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FRUIT AND SEEDLING DIVERSITY AMONG SWEET CHESTNUT (*Castanea sativa* Mill.) POPULATIONS IN TURKEY

RAZNOLIKOST PLODOVA I SADNICA U POPULACIJAMA PITOMOGA KESTENA (*Castanea sativa* Mill.) U TURSKOJ

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Summary

Sweet chestnut, *Castanea sativa* Mill., is an important multipurpose tree species in Asia Minor and Europe. The objective of this study was to investigate variation among eight sweet chestnut populations in Turkey by using different morphological characteristics of fruits and seedlings. A total of four fruit characteristics were analysed: fruit length, width and thickness, and fruit shape, i.e. the ratio of fruit length and width. Additionally, 1000 fruit mass and fruit moisture content were determined as well. Measurements of seedling length, root collar diameter and sturdiness quotient were carried out at one-year old seedlings. The highest values of fruit length, width and thickness were found in İzmir population, while the highest values of seedling length, root collar diameter and sturdiness quotient were found in Balıkesir population. The 1000 fruit mass ranged between 3815.1 g and 10516.5 g, and the highest average fruit moisture content was 52.21 %. In general, the fruit size increased from eastern to western populations. Furthermore, the results of statistical analyses showed that there were significant differences between analysed populations for measured morphological characteristics related to both fruit and seedling. Application of cluster analysis revealed grouping of populations according to the eco-geographic principle. However, human influence on the population structure cannot be excluded as well.

Key words: *Castanea sativa*, sweet chestnut, Turkey, morphology, fruit, seedling

INTRODUCTION
UVOD

Chestnut species are an important forest trees and shrubs belonging to the *Fagaceae* family. The *Castanea* Mill. genus encompasses seven economically and ecologically significant species, widely spread in the temperate forest zone of the northern hemisphere (Johnson 1988; Lang *et al.* 2007), where the sweet chestnut (*Castanea sativa* Mill.) is the only species naturally found in Europe and Asia Minor (Kayacık 1981). Sweet chestnut is distributed across the Mediterranean region, from the Caspian Sea to the Atlantic Ocean (Fernández-López and Alía 2003). In Turkey, it can be found mainly over the North and Western Anatolia (Black Sea Coast), and the Marmara Region. Chestnut grows up to 1200 m above sea level in the Black Sea region. In addition, it can also rise up to 1700 m in Rize region and up to 1800 m in the Aegean region (Kütahya-Simav).

Naturally it forms mixture stands with other tree species (Davis 1982; Soylu 2004; Turna 2013). According to Mattioni *et al.* (2008) three main managing types (domestication levels) may be identified for sweet chestnut: (1) naturalized stands; (2) managed coppice; and (3) orchards. Additio-

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nally, in the Mediterranean countries there are a large number of old, grafted cultivars of sweet chestnut (Goulão *et al.* 2001; Pereira-Lorenzo *et al.* 2001, 2010; Botta *et al.* 2005; Martin *et al.* 2007; Idžojtić *et al.* 2012; Poljak *et al.* 2016, 2017), i.e. varieties of the sweet chestnut with the best quality, tasty and large fruits.

In Europe, there are three main areas (Georgia, eastern Turkey and Italy) having particular biological value for conservation of genetic resources of sweet chestnut (Villiani *et al.* 1999; Mattioni *et al.* 2017). Likewise, areas particularly rich in genetic diversity were detected in the Iberian (Martin *et al.* 2012) and Balkan Peninsula (Lusini *et al.* 2014; Poljak *et al.* 2017). It is important to note that some of these areas (Italy, Turkey, Iberian Peninsula) are the leading European chestnuts producers (Goulão *et al.* 2001). Sweet chestnut cultivars in Turkey were not accurately characterized and classified according to their origins. In addition, cultivars having the same name and different genotype emerged in many regions (Ertan 2007).

Sweet chestnut is an important multipurpose tree species used for its wood, fruit, honey, and tannin (Idžojtić *et al.* 2009). It is also a valuable species in ecosystems and landscapes. For example, chestnuts are rich in carbohydrates, proteins, vitamins and minerals. In addition, sweet chestnut honey has antioxidant and antimicrobial properties, and branches can be used in painting. From the perspective of the global and national forestry, sweet chestnut is of great importance with regard to versatile usage possibilities. However, chestnut forests have been seriously degraded since the introduction of chestnut diseases (Akdogan and Erkam 1968; Heiniger and Rigling 1994; Gurer 2001; Krstin *et al.* 2017). In addition, stand structure of chestnut forests are frequently degraded because of inappropriate silvicultural treatments (Turna *et al.* 2014). In studies conducted in Europe in recent years, researchers have expressed that the genetic diversity of sweet chestnut is endangered, and that it is important to ensure the conservation and sustainable use of chestnut genetic resources (Mellano *et al.* 2012).

In many fields of plant sciences, morphological information's are still very important (Douaihy *et al.* 2012; Poljak *et al.* 2015) such as different taxon delimitation (Mac-Key 1988; Poljak *et al.* 2014a; Sękiewicz *et al.* 2016), population variability (Brus *et al.* 2011, 2016; Douaihy *et al.* 2012; Poljak *et al.* 2012, 2014a, 2018; Zebec *et al.* 2014, 2015), cultivar characterization (Ertan 2007; Ertan *et al.* 2007; Poljak *et al.* 2016) and selection (Polat and Özkaya 2005; Solar *et al.* 2001, 2005), morphological and physiological seed characterization (Powell 2010; Yilmaz and Yüksel 2014; Drvodelić *et al.* 2015; Daneshvar *et al.* 2016), and trends in leaf morphology regarding the branch position and patterns of crown plasticity (Bruschi *et al.* 2003; Bednorz 2006; Poljak *et al.* 2014b). The studies of European and Turkish sweet chestnut populations revealed high morphological variation within populations and low differentiation between populations (Villani *et al.* 1991; Pereira-Lorenzo *et al.* 1996; Serdar 1999; Podjavorsek *et al.* 1999; Serdar and Soylu 1999; Solar *et al.* 2001, 2005; Miguelez *et al.* 2004; Bolvanský and Užík 2005; Ertan 2007; Idžojtić *et al.* 2009; Mujić *et al.* 2010; Poljak *et al.* 2012). Ertan (2007) pointed out that morphological and phenological characteristics can be used to improve quantitative estimates of genetic similarities and relationships. In addition, morphological characterization is still official method for protection and registration of new cultivars (Pereira-Lorenzo *et al.* 1996). Moreover, information's about the seed quality (morphological and physiological characteristics), and population diversity should be used in order to grow quality and healthy seedlings (Powell 2010).

The aim of this study was to assess variation among eight sweet chestnut populations in Turkey by using nine different morphological characteristics of fruits and seedlings.

MATERIALS AND METHODS
MATERIJALI I METODE

Chestnut fruits were collected from Adapazarı, Artvin, Aydın, Balıkesir, Bartın, İzmir, Kütahya and Sinop located in natural distribution area of *Castanea sativa* in Turkey (Figure 1, Table 1). An average distance of 150–200 m was established between sampled trees. Fruits were sampled from 15 to 20 trees per population.

Table 1. Coordinates and altitudes of the analysed populations.
Tablica 1 Koordinate i nadmorske visine analiziranih populacija.

Population acronym Akronim Populacije	Population name Naziv populacije	Latitude Geografska širina	Longitude Geografska dužina	Altitude Nadmorska visina
A1	Aydın	37° 56′ 28″	28° 18′ 56″	230
A2	Adapazarı	40° 41′ 27″	30° 48′ 22″	1000
A3	Artvin	41° 22′ 09″	41° 32′ 42″	800
B1	Balıkesir	39° 22′ 25″	27° 15′ 41″	840
B2	Bartın	41° 46′ 45″	32° 31′ 09″	980
İ1	İzmir	38° 16′ 55″	28° 01′ 34″	550
K1	Kütahya	39° 05′ 54″	28° 55′ 03″	900
S1	Sinop	41° 53′ 51″	34° 53′ 04″	370

After the collection, fruits from eight populations were measured in the laboratory. A total of three characteristics were measured by using a digital calliper: fruit length (FL), fruit width (FW), and fruit thickness (FT). In order to quantify the fruit shape, the ratio of fruit length and width (FL/FW) was derived. Measurements were made with millimetre (mm) sensitivity. In addition, 1000 fruit mass (1000FM)

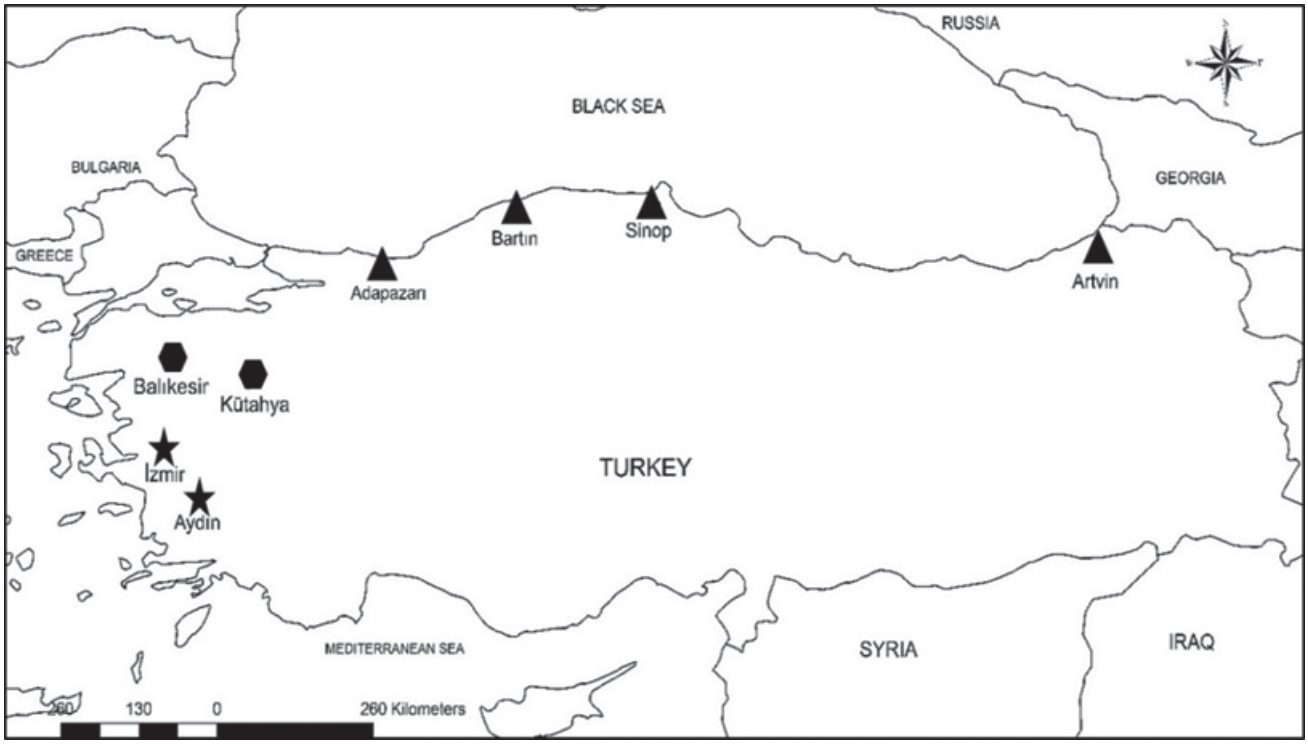

Figure 1. The geographical locations of the sampled sweet chestnut populations, and distribution of groups obtained by cluster analysis.
Slika 1. Geografski lokaliteti istraživanih populacija pitomoga kestena i prostorni raspored skupina dobivenih kalsterskom analizom.

were calculated based on ISTA rules (ISTA 1993) - 800 (8×100) fruits were collected randomly from eight populations and weighed using a precision balance.

Fruit moisture content (FM) (%) was determined on a fresh mass basis using four replicates of 50 g each; fruits were weighed, oven dried at 103±1°C for 16±1 h, and reweighed (ISTA 1993).

After the measurements, fruits were sown, using a randomised sampling design, in nursery seedbeds in October. Seedling length (SdL), root collar diameter (RCD) and sturdiness quotient (SQ) were carried out at 1-year-old seedlings grown in seedbed. Measurements were made on total of 720 seedlings to be 3×30 seedlings from each populations. The sturdiness quotient refers to the ratio of the height of the seedling to the root collar diameter and expresses the vigour and robustness of the seedling (Thompson 1985; Aldhous 1994; Jaenicke 1999).

Finally, data were analysed using the SPSS 23.0 statistical program. The conducted analyses included ANOVA, Duncan's Test, Pearson's correlation coefficient, hierarchical cluster analysis, and discriminant analysis.

RESULTS
REZULTATI

The highest values of FL, FW and FT were obtained in İzmir population. While the highest FL/FW ratio was recorded in Aydın population. Balıkesir population was characterized by the highest values of SdL, RCD and SQ. The values of fruit length ranged from 21.56 to 31.21 mm, and the mean length was 25.96 mm in all analysed populations. The fruit width in all populations ranged from 22.92 to 32.43 mm, with a mean value of 27.75 mm. The fruit thickness ranged from 14.74 to 18.99 mm, and the mean value of fruit thickness was 16.51 mm. The fruit length/width ratio varied between 0.83 and 0.98 for all populations. Average seedling length, root collar diameter and sturdiness quotient were detected to vary between 10.32-19.17 cm, 4.61-6.94 mm and 1.85-3.20, respectively. The mean values, standard deviations, maximum and minimum values of the fruit and seedling sizes are presented in Figure 2. The coefficients of variation for the studied characteristics ranged from 7.17 % to 54.04 % (Figure 3).

The ANOVA revealed that there are statistically significant differences (P<0,05) among the analysed populations in terms of all measured morphological characters. In addition, Duncan's test was performed. For the variables: fruit width (I1-A1; K1-B1; A3-A2; B2; S1), fruit length/fruit width ratio (I1-A1-K1-A3; B1; B2; S1; A2), and the root collar diameter (B1; A3-S1; A1; I1-B2-A2; K1) five groups were revelled. Furthermore, fruit (I1-A1; K1-B1; B2-S1; A2-A3) and seedling length (K1-B2-S1-A3; I1-A1; B1; A2), as well the sturdiness quotient (B1-A2-K1; S1-B2; I1-A3; A1) formed four groups. When the groups with regard to the fruit thickness were examined: I1 and A1 populations were

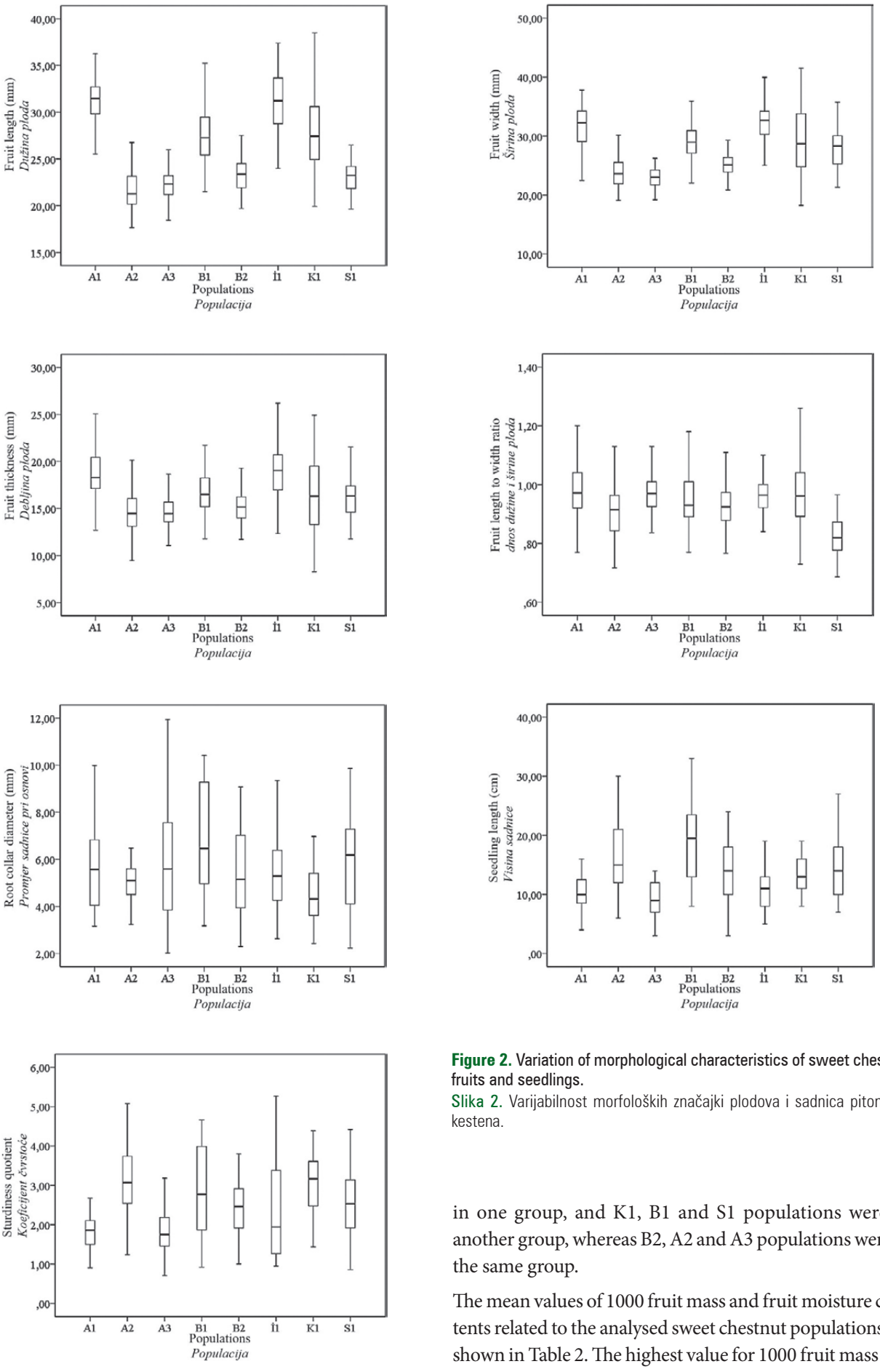

Figure 2. Variation of morphological characteristics of sweet chestnut fruits and seedlings.

Slika 2. Varijabilnost morfoloških značajki plodova i sadnica pitomoga kestena.

in one group, and K1, B1 and S1 populations were in another group, whereas B2, A2 and A3 populations were in the same group.

The mean values of 1000 fruit mass and fruit moisture contents related to the analysed sweet chestnut populations are shown in Table 2. The highest value for 1000 fruit mass was

Table 2. The mean values of 1000 fruit mass and fruit moisture content.
Tablica 2 Srednje vrijednosti težine 1000 plodova i sadržaj vlage u plodovima.

Characters (unit) *Značajke (jedinica)*	Population *Populacija*							
	A1	A2	A3	B1	B2	İ1	K1	S1
1000FM (g)	10360.9	4451.4	3815.1	6155.1	6592.5	10516.5	7210.6	6923.7
FM (%)	44.80	44.45	38.46	39.64	41.32	47.34	42.81	52.21

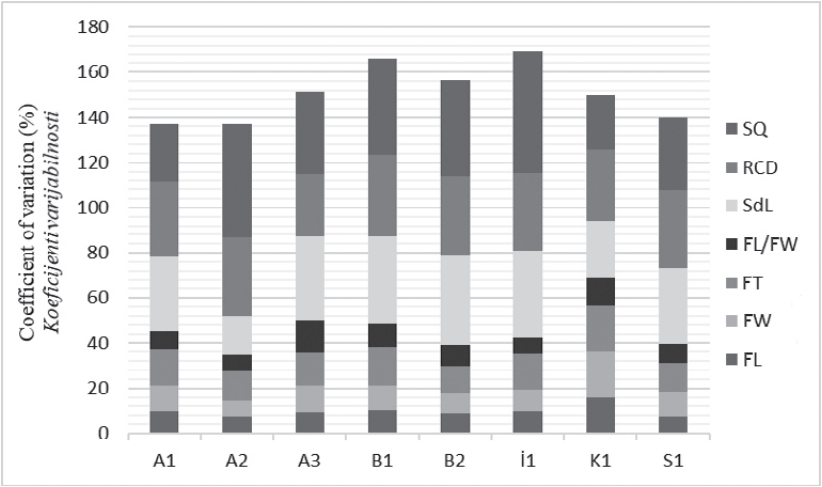

Figure 3. Coefficients of variation (%) of fruit and seedling studied characteristics in eight sweet chestnut populations.
Slika 3. Koeficijenti varijabilnosti (%) analiziranih značajki plodova i sadnica iz osam populacija pitomoga kestena.

determined in İzmir population (10516.5 g), and the lowest value in Artvin population (3815.1 g). The highest fruit moisture content was recorded in the Sinop population (52.21 %), and the lowest in the Artvin population (38.46 %).

The cluster analysis was conducted in order to determine degree of similarity or dissimilarity among populations with regard to the fruit and seedling morphological characteristics (Figure 4). The first group was made of the İzmir and Aydın populations. Balıkesir population took place in the second group with Kütahya population. The populations from the northern region of Turkey were in third group. Furthermore, discriminant analysis was used to find out the variables which best discriminate the groups obtained by cluster analysis (I1-A1; K1-B1; B2-S1-A2-A3). The results of the discriminant analysis suggested that the differentiation between the analysed groups is significant (P<0.05). The following variables had the highest discrimination power between researched groups: FL (P=0.000), 1000FM (P=0.000), FT (P=0.001) and FW (P=0.012) exhibited the

Table 3. Pearson correlation coefficients between pairs of morphological characters.
Tablica 3 Pearsonovi koeficijenti korelacije između parova morfoloških značajki.

	FL	FW	FT	FL/FW	1000FM	FM	SdL	RCD	SQ
FL	1	0.938**	0.947**	0.536	0.955**	–0.054	–0.442	0.290	–0.234
FW		1	0.961**	0.213	0.914**	0.213	–0.387	0.306	–0.165
FT			1	0.316	0.965**	0.153	–0.502	0.321	–0.365
FL/FW				1	0.454	–0.701	–0.282	0.050	–0.215
1000FM					1	0.126	–0.600	0.145	–0.363
FM						1	–0.286	–0.243	–0.075
SdL							1	0.478	0.818*
RCD								1	0.267
SQ									1

**Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level
** Korelacija je značajna na razini 0,01; * Korelacija je značajna na razini 0,05

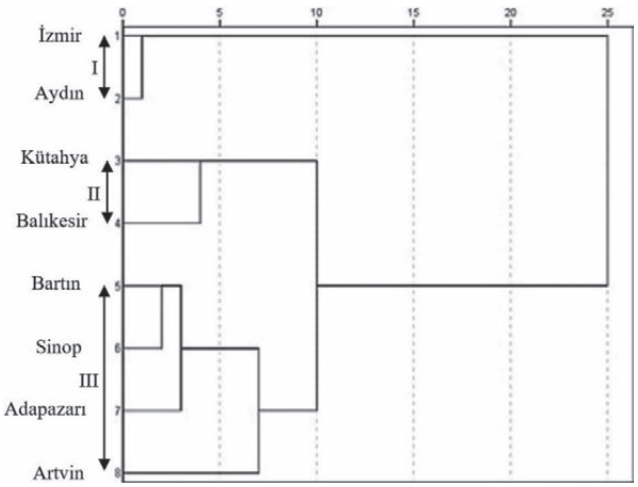

Figure 4. Dendrogram obtained from hierarchical cluster analysis.
Slika 4 Dendrogram dobiven hijerarhijskom klasterskom analizom.

best distinguish, respectively. The spatial distribution of these three groups is presented in Figure 1.

Relationships among all morphological characteristics were expressed in a correlation matrix in Table 3. Accordingly, it was determined that there was statistically significant positive correlation at the 99% confidence level between fruit length, fruit width, fruit thickness and 1000 fruit mass, and at the 95% confidence level between seedling length and sturdiness quotient.

DISCUSSION AND CONCLUSIONS
RASPRAVA I ZAKLJUČCI

In this study we analysed fruit length, width, and thickness, fruit length/width ratio, 1000 fruit mass, fruit moisture content, seedling length, root collar diameter and sturdiness quotient in eight sweet chestnut populations from different regions of Turkey.

Fruit characteristics observed in this study were similar to those previously reported for the sweet chestnut populations from the following countries: Bosnia and Hercegovina, Croatia, Slovakia, Slovenia and Turkey. Mujić *et al.* (2010) analysed fruit morphometric characteristics in four populations in Bosnia-Herzegovina. Authors reported the following morphological characteristics: fruit length (20.62-24.70 mm), width (22.11-27.01 mm) and thickness (23.60-26.80 mm). Furthermore, Idžojtić *et al.* (2009) and Poljak *et al.* (2012) researched morphological variation of sweet chestnut populations from Croatia, and revealed high degree of morphological variability. The highest values for fruit length, width and thickness were reported for the Mediterranean and North-Western Croatian populations. In a study conducted in Slovenia on phenotypic and genotypic diversity of sweet chestnut, Solar *et al.* (2005) stated that the average length, width and thickness of fruits obtained from 244 trees was 27 mm, 39 mm and 19 mm, respectively. Additionally, Solar *et al.* (2001) compared morphological characteristics of fruits obtained from sweet chestnut populations located in three different regions in Slovenia. Authors determined the following ranges: fruit length (2.5-3.0 cm), fruit width (2.7-3.4 cm), fruit thickness (1.9-2.0 cm,), and number of fruits per kilogram (85-152). From the established variables conclusions about the fruit shape can be made. It was reported that the nut width is larger than nut height in the majority of Croatian (Idžojtić *et al.* 2009; Poljak *et al.* 2012), Slovenian (Solar *et al.* 2001, 2005), Slovakian (Bolvanský and Užík 2005), Bosnian Herzegovinian (Mujić *et al.* 2010), and Turkish (Villani *et al.* 1991) populations. Likewise, we found out that fruit length/width ratio for Turkish sweet chestnut populations varied between 0.8 and 0.9. Ertan *et al.* (2007) determined high-yielding and good-quality chestnut genotypes within naturally grown sweet chestnut populations located in Nazilli district, Aydın province. Fruit samples were collected from 80 trees, and the following values for the fruit width, length and height were observed: 18.95-23.70 mm; 35.17-41.18 mm and 30.39-34.31 mm, respectively. Similar results were reported by Ertan (2007) were fruit samples were collected from 10 sweet chestnut accessions from 10 different areas, which were selected among 80 accessions at the end of a selection study for high nut quality and high yield among natural populations in the Nazilli district. In addition, in the same study leaf morphological and fruit chemical analysis were studied as well.

The mean mass of chestnut fruits from four populations in Bosnia-Herzegovina was 4.42-6.47 g (Mujić *et al.* 2010). The average nut mass for Croatian populations was 7.1 g and 8.3 g (Idžojtić *et al.* 2009; Poljak *et al.* 2012). According to Ertan *et al.* (2007), chestnut fruits grown in Aydın-Nazilli ranged from 13.45 g to 19.96 g. The average fruit mass values for Slovenian populations ranged between 7.1 and 14.3 g (Solar *et al.* 2001, 2005). Serdar and Soylu (1999) pointed out that the mean mass values of fruits from the Samsun vicinity in Turkey varied between 5.3 and 15.1 g. In addition, the mass of the nuts from six natural sweet chestnut Turkish populations varied from 3.4 to 5.2. g (Villani *et al.* 1991). Similarly, 1000 fruit mass in our study ranged from 3815.1 g (fruit mass 3.81 g) to 10516.5 g (fruit mass 10.52 g). Chestnut forests in Turkey have a high degree of variation. In general, in our study the smaller nut mass values were observed in natural populations with undegraded stand structure. Furthermore, decrease in fruit sizes from west to east was also confirmed.

The multivariate statistical methods revelled that the populations with similar ecological conditions and being close to each other were in the same group. Solar *et al.* (2005) and Poljak *et al.* (2012) stated that sweet chestnut populations are well adapted to the climatic and soil conditions, but they

differ in numerous morphological traits, productivity, and fruit quality. In addition, almost all of the populations in the third group are of natural structure. The mentioned populations, from the North Anatolia Region (Black Sea Coast) of Turkey, are in general characterized with higher genetic (Villani *et al.* 1999; Mattioni *et al.* 2017) and morphological variation. Populations in the first and second group are both natural and grafted.

Miguelez *et al.* (2004) found that moisture contents were over 50% in chestnut seeds obtained from 15 different populations spread over Galicia region in Spain. In our study, the fruit moisture content ranged from 38.46% to 52.21%. Differences between these two studies are probably the result of different methodologies. In our research, whole fruits were used, while Miguelez *et al.* (2004) analysed only the edible part of the fruit, i.e. the kernel.

In our research, sturdiness quotient ranged from 1.85 to 3.20. Sturdiness quotient is a criterion commonly used for seedling quality classification (Bacon 1979; Aldhous 1994; Genç and Yahyaoglu 2007). The ideal value for a seedling to be considered as sturdy is less than six (Jaenicke 1999). Seedlings with sturdiness ratio greater than six were actually thin, tall and etiolated, while a small quotient indicates sturdy plants with a greater chance of survival, particularly on windy or dry sites (Takoutsing *et al.* 2013).

As a conclusion, specific east-west increase in fruit sizes in the chestnut forests of Turkey is probably the result of ecological conditions and human influence. Those findings are in the line with the result of the previously published paper by Villani *et al.* (1991). Authors concluded that human influence could have enhanced the genetic, morphometric, and physiological differentiation of natural western chestnut populations with respect to the central and eastern ones. In the direction of sustainable forestry principles, the stand structure of the natural sweet chestnut forests must always be protected, and applications such as grafting works can caused decreasing of genetic diversity.

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SAŽETAK

Pitomi kesten, *Castanea sativa* Mill., plemenita je vrsta drveća od koje imamo višestruku gospodarsku korist (kvalitetno drvo, jestivi plodovi, med, ogrijev, listinac i dr.). Rasprostranjen je u mediteranskom području, od Kaspijskog jezera do Atlantskog oceana. U Turskoj najveće površine pod kestenovim šumama nalazimo na području sjeverne i zapadne Antolije te u regiji Marmara. U posljednjih nekoliko desetljeća pitomi kesten je ugrožen od raka kestenove kore i negativnih antropogenih utjecaja. S obzirom na to provedena su brojna istraživanja s ciljem očuvanje genofonda ove plemenite vrste drveća.

Glavni cilj ovoga istraživanja bio je utvrditi morfološku varijabilnost plodova i jednogodišnjih sadnica pitomoga kestena u Turskoj. Plodovi za morfometrijsku analizu skupljeni su tijekom listopada u osam populacija pitomoga kestena na području istočne, središnje i zapadne Turske (slika 1, tablica 1). Unutar svake populacije sakupljeni su uzorci sa po 15 do 20 stabala. Ukupno su određene po četiri značajke na svakom plodu: dužina, širina i debljina ploda te omjer dužine i širine ploda. Osim toga, određen je i udio vlage u plodovima, kao i masa 1000 zračno suhih plodova. Plodovi su u proljeće posijani na unaprijed pripremljene gredice. Na kraju prvog vegetacijskog razdoblja mjerene su sljedeće značajke: visina sadnice, promjer sadnice pri osnovi i koeficijent čvrstoće. Za utvrđivanje varijabilnosti populacija korištene su deskriptivne i multivarijatne statističke metode. Podaci su obrađeni u programskom paketu SPSS 23.0.

Rezultati deskriptivne statističke analize prikazani su na slici 2. Najviše vrijednosti za dužinu, širinu i debljinu ploda utvrđene su u populaciji Izmira, dok su najviše prosječne vrijednosti za visinu sadnice, promjer sadnice pri njenoj osnovi i koeficijent čvrstoće utvrđene u populaciji Balikesira. Masa 1000 zračno suhih plodova kretala se je od 3815,1 g do 10516,5 g, a udio vlage u plodovima od 38.46 % do 52,21 % (tablica 2). Istraživanjem je utvrđen visok stupanj varijabilnosti populacija pitomoga kestena u Turskoj (slika 3). Rezultati provedene analize varijance pokazali su da se populacije međusobno signifikantno razlikuju za sve istraživane značajke. Pearsonovim koeficijentom korelacije utvrđena je statistički značajna korelacija između dužine, širine i debljine ploda, kao i mase 1000 zračno suhih plodova (tablica 3). Osim toga, pozitivna korelacija utvrđena je i između visine sadnice i koeficijenta čvrstoće. Duncanovim testom i hijerarhijskom klasterskom analizom utvrđeno je da se one populacije koje su geografski bliže i ekološki sličnije nalaze u istoj skupini (slika 1 i 4). Također je utvrđeno da se veličina plodova u kestenovim šumama Turske povećava od istoka prema zapadu.

KLJUČNE RIJEČI: *Castanea sativa*, pitomi kesten, Turska, morfologija, plodovi, sadnice