Mugla Journal of Science and Technology

GENETIC CHARACTERIZATION OF RELICT ENDEMIC LIQUIDAMBAR ORIENTALIS (ALTINGIACEAE) POPULATIONS

Ömer YÜZER*, Department of Medical Laboratory Techniques, Vocational School, Doğuş University, Istanbul, Turkey, oyuzer@dogus.edu.tr
(https://orcid.org/0000-0003-0209-324X)
Alper TONGUÇ, Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey, alpertonguc@mu.edu.tr
(https://orcid.org/0000-0002-2613-3114)
Ersin DOĞAÇ, Department of Molecular Biology and Genetics, Faculty of Science, Muğla Sıtkı Koçman University, Muğla, Turkey, ersindogac@mu.edu.tr
(https://orcid.org/0000-0003-4426-2187)

Received: 15.01.2024, Accepted: 03.04.2024
*Corresponding author
Research Article
DOI: 10.22531/muglajsci.1419958

Abstract

Liquidambar orientalis, a relict endemic species, is distributed in the south-west of Turkey. The natural spread areas of the species have been decreasing since the early 1900s. This poses a great threat to the future and genetic diversity of the species. In this study, the genetic diversity of a total of 190 individuals in ten populations was examined with ten polymorphic ISSR primers. Data analysis was performed using POPGENE ver. 1.32 and GenAlEx 6.503 softwares. According to the results of the analysis, a total of 271 loci were identified. For the ten populations, the rate of polymorphic loci ranged from 21.03% to 34.69%. The mean number of observed alleles (Na) and mean number of effective alleles (Ne) were found as 1.98 ± 0.13 and 1.47 ± 24 0.37, respectively. Nei's genetic diversity values (h) ranged from 0.07 ± 0.15 to 0.12 ± 0.18. According to the results of Molecular Analysis of Variance (AMOVA) and POPGENE ver. 1.32 analysis, it was determined that the main contribution to the genetic variance originated from among the populations. In addition, the gene flow level (NM) was found to be low with a value of 0.27 per generation.

Keywords: Genetic Diversity, ISSR Marker, *Liquidambar orientalis*, Muğla, Polymorphism

RELİKT ENDEMİK LIQUIDAMBAR ORIENTALIS (ALTINGIACEAE) POPULASYONLARININ GENETİK KARAKTERİZASYONU

Özet

Relikt endemik bir tür olan Liquidambar orientalis, Türkiye'nin güneybatısında yayılış göstermektedir. Türün doğal yayılış alanları 1900'lü yılların başlarından itibaren giderek azalmaktadır. Bu durum türün geleceği ve genetik çeşitliliği açısından büyük bir tehdit oluşturmaktadır. Bu çalışmada on popülasyondaki toplam 190 bireyin genetik çeşitliliği on adet polimorfik ISSR primeri ile incelenmiştir. Verilerin analizi POPGENE ver. 1.32 ve GenAlEx 6.503 yazılımları ile gerçekleştirilmiştir. Analiz sonuçlarına göre toplam 271 lokus tespit edildi. On popülasyon için polimorfik lokusların oranı %21,03 ile %34,69 arasında değişmektedir. Gözlenen ortalama alel sayısı (Na) ve etkili alellerin ortalama sayısı (Ne) sırasıyla 1,98±0,13 ve 1,47±24±0,37 olarak bulundu. Nei'nin genetik çeşitlilik değerleri (h) 0,07 ± 0,15 ila 0,12 ± 0,18 arasında değişmektedir. Moleküler Varyans Analizi (AMOVA) ve POPGENE ver. 1.32 analizinde genetik varyansa asıl katkının popülasyonlar arasında kaynaklandığı belirlendi. Ayrıca gen akış düzeyinin (NM) nesil başına 0,27 değeriyle düşük olduğu tespit edildi.

Anahtar Kelimeler: Genetik Çeşitlilik, ISSR Markörü, *Liquidambar orientalis*, Muğla, Polimorfizm

Cite

Yüzer, Ö., Tonguç A., Doğaç E., (2024). "Genetic Characterization of Relict Endemic *Liquidambar orientalis* (Altingiaceae) Populations", Mugla Journal of Science and Technology, 10(1), 42-50.

1. Introduction

Species belonging to the genus *Liquidambar* classified in the Altingiaceae family are called sweetgum trees. *Liquidambar* means fragrant liquid, formed by the combination of Latin word liquidus (liquid) and Arabic word amber (fragrant) [1]. *Liquidambar* genus is represented by four different species located on approximately the same latitudes on Earth. These species are distributed in three continents in the Northern Hemisphere, America, South East Europe and Asia. These species belonging to the genus *Liquidambar* are: "*L. acalycina*", which is distributed in Central and Southern China; "*L. formosana*", which is distributed in

South China, Democratic People's Republic of Korea, Republic of Korea, Republic of China, Lao People's Democratic Republic and North Viet Nam; "*L. styraciflua*", which is distributed in Southeast-Central America and Mexico, and "*L. orientalis*", which is distributed in South Western Türkiye [2]. The genus *Liquidambar* is also thought to have existed for about 65 million years. Because of this situation, species in the *Liquidambar* genus are called relict species. *L. orientalis*, also known as Anatolian Sweetgum Tree, is a relict and endemic species for Türkiye. [3-9]. According to [10], there are two varieties of *L. orientalis*. These are *L. orientalis* Mill. var. *orientalis* and *L. orientalis* Mill. var. *integriloba* Fiori. Relict and endemic *L. orientalis* species is spreaded in South Western Türkiye. Although it generally spreads in Köyceğiz, Marmaris, Fethiye, Ula and Dalaman districts within Muğla province, there are sweetgum trees in certain regions in Aydın, Denizli, Antalya, Burdur, Isparta. *L. orientalis* are found in humid environments such as streams and small streambeds as a habitat. It spreads in regions with high climatic rainfall, sandy wetlands and acidic soils. One of the biggest factors in its development is the availability of water. For this reason, sweetgum forests may spread along the area where water spreads on flat lands, while in rough lands where there is a land slope, it can only spread along the line where the water is located. It spreads in the regions where Mediterranean Climate is dominant in Southwestern Türkiye. In this case, it can be said that temperature is an important factor for the spread areas. In addition, it has been stated in different studies that the spread area can vary from sea level to 1000 meters elevation [3-5,7-9]. The sweetgum tree is a type of plant that is about 20-25 meters tall, has a thick branch structure, has leaves connected to the branches with thin long stems and has a wide top. Sweetgum tree is very similar to the plane tree. It is a deciduous species. Although the leaves generally appear with 5 lobes, they can rarely be found as 3 or 7 lobes. These lobes can have blunt and pointed tips. In addition, the lobes on the leaf are divided into several lobes again. Leaves have an aromatic scent. They can live to be about 200-300 years old. When the tree is young, it has a thin and long trunk structure. A thicker trunk structure is observed in old trees. As the tree ages, its bark darkens and its trunk thickens. There are balsam channels to repair the injuries that may occur under the trunk of the tree. The liquid obtained from these channels is called sweetgum oil. In order to obtain this sweetgum oil, which has medical and economic value, wounds are created on the tree trunk. This oil is used in the treatment of diseases such as asthma, bronchitis, lung, ulcer and gastritis. In addition, it is used as an odor stabilizer in the perfume industry. For this reason, as the tree ages, large and deep wounds occur on its trunk [5,7,1112]. The spreading areas of sweetgum trees have decreased significantly from past to present. The sweetgum forests, which spread over an area of 7000 hectares in 1947, are thought to have a spread between 1500 and 2000 hectares today. Sweetgum oil is a product that has been produced and exported for years due to its medicinal and economic value. Over the years, the production and export of sweetgum oil has decreased in proportion to the decrease in the spreading areas of sweetgum forests. When the past records are examined, sweetgum oil, which was produced as 200 tons per year, can be produced in much less amount today. The main reasons for this situation are the unconscious destruction of sweetgum trees in order to generate economic income, the cutting of trees for agricultural and tourism purposes, and forest fires [1, 13]. Decreasing areas of spread threaten the future of *L. orientalis*. Genetic diversity increases the chances of survival of species against changing environmental conditions. It also contributes to the saving of species in danger of extinction through the implementation of effective conservation programs. For this reason, it is very important to determine the genetic diversity of the species. The study aimed to determine the genetic diversity of *L. orientalis* species by using ISSR primers. Allozyme [14,15], chloroplast DNA (cpDNA) [16-18] and randomly amplified polymorphic DNA (RAPD) [9,12] studies were conducted to understand the genetic structure of the species. However, there are no studies in the literature using simple sequence repeat (ISSR) markers. Therefore, this study is the first attempt to determine the genetic diversity of South-West Anatolian *L. orientalis* populations by using ISSR markers.

2. Materials and Methods

2.1. Plant Materials

Natural sweetgum forests spreading in Muğla province were determined as the study area. Field studies were carried out between April and June 2019, and firstly, general spread areas and boundaries of the population where the field study will be conducted were determined in natural forest. Then, transects were determined to divide the entire population area at equal intervals. Transect refers to a linear straight line. Ten populations were sampled with reference to the transects determined for each population. This sampling was carried out with approximately 50 to 100 meters between each individual, depending on the population size. In order to obtain 20 individuals from each population (excluding Kızılyaka 2 population), different numbers of transects were created according to the geographical features of the populations (land slope, topography, etc.). Information on the populations where the fieldwork was conducted and their population numbers are given in Table 1 and Figure 1.

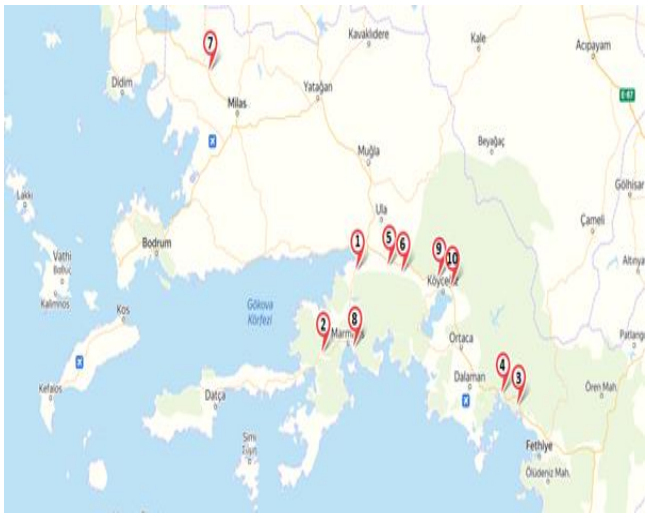

Figure 1. Location information on studied populations.

2.2. Genomic DNA Extraction

Twenty individuals from each of the nine populations (Marmaris Cetibeli, Marmaris Degirmenyanı, Fethiye Gunluklu, Fethiye Inlice, Ula Kızılyaka 1, Milas Selimiye, Marmaris National Park, Koycegiz Toparlar, Koycegiz Zeytinalanı) and ten individuals from a population (Ula Kızılyaka 2) were used for DNA extraction. Sweetgum leaves taken from individuals constituting the population were crushed using approximately 5 ml of liquid nitrogen in order to obtain gDNA. Sweetgum leaves, pulverized by the crushing process, were weighed and used at approximately 0.03 grams to initiate DNA extraction. Then the optimized Soltis Lab DNA CTAB extraction protocol was followed [19,20]. DNA samples, whose purity and concentration were determined by spectrophotometer, were diluted to 10 ng/µl and stored at -20°C for PCR.

Table 1. Varieties, individual numbers and location information of studied populations.

Population numbers	Populations	Variety information	Number of individuals	Location
1	Marmaris–Cetibeli	*L. orientalis* Mill. var.*integriloba* Fiori	20	36° 58' N/28° 17' E, 30m
2	Marmaris-Degirmenyanı	*L. orientalis* Mill. var.*integriloba* Fiori	20	36° 49' N/28° 08' E, 22m
3	Fethiye–Gunluklu	*L. orientalis* Mill. var.*orientalis*	20	36° 43' N/29° 01' E, 15m
4	Fethiye–Inlice	*L. orientalis* Mill. var.*orientalis*	20	36° 43' N/28° 57' E, 12m
5	Ula–Kızılyaka 1	*L. orientalis* Mill. var.*integriloba* Fiori	20	37° 01' N/28° 27' E, 112m
6	Ula–Kızılyaka 2	*L. orientalis* Mill. var.*integriloba* Fiori	10	37° 01' N/28° 27' E, 111m
7	Milas–Selimiye	*L. orientalis* Mill. var.*orientalis*	20	37° 26' N/27° 39' E, 92m
8	Marmaris–National Park	*L. orientalis* Mill. var.*integriloba* Fiori	20	36° 50' N/28° 17' E, 8m
9	Koycegiz–Toparlar	*L. orientalis* Mill. var.*integriloba* Fiori	20	36° 59' N/28° 38' E, 11m
10	Koycegiz-Zeytinalanı	*L. orientalis* Mill. var.*integriloba* Fiori	20	36° 57' N/28° 43' E, 20m

2.3. Polymerase Chain Reaction

PCR was performed in a total volume of 25 µl. The reaction consisted of 12.3 µl of dH_2O, 10.5 µl of Thermo Scientific DreamTaq PCR Master Mix (2×), 1 µl of primer, 0.2 µl of Tween®20 and 1 µl of DNA. PCR cycling conditions were performed at 95 °C for 15 seconds, 55 °C for 30 seconds, and 68 °C for 3 minutes for 35 cycles. In addition, a pre-denaturation at 95 °C for 3 minutes and a final elongation at 72 °C for 10 minutes were performed. Ten ISSR primers were used for amplification: $(ACC)_6CC$, $CCA(TG)_7T$, $GCA(AC)_7$, $GGG(AC)_7$, $(GA)_8GG$, $(AG)_8TA$, $(GT)_8C$, $(AC)_8T$, $(GGGTG)_3$ and $(GA)_8TC$. PCR products were run for 3 hours on a 1% agarose gel containing ethidium bromide (0.5 µg/mL) at 70V. PCR products run in the gel were visualized and photographed under UV light with the gel imaging system (Figure 2).

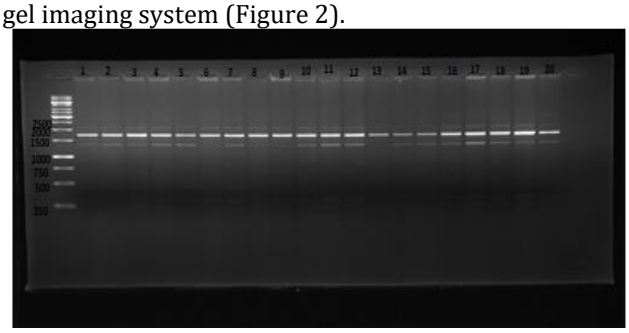

Figure 2. PCR amplification products generated from Fethiye Inlice population using primer number 10 [$(GA)_8TC$] were visualized by staining with ethidium bromide on 1% agarose gel.

2.4. Analysis of data

Gel images obtained as a result of screening a total of one hundred and ninety individuals from ten different populations with ten primers were scored according to the band profiles created by the Thermo Scientific™ GeneRuler 1 kb DNA Ladder used during electrophoresis. According to the band profile created by the DNA ladder used, the lengths of the band profiles formed by all individuals were determined and scored as 1 if there is band formation in the specified length, and 0 if there is no band formation. After 1-0 scoring was processed on Microsoft Excel, the data were analyzed using POPGENE ver. 1.32 [21] and GenAlEx 6.503 computer software [22]. As a result of the POPGENE ver. 1.32 analysis; allele number [23], effective allele number [24], polymorphic locus rate and genetic distance [23] are also revealed using appropriate formulas. Genetic variation within and among populations was calculated as a result of AMOVA analysis performed with GenAlEx 6.503 software. Phylogenetic relationships for populations were created using TreeView X software [25].

3. Results

Genetic characterization of one hundred and ninety individuals from ten different populations of Muğla province was created using ten different ISSR primers. Two hundred and seventy-one loci were identified from the ten primers used. Information on the primers used and the number of loci detected with these primers are given in Table 2.

Table 2. Information on primers sequence, number of total bands, percent of polymorphic bands and fragment size used for the analysis of the genetic variation of *Liquidambar orientalis* populations.

Primer Numbers	ISSR-Primer Sequence (5'-_3')	Number of total bands	Number of polymorphic bands	Number of monomorphic bands	Percent of polymorphic bands	Range of fragment sizes (bp)
1	$(ACC)_6CC$	26	26	0	100	250-1550
2	$CCA(TG)_7T$	32	32	0	100	450-2800
3	$GCA(AC)_7$	38	38	0	100	250-3000
4	$GGG(AC)_7$	34	34	0	100	250-2500
5	$(GA)_8GG$	17	17	0	100	350-1500
6	$(AG)_8TA$	30	30	0	100	300-3000
7	$(GT)_8C$	25	25	0	100	300-2400
8	$(AC)_8T$	26	25	1	96.15	250-2600
9	$(GGGTG)_3$	27	24	3	88.88	300-2500
10	$(GA)_8TC$	16	15	1	93,75	250-3000
	Total	271	266	5	-	-
	Mean	27.1	26,6	0,5	100	295-2485

According to Table 2, it was determined that primer number 3 formed the most band formation and primer 5 numbered the least band formation. It was determined that ten primers used produced an average of 27 bands. In addition, it was determined that an average of 26 of these bands were polymorphic. Five of the identified loci are monomorphic loci, and 266 are polymorphic loci. The rate of polymorphism in all loci was found to be 98.15%. Primers used formed at least 88.88% polymorphic band. The length of the bands formed varied between 250 and 3000 bp.

Table 3. Number of observed, polymorphic and private bands and percentage of polymorphic loci of all populations.

Population	Sample Size	Number of bands	Number of polymorphic bands	Number of private bands	%Polymorphism
Marmaris Cetibeli	20	130	70	8	25.83
Marmaris Degirmenyanı	20	145	83	6	30.63
Fethiye Gunluklu	20	129	94	1	34.69
Fethiye Inlice	20	129	87	5	32.10
Ula Kızılyaka 1	20	112	86	6	31.73
Ula Kızılyaka 2	10	130	57	5	21.03
Milas Selimiye	20	118	75	4	27.68
Marmaris National Park	20	151	82	10	30.26
Koycegiz Toparlar	20	133	63	4	23.25
Koycegiz Zeytinalanı	20	123	74	7	27.31
Mean	19	130	77.1	5.6	28.45

The data on the number of bands obtained as a result of the GenAlEx 6.503 analysis are given in Table 3. Accordingly, it has been determined that the population with the most bands is the Marmaris National Park location, which creates 151 bands. The location with the least bands is Ula Kızılyaka 1 location, which creates 112 bands. The location with the highest number of polymorphic bands in the bands obtained by

using ten primers is Fethiye Gunluklu location. The location that creates the least polymorphic band is Ula Kızılyaka 2 location. According to the Nei's genetic diversity values obtained as a result of POPGENE ver. 1.32 analysis, the percentage polymorphism rate of the populations varied between 23.25% to 34.69%.

Table 4. Genetic diversity parameters between populations. *Na*: the mean number of observed alleles; *Ne*: the mean number of effective alleles; *h*: Nei's gene diversity; *I*: Shannon's information index.

Populations	*Na*	*Ne*	*h*	*I*
Marmaris-Cetibeli	1,25±0,43	1,13±0,28	0,07±0,15	0,12±0,22
Marmaris-Degirmenyanı	1,30±0,46	1,17±0,32	0,10±0,17	0,15±0,25
Fethiye-Gunluklu	1,34±0,47	1,21±0,34	0,12±0,18	0,18±0,27
Fethiye-Inlice	1,32±0,46	1,19±0,33	0,11±0,18	0,16±0,26
Ula-Kızılyaka 1	1,31±0,46	1,19±0,34	0,11±0,18	0,16±0,26
Ula-Kızılyaka 2	1,21±0,40	1,15±0,32	0,08±0,17	0,12±0,24
Milas-Selimiye	1,27±0,44	1,16±0,31	0,09±0,17	0,14±0,24
Marmaris-National Park	1,30±0,46	1,16±0,29	0,10±0,16	1,15±0,24
Koycegiz-Toparlar	1,23±0,42	1,12±0,26	0,07±0,15	0,10±0,22
Koycegiz-Zeytinalanı	1,27±0,44	1,16±0,31	0,09±0,17	0,14±0,24

Genetic variation statistics obtained for all population loci using POPGENE ver 1.32 software are given in Table 4. The average number of observed alleles (*Na*), the average number of effective alleles (*Ne*), Nei's gene diversity (*h*) and Shannon's information index (*I*) values were estimated using POPGENE ver 1.32. Fethiye Gunluklu, Ula Kızılyaka 1 and Fethiye İnlice populations generally had the highest genetic diversity values. Information on genetic diversity for all populations is given in Table 5.

Table 5. Genetic diversity, genetic differentiation and gene flow values for all populations.

	H_T	H_S	D_{ST}	G_{ST}	N_M
Average of All Populations	0,27±0,03	0,09±0,007	0,18	0,64	0,27

The total genetic diversity (H_T) in all populations was found to be 0.27±0.03. A low portion of this diversity, 0.09 ± 0.007, was owing to within population genetic variation (H_S). Genetic diversity among populations (D_{ST}), was found as 0.18 from H_T and H_S values (Table 5). According to these values, approximately 33% of the genetic diversity consists of within population genetic variation, while approximately 67% is due to among population genetic variation. According to the Analysis of Molecular Variance (AMOVA) obtained from GenAlEx 6.503 software results, the major contribution to genetic variance (71%) was due to variation among populations. Genetic differentiation level (G_{ST}) was calculated as 0.64 and gene flow value (N_M) was calculated as 0.27 with the POPGENE ver. 1.32 software. Genetic distance value (D_N) of populations was calculated using POPGENE ver. 1.32 software.

Table 6. Estimates of Nei's genetic distance (D_N) coefficients among the *L. orientalis* populations.

	Marmaris Cetibeli	Marmaris Degirmenyanı	Fethiye Gunluklu	Fethiye Inlice	Ula Kızılyaka 1	Ula Kızılyaka 2	Milas Selimiye	Marmaris National Park	Koycegiz Toparlar	Koycegiz Zeytinalanı
Koycegiz Zeytinalanı										*****
Koycegiz Toparlar									*****	0,2598
Marmaris National Park								*****	0,2353	0,3037
Milas Selimiye							*****	0,2245	0,2600	0,2186
Ula Kızılyaka 2						*****	0,2258	0,2763	0,2791	0,2376
Ula Kızılyaka 1					*****	0,1615	0,2008	0,2593	0,2477	0,1898
Fethiye Inlice				*****	0,1851	0,2771	0,2113	0,2652	0,2392	0,2066
Fethiye Gunluklu			*****	0,1866	0,1969	0,3111	0,2405	0,2363	0,2589	0,2483
Marmaris Degirmenyanı		*****	0,1893	0,2451	0,2715	0,3348	0,2719	0,2743	0,2675	0,3090
Marmaris Cetibeli	*****	0,2475	0,2655	0,2665	0,2168	0,2734	0,2385	0,2599	0,2394	0,2677

The genetic distance of each population from other populations is given in Table 6. Accordingly, the two genetically closest populations were determined as Ula Kızılyaka 1 and Ula Kızılyaka 2 populations with genetic distance value (D_N): 0.1615. The two populations with the most genetic distance from each

other were observed as Marmaris Degirmenyani and Ula Kızılyaka 2 populations with genetic distance value (D_N): 0.3348 (Table 6). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram studied populations were constructed according to Nei's [26] standard genetic distances and the dendrogram was given in Figure 3.

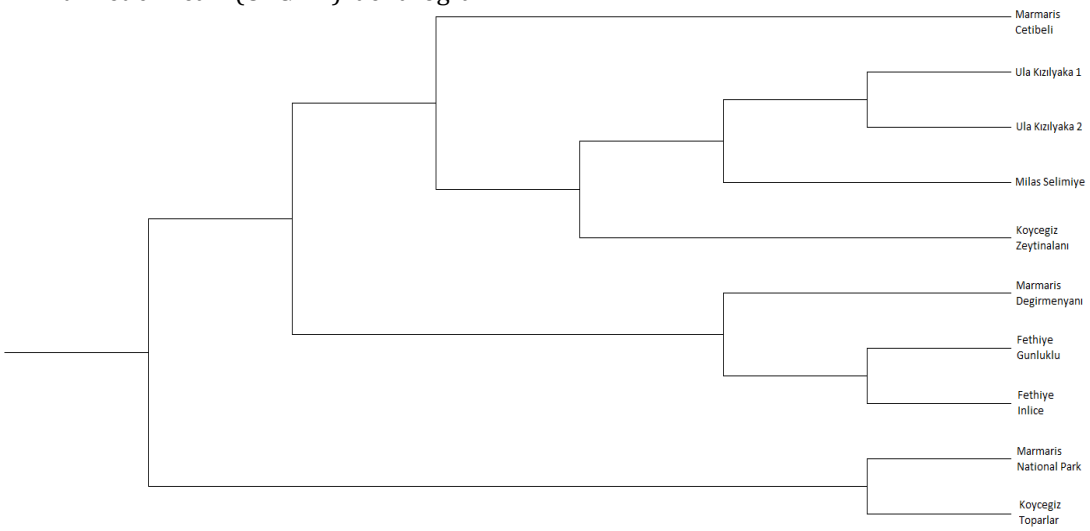

Figure 3. UPGMA dendrogram based on Nei's (1972) genetic distance among *L. orientalis* populations.

The tree structure is divided into three groups. The first of these groups consisted of the Marmaris Cetibeli, Ula Kızılyaka 1, Ula Kızılyaka 2, Milas Selimiye and Koycegiz Zeytinalanı populations, the second from the Marmaris Degirmenyanı, Fethiye Inlice, and Fethiye Gunluklu populations, and the third from the populations of Marmaris National Park and Koycegiz Toparlar.

4. DISCUSSION

L. orientalis, which is a relict endemic species in Türkiye, is one of the most important and remarkable species of Türkiye in terms of biodiversity. Determining the genetic diversity of populations is very important to protect species biodiversity. As a result of the literature search, it was determined that several different methods were used to determine the genetic diversity of *L. orientalis* populations. On the other hand, there are no studies in the literature using ISSR markers, which also constitute the originality of this study. When the molecular studies made with sweetgum are examined in the literature, there are some studies using isoenzymes that are biochemical markers [14,15]. In addition, with the introduction of molecular markers, the use of biochemical markers has become less preferred. The reason for this is that molecular markers are more successful in detecting polymorphism than biochemical markers [27]. In the study carried out by [15], genetic diversity was investigated over 320 individuals in 14 sweetgum populations with 8 isoenzyme systems. For all populations, the average number of observed alleles (*Na*) was 1.84 and the average number of effective alleles (*Ne*) was 1.58. Although the average number of alleles from these values is lower than the value in this study, the average effective allele number is higher. In [12] study, 320 individuals from 14 *L. orientalis* populations and 30 RAPD primers were used. As a result of the study, a total of 453 loci were identified and 6 of these loci were found to be monomorphic for all populations. According these data, it was determined that the average number of polymorphic loci detected for each primer was higher in ISSR primers (while an approximately of 15 polymorphic loci per primer was determined in RAPD primers, an approximately of 27 polymorphic loci per primer was detected with ISSR primers). The eight populations used in this study and [12] are the same. These are Milas Selimiye, Köyceğiz Zeytinalanı, Köyceğiz Toparlar, Ula Kızılyaka, Marmaris Çetibeli, Marmaris Değirmenyanı, Fethiye Gunluklu and Fethiye Inlice populations. The observed average allele number, average effective allele number, genetic diversity in the population, polymorphic loci number and percent polymorphism values for these locations were found to be lower than the study of [12]. The main reason for these values being higher than our study is thought to be the higher number of individuals and primers used in the study with RAPD. Although the sampling sites in both studies are the same, the sampling in this study was carried out approximately 15 years after the sampling conducted by [12]. The loss of genetic diversity in populations during this period may also be another reason for the lower values found. [9] investigated the genetic diversity of *L. orientalis* populations using 10 RAPD primers and 25 individuals from each population in 18 populations. It was determined that all genetic diversity values obtained as a result of the study were lower than our study. Since both ISSR and RAPD primers are dominant markers, it is very important to determine the choice of which

primer to use in determining the polymorphism. When the genetic diversity values obtained in this study were compared with the values obtained by both [9] and [12], it was thought that ISSR primers were more successful in detecting polymorphism within the same population. It is thought that screening an equal number of samples collected from the same individual at the same locations with the same number of ISSR and RAPD primers will provide more accurate data to support this idea or to prove the opposite. When the Nei's genetic diversity values were examined in the results of the analysis, total genetic diversity values (H_T) = 0.27±0.03 in based of 271 loci for all populations. It was found that 33% of the total genetic diversity in 271 loci was due to within population genetic diversity values (H_S) = 0.09±0.007, and 67% of genetic diversity was found to be due to among population genetic diversity with D_{ST} = 0.18 values. As a result of the molecular analysis of variance (AMOVA), it was observed that 29% of the genetic diversity was due to within population genetic diversity and 71% to among population genetic diversity. Therefore, according to the results obtained from both POPGENE ver. 1.32 and GenAlEx 6.503 software, it has been clearly revealed that the main source of genetic diversity is among the populations. The main reason for this is thought to be limited gene flow among populations. G_{ST} is one of the values that reveal genetic differentiation. G_{ST} is observed in values ranging from 0 to 1 and expresses the level of genetic differentiation. If the G_{ST} value is 0.05 or less, genetic differentiation within the population is negligible. If it is above 0.25, the level of genetic differentiation is high. In this study, the G_{ST} value was found to be 0.63. That is, the genetic differentiation among the studied populations is quite high. N_M, is gene flow value, was calculated. It has been reported by [28] that the species with a gene flow (N_M) value of 0.265 are self-pollinating and can spread their seeds and pollen to short distances such as 2-3 meters. The gene flow (N_M) value of 4,750 has been reported to be the values used for species that pollinating from long distances with various seed and pollen carriers and can also spread their seeds and pollen to long distances. In addition, it has been reported that the gene flow level (N_M) of 0.50 is the critical value and the values above this value are the amounts that prevent genetic drift. If the N_M value is less than 1, it is considered that there is differentiation among populations [29]. In our study, the gene flow level (N_M) was found to be 0.29. The fact that this value is above 0.265 indicates that the population members pollinating with the trees that are close neighbors in the distribution area, while the value below 4.750 indicates that the pollinating caused by long distances is not too much or not at all. The fact that the genetic diversity (H_S) value within the population is already as low as 0.09 reveals that there is a limited gene flow (N_M: 0.29). The results of the G_{ST} and N_M values found and the AMOVA analysis are an indication that the studied populations may be subject to genetic drift and that individuals in the studied populations pollinating within the population and form the next generation. The genus Liquidambar is represented by four different species on Earth. There are some studies in the literature to determine genetic diversity by using ISSR markers in *L. formosana*, one of these species. [30] found the G_{ST} value of the L. formosana populations to be 0.185 and the N_M value to be 2.194. In addition, as a result of molecular variance analysis, it was determined that the genetic variation originates from among populations with a rate of 14.51% and within the population at a rate of 85.49%. [31], according to the results of AMOVA, genetic variation in *L. formosana* populations originates from within the population with a rate of 94.02% and among populations with a rate of 5.98%. [30] and [31] revealed that genetic diversity is mostly caused by within the population, with AMOVA, G_{ST} and N_M values. These values indicate that, unlike the *L. orientalis* species, *L. formosana* populations are not at risk of genetic drift. [30] and [31], the most important reason for the higher genetic diversity values and the absence of genetic drift risk for the species is that the *L. formosana* species has populations that spread over very large and undivided areas. For this reason, it is very important to protect the *L. orientalis* populations whose spread areas are decreasing day by day. *L. orientalis* species is considered by some researchers to have two varieties, *L. orientalis* Mill. var. *orientalis* and *L. orientalis* Mill. var. *integriloba* Fiori, in terms of morphological character. It can be said that *L. orientalis* Mill. var. *orientalis* and *L. orientalis* Mill. var. *integriloba* Fiori varieties do not differ from each other within the determined 271 loci. The second location pair with the least genetic distance from each other is Fethiye İnlice (*L. orientalis* Mill. var. *orientalis*) and Ula Kızılyaka 1 (*L. orientalis* Mill. var. *integriloba* Fiori) populations (Table 6). In other words, *L. orientalis* species, which is morphologically divided into two varieties, could not be genetically separated into two different varieties after this study. When Nei's genetic distance values were examined, the two populations with the least genetic distance to each other were found with a value of 0.1615, Kızılyaka 1 and Kızılyaka 2. Kızılyaka 1 and Kızılyaka 2 populations were separated from each other due to the tree cuttings and roads made by humans. For this reason, it is an expected result that the genetic distance between them is the least compared to other populations. Among the studied populations, the locations with the furthest distance are the Selimiye population in Milas district and the İnlice and Gunluklu populations in Fethiye district. Therefore, it was expected that the genetic distance from each other would be the highest between these populations in the analysis results. However, in the analysis results, the populations with the highest genetic distance from each other were determined as Marmaris Değirmenyanı and Kızılyaka 2 populations with a value of 0.3348. Although they are located in

neighboring districts, these two populations are quite far from each other (Figure 1). In addition, the reason for the highest genetic distance value may be the geographical isolation between the two populations created by the mountains and the sea. The UPGMA method divided the populations into three groups. The first of these groups consisted of the Marmaris Cetibeli, Ula Kızılyaka 1, Ula Kızılyaka 2, Milas Selimiye and Koycegiz Zeytinalanı populations, the second from the Fethiye Gunluklu, Fethiye Inlice and Marmaris Degirmenyanı populations, and the third from the populations of Marmaris National Park and Koycegiz Toparlar. When the geographical locations and distances of the populations are examined, it is clear that the closest locations to each other are the Kızılyaka 1-Kızılyaka 2 population pairs and the Fethiye Inlice-Fethiye Gunluklu population pairs (Figure 1). Therefore, these two population pairs were expected to be in the same tree branches in the UPGMA dendrogram. The obtained UPGMA dendrogram supports the expected result (Figure 3). Species must be protected in order to preserve genetic diversity and transfer it to the next generations. In situ conservation should be initiated primarily for all natural *L. orientalis* populations to preserve the current genetic diversity of the species. As a result of this study, the three populations with the highest parameters (average number of alleles, number of effective alleles, Nei's genetic diversity, Shannon's constant, number of polymorphic loci and polymorphic loci) revealing genetic diversity were determined as Fethiye Gunluklu, Fethiye İnlice and Kızılyaka 1. In the studies conducted by [12] and [17], it was determined that the Gunluklu and Kızılyaka populations were among the populations with the highest genetic diversity values. In this context, it is suggested that priority should be given to these three populations in a conservation program to be initiated for *L. orientalis* species. It is recommended that ex-situ protection programs be activated following the in-situ protection programs initiation.

5. Acknowledgment

This work was supported by the Scientific Research Projects (BAP) Muğla Sıtkı Koçman University under Grant numbers [19/080/02/1/2 and 21/121/02/1].

6. References

[1] Arslan, M.B. and Şahin, H.T., "Unutulan bir orman ürünü kaynağı: anadolu sığla ağacı (*Liquidambar orientalis* Miller)", *Bartın Orman Fakültesi Dergisi*, 18(1): 103-117, 2016.

[2] Ickert-Bond, S.M., Pigg, K.B. and Wen, J., "Comparative infructescence morphology in *Liquidambar* (Altingiaceae) and its evolutionary significance", *American Journal of Botany*, 92(8): 1234–1255, 2005. https://doi.org/10.3732/ajb.92.8.1234

[3] Acatay, A., "Sığla Ağacı'nın (*Liquidambar orientalis* Mill.) Türkiye'de yayılışı, yeni tespit edilen *Liquidambar orientalis* var. *suber* varyetesi ve sığla ağacına musallat olan böcekler", *İstanbul Üniversitesi Orman Fakültesi Dergisi*, 8(2), 40-56, 1963.

[4] Atay, I., "Sığla ağacının (*Liquidambar orientalis* Mill.) önemi ve sivikültürel özellikleri", *İstanbul Üniversitesi Orman Fakültesi Dergisi,* 35: 15-21, 1985.

[5] Efe, A., "*Liquidambar orientalis* Mill. (sığla ağacı)'ın morfolojik ve palinolojik özellikleri üzerine araştırmalar", *İstanbul Üniversitesi Orman Fakültesi Dergisi,* 37 (2): 273-286, 1987.

[6] Akman, Y., Ketenoğlu, O. and Kurt, L., "Fethiye-Marmaris ve Bucak çevrelerinde yetişen *Liquidambar orientalis* Mill. topluluklarının floristik yapısı", *Doğa-Turkish Journal of Botany*, 16, 273-86 1992.

[7] Günal, N., "*Liquidambar orientalis* (Anadolu sığla ağacı)'in güneybatı Anadolu'daki yayılışında relief iklim ilişkileri", *Türk Coğrafya Dergisi*, 29: 175-190, 1994.

[8] İstek, A. and Hafızoğlu, H., "Sığla ağacı (*Liquidambar orientalis* Mill.) odununun anatomik özelliklerinin belirlenmesi", *Bartın Orman Fakültesi Dergisi,* 1(1): 18-26, 1998.

[9] Velioğlu, E., Kandemir, G., İcgen, Y., Çengel, B., Alan, M. and Kaya, Z., "Türkiye'deki sığla *(Liquidambar orientalis* Mill*.)* populasyonlarının genetik yapısının moleküler belirteçlerle belirlenmesi ve koruma stratejileri geliştirilmesi", *Orman Ağaçları ve Tohumları Islah Araştırma Müdürlüğü Teknik Bülten No: 20*, Ankara, 2008.

[10] Davis, P.H., "Flora of Turkey and The East Aegean Islands Vol. 4.", *Edinburgh University Press*, Edinburgh, 1972.

[11] Aydıngöz, M. and Bulut, S., "Egenin gizli kalmış şifa iksiri: Sığla", *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, 14: 1-6, 2014.

[12] Doğaç, E., "Türkiye'deki relikt endemik sığla ağacı (Liquidambar orientalis Mill. var. orientalis ve L. orientalis Mill. var. integriloba Fiori) populasyonlarındaki genetik çeşitliliğin RAPD (Rastgele Üretilen Polimorfik DNA) belirteçleri yardımıyla belirlenmesi", Master thesis, *Muğla Üniversitesi Fen Bilimleri Enstitüsü*, Muğla, Türkiye, 2008.

[13] Ürker, O., "Çevre etiği bakımından Anadolu sığla ormanları", PhD, *Ankara Üniversitesi Sosyal Bilimler Enstitüsü*, Ankara, Türkiye, 2014.

[14] Hoey, M.T. and Parks, C.R., "Isozyme divergence between eastern Asian, North American, and Turkish species of *Liquidambar* (Hamamelidaceae)", *American Journal of Botany*, 78: 938-947, 1991. https://doi.org/10.1002/j.1537-2197.1991.tb14497.x

[15] Öztürk, E., "Türkiye'deki relikt endemik sığla ağacı (*Liquidambar orientalis* Mill. var *orientalis* ve *L. orientalis* Mill. var*. integriloba* Fiori) populasyonlarında izoenzim polimorfizminin polyakrilamid jel elektroforezi (Page) yöntemi ile

saptanması", Master thesis, *Muğla Üniversitesi Fen Bilimleri Enstitüsü*, Muğla, Türkiye, 2008.

[16] Or, M., "Kloroplast genomundaki kodlanmayan trn bölgelerinin karşılaştırılması yapılarak *Liquidambar orientalis* Mill. varyetelerinin filogenetik analizi", Master thesis, Orta Doğu Teknik Üniversitesi, *Fen Bilimleri Enstitüsü*, Ankara, Türkiye, 2007.

[17] Özdilek, A., "Kloroplast genomundaki matk gen bölgesine göre *Liquidambar orientalis* Mill. varyetelerinin genetik farklılaşması", Master thesis, *Orta Doğu Teknik Üniversitesi Fen Bilimleri Enstitüsü*, Ankara, Türkiye, 2007.

[18] Doğaroğlu, T., Günenç, E., Manap, R.Y., Taşkın, V., Taşkın, B.G., and Doğaç, E., "Genetic Structure and Phylogenetic Analysis of Liquidambar orientalis Mill.(Altingiaceae) Populations Based on Non-Coded psaA/ycf3 Intergenic Region in The Chloroplast Genome in Türkiye", *Turkish Journal of Bioscience and Collections*. *7*(2), 45-58, 2023.

[19] Doyle, J.J. and Doyle, J.L., "A rapid DNA isolation procedure for small quantities of fresh leaf tissue", *Phytochemical Bulletin*, 19, 11-15, 1987.

[20] Cullings, K.W., "Design and testing of a plant-specific PCR primer for ecological and evolutionary studies", *Molecular Ecology*, 1: 233-240, 1992. https://doi.org/10.1111/j.1365-294X.1992.tb00182.x

[21] Yeh, F.C., Yang, R.C. and Boyle, T., POPGENE Version 1.32: Microsoft Window-Based Freeware for Population Genetics Analysis, *University of Alberta*, Edmonton, 1999.

[22] Peakall, R. and Smouse, P.E., "GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research: an update", *Bioinformatics*, 28: 2537-2539, 2012. https://doi.org/10.1093/bioinformatics/bts460

[23] Nei, M., "Molecular Evolutionary Genetics", *Columbia University Press*, New York, 1987.

[24] Kimura, M. and Crow, J.F., "Effect of overall phenotypic selection on genetic change at individual loci", *Proceedings of the National Academy of Sciences,* 75(12): 6168-6171, 1978. https://doi.org/10.1073/pnas.75.12.6168

[25] Page, R.D.M., "Visualizing Phylogenetic Trees Using TreeView". *Current protocols in bioinformatics*, 6 (2): 1-15, 2003. https://doi.org/10.1002/0471250953.bi0602s01

[26] Nei, M., "Genetic Distance Between Populations", *American Naturalist*, 106: 283-292, 1972. https://doi.org/10.1086/282771

[27] Ovesna, J., Polakova, K. and Leisova, L., "DNA analyses and their applications in plant breeding", *Czech Journal of Genetics and Plant Breeding*, 38(1): 29–40, 2002. https://doi.org/10.17221/6108-CJGPB

[28] Hamrick, J.L., Godt, M.J.W. and Sherman-Broyles, S.L., "Factors influencing genetic diversity in woody plant species", New Forests, 6: 95–124, 1992. https://doi.org/10.1007/BF00120641

[29] Wright, S., "The Genetical Structure of Populations". *Annals of Eugenics*, 15: 323-354, 1951. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x

[30] Bi, X.Q., Jin, X.Z. and Li, H.J., "Genetic diversity in the natural populations of *Liquidambar formosana* revealed by ISSR molecular markers", *Bulletin of Botanical Research*, 30: 120–125, 2010. https://doi.org/10.3390/f11020203

[31] Sun, R., Lin, F.R., Huang, P. and Zheng, Y., "Moderate genetic diversity and genetic differentiation in the relict tree *Liquidambar formosana* Hance revealed by genic simple sequence repeat markers", *Frontiers in Plant Science*, 7:1411, 2016. https://doi.org/10.3389/fpls.2016.01411