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Fruit and seed diversity of *Smilax excelsa* in the Black Sea Region, Turkey

Türkiye'nin Karadeniz Bölgesi'ndeki *Smilax excelsa*'nın meyve ve tohum çeşitliliği

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Abstract

Smilax excelsa L., a member of the Smilacaceae family, can be used as a non-wood forest product because it is a medicinal and aromatic plant. In this study, fruits obtained from six different regions (Araklı-Arsin-Sarıca populations in Trabzon province, Yakakent population in Samsun province, Erfelek population in Sinop province, Amasra population in Bartın province) were used. After measuring the fruit length and width values on the collected fruits, the fruit fleshes were removed. Then, on the obtained seeds, the seed length, seed width, and 1000 seed weight values were determined. As a result of the study, Trabzon-Sarıca population had the highest value (117.55 g) in terms of 1000 seed weight, while the Samsun-Yakakent population had the highest values in terms of all measurements made on fruits and seeds (fruit length and width, seed length and width). In addition, statistical differences were determined depending on all characteristics measured and it was found that three different groups of populations were formed as a result of hierarchical cluster analysis. These differences show that there are morphogenetic variations within the natural distribution areas of the species. Detailed consideration of the chemical contents of Samsun-Yakakent and Trabzon-Sarıca populations can be suggested because of the high values in terms of fruit and seed dimensions for the use of these species as non-wood forest products.

Keywords: *Smilax excelsa*, non-wood forest product, morphological characteristics, variation

Öz

Smilacaceae familyasının bir üyesi olan *Smilax excelsa* L. türü, tıbbi ve aromatik bir bitki olduğundan odun dışı orman ürünü olarak kullanılabilmektedir. Bu çalışma kapsamında altı farklı bölgeden (Trabzon/Araklı-Arsin-Sarıca popülasyonları, Samsun/Yakakent popülasyonu, Sinop/Erfelek popülasyonu, Bartın/Amasra popülasyonu) elde edilen meyveler kullanılmıştır. Toplanan meyveler üzerinde meyve boyu ve eni değerleri ölçüldükten sonra meyve etleri uzaklaştırılmıştır. Elde edilen tohumlar üzerinde de tohum boyu, tohum eni ve 1.000 tane ağırlığı değerleri tespit edilmiştir. Çalışma sonucunda, 1.000 tane ağırlığı bakımından Trabzon-Sarıca popülasyonu en yüksek (117.55 g) değere sahip olurken meyve ve tohumlar üzerinde yapılan tüm ölçümler açısından (meyve boyu ve eni, tohum boyu ve eni) Samsun-Yakakent popülasyonu en yüksek değerlere sahip olmuştur. Ayrıca, ölçülen tüm karakterlere bağlı olarak istatistiki farklar belirlenmiş ve hiyerarşik kümeleme analizi sonucunda popülasyonlara ilişkin üç farklı grup meydana geldiği tespit edilmiştir. Bu farklılıklar türün doğal yayılış alanı içerisinde morfogenetik varyasyonların olduğunu ortaya koymaktadır. Bu türün odun dışı orman ürünü olarak kullanılması için meyve ve tohum boyutları bakımından yüksek değerlere sahip olması nedeniyle Samsun-Yakakent ve Trabzon-Sarıca popülasyonlarının kimyasal içeriklerinin detaylı olarak ele alınması önerilebilir.

Anahtar Kelimeler: *Smilax excelsa*, odun dışı orman ürünü, morfolojik karakterler, varyasyon

1. Introduction

For the human being, the usage about plant traditional medicines is based on a knowledge passed orally from generation to generation, and there are only a few written documents (Neves et al., 2009). People have gathered plant and animal resources for their needs since ancient times. Edible nuts, mushrooms, fruits, herbs, spices, gums, game, fodder; fibers used for the construction of shelter and housing, clothing or utensils; plant or animal products for medicinal, cosmetic or cultural uses can be given as examples. Even today, millions of people mostly in developing countries meet their needs from these plants and animal products (Iqbal, 1993; Walter, 2001). Medicinal plants, containing inherent active ingredients tending or used to cure disease or relieve pain, and aromatic plants, having strong characteristic smell or fragrance (King, 1992), are produced and offered on a large scale of products, from crude materials to processed and packaged products such as pharmaceuticals, herbal remedies, teas, spirits, cosmetics, sweets, dietary supplements, varnishes and insecticides (Ohrmann, 1991; Lange, 1996; Gorecki, 2002). In studies on the importance of the subject, Rao et al. (2004) reported that medicinal and aromatic plants (MAPs) play an important role in the healthcare of people around the world, especially in developing countries. Balandrin and Klocke (1988) also stated that plant-derived products will be extremely important for mankind as sources of drugs, foods, pesticides, and other raw materials in the future as in the past.

The Smilacaceae family, including two genera to be *Smilax* L. and *Heterosmilax* Kunth., is one of the most abundant and diverse climbing plants in many ecosystems of the Old and New Worlds (Gentry, 1991). *Smilax* is the core genus of the family, containing approximately 200 species (Cameron and Fu, 2006). This genus, native to tropical and temperate parts of the world, is generally characterized as dioecious, climbing, woody vines with prickles, alternate leaves exhibiting reticulate venation, paired stipular tendrils, and fleshy berries (Koyama, 1984; Baytop, 1999; Chen and Koyama, 2000; Özsoy et al., 2008; Özsoy et al., 2013). It spreads in Bulgaria, Greece, Transcaucasia and northern Iran, north, southwest, and south of Turkey, and also grows up to 1000 m elevations above sea level in coniferous and deciduous forests and shrubs, in flood areas and valleys near the water. *Smilax excelsa* is one of the characteristic plants of Black Sea region distributed in Northern Anatolia, Thrace and the Mediterranean Sea coast including Artvin, Trabzon, Samsun, Sinop, Zonguldak, Bolu, İstanbul, Tekirdağ, Aydın, Muğla, Antalya and Hatay provinces (Baytop, 1984; Davis, 1984; Güner et al., 2012; Eminağaoğlu and Aksu, 2018).

Smilax species are used to treat some diseases such as syphilis, acute bacillary dysentery, acute and chronic nephritis, eczema, dermatitis, cystitis, and mercury and silver poisoning (Ivanova et al., 2010). It is well-known that *Smilax* rhizomes have various pharmacological activities (Ban et al., 2006) including immunomodulatory (Jiang and Xu, 2003), antibacterial, antifungal, antioxidant, antitumor, antimutagenic, antiinflammatory (Navarro et al., 2003; Azap et al., 2017) and hepatoprotective (Chen et al., 1999) ones. On the other hand, the plant shoots are consumed as vegetables and other parts such as leaves (Baytop, 1999) have been used in folk medicine (Baytop, 1984; Asımgil, 2003). Leaves and fruits of *Smilax excelsa* include natural antioxidants and are also rich in unsaturated fatty acids (Yıldız et al., 2018). In this study, it is aimed to determine some morphological variations related to fruits and seeds obtained from different regions for *Smilax excelsa*, which is important in many aspects.

2. Material and Method

Fruits of *Smilax excelsa* used in the conduct of this study were collected from natural distribution areas of the species. For this aim, Araklı-Arsin-Sarıca populations in Trabzon province, Yakakent population in Samsun province, Erfelek population in Sinop province and Amasra population in Bartın province were selected. Information about these populations are given in Table 1, and distributions are shown in Figure 1.

The study material was obtained in October. Fruit length (FL), fruit width (FW) and fruit length to width ratio (FL/FW) were measured on the fruits. Measurements were made using 3x50 fruits from each population. After that, the fruit flesh was removed and the seeds were obtained. About the seeds, seed length (SL), seed width (SW) and seed length to width ratio (SL/SW) were determined on 3x50 seeds from each population. Measurements were performed using digital caliper in millimeter sensitivity. 1000 seed weight (1000 SW) was also determined in the study. For this purpose, weights of 800 seeds (8x100) were weighed with precision balance, and 1000 SW were calculated based on ISTA rules (Anon., 1993). 1000 seed weight is calculated with the formula stated below.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} = \frac{8 \times 100}{n}$$

$$1000\ SW = 10 \times \bar{x}$$

Using SPSS 23 statistical program, variations among populations related to morphological characteristics (FL, FW, FL/FW, SL, SW, SL/SW) were analyzed with variance analysis (One Way ANOVA) and Duncan's test (to determine occurring groups). Correlation between pairs of morphological characteristics was evaluated using Pearson's correlation coefficient. The overall variations of morphological characteristics among populations was also examined using hierarchical cluster analysis (Özdamar, 1999; Özkan, 2003).

Table 1. Coordinates and altitudes of the populations
Tablo 1. Popülasyonlara ilişkin koordinat ve yükseltiler

No	Population	Latitude	Longitude	Altitude (m)
1	Trabzon-Araklı	40° 49' 36''	39° 58' 13''	700
2	Trabzon-Arsin	40° 52' 46''	39° 53' 39''	670
3	Trabzon-Sarıca	41° 03' 38''	39° 26' 01''	200
4	Samsun-Yakakent	41° 39' 33''	35° 26' 39''	50
5	Sinop-Erfelek	41° 54' 13''	34° 50' 08''	285
6	Bartın-Amasra	41° 45' 04''	32° 30' 13''	345

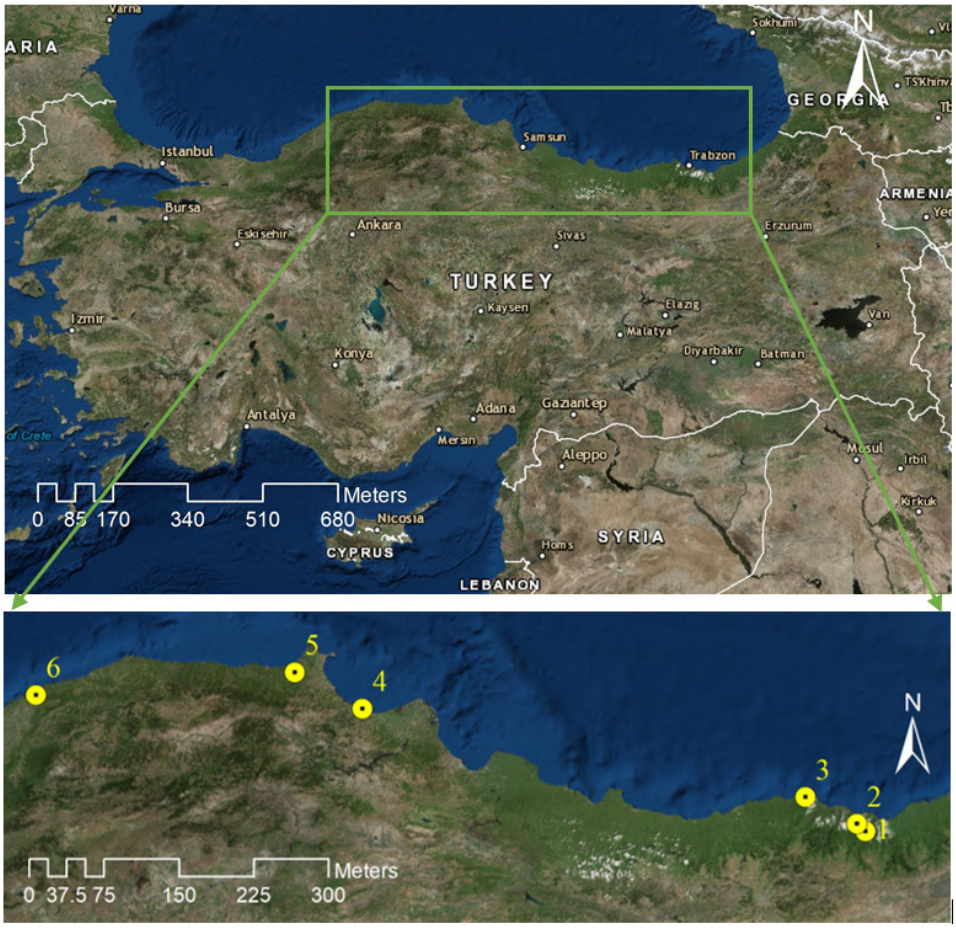

Figure 1. Distributions of the populations
Şekil 1. Popülasyonların harita üzerindeki dağılımları

3. Results

The mean values of 1000 seed weight related to the analyzed *Smilax excelsa* populations are shown in Figure 2. While the highest value for 1000 seed weight was determined in Sarıca population (117.55 g), the lowest value was in Yakakent population (50.7 g).

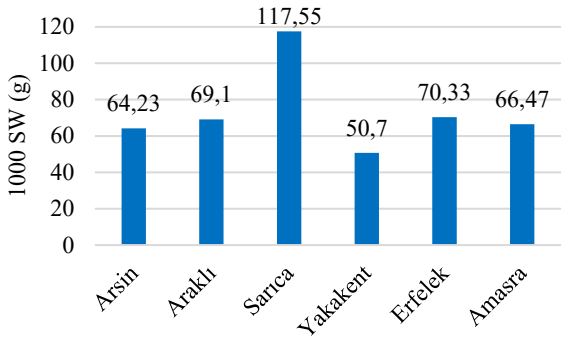

Figure 2. The mean values of 1000 seed weight
Şekil 2. Ortalama 1000 tane ağırlığı değerleri

The values of mean, standard deviation, maximum and minimum related to the fruit and seed sizes are given in Figure 3.

The highest values of all measured characteristics (FL, FW, SL and SW) were obtained in Yakakent population. Additionally, the highest ratio of FL/FW and SL/SW were recorded in Arsin population. The fruit length in all populations ranged from 8.16 to 9.21 mm, with a mean value of 8.81 mm. The values of fruit width ranged from 8.47 to 9.78 mm, and the mean length was 9.25 mm in all analyzed populations. The seed length ranged from 3.50 to 6.49 mm, and the mean value of seed length was 5.03 mm. The seed width varied between 3.53 and 6.85 for all populations, with a mean value of 5.30 mm. The averages of fruit length/width and seed length/width ratio were detected to vary between 0.92-1.02 mm and 0.95-1.04 mm, respectively.

Table 2. The results of variance analysis about morphological characteristics
Tablo 2. Morfolojik özelliklere ilişkin varyans analizi sonuçları

	FL	FW	SL	SW	FL/FW	SL/SW
F	8.268	13.204	137.532	258.691	5.503	4.665
P	0.000*	0.000*	0.000*	0.000*	0.000*	0.001*

* $P<0.01$ (There is a statistically significant difference.)

The ANOVA analysis revealed that there are statistically significant differences ($P<0.01$) among the analyzed populations in terms of all measured morphological characteristics (Table 2). Besides, when Duncan's test was performed, there were four groups in terms of FL and FL/FW ratio, and two groups in terms of FW among the populations. On the other hand, it occurred six, five and three groups in terms of SL, SW and SL/SW ratio, respectively, among the populations. The coefficients of variation for the studied characteristics ranged from 2.23% to 17.81% (Figure 4).

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

The hierarchical cluster analysis was conducted to determine the degree of similarity or dissimilarity among populations concerning the morphological characteristics of the fruits and seeds. Regarding Figure 5, two main groups were formed. The first group was Araklı, Arsin, Sarıca and Erfelek populations. The second one was Yakakent and Amasra populations.

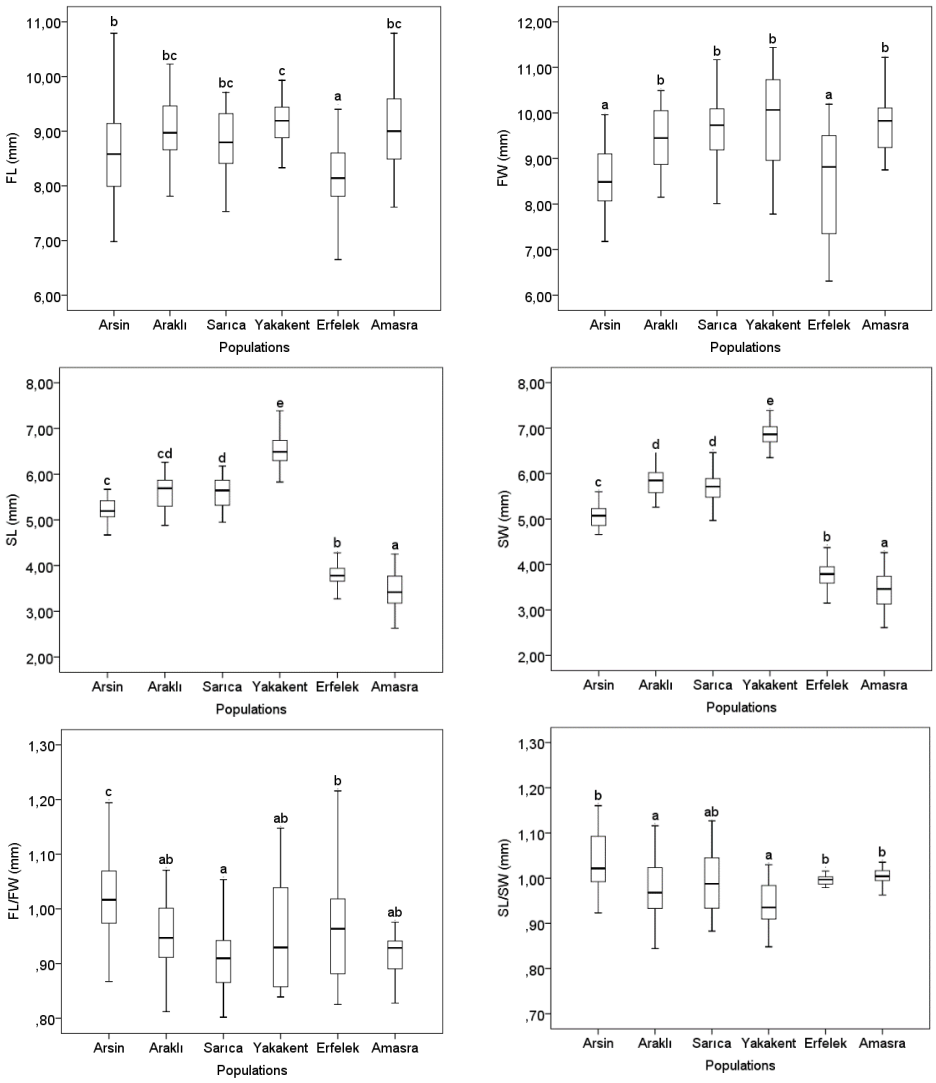

Figure 3. Mean values and Duncan test results about fruit and seed characteristics
Şekil 3. Meyve ve tohumlara ilişkin ortalama değerler ve Duncan testi sonuçları

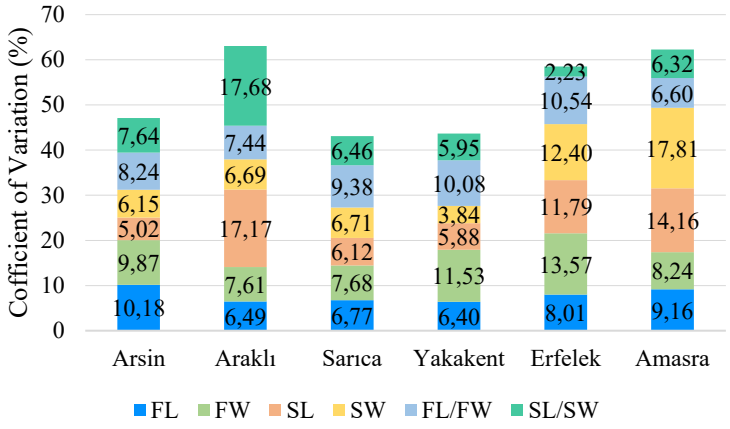

Figure 4. Coefficients of variation (%) of fruit and seed characteristics
Şekil 4. Meyve ve tohum karakterlerinin varyasyon katsayıları

Table 3. Pearson correlation coefficients between pairs of morphological characteristics
Tablo 3. Morfolojik karakterlerin ikili karşılaştırmaları arasındaki Pearson korelasyon katsayıları

	FL	FW	SL	SW	FL/FW	SL/SW
FL	1	0.633**	0.197**	0.262**	0.131	-0.161*
FW		1	0.107	0.197**	-0,680**	-0,203**
SL			1	0.892**	0.060	0.121
SW				1	0.010	-0,337**
FL/FW					1	0.098
SL/SW						1

**Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level

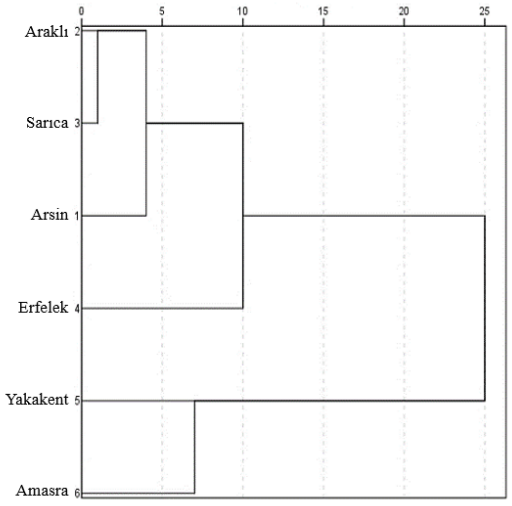

Figure 5. Dendrogram about hierarchical cluster analysis
Şekil 5. Hiyerarşik kümeleme analizine ilişkin grafik

4. Discussion

In this study which was conducted on the fruit and seed sizes taken from different regions in the natural distribution area of *Smilax excelsa* L., the highest results related to the morphological characteristics were obtained from the Yakakent population. But, the highest 1000 seed weight was determined as 117.55 g in Sarıca population. Herrera (1981) reported that the berries of *Smilax aspera* may contain one, two or three seeds, and one-seed fruits have the largest and three-seed the smallest, pulp/seed ratio. Compared to the other populations, the reason for the high 1000 seed weight value in Sarıca population can be explained by the number of seeds in a fruit. According to this study, although there were two seeds in each fruit in Sarıca region, there were two-three seeds in each fruit in other regions.

As can be seen from Table 3, a positive correlation was determined between the measured seed and fruit characters in terms of Pearson correlation coefficients. Similar results have been obtained in the studies conducted on the species having the fleshy fruit such as *Cornus mas* L. (Hassanpour et al., 2012; Mratinić et al., 2015), *Celtis australis* L. (Ammari et al., 2016) and *Myrtus communis* L. (Melito et al., 2016).

Yıldız et al. (2018) reported that the highest 100 seed weight was determined as 4.14 g in Iskenderun/Hatay location and the lowest value was obtained as 3.91 g in Defne/Hatay location for *Smilax excelsa*. On the other hand, they also stated that the highest 100 seed weight occurred as 6.12 g in Yayladagi 1/Hatay and the lowest was in Yayladagi 2/Hatay as 5.70 g for *Smilax aspera*. In another study, 100 seed weight for *S. aspera* was 6.00 g (Özgül-Yücel, 2005). Compared to the previous studies conducted, Sarıca population in the present study has a higher value in terms of 1000 seed weight. The morphological characteristics of this population were generally very close to Yakakent population. For further studies of the species, Trabzon-Sarıca populations can be good research and interest areas. As the population of Samsun-Yakakent has high values for fruit characteristics, having an important place of the fruit especially in terms of the species' usage areas highlights this population.

In other respects, in a study made to determine morphological, phytochemical and cytological variations *Smilax* species of Java, it was found that there were variations in morphological characters, phenolic and saponin spots numbers and chromosome length, but not in chromosome number (Ungson and Sastrapradja, 1976). Seed and fruit characteristics measured in *Smilax excelsa* show significant differences in different populations in this study. Geographic variations, forming the basis of studies on variation, can be determined according to the phenotypic characteristics, regardless of the hereditary differences that require long-term studies.

Geographic variation consists of hereditary and the common effects of external factors including physical and biotic factors, and their interrelationships (Ürgenç, 1982; Şimşek, 1993). As a result of this data, it can be clearly understood that the species has a genetic variation in its natural distribution areas.

In addition, as a result of the observations, *Smilax excelsa* can be evaluated in many usage areas including a non-wood forest product, border plant as climbing shrub and visual effect thanks to the color of its leaves and fruits.

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