



A Homonym Determination of *Olea europaea* L. by using Molecular Identification and Image Analysis

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ABSTRACT

Background: The study was conducted at the various locations in Antalya province, where the Tavşan Yüreği olive variety is registered as the Tavşan Yüreği olive variety, which is spreading especially in Kepez and Manavgat districts.

Methods: The objective of this study was to determine a homonym of *Olea europaea* var. Tavşan Yüreği by using molecular identification and image analysis. Based on molecular analyses and morphological measurements made on collected leaf and fruit samples collected, the morphological identification method is used based on imaging techniques from high-resolution photos.

Result: The results showed that, according to the molecular definition and image analysis measurements, the source of the difference between all locations, including the BATEM sample, may be genotype or phenotypic diversity within the variety.

Key words: Homonym, Image analysis, Molecular identification, Olive, Tavşan Yüreği.

INTRODUCTION

The homeland of the olive (*Olea europaea* L.), a member of the Oleaceae family with a lifespan of 3,000 years, is Upper Mesopotamia and South Pre-Asia, including the Southeastern Anatolia Region.

Many olive (*Olea europaea* L.) varieties grown in Anatolia have not been characterized (morphologic and biologic characteristics), but some have been identified by morphologic and biological characteristics, but still there are some variations due to unknown genotypic or phenotypic causes (Özkaya, 2004). Its spread has been in three ways. The first is to Tunisia and Morocco via Egypt, the second to the Aegean islands through Anatolia, Greece, Italy and Spain, the third to Pakistan and China via Iran. Archaeological studies show that olive cultivation dates to 4000 BC. The first detailed study on the determination of olive gene sources was carried out in the 1960s with the support of FAO. As a result of this long-term study, the varieties and types selected by mass selection from olive tree growing countries were both put in collections in their own country collection and some of them were placed in the World Olive Collection in Spain. Italian Researcher Giorgio Bartolini founded the FAO-Olive Gene Resources Knowledge Base in 1997, where he collected information on 1,200 olive varieties and 1,250 studies on them. Although he has turned it into a book and he has presented it in a format that can be corrected on his website (Anonymous, 2021a).

Many studies have been carried out to determine the geographical and variety-derived variations of olive varieties. Although it was stated that there are close to 500 varieties as an olive gene source in Italy, the importance of determining the characteristics of these varieties and preserving them has been emphasized. According to studies of Bari *et al.* (2003) and Al Ibrahim *et al.* (2008) olive stone is one of the most important criteria for variety identification

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and they identified a total of 19 varieties using the g pattern analysis method and classical identifiers, they stated that the use of olive stones as a descriptor was 60% successful in finding variations of the varieties. They also stated that the purpose of their study was to create and publish a gene bank for the Palmyra oasis variety and other varieties. Bari *et al.* (2003) emphasized the morphological descriptive properties of olive stone in their studies and stated that these properties are an auxiliary element in determining olive variety. They stated that an application technique based on visual experiences is used in the identification of olive varied, but the success rate varies depending on the expertise of the person who makes the identification. At the same time, they found that there was a visual limit on the stone structure of the eye and the shapes with spherical, elliptical and soft lines on this structure. In their studies, they aimed to classify olive varied using mathematical tools and image processing techniques of the geometries that make up olive stone characteristics (Fig 1). They stated that 90% accuracy estimates can be made using the Mahalanobis distance.

They also made genetic identifications in some trees, comparing the results with the results found from the image analysis method and emphasizing that there was no significant statistical difference between them. Therefore the research is aimed to determine the homonyms of Tavşan Yüreği olive variety by using the morphological parameters with the help of image analysis measurements.

MATERIALS AND METHODS

Due to a variety of factors such as incorrect labeling, synonyms and local naming, the phylogenetic relationships of Turkish varieties and these various forms have not been fully elucidated. Until the 1990s, varieties were identified and separated based on their morphological characteristics. With the advancement of technology and the use of biotechnological methods in agriculture, this problem has begun to be addressed to some extent. Many studies have been carried out around the world and in our country to identify cultivars and determine the relationships between cultivars using molecular markers (Topaklı and Hepaksoy, 2019). In the light of this information, olive fruits and leaves were collected from seven orchards for molecular and morphological analysis. The orchards were selected according to shape of fruits.

Molecular analysis

In the study, we used 7 olive orchards or groves of Tavşan

Yüreği in Antalya. Leaf samples of selected 7 olive genotypes were included in SSR analysis. DNA extraction. Genomic DNA was extracted from young leaf tissue using the DNA Purification Kit (Promega, Madison, WI) according to the instructions provided by the manufacturer. Subsequently, aRNase treatment was performed on the eluted DNA samples. The purity and concentration of the DNA were checked both on 1% (w/v) agarose gels and by NanoDrop®ND-1000.

Seven widely used SSR loci (DCA9, UDO26, DCA13, DCA11, DCA15, DCA18, UDO12, UDO24, UDO4, UDO9) were used in Polymerase Chain Reaction (PCR) studies. PCR was conducted in a volume of 10 µL and contained 15 ng genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA polymerase (Promega), 1.5 mM MgCl₂ and 2 µL 5X buffer. The forward primers were labeled with Well RED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). Reactions without DNA were included as negative controls. PCR amplification was performed by using the Biometra®PCR System. The amplification conditions consisted of an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 52-56°C and 2 min at 72°C with a final extension at 72°C for 10 min. The PCR products were first separated on a 3% (w/v) agarose gel run at 80 V for 2 h. The gel was then stained with ethidium bromide at a concentration of 10 mg/mL. A DNA ladder (100 bp) (Promega) was used for the approximate quantification of the bands. The results were then converted to a similarity matrix and a dendrogram was constructed with the UPGMA method using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System).

RESULTS AND DISCUSSION

Registration procedures for the Tavşan Yüreği olive variety were made in 1990 by the Directorate of The Republic of Turkey Ministry of Agriculture and Forestry Batı Akdeniz Agricultural Research Institute (BATEM) in Antalya (Table 1).

The results were evaluated from leaf and fruit samples collected from experience gardens that placed in different regions where Tavşan Yüreği was grown in Antalya were evaluated (Table 2). On the other hand, measurements and

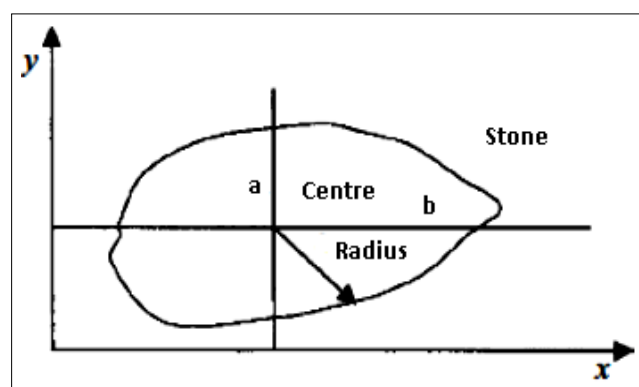


Fig 1: Geometric parameters used to define the stone (Bari *et al* 2003).

Table 1: Information about the registration of the Tavşan Yüreği olive variety by BATEM (Anonymous, 2021b).

REPUBLIC OF TURKEY						
MINISTRY OF FOOD, AGRICULTURE AND LIVESTOCK						
GENERAL DIRECTORATE OF PLANT PRODUCTION						
Variety Registration and Seed Certification Center						
(REGISTERED VARIETIES)						
This bulletin was issued following Article 39 of the "Regulation on the Registration of Plant Varieties" published in the Official Gazette dated 08.11.2006- dated 26340 and prepared based on this law with the "Seed Law" no. 5553 and published in the Official Gazette dated January 13, 2008 and numbered 26755.						
No	Variety name	Identification of variety	Maintainer	Registration date	Variety name	Variety name in Latin
645	Tavşan Yüreği		BATEM	3.05.1990	OLIVE	<i>Olea europaea</i> L.

analysis were only made in the leaf samples collected from BATEM. The olive fruit samples were collected and re-analyzed by the method of BATEM. We have focused on the differences in the shape of the olive fruits of Tavşan Yüreği collected from 7 locations. Since the olive cultivars located in National Olive Collections located in İzmir-Kemalpasa belong to Olive Research Institute. It is determined that there are differences between fruits and stones, we can say that there is Homonym Tavşan Yüreği. It means that two different cultivars but under the same name.

As a result of the molecular identification of the leaves (Fig 2), it was observed that the Tavşan Yüreği samples (number 4) collected from the Manavgat Gundogdu region differed in terms of the primary ones examined. In the present study, we used 10 highly polymorphic simple sequence repeat (SSR) primer pairs for DNA fingerprinting of 7 Tavşan Yüreği orchards or groves. According to molecular analysis, BATEM and Vakıflar with Kepez Kirişçiler have very closed more than 95%.

This was also detected in morphological measurements of fruits, leaves and stones (Table 3). Accordingly, the largest

Table 2: Coordinates of Tavşan Yüreği variety samples collected from different locations of Antalya.

Genotype	Sample	Latitude	Longitude	Altitude
4	Manavgat Gündoğdu	36.8939°N	031.3039°E	143 m
13	Kepez Kirişçiler	37.0449°N	030.7043°E	288 m
15	Kepez Kirişçiler Çil Tavşan Yüreği Yuvarlak	37.0519°N	030.7102°E	302 m
16	Kepez Kirişçiler	37.0448°N	030.7044°E	288 m
17	Vakıflar	36.9263°N	030.6508°E	90 m
18	Vakıflar	36.9250°N	030.6537°E	87 m
19	BATEM	36.8762°N	030.7216°E	33 m

Table 3: Morphological measurements of Tavşan Yüreği variety samples collected from different locations of Antalya in stones, fruits and leaves.

Genotype	Location	Fruit				Leaf			Stone	
		100 Weights (gr)	Hight (mm)	Radius 1 (mm)	Radius 2 (mm)	Width (mm)	Height (mm)	Height (mm)	Radius 1 (mm)	Radius 2 (mm)
4	Manavgat Gündoğdu	483.60	23.62	19.12	19.32	8.19	51.68	13.74	8.06	8.13
13	Kepez Kirişçiler	489.70	22.51	18.70	18.67	8.80	63.96	15.18	10.21	9.17
15	Kepez Kirişçiler Çil Tavşan Yüreği Yuvarlak	270.00	18.61	15.03	15.08	9.53	68.70	14.40	9.42	9.19
16	Kepez Kirişçiler	370.70	21.04	16.61	16.29	9.21	60.90	16.98	10.33	10.21
17	Vakıflar	322.90	22.68	18.76	18.58	8.52	61.79	14.72	9.85	9.36
18	Vakıflar					8.16	58.80			
19	Batem					8.49	68.73			

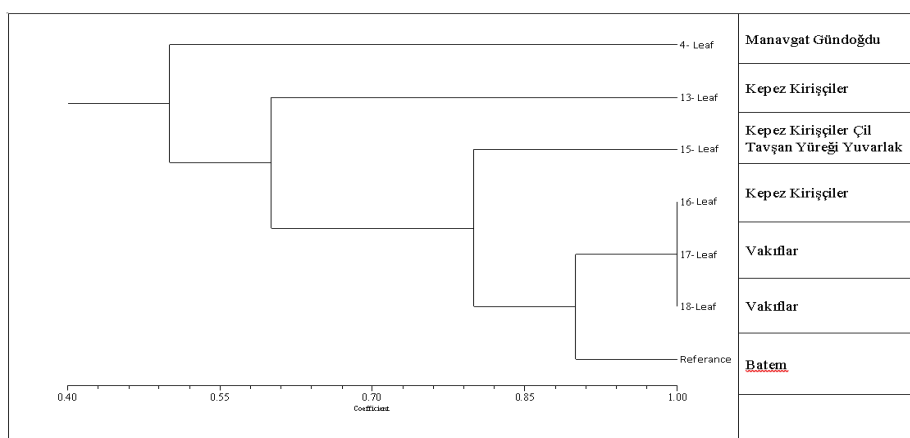


Fig 2: Dendrogram showing genetic kinship relationship in leaf samples of Tavşan Yüreği variety collected from different regions of Antalya.

fruit (based on the width and height measurements), the smallest stone (based on the width and height measurements) were obtained in the genotype selected from Manavgat Gundogdu. Because of Homonym, there is one name, but different because of the shape of fruit, stone and leaves.

However, this difference occurred both from number 4 and in the samples collected from the Kepez Beamers region. According to the molecular identification results, it was determined that there was a low similarity between 60% and 80% in terms of the primary examined in samples 13, 15 and 16. In morphological measurements, number 13 is at the forefront of the weight of 100 fruits, but number 15 is found to have the least and smallest fruit (based on the width and height size measurements) in terms of the weight of 100 fruits. Fruit number 16 was determined to have the largest stone (based on the width and height size measurements). Although the size of fruit and stone is different because of homonym.

When the photos of fruits, leaves and stones measured in morphological properties (Table 4) are examined, it can be observed that there is a significant difference in the stone structure of genotype number 4. The lines are in a deeper state in the stone, which is heart-shaped but has a pointed tip. In genotypes 13, 15 and 16, it is seen that the stones are heart-shaped, but the tip is not pointed. It is observed that there is some difference in fruit shape between genotype number 4 and others. In terms of leaf shapes, there is no significant visual difference. Since there is some variation between locations, we can accept that "Antalya Tavşan Yüreği" is a candidate as geographical indication. Table 4 also shows that the stone of the Tavsan Yüreği olive variety in the National Collection Garden has an elliptical pointed structure, whereas the stone of the 'Antalya Tavsan Yüreği' olive variety is heart-shaped.

The fact that the Tavşan Yüreği trees that were registered and applied by BATEM in 1990 were more similar to genotypes 16 from Kepez region, genotypes 17 and 18 from the Foundation's land in terms of the primers examined

in molecular identification indicates that there will be no morphological difference (Fig 2). In the literature, Ercisli *et al.* (2012) stress that similarity matrix indicated that the 'Ayvalik' and 'Tavşan Yüreği' were the most distant cultivars among Turkish samples with 0.45 similarity ratio. Ozkaya *et al.* (2009)



Fig 3: Fruits, stones and leaves of Tavşan Yüreği in the book of Canözner (1991).



















Fig 4: Fruits, stones and leaves of Tavşan Yüreği in the book of Özlübay (2011).



Fig 5: Mobile olive oil factory truck (Anonymous, 2021c).

Table 4: Seeds, fruits and leaves of Tavşan Yüreği samples were collected from different locations in Antalya.

No	Variety	Location	Stone	Fruit	Leaf (front)	Leaf (rear)
4-Leaf	Tavşan Yüreği	Manavgat Gündoşdu				
13-Leaf	Tavşan Yüreği	Kepez Kirişçiler				
15-Leaf	Çil Tavşan Yüreği Yuvarlak	Kepez Kirişçiler				
16-Leaf	Tavşan Yüreği	Kepez Kirişçiler				

imply that the comparison were done with 'Memecik' and 'Tavşan Yüreği' cultivars which are important olive oil and table olive cultivars, respectively. Since 'Memecik' and 'Tavşan Yüreği' were 100% similar. Yegenoglu *et al.* (2017) told that there was a moderate correlation between pairwise distances estimated from ISSR data and distances from morphological characters (0.511). The euclidean distance matrix represented that the lowest value was between Tavşan Yüreği and Çilli (1.62), while the highest value was between Manzanilla and Cekiste (7.91).

However, in every 2 catalogs published by Bornova Olive Grove Research Institute (Fig 3), there is no similarity with the Tavşan Yüreği examined, especially in terms of its stone structure (Fig 4).

As stated in the book 'Our Olive Varieties' prepared by Dr. Nejat Özlübey. The Tavşan Yüreği olive variety in the National Collection Garden and the Tavşan Yüreği variety registered by BATEM are different from each other. So, both Tavşan Yüreği varieties are Homonym. (Homonym: Genotypes named by the same name but genetically disparate. Synonym: The same genotypes named by different names but genetically identical).

CONCLUSION

Tavşan Yüreği olive variety, which is spreading especially in Kepez and Manavgat districts in Antalya province, has been determined to be different from the Tavşan Yüreği olive variety in the catalogs prepared by the Olive Research Institute, based on molecular analyses and morphological measurements made from leaf and fruit samples collected from different locations in Antalya province, including BATEM, where the Tavşan Yüreği olive variety is registered (Anonymous, 2021b). Introducing to the world the olive oil varieties produced from olives that are in danger of disappearing, the Anatolian Olive Oils Project processes rare olives native to Anatolia in a mobile olive oil processing machines are using after harvesting and makes them part of the tables (Fig 5).

Conflict of interest: None.

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