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AGRO-MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF DISEASE-FREE SEED PROPAGATED EUROPEAN CHESTNUTS (*Castanea sativa* Mill.)

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Disease and pest free chestnut (*Castanea sativa* Mill.) fruits were collected from 13 different seedling origin genotypes during the 2016 harvest season from the Mediterranean region of Turkey. The tree growth habit, nut weight, kernel ratio, kernel color, moisture, crude protein, crude fat, dietary fiber, total polyphenols, antioxidant activity, and fatty acid content of chestnuts were determined. The results showed that most of the genotypes had a semi-upright tree growth habit. The nut weight and kernel ratio were 5.87 g (A-9), 11.13 g (A-1), 73.38% (A-1), and 82.84% (A-12) among genotypes. The total crude fat content ranged from 0.80% (A-11) to 2.14% (A-12) while the crude protein content was between 4.78% (A-10) and 7.96% (A-9). Total polyphenols varied from 78 (A-12) to 124 (A-3) µg GAE/g and antioxidant activity was found to vary between 5.33 (A-12) and 9.83 (A-3) µmoles Trolox equivalent/g dry weight basis. Oleic and linoleic acid were the major fatty acids in all chestnut fruits, followed by palmitic and linolenic acids. The introduction of these genotypes as new cultivars by vegetative propagation may result in an increase in the quality of the chestnuts from the Mediterranean region of Turkey.

Keywords: Chestnut, bioactive content, diversity, fatty acids.

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INTRODUCTION

Nuts together with other fruit species play an important role in human nutrition and health, as sources of vitamins, minerals, antioxidants, dietary fiber and phytonutrients (plant-derived micronutrients). They are genetically very diverse group and distributed throughout the world (ERCISLI *et al.*, 2008a,b; SERCE *et al*., 2010; FAZENDA *et al*., 2019; GUNEY *et al*., 2019; OZKAN *et al*., 2019; GECER *et al*., 2020).

Chestnuts (*Castanea sativa* Mill.) are cool-season crops; available in the markets from October through March, accepted as one of the oldest and the most important nut crop found throughout the temperate zone, worldwide. With species indigenous to all three continents the chestnuts have been cultivated and consumed throughout Asia, Europe, and America for a long time (BOUNOUS, 2005). They have a quite remarkable nutritional composition that sets them apart from all the other nuts and makes them an outstanding food source, which can be a dietary staple. The nuts have nearly 50% water content when fresh, which makes them highly perishable. Chestnuts are made up of primarily complex carbohydrates, low in protein (mostly high-quality), and very low in fat, have reasonable quantities of vitamin C and potassium (MUJIC *et al.,* 2010; VASCONCELOS *et al.,* 2010).

European chestnut (*C. sativa*) is a large growing (around 20-30 m tree height) and wide spreading tree which originated from around Turkey and the Black Sea region of southern Russia. The nuts are quite variable but superior fruiting possesses good size, sweet taste and has easy to remove inner skins (BOUNOUS, 2002).

In the Mediterranean region, chestnuts have been cultivated for at least 3.000 years. It is thought that European chestnut (*C. sativa*) was introduced from Asia Minor (Anatolia), via Turkey, to southern Europe and North Africa. Later European chestnut cultivation extended into northwest and central Europe and it was the Romans who named chestnuts 'Castanea', possibly after the name of the town where chestnuts were once very common. In Asia, the Japanese chestnut (*Castanea crenata*) has been cultivated at least since the 11th century, while the Chinese chestnut (*Castanea mollissima*) has been possibly planted for 2.000-6.000 years. In the USA, the American chestnut (*Castanea dentate*) was once a major component of the indigenous forests. All Castanea species and their hybrids are edible and some are used in commercial nut production around the world (BOUNOUS, 2005; LANG *et al.,* 2006).

In Turkey, especially in the last decade, a gradual return of the interest in the sweet chestnut cultivation was observed. In rural forests in Turkey, chestnuts are providing multiple benefits to livelihood to the population of the region for centuries. Rural people use chestnuts for wood, fruit, honey, tannin, preservation of ecological and landscape values (ORMECI *et al.*, 2016). Main chestnut growing regions in Turkey are: Aegean Region, the Mediterranean Region, the Marmara region and east of the Black Sea Region (ERTAN, 2007). In all these regions there are valuable chestnut populations with the vast genetic variations in Turkey due to continuing sexual propagation for a long time. Chestnut populations show considerable variations in nut quality, tree characteristics, productivity, health status, and climatic adaptability. These genetic resources provide a good opportunity for genetic improvement. It seems that there were a lot of chestnut genotypes that were free of chestnut blight (*Cryphonectria parasitica*) and ink disease (*Phytopthora cambivora*) damage in Turkey's forests (ORMECI *et al.,* 2016).

Chestnuts stand out from other edible nuts because of their distinctive nutrition profile. Chestnuts, like hazelnuts and almonds, etc., are gluten-free food. Moreover, for the same reason, they are popular raw ingredients in the preparation of gluten-free food formulas intended for use in gluten-sensitive, wheat allergy, and celiac disease patients. The nuts are consumed directly in roasted or boiled form or evaluated as value-added products such as chestnut desserts and candied chestnut (MERT and ERTURK, 2017). Composition and health studies clearly indicated that chestnut fruits have considerable potential as functional foods or as food ingredients, e.g. chestnut polyphenolic extracts as a natural source of antioxidants and other beneficial compounds (DE LA MONTANA MIGUELEZ *et al*., 2004; PEREIRA-LORENZO *et al.*, 2006).

The main objective of the present study was to determine the agro-morphological characteristics and biochemical content of 13 selections of European chestnut identified in the Mediterranean region of Turkey in order to select the genotypes of higher quality as seed sources for reforestation and as bud sources for grafting propagation as new cultivars.

MATERIAL AND METHODS

Plant material

This research was conducted in the Antalya province of Turkey in 2016. Among the population, a total of 13 seed propagated promising chestnut genotypes were marked in terms of yield, nut size, earliness and resistance to chestnut blight and ink disease characteristics. The nut samples were collected from 13 promising genotypes. The A letter (A-1 to A-13) was assigned to the 13 genotypes.

Agro-morphological traits

Agro-morphological characteristics and biochemical content were conducted with four replications on a total of 40 nuts per genotype. Nut weight was measured by using a digital balance with a sensitivity of 0.001 g. Kernel ratio (%) was counted considering the weight of the nut and the kernel (ERTAN *et al.*, 2007).

Proximate analysis

The kernels of the samples were used to assess the moisture, crude protein, crude fat, dietary fiber, total carbohydrates, total polyphenols, and antioxidant activity. The moisture content of the chestnuts was determined by the gravimetric method using a drying oven at 105±2 °C. The total nitrogen was analyzed using the Kjeldahl method, and crude protein content was calculated using a nitrogen conversion factor of 5.30, which is specific for chestnut fruits (AOAC, 2000). Total fat was determined after extraction with ether for 16 h in a soxhlet device (AOAC, 2000). The dinitrophenol method was utilized in the analysis of total carbohydrates (ROSS, 1959) using a spectrophotometer.

Total polyphenol and antioxidant activity

For the total polyphenol analysis, the sample extraction was carried out by combining 3 g of the sample with 6 mL of 70% (v/v) ethanol and homogenizing with an Ultra-Turrax homogenizer. The extract was shaken at 210 rpm under the refrigerated conditions for 15 min and then centrifuged for 15 min at 2346 x g (5˚C). Before the analysis, phenolic compounds were

extracted by solid-phase extraction because substances such as reducing sugars, alcohol and tartaric acid, as well as antioxidant compounds (ascorbic acid) could interfere in the determination of polyphenols with the Folin-Ciocalteu reagent (NACZK and SHAHIDI, 2004). Commercially available octadecyl C18 cartridges (1 g, 6 mL) were used for the extraction of the phenolic fraction according to the following protocol: 2 mL of sample was loaded onto the column previously conditioned with 5 mL of methanol and 10 mL of water. The column was eluted with 4 mL of 0.02 N sulphuric acid to eliminate all the water-soluble compounds. The compounds retained by the column were recovered by eluting with 4 mL of 60% (v/v) methanol solution. Total polyphenol content was determined by the colorimetric reaction with the Folin-Ciocalteu reagent (SINGLETON and ROSSI, 1965). Gallic acid was used as an external standard for the calibration curve and results were expressed as mg of gallic acid equivalents (GAE) per g of dry weight.

For antioxidant activity determination, the sample extraction was carried out combining 3 g of sample with 6 mL of 70% (v/v) ethanol and homogenizing with an Ultra-Turrax homogenizer. The extract was shaken at 180 rpm under refrigerated condition for 15 min then centrifuged for 15 min at 2346 x g (5°C). The radical-scavenging activity was determined soon after extraction by the ABTS+ radical cation decolorization assay, as described by RE *et al.* (1999). The bleaching rate of ABTS+ in the presence of the sample was monitored at 734 nm by a spectrometer. A volume of 2.97 mL of ABTS+ solution was used. The reaction was started by the addition of 30 mL of the ethanolic extract diluted up to 1:10. ABTS+ bleaching was monitored at 25°C for at least 30 min and the percentage of decoloration after 7 min was used as the measure of antioxidant activity. In this dilution range, the ABTS+ bleaching was proportional to the concentration of the sample added to the medium, and a linear model fit the dose-response curve. Antioxidant activity was calculated by the ratio of the regression coefficient of the dose-response curve of the sample and the regression coefficient of the dose-response curve of Trolox (hydrophilic homolog of a-tocopherol) and was expressed as μmoles of Trolox equivalents per g of sample.

Fatty acid analysis

FAME was prepared by hydrolysis with a 2 M methanolic potassium hydroxide solution, and extraction with n-heptane, in accordance with ISO 5509 method (ISO, 2000) and following a procedure described in previous work (BARREIRA *et al.*, 2009). The fatty acids profile was evaluated with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, a flame ionization detector (FID), and a Chrompack CP-9050 auto-sampler. Separation was achieved on a 50 m, 0.25 mm i.d. fused silica capillary column coated with a 0.19 lm film of CP-Sil 88 (Chrompack). Helium was used as a carrier gas at an internal pressure of 120 kPa. The results are expressed in the relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area, and assuming that the detector response was the same for all compounds.

Statistical analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by LSD test at $p < 0.05$ significant level. SPSS software was used for analysis.

RESULTS AND DISCUSSION

Agro-morphological traits

The result showed that 10 out of 13 genotypes had semi-upright growth habit and 8 out of 13 genotypes had light cream kernel color (Table 1). SERDAR *et al.* (2011) reported that the new chestnut cultivar Serdar had semi-upright tree growth habit and light cream kernel color.

Table 1. Some agro-morphological characteristics of 13 chestnut genotypes

Genotypes	Tree growth habit	Nut weight (g)	Kernel ratio (%)	Kernel color
A-1	Semi upright	11.13	73.38	Light cream
A-2	Semi upright	8.76	77.56	Cream
A-3	Semi upright	10.03	80.03	Light cream
A-4	Semi upright	8.14	77.56	Light cream
A-5	Spreading	7.44	80.55	Light cream
A-6	Semi upright	6.63	76.36	Cream
A-7	Semi upright	9.11	75.23	Light cream
A-8	Semi upright	6.21	74.50	Light cream
A-9	Semi upright	5.87	81.21	Cream
A-10	Spreading	10.11	74.90	Light cream
A-11	Semi upright	6.39	77.33	Dark cream
A-12	Spreading	7.03	82.84	Light cream
A-13	Semi upright	7.25	78.20	Cream
LSD_{05}		3.22	2.66	

Statistically, differences in nut weight and kernel ratio were found among genotypes at $p<0.05$ (Table 1). The nut weight is highly variable among chestnut genotypes, which ranged from 5.87 g (A-9) to11.13 g (A-1) (Table 1). A study on seed propagated chestnuts was conducted in Northeastern Turkey and 13 promising chestnut genotypes were selected. The average nut weight varied between 4.79 to 7.45 g among those 13 genotypes, respectively (SERDAR, 2002). In Bosnia and Herzegovina, the average nut weight was between 4.49 and 6.25 g in natural populations (MUJIC *et al.,* 2010). In Slovenia, there was regionally great variation among seed propagated chestnut genotypes on nut weight which varied from 3.5 to 18.5 g (SOLAR *et al.,* 2005). In Spain also great variability on nut weight (from 8.40 to 22.72 g) was observed in populations found in different geographical regions (PEREIRA-LORENZO and FERNÁNDEZ-LÓPEZ, 1997; PEREIRA-LORENZO *et al.,* 2006). BOUNOUS (2002) categorizes chestnut cultivars in two main groups: 'Marroni' and chestnut types and according to the Italian region, nut weight is grouped from 6.25 to 14.28 g for chestnut, and 10.00 to14.28 g for marroni type. Chestnut weight depends on the number of fruits that are formed in the burr. High variability in

nut weight (2.8-19.1 g) has been reported for 21 Romanian chestnut genotypes (BOTU *et al.*, 1999) and the nuts (5.3–15.1 g) from the Samsun provinces in Turkey (SERDAR and SOYLU, 1999).

Kernel ratio of the 13 chestnut genotypes varied from 73.38% (A-1) to 82.84% (A-12), respectively (Table 1). ERTAN (2007) reported a percentage of kernels among European chestnuts from ten different areas in Turkey between 75.9% and 86.1%. MUJIC *et al.* (2010) determined that the percentage of the kernel was ranged from 78.5 to 87.3 % in non-grafted and 81.0% in grafted chestnuts in Bosnia and Herzegovina. Environmental influence and genetic factors can affect chestnut morphological features connected with a tree, but also connected with a nut (MUJIC *et al.*, 2010).

Proximate composition

The moisture, total crude protein, crude fat, total carbohydrates, and dietary fiber content of 13 chestnut genotypes from the Mediterranean region in Turkey were shown in Table 2. All parameters were statistically variable at $p<0.05$. Moisture, crude protein, crude fat, carbohydrate, and dietary fiber content ranged from 45.26% (A-3) to 52.04% (A-5); 4.78% (A-10) to 7.96% (A-9); 0.80% (A-11) to 2.14% (A-12); 54.10% (A-5) to 62.74% (A-3), and 1.93% (A-11) to 3.24% (A-3), respectively (Table 2).

Table 2. Proximate composition of chestnut fruits of 13 genotypes (dry weight basis per 100 g)

Genotypes	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Carbohydrate (%)	Dietary Fiber (%)
A-1	47.27	7.24	1.88	59.21	2.87
A-2	50.14	4.90	1.06	58.02	2.00
A-3	45.26	7.75	1.67	62.74	3.24
A-4	50.23	5.11	1.55	57.63	2.04
A-5	52.04	4.87	1.95	54.10	2.08
A-6	47.55	7.22	1.34	59.58	3.01
A-7	50.20	5.53	1.24	57.10	2.20
A-8	46.05	7.87	1.02	60.10	2.61
A-9	48.26	7.96	1.30	59.60	2.25
A-10	51.18	4.78	1.01	55.82	2.18
A-11	50.76	5.43	0.80	56.10	1.93
A-12	45.48	7.36	2.14	60.97	2.19
A-13	49.23	6.91	2.06	58.12	2.25
LSD_{05}	3.78	2.07	0.29	3.02	0.36

ER *et al.* (2013) reported moisture, crude oil, crude protein, crude fiber, and total carbohydrates values as 51.7-56.9%, 2.2-3.5%, 5.1-6.3%, 2.3-3.7%, and 73.2-81.3%, respectively in chestnut fruits collected from different regions of Turkey. OZEL (2015) determined moisture, crude protein, and crude oil in chestnut genotypes from Northeastern Turkey between 50.8-58.4%, 5.5-7.2%, and 2.7-4.6%. MERT and ERTURK (2017) studied the kernel composition of 19 local and foreign chestnut cultivars and found out 5.58-7.35 g/100 g

protein and 58.18-66.21 g/100 g total carbohydrates (on the dry matter basis). BERNARDEZ *et al*. (2004) reported moisture contents of chestnuts in Spain between 48.37-59.35%. The total protein content was reported between 4.50 and 10.87 g/100 g by different researchers in *C. sativa* Mill (DE LA MONTANA MIGUELEZ *et al.*, 2004; ERTÜRK *et al.*, 2006; PEREIRA-LORENZO *et al.*, 2006; MERT and ERTURK, 2017). MERT and ERTURK (2017) reported total carbohydrate quantities of chestnuts between 58.18 and 66.21% depending on the cultivar. Our results are in agreement with previous results. When compared to the other nuts such as walnuts, hazelnuts etc., chestnuts differed from other nuts and had lower fat (2.0-5.0%) content. The crude fat content was significantly different among the cultivars ($p < 0.05$). MERT and ERTURK (2017) reported the crude fat amount chestnut cultivars ranged from 0.87 to 2.61%. Our results are in accordance with those obtained by ERTÜRK *et al.* (2006) for *C. sativa* and hybrid cultivars, for Italian cultivars (SACCHETTI and PINNAVAIA, 2005) and for Spanish cultivars (DE LA MONTANA MIGUELEZ *et al*., 2004; PEREIRA-LORENZO *et al., 2006*).

Total polyphenols and antioxidant activity

There were statistical differences among chestnut genotypes on these parameters ($p<0.05$) and total polyphenols were found between 78 (A-12) and 124 (A-3) µg GAE/g on a dry weight basis and antioxidant activity was between 5.30 (A-9) and 9.83 (A-3) µmoles Trolox equivalent/g on a dry weight basis (Table 3).

Table 3. Total polyphenol content and antioxidant activity of 13 chestnut genotypes (dry weight basis per g fruit)

Genotypes	Total polyphenols (µg GAE/g)	Antioxidant activity (µmoles Trolox equal/g)
A-1	89	5.96
A-2	107	7.96
A-3	124	9.83
A-4	111	8.83
A-5	96	6.56
A-6	119	9.17
A-7	80	6.06
A-8	102	8.11
A-9	90	5.30
A-10	85	5.41
A-11	106	8.20
A-12	78	5.33
A-13	93	6.07
LSD_{05}	10.2	1.89

According to our results, the chestnut could be classified among fruits with low polyphenol content (VINSON *et al.*, 2001). With respect to the total phenols yields referred to the chestnut fruit, total phenol contents were reported between 21.8-24.7 g GAE/100 g (ER *et al.*, 2013). Total phenol contents of chestnut extracts ranged between 2.15-10.26 g GAE/100 g

(BARREIRA *et al.*, 2009). SUAREZ *et al.* (2012) reported the total phenol contents (1.96-4.31 g gallic acid per kg DW). Our results are also comparable to those (1.27-2.35 g/kg DW) reported by VEKIARI *et al.* (2007) who found the highest values in Spanish chestnuts followed by Greek nuts. OTLES and SELEK (2012) reported total phenolic contents between 5 mg GAE per g DW and 32.82 mg GAE per g DW in chestnut fruit samples.

In this study, the antioxidant activity of chestnuts was defined with a method extensively used in the literature, expressed in terms of μmoles of Trolox equivalents on g, and the values were compared with those obtained on other fruit and nuts by the same method. The antioxidant activity of the 13 chestnut genotypes represented a median value between the TEAC values of other fruit as reported by PELLEGRINI *et al.* (2003).

Fatty acids

Table 4 shows the fatty acid profiles of each chestnut genotype. Chestnuts genotypes had significant variation among the fatty acid composition of the samples ($p<0.05$). Oleic acid is the most abundant fatty acid, varying from 33.2% (A-7) to 41.4% (A-3) among genotypes. Polyunsaturated fatty acids (PUFA) seemed to be favored for most of the genotypes and linoleic acid was evidently the major PUFA, with contents ranging from 32.2% (A-3) and 37.8% (A-1) (Table 4). The high amount of PUFA represents a well-known advantage since it is classified as an essential fatty acid (EMKEN *et al.*, 1994). Therefore, the oil composition of these local Turkish chestnuts indicated that these seedlings may be of great benefit to health in the human diet due to their fatty acid profile. BADA *et al.* (2010) revealed that the fatty acid composition depended primarily on the genotype, the environment, the geographical origin, and agricultural practices.

Table 4. Means of the fatty acids of 13 chestnut genotypes (dry weight basis per g fruit)

Genotypes	Palmitic acid (16:0) (%)	Oleic acid (18:1) (%)	Linoleic acid (18:2) (%)	Linolenic acid (18:3) (%)
A-1	15.3	37.6	37.8	3.5
A-2	13.7	37.3	34.1	3.3
A-3	14.6	41.4	32.2	3.5
A-4	11.0	35.3	35.2	3.6
A-5	16.2	38.7	34.6	4.1
A-6	16.8	35.3	35.1	3.7
A-7	18.9	33.2	33.3	4.0
A-8	15.0	34.8	33.9	3.0
A-9	14.3	40.1	32.9	4.2
A-10	12.9	38.0	36.0	4.0
A-11	14.5	36.1	35.5	4.0
A-12	12.6	34.2	37.1	3.8
A-13	16.0	37.1	34.0	4.0
LSD_{05}	2.1	2.6	2.4	0.4

Results demonstrated that the chestnut lipid fraction is mainly constituted by three fatty acids: linoleic, oleic, and palmitic acids accounting for more than 90% of the total fatty acid

content, a value slightly higher or similar when compared to the results obtained by other research groups (BORGES *et al*., 2007; BARREIRA *et al.,* 2012). We suggest that the differences between our results and those obtained by the other researchers are due to the different origins of chestnut samples. Currently, the increasing consumer's interest in chestnuts could be explained with their nutritional value and potentially beneficial health effects, including the well-known advantages of omega-3 and omega-6 PUFAs, whose intake is insufficient in Western diets (SIMOPOULOS, 1991).

CONCLUSION

To the best of our knowledge, the present study is the first report on the agro-morphological and biochemical composition of the chestnuts from the Mediterranean region of Turkey. The chestnut oil was characterized with medium to high oil yield and concentration of PUFAs, mainly represented by oleic, linoleic, and linolenic acids. This composition is similar to that of different commercial chestnut cultivars. Thus, this oil may be considered with high nutraceutical value and a good source for the human diet. These findings confirm that the genotype as a major factor effects on the composition of the chestnut agro-morphological and biochemical characteristics. Some genotypes had bigger nut weight and better biochemical content. The introduction of these genotypes as new cultivars through vegetative propagation can lead to an increase in the quality of chestnuts from the Mediterranean region in Turkey. In addition, these genotypes could be used as a seed source for chestnut propagation in the same region, as the choice of the seed source is considered crucial for the success of future plantings in the orchard management.

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AGRO-MORFOLOŠKE I BIOHEMIJSKE KARAKTERISTIKE EVROPSKOG KESTENA (*Castanea sativa* Mill.) DOBIJENOG PROPAGACIJOM IZ SEMENA BEZ BOLESTI

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Izvod

Plodovi kestena (Castanea sativa Mill.) bez bolesti i štetočina sakupljeni su od 13 različitih genotipova poreklom iz sadnica tokom sezone berbe 2016. iz mediteranskog regiona Turske. Određeni su parametric rasta drveća, težina ploda, odnos zrna, boja zrna, vlaga, sirovi proteini, sirova masnoća, dijetalna vlakna, ukupni polifenoli, antioksidativna aktivnost i sadržaj masnih kiselina u kestenu. Rezultati su pokazali da je većina genotipova imala polu-uspravni rast drveta. Masa ploda i odnos zrna bili su 5,87 g (A-9), 11,13 g (A-1), 73,38% (A-1) i 82,84% (A-12)kod različitih genotipovima. Ukupni sadržaj sirovih masti kretao se u rasponu od 0,80% (A-11) do 2,14% (A-12), dok je sadržaj sirovih proteina bio između 4,78% (A-10) i 7,96% (A-9). Otkriveno je da ukupni polifenoli variraju od 78 (A-12) do 124 (A-3) µg GAE / g, a antioksidativna aktivnost varira između 5,33 (A-12) i 9,83 (A-3) µmoles Trolox ekvivalent / g suve težine. Oleinska i linoleinska kiselina bile su glavne masne kiseline u svim plodovima kestena, a slede palmitinska i linolenska kiselina. Uvođenje ovih genotipova kao novih kultivara vegetativnim razmnožavanjem može rezultirati povećanjem kvaliteta kestena iz mediteranskog regiona Turske.

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