

## Some Leaf Characteristics are Better Morphometric Discriminators for Chestnut Genotypes

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### ABSTRACT

This study was carried out in order to determine the leaf characteristics of some chestnut genotypes in the Central Black Sea Region of Turkey and also to determine whether the leaf morphometric characteristics could be used for differentiation of genotypes. In this study, seven chestnut (*Castanea sativa* Mill.) genotypes (SA5-1, SE 3-12, SE 21-2, SE 21-9, 552-8, 556-7 and 556-8) and one cultivar (Sariaslama) were used. Some leaf parameters such as lamina length, lamina width, leaf length, leaf area, petiole length, teeth width, teeth length, stomatal density, stomata width, stomata length, lamina width/lamina length, lamina width/leaf length, petiole length/lamina length, stomatal index, distance between the lateral veins and teeth width/teeth length were measured. Most of the chestnut genotypes could be differentiated easily by using leaf morphometric characteristics. The lamina width, lamina length, leaf length, distance between the lateral veins, leaf area, stomata width, stomata length and the ratios of teeth width/teeth length, lamina width/lamina length and lamina width/leaf length were better discriminators for chestnut genotypes.

**Keywords:** *Castanea sativa*, Cultivar, Discrimination, Morphometric traits, Selection,

### INTRODUCTION

Phenotypic evolution is a comprehensive term in plant biology applied to variation within and among species of an array of morphological characters (Pigliucci *et al.*, 1991). Furthermore, morphological and phenological characteristics are traditionally used to develop quantitative estimates of genetic similarities and relationships (Ertan, 2007). Studies of plant ecotypic differentiation have widely demonstrated genetic variation in allocational and growth related traits (Sultan, 1995). Lang and Huang (1999) noted that evaluation of genetic diversity and population structure of the species in natural populations is crucial for developing a conservation strategy and sustainable utilization of the natural resources.

China, Korea, Turkey, Italy, Bolivia, Japan, Portugal and Spain are the leading countries in

the World's chestnut production. However, there are two main areas of particular biological value for European chestnut genetic resources in Europe: Turkey and the Iberian Peninsula, with the former being one of the original centers of chestnut (Villani *et al.*, 1999). Chestnut growing areas in Turkey are spread from the Eastern Black Sea Region, through Marmara and the Aegean Regions and then reach to Antalya in the Mediterranean Region in Anatolia (Davis, 1982; Soylu, 2004). Moreover, the Black Sea Region has one third of the total chestnut production in Turkey (Turkstat, 2010). Therefore, selection studies for yield, earliness and fruit quality have been made to improve the chestnut cultivars in the Marmara (Ayfer *et al.*, 1977; Ayfer and Soylu, 1995) and Aegean Regions (Ozkarakas *et al.*, 1995; Ertan *et al.*, 2007; Koyuncu *et al.*, 2008) and especially in the Black Sea Region (Serdar, 1999; Serdar and Soylu, 1999; Serdar, 2002; Ozkan, 2003;

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Yarilgac *et al.*, 2009). After some selection studies in the Black Sea Region, experimental orchards were established in two locations (Samsun and Ordu) in 1998. Preliminary results for especially the number of unproductive years, plant growth and some pomological and phenological traits under the same ecological conditions have been reported (Serdar and Soyulu, 2005). The yield and some fruit traits of the genotypes were determined in 2000-2005. This information was used by Turkey Variety Registration and Seed Certification Centre for variety registration of these genotypes in 2006. Indeed, during these studies a number of problems arose in evaluating some morphological leaf traits of the genotypes such as shape of base of blade, color of lower side, incisions of margin, leaf hairiness etc., because these leaf traits were very changeable depending upon years, shoots, and sampling points in the shoots.

Chestnut leaves are simple, arranged alternately, dark green in color, and shiny in appearance. The margins of leaf are coarsely toothed. Advances in genetic selection and growing system are likely to affect leaf parameters. The cultivar identification is traditionally based on the observation of morphological characteristics of which expressions are largely influenced by developmental, environmental and cultivation

factors (Pereira-Lorenzo *et al.*, 1996a; Oraguzie *et al.*, 1998). However, there is not enough knowledge related to effects of genetic factors and environmental conditions on stability of leaf parameters in chestnut. On the other hand, determining the morphological and phenological differences amongst the selected chestnut genotypes has to be requested in cultivar registration. Thus, it seemed worthwhile to evaluate leaf characteristics. Accordingly, the objective of this research was to evaluate the leaf characteristics of seven chestnut (*Castanea sativa* Mill.) genotypes and one cultivar grown under the same conditions and also to determine whether leaf morphometric characteristics could be used for differentiation of genotypes.

## MATERIAL AND METHODS

Field studies were carried out in August, 2006-2007 in an orchard (7 x 7 m) established in 1998 located in Fatsa county of Ordu province. This place is located in the North of Turkey (40°58'38''N and 37°36'35''E, 240 m a.s.l.) in the Central Black Sea Region (Figure 1). According to data obtained from Turkish State Meteorological Service (TSMS, 2008), the climate of the area in the experiment years is characterized by annual mean temperature

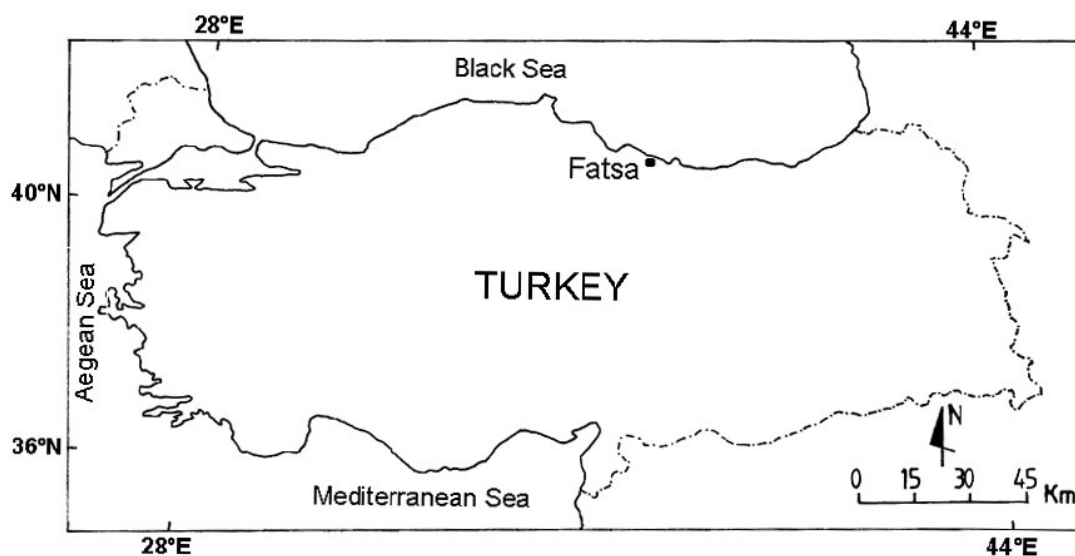
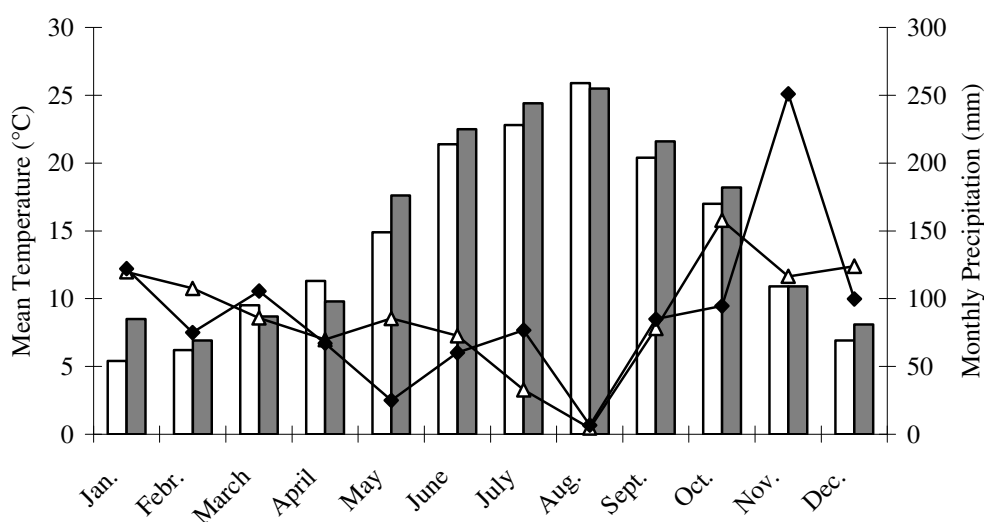


Figure 1. Map showing Fatsa from which samples were obtained.

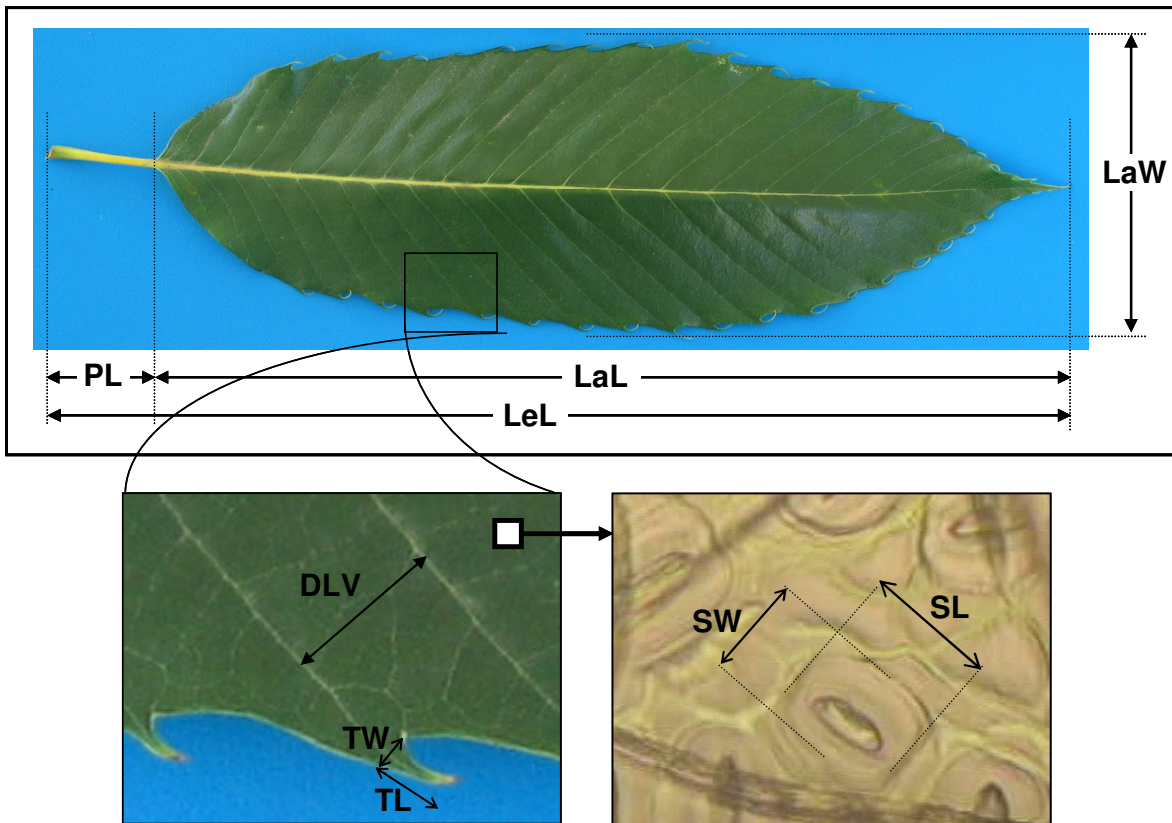
of 14.4-15.2°C, and total rainfall of 1 053.9-1 068.5 mm (Figure 2). The soil was clay loam with 1.14 % organic matter and a pH of 5.75. Trees were 8 years old, grown under same conditions.

In the study, chestnut genotypes with superior fruit characteristics (not merely timber-type) were preferred. For this aim, in the study, the SA5-1, SE 3-12, SE 21-2, SE 21-9, 552-8, 556-7, and 556-8 chestnut (*Castanea sativa* Mill.) genotypes selected from the Black Sea Region (Serdar, 1999; Serdar and Soylu 1999) and Sariaslama as standard cultivar selected from the Marmara Region, Turkey (Ayfer and Soylu, 1995) were used. Ten leaves per tree and three trees per genotype were sampled. Leaf samples were taken from 5<sup>th</sup>-7<sup>th</sup> nodes in well developed lateral shoots at the closing time of stomata, 09.00-11.00 a.m (Sahin and Soylu, 1991). Leaf morphologic parameters were determined in adult leaves from the external part of the crown, which were collected on 9 and 10 August. After leaf sampling, 6 leaf pieces containing the membrane of stomatal surface were taken from each leaf. In this preparation, firstly nail polish was applied to between the lateral veins of lower surface containing stomatas (Sahin and Soylu, 1991).

After drying out for approximately five minutes, leaf pieces (2.0-2.5 cm<sup>2</sup>) containing membrane of stomatal surface taken out with adherent acetate (Bozoglu and Karayel, 2006). After taking of stomatal surface, dimensions of leaf, lamina and teeth, and distance between the lateral veins were measured in the leaf samples, and some ratios of these traits were determined. Stomata count and measurements of dimensions including width and length were done in three regions having an area of 180 mm<sup>2</sup> per sampling using 40x100 or 20 x100 magnifying lenses. In this study, lamina length, lamina width, leaf length, petiole length, teeth width, teeth length, distance between the lateral veins, stomatal density, stomata width, and stomata length were measured (Figure 3) and lamina width/lamina length, lamina width/leaf length, petiole length/lamina length, stomatal index (width/length) and teeth width/teeth length were calculated (UPOV 1989; Pigliucci *et al.*, 1991; Kotobuki, 1996; Oraguzie *et al.*, 1998). Leaf area was determined according to Serdar and Demirsoy (2006). All measurements were repeated in both years (2006 and 2007) in order to determine effects of environmental conditions on studied leaf parameters.



**Figure 2.** Average monthly readings during the experiments. The bars show temperature (□) 2006 and (■) 2007, while the lines show precipitation (△) 2006 and (◆) 2007.



**Figure 3.** Diagrammatic explanation of some of the leaf traits measured. LaW: Lamina width, PL: Petiole length, LaL: Lamina length, LeL: Leaf length, DLV: Distance between the lateral veins, TW: Teeth width, TL: Teeth length, SW: Stomata width, SL: Stomata length. Other traits discussed in the text.

Leaf characteristics of the genotypes, including the effects of year, genotype, and their interaction, were studied by two-factor ANOVA. Therefore, data were analyzed in a randomized block design as factorial arrangement (2 x 8) of treatments. For data on leaf characteristics, individual leaves were considered as experimental unit ( $n = 30$  per genotype). When the F test was significant, differences were determined by Duncan's multiple range tests. All analyses were performed using the SPSS statistical package (SPSS, 1999). Results are presented as means and a pooled SEM.

## RESULTS AND DISCUSSION

The results show that genotype generates more difference in studied leaf parameters than

year, except for stomatal index, and that a significant interaction between genotype and year was observed for the petiole length, teeth width, teeth length, stomatal density and petiole length to lamina length ratio. Leaf parameters such as size and shape are a frequently recorded variable in plant research, as they can be important indicators of variability within and among populations (Aravanopoulos, 2005). Some leaf characteristics related to leaf and stomata size and stomata density is presented in Table 1. Lamina width was the lowest in SE 3-12 and highest in 552-8 and SE 21-9 ( $p < 0.01$ ). The lamina width of 556-7, Sariaslama, 556-8 and SE 21-2 had similar values ( $p < 0.01$ ). The 552-8 genotype had the highest leaf dimensions and distance between the lateral veins. The SE 3-12 and Sariaslama genotypes had the lowest distance between the lateral veins ( $p < 0.01$ ). The leaf length was lowest in 556-7 and SA 5-1 genotypes. The area of leaves from the

**Table 1.** Some leaf characteristics related to leaf and stomata size and stomata density of chestnut genotypes

Genotype	LA (cm <sup>2</sup> )	LaL (cm)	LaW (cm)	LeL (cm)	PL (cm)	TW (mm)	TL (mm)	SD (no. per mm <sup>2</sup> )	SW (µm)	SL (µm)	DLV (mm)
SA 5-1	80.5bc	18.1de	6.03b	20.2d	2.13bc	2.87a	4.10c	395.2c	17.4c	23.9bc	10.00b
SE 3-12	66.9c	21.6b	4.10d	24.4ab	2.83a	1.73c	4.13c	322.1d	17.1c	23.6bc	7.73e
SE 21-2	70.7c	19.5cd	5.10c	21.8cd	2.33bc	1.63cd	3.06d	420.6b	17.6bc	24.0bc	9.10c
SE 21-9	94.4b	21.1b	6.30ab	23.0bc	1.90d	1.23d	2.16e	398.9c	17.4c	23.3c	8.76cd
552-8	112.2a	23.4a	6.77a	25.5a	2.10cd	1.23d	2.20e	431.4b	17.7bc	24.1b	11.56a
556-7	67.8c	17.7e	5.37c	20.6d	2.90a	3.10a	4.96a	321.1d	18.2ab	25.1a	8.16de
556-8	81.6bc	21.3b	5.33c	23.7b	2.36b	1.73c	2.90d	457.3a	17.5c	23.8bc	8.86cd
Sariaslama	79.9c	20.9bc	5.37c	23.2bc	2.26bc	2.36b	4.53b	385.8c	18.7a	25.9a	7.46e
SEM	3.65	0.45	0.18	0.43	0.08	0.17	0.22	12.03	0.20	0.22	0.29
Main effects											
Year	NS	NS	*	NS	**	**	**	**	**	**	NS
Genotype	**	**	**	**	**	**	**	**	**	**	**
Year x Genotype	NS	NS	NS	NS	*	*	**	**	NS	NS	NS

a, b, c: Means within a column lacking a common superscript differ (NS:  $p > 0.05$ , \*,  $p < 0.05$ , \*\*,  $p < 0.01$ ). SEM: Standard error of the mean. LA: Leaf area, LaL: Lamina length, LaW: Lamina width, LeL: Leaf length, PL: Petiole length, TW: Teeth width, TL: Teeth length, SD: Stomatal density, SW: Stomata width, SL: Stomata length, DLV: Distance between the lateral veins.



SE 3-12, 556-7, SE 21-2, Sariaslama, SA 5-1, 556-8, which had similar leaf areas, and SE 21-9 genotype were significantly smaller than those from 552-8 genotype. The leaf area of 552-8 genotype was 40.4 and 28.8% larger than those of the SE 3-12 genotype and Sariaslama cultivar, respectively. Radersma *et al.* (2008) reported that leaf area may be an important determinant of water use in trees. Pigliucci *et al.* (1991) found significant variations among populations of *Castanea sativa* in both the pattern and magnitude of integration of leaf and fruit traits.

Stomata are used for gas exchange, and have important role in the environmental stress, ploidy levels and resistance to drought, disease and insects (Franks and Farquhar, 2007; Fernandez *et al.*, 2008; Gomes-Laranjo *et al.*, 2008; Chen *et al.*, 2009; Lukovic *et al.*, 2009; Mehri *et al.*, 2009). The leaf stomata frequency of the genotypes can be related to adaptation processes of the trees. The 556-8 genotype having the highest stomata density has also higher productivity and more resistance to chestnut blight. Stomata dimensions were the lowest in SE 21-9 and largest in Sariaslama and 556-7 genotypes, which originated from the Marmara Region of Turkey. Sahin and Soyulu (1991) noted that Sariaslama was one of the standard cultivars having big stomatas in this region. In the present study, chestnut genotypes having wide stomatas also had long stomatas. This finding is in consistence with that of Sahin and Soyulu (1991).

Some ratios in relation to dimension of leaf, teeth and stomata of different chestnut genotypes are presented in Table 2. The SA 5-1 genotype had the highest ratios for lamina width/lamina length, lamina width/leaf length and also teeth width/teeth length. SE 3-12 genotype, having the narrowest leaf, had also the lowest values for these ratios. But 552-8 and SE 21-9 genotypes, having the widest leaf, did not have the highest values for these ratios (Table 2).

Table 1 and Table 2 show that there was a strong genetic impact on leaf and stomata size and shape parameters (ratios) except for stomatal index. This result indicates that leaf parameters studied in the present study may be

appropriate variables in order to detect levels of phenotypic variability among genotypes. Furones and Fernandez-Lopez (2005) and Aravanopoulos (2005) stated that leaf traits, such as leaf size and shape may be useful as descriptors in chestnut and may be suitable variables in order to detect levels of phenotypic variability among populations. However, the use of a group of parameters (petiole length, teeth width, teeth length, stomatal density and petiole length/lamina length) for detecting the levels of phenotypic variability among genotypes may not be considered reliable because these parameters can be variable due to the effect of year x genotype interaction. Bozoglu and Karayel (2006) reported that there was not enough evidence for using stomatal density characteristic for cultivar differentiation in pea. Morphological characterization is the official method accepted for registration and protection of new cultivars (Pereira-Lorenzo *et al.*, 1996a). Therefore, these results support the idea that it is necessary to find morphological descriptors that are able to distinguish new chestnut cultivars and to make observations in different years and regions to reduce environmental interactions (Pereira-Lorenzo *et al.*, 1996a; Oraguzie *et al.*, 1998). Although morphological traits depend on environmental conditions, the genetic effects are expected to be more important than the environmental ones (Pereira-Lorenzo *et al.*, 1996a,b) as was observed in the present study. Goulao *et al.* (2001) reported that although significant differences among chestnut trees were observed for some traits, the intercultural differences were higher than intracultural variations, which is in agreement with the findings of our experiment. Some leaf parameters (lamina length, leaf length, leaf area and distance between the lateral veins) did not vary between years although year had a significant impact on other leaf parameters (Table 1 and Table 2). There were significant effects of year x genotype interactions on the petiole length, teeth width, teeth length, and stomatal density (Table 1). Leaf dimensions can be slightly changeable according to

**Table 2.** Some ratios in relation to dimensions of leaf, teeth and stomata of chestnut genotypes.

Genotype	LaW/LaL	LaW/LeL	PtL/LaL	SW/SL	TW/TL
SA 5-1	0.33a	0.30a	0.12c	0.73	0.69a
SE 3-12	0.19d	0.17d	0.13b	0.73	0.42e
SE 21-2	0.26c	0.23c	0.12c	0.73	0.52cd
SE 21-9	0.30b	0.27b	0.09d	0.75	0.56bcd
552-8	0.29b	0.27b	0.09d	0.74	0.55bcd
556-7	0.30b	0.26b	0.17a	0.72	0.62ab
556-8	0.25c	0.23c	0.11c	0.73	0.59bc
Sariaslama	0.25c	0.23c	0.11c	0.72	0.51d
SEM	0.009	0.008	0.002	0.052	0.020
Main effects					
Year	**	**	**	**	**
Genotype	**	**	**	NS	**
Year x Genotype	NS	NS	**	NS	NS

a, b, c: Means within a column lacking a common superscript differ (NS:  $p>0.05$ , \*:  $p<0.05$ , \*\*:  $p<0.01$ , \*\*\*:  $p<0.001$ ). SEM: Standard error of the mean. LaW/LaL: Lamina width/lamina length, LaW/LeL: Lamina width/leaf length, PtL/LaL: Petiole length/lamina length, SW/SL: Stomatal index, TW/TL: Teeth width/teeth length.



environmental conditions and cultural practices. However, the ratios obtained from leaf dimensions may be more stable. There were no significant effects of year x genotype interaction on the ratios obtained from leaf dimensions except for the petiole length/lamina length (Table 2). Responses of plant populations to environmental changes clearly depend upon the interaction between individual phenotypic plasticity and genetic variation (Nicotra *et al.*, 1997). The significant effects of year or year x genotype interaction may be related to the climatic conditions, including homogeneous irradiation during the daylight in each year and/or with the developmental age (Mediavilla and Escudero, 2003; Covone and Gratani, 2006).

Four of chestnut genotypes (SE 21-2, SE 3-12, SE 21-9 and 552-8) used in the present study were registered by Turkey Variety Registration and Seed Certification Centre and were named as Ersinop, Unal, Erfelek and Eryayla, respectively in 2009. This study was the first effort to evaluate the leaf characteristics of four novel chestnut cultivars.

### CONCLUSIONS

It is concluded that leaf parameters may be suitable variables in order to detect levels of phenotypic variability among chestnut (*Castanea sativa* Mill.) genotypes. In the present study, the traits of lamina width, lamina length, leaf length, distance between the lateral veins, leaf area, stomata width, stomata length and the ratios of teeth width/teeth length, lamina width/lamina length and lamina width/leaf length were determined as more reliable characteristics for discrimination of the chestnut genotypes. In this study, most of the chestnut genotypes were easily distinguished by morphometric leaf traits. However, discrimination of some genotypes such as SE 21-2 and 556-8 was difficult with these leaf parameters. Hence, other morphological traits for catkins, nuts, burs and buds should be considered. On the other hand, genetic diversity among these

chestnut genotypes should be investigated by using molecular markers.

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## برخی مشخصات برگ، ممیزه بهتری در اندازه گیری های ریخت شناسی ژنوتیپ های شاه بلوط می باشند

او. سردار و ن. کورت

### چکیده

این بررسی به منظور ارزیابی مشخصات برگ در تعدادی از ژنوتیپ های شاه بلوط که در اندازه گیری ریخت شناسی برگ جهت تشخیص ژنوتیپ ها می تواند کاربرد داشته باشد در منطقه مرکزی دریای سیاه در ترکیه اجرا شد. در این بررسی هفت ژنوتیپ شاه بلوط (*Castanea sativa* Mill.) شامل ژنوتیپ های (Sariaslama) مورد استفاده قرار گرفت. برخی مشخصات برگ مانند طول پهنک، عرض پهنک، طول برگ، سطح برگ، طول دم برگ، عرض دندان های برگ، طول دندان های برگ، تراکم روزنه ها، عرض روزنه ها، طول روزنه ها، عرض پهنک به طول پهنک، عرض پهنک به طول برگ، طول دم برگ به طول پهنک، ضریب روزنه ای، فاصله بین رگبرگ های جانبی و عرض دندان به طول دندان اندازه گیری شدند. بر این اساس برخی از ژنوتیپ های شاه بلوط را می توان به سهولت با استفاده از ویژگی های مربوط به اندازه گیری ریخت شناسی برگ مشخص نمود. در این میان ویژگی های پهنک برگ، طول پهنک برگ، طول برگ، فاصله بین رگبرگ های جانبی، سطح برگ، عرض روزنه، طول روزنه و نسبت عرض دندان برگ به طول دندان، عرض پهنک به طول پهنک و عرض پهنک برگ به طول برگ به عنوان بهترین ممیزه در تفکیک ژنوتیپ های شاه بلوط تشخیص داده شدند.