.247	0.504
Nut length	0.126	0.482	0.037	0.417
Nut height	0.300	0.047	-0.289	0.121
NH/NL	0.279	-0.155	-0.338	0.012
Scar width	-0.188	0.392	-0.110	-0.494
Scar length	0.246	0.343	0.371	-0.121
SL/SW	0.350	-0.032	0.268	0.110
SL/NL	0.239	0.221	0.360	-0.332
DBLS	0.276	0.317	-0.063	-0.109
LSB	0.051	0.330	-0.534	0.115
Nut weight	-0.354	0.159	-0.034	-0.234
Kernel weight	-0.368	0.070	0.083	-0.097
Kernel percentage	-0.293	-0.110	0.217	0.272
Shell thickness	0.280	-0.297	0.212	-0.115
Eigenvalue	6.85	2.51	1.81	1.28
Variance (%)	48.93	17.95	12.96	9.14
Total variance (%)	48.93	66.88	79.83	88.98

In our study, similar to the results of previous studies (Pereira-Lorenzo et al., 1996; Ertan, 2007; Bostan et al., 2018; Poljak et al., 2021; Poljak et al., 2022), it was found that component 1 had a significant share in total variation and traits such as nut size and weight represented component 1. Atefe et al. (2015) also reported that the first three principal components represented the highest cumulative variance, and that nut weight and size traits were prominent.

Cluster Analysis

Cluster analysis for intrapopulational relationships revealed that genotypes were divided into 2 main groups and these groups were divided into 2 subgroups and branching occurred and a total of 12 clusters were formed (Figure 2).

The most genotypes were in cluster 1 (19) and the least in cluster 9 (3). Genotypes 62 and 67 were the most similar to each other in terms of fruit characteristics.

Genotypes 31, 32 and 37 in cluster 9 had the highest values in terms of fruit width, NH/NL, nut weight, kernel weight and kernel percentage, which were significantly different from the other clusters (Table 7).

Table 7. Number of genotypes and averages of the traits of the clusters intrapopulational
Çizelge 7. Populasyon içi kümelerin genotip sayısı ve incelenen özelliklerinin ortalamaları

Cluster	Count	NWI	NL	NH	FH/FL	SW	SL	SL/SW	SL/NL	DBLS	LSB	NW	KW	KP	ST
1	19	12.44	18.91	18.25	0.97	9.10	15.18	1.75	0.80	9.43	5.79	4.02	2.90	71.45	1.08
2	13	12.25	20.15	18.58	0.93	8.48	14.56	1.79	0.72	9.57	6.16	3.78	2.81	72.67	0.83
3	5	12.27	17.70	16.13	0.92	8.51	10.82	1.29	0.62	8.82	5.77	3.29	2.30	67.60	0.86
4	12	11.73	17.30	17.13	1.00	7.79	13.91	1.86	0.81	8.54	5.40	2.89	1.87	62.97	0.92
5	8	15.03	22.66	22.53	1.00	11.32	18.31	1.66	0.81	12.06	6.40	4.93	3.59	72.32	1.30
6	6	16.36	23.03	21.49	0.94	10.34	15.77	1.59	0.69	10.35	7.62	4.92	3.90	78.03	1.20
7	5	14.03	20.96	19.54	0.93	11.96	13.91	1.30	0.66	9.87	7.26	4.28	3.20	73.64	0.92
8	9	13.85	21.54	20.46	0.95	10.29	15.72	1.59	0.73	10.82	5.24	4.27	3.27	75.57	0.89
9	3	17.32	21.68	17.52	0.81	10.89	15.52	1.45	0.72	9.99	4.41	5.83	4.85	81.91	1.05
10	6	12.77	19.13	20.03	1.05	9.29	15.97	1.78	0.84	10.93	6.50	4.12	3.05	71.64	0.82
11	12	13.76	21.18	20.42	0.97	10.37	18.14	1.81	0.86	11.17	6.26	4.86	3.78	76.21	0.96
12	7	13.91	21.34	19.09	0.90	9.06	17.67	1.98	0.83	10.52	5.68	3.92	2.85	71.92	1.16

In previous studies on this subject, it was reported that different clusters were formed in genotypes in terms of similar nut characteristics (Oraguzie et al., 1998; Serdar et al., 2014; Bostan et al., 2018; Stojanović and Magazin, 2020; Poljak et al., 2022); it has been reported that traits such as nut weight, nut size, shape and scar size are important distinguishing characteristics of clusters (Pereira-Lorenzo et al., 1996; Solar et al., 2001; Ertan, 2007; Cutino et al., 2010; Atefe et al., 2015; Grygorieva et al., 2017).
Cluster analysis for interpopulational relationships revealed that the populations formed 2 main groups, with 5 populations in group 1 and 2 populations in group 2 (Figure 4).

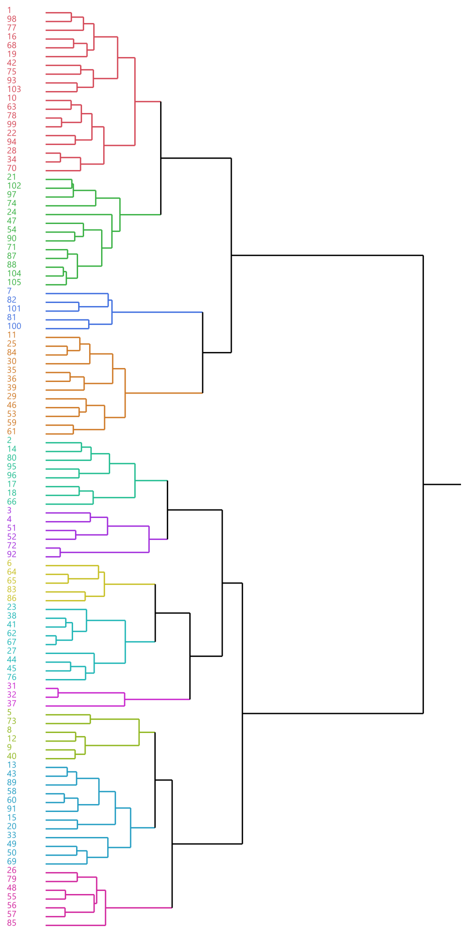

Figure 3. Dendrogram of chestnut genotypes according to fruit characteristics determined by cluster analysis
Şekil 3. Kestane genotiplerinin meyve özelliklerine göre kümeleme analizi ile belirlenen dendrogramı

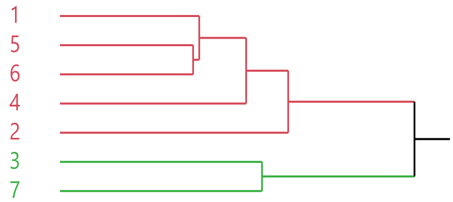

Figure 4. Dendrogram of chestnut populations according to fruit characteristics determined by cluster analysis
Şekil 4. Kestane populasyonlarının meyve özelliklerine göre kümeleme analizi ile belirlenen dendrogramı

Populations 5 and 6 were the most similar to each other in terms of nut characteristics, while populations 1 and 3 were the most distant.
Populations 3 and 7 in cluster 2 had the highest values in terms of nut width, scar width, nut weight, kernel weight and kernel percentage and were significantly different from cluster 1 (Table 8).

Table 8. Number of genotypes and averages of the traits studied in interpopulation clusters
Çizelge 8. Populasyonlararası kümelerde genotip sayısı ve incelenen özelliklerinin ortalamaları

Variables	**Cluster**	1	2
	Count	5	2
NWI		13.33	13.60
NL		20.37	20.00
NH		19.58	18.52
NH/NL		0.97	0.93
SW		9.49	9.69
SL		15.85	15.19
SL/SW		1.75	1.63
SL/NL		0.78	0.76
DBSL		10.27	9.80
LSB		6.18	5.70
NW		4.04	4.31
KW		2.96	3.27
KP		71.58	73.70
ST		1.00	0.98

Similar results were obtained from previous studies. In Slovenia, significant genetic differences were found among 6 subpopulations within 4 main regions (Solar et al., 2001); in Greece, two distant regions showed significant variation in almost all nut traits and Mount Paiko region had higher mean values for nut weight, nut length, nut width, nut height, nut length to the widest point (DBSL) and nut weight (Alizoti and Aravanopoulos, 2005); It was reported that populations consisting of 15-20 genotypes selected from 8 provinces in different parts of Türkiye and from each province formed 3 groups and nut length, 1000 nut weight, nut height and nut width were the traits with the highest discrimination power among the groups (Atar and Turna, 2018) and a total of 8 populations (160 genotypes), 5 from Croatia, 1 each from Italy, Slovenia and Bosnia-Herzegovina, were divided into two groups in terms of 10 fruit morphometric traits (Poljak et al., 2022).

Conclusions

As a result, principal component and clustering analyses explained the phenotypic variation of 105 chestnut genotypes in the natural population in Yağlıdere district according to nut traits and population relationships explained all the phenotypic variation among genotypes. Since the variation of the nut traits examined among both genotypes and populations is significant, it can be said that these variations of nut traits can be used in further genetic studies. Among the parameters examined in the whole population, especially nut and kernel weight had high coefficient of variation.

It can be said that the sizes of chestnut genotypes of Yağlıdere region are generally small and therefore have the potential to be used for secondary food products.

This study is preliminary research, and it can be said that expanding it based on years and populations can contribute to future breeding studies.

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